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ÉTICA

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Contenido

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Avaliação da qualidade do ar e pesquisa de fungos em uma clínica de hemodiálise no município de Caicó-RN, Brasil

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Resumo

Introdução: os fungos anemófilos possuem a capacidade de liberar no ambiente suas estruturas reprodutivas chamadas de esporos, os quais são transportadas pelo ar e ao encontrarem uma área propícia, depositam-se, colonizam e reproduzem-se. Diante dos fatos, este trabalho teve por objetivo isolar, quantificar e identificar a microbiota fúngica presente no ar do interior de uma clínica de hemodiálise situada no Rio Grande do Norte. **Metodologia:** para isso foi utilizado o método de sedimentação espontânea para a deposição desses esporos em placas de Petri com meio de cultura Agar Sabouraud Dextrose as quais foram expostas nos locais por 15 minutos, e incubadas a 28°C por até 14 dias. Após esse período, as colônias foram contadas, repicadas e identificadas macro e microscopicamente utilizando a técnica de microcultivo. **Resultados:** com a identificação foi possível notar a presença dos fungos filamentosos *Cladosporium* spp. (42,85%) o qual houve maior prevalência, *Aspergillus* spp. (7,14%), *Bipolaris* spp. (3,54%) e *Mycelia sterilia* (3,57%), além de uma quantidade de leveduras (42,85%) cujos gêneros não foram identificados. Além disso, foi utilizada uma equação para medir a qualidade do ar, e pode-se observar que a quantidade máxima de Unidade Formadoras de Colônias por metro cúbico (UFC/m^3) encontrada e a relação I/E (quantidade de fungos no interior do ambiente/quantidade de fungos no exterior do ambiente) estavam dentro dos limites estabelecidos pela ANVISA. **Conclusão:** assim sendo, a qualidade do ar foi

avaliada de acordo com os parâmetros da ANVISA e foi considerada satisfatória, pois a quantidade de UFC/m^3 e a relação I/E estão dentro dos limites estabelecidos. Esses dados são importantes para monitorar a saúde dos pacientes e dos profissionais que frequentam a clínica, bem como para prevenir possíveis infecções fúngicas.

Palavras-chave: Hemodiálise, fungos filamentosos, qualidade do ar.

Summary

Air quality assessment and fungus research in a hemodialysis clinic in the city of Caicó-RN, Brazil

Introduction: Airborne fungi have the ability to release into the environment their reproductive structures called spores, which are transported by the air and when they find a suitable area, they deposit, colonize, and reproduce. Given the facts, this work aimed to isolate, quantify and identify the fungal microbiota present in the air inside a hemodialysis clinic located in Rio Grande do Norte. **Methodology:** For this, the spontaneous sedimentation method was used for the deposition of these spores in Petri dishes with Sabouraud Dextrose Agar culture medium, which were exposed in the places for 15 minutes, and incubated at 28°C for up to 14 days. After this period, colonies were counted, picked and identified macro and microscopically using the microculture technique. **Results:** With the identification it was possible to notice the presence of the filamentous fungi *Cladosporium* spp. (42.85%) which had the highest prevalence, *Aspergillus* spp. (7.14%), *Bipolaris* spp. (3.54%), and *Mycelia sterilia* (3.57%), in addition to a number of yeasts (42.85%) whose genera were not identified. In addition, an equation was used to measure air quality, and it can be seen that the maximum amount of Colony Forming Unit per cubic meter (CFU/m^3) found and the I/E (amount of fungus inside the environment/amount of fungus outside the environment) ratio were within the limits established by ANVISA. **Conclusion:** therefore, the air quality was evaluated according to the ANVISA parameters and was considered satisfactory, since the amount of CFU/m^3 and the I/E ratio are within the established limits. These data are important to monitor the health of patients and professionals who attend the clinic, as well as to prevent possible fungal infections.

Keywords: Hemodialysis, filamentous fungi, air quality.

Resumen

Evaluación de la calidad del aire e investigación de hongos en una clínica de hemodiálisis en la ciudad de Caicó-RN, Brasil

Introducción: los hongos anemófilos tienen la capacidad de liberar en el ambiente sus estructuras reproductoras llamadas esporas, que son transportadas por el aire y cuando encuentran una zona adecuada, se depositan, colonizan y reproducen. Dados los hechos, este trabajo tuvo como objetivo aislar, cuantificar e identificar la microbiota fúngica presente en el aire dentro de una clínica de hemodiálisis ubicada en Rio Grande do Norte. **Metodología:** para ello, se utilizó el método de sedimentación espontánea para la deposición de estas esporas en placas Petri con medio de cultivo Agar Sabouraud Dextrosa que fueron expuestas en los lugares durante 15 minutos, e incubadas a 28°C por hasta 14 días. Tras este periodo, se contaron las colonias, se trasplantaron y se identificaron macro y microscópicamente mediante la técnica del microcultivo. **Resultados:** La identificación reveló la presencia de hongos filamentosos, *Cladosporium* spp. (42,85%) con la mayor prevalencia, *Aspergillus* spp. (7,14%), *Bipolaris* spp. (3,54%) y *Mycelia sterilia* (3,57%), además de una cantidad de levaduras (42,85%) cuyos géneros no fueron identificados. Además, se utilizó una ecuación para medir la calidad del aire, y se pudo observar que la cantidad máxima de Unidades Formadoras de Colonias por metro cúbico (UFC/m^3) encontrada y la relación I/E (cantidad de hongos dentro del ambiente / cantidad de hongos fuera del ambiente) estaban dentro de los límites establecidos por la ANVISA. **Conclusión:** por lo tanto, la calidad del aire fue evaluada según los parámetros de ANVISA y fue considerada satisfactoria, ya que la cantidad de UFC/m^3 y la relación I/E se encuentran dentro de los límites establecidos. Estos datos son importantes para vigilar la salud de los pacientes y profesionales que acuden a la clínica, así como para prevenir posibles infecciones fúngicas.

Palabras clave: Hemodiálisis, hongos filamentosos, calidad del aire.

INTRODUÇÃO

Os fungos são microrganismos importantes para natureza, desempenham um papel indispensável na decomposição de materiais orgânicos, na degradação de alimentos, porém algumas espécies podem atuar como parasitas em seres vivos, podendo, eventualmente, causar a morte destes. Estão em contato conosco todos os dias e são encontrados em praticamente qualquer local que nos cerca, inclusive no ar, o qual representa o

meio de dispersão mais bem-sucedido para que se reproduzam, espalhando suas estruturas reprodutoras em forma de conídios e esporos [1, 2].

Aqueles fungos que possuem a capacidade de dispersão no ar são chamados de anemófilos, e estão intimamente ligados a saúde do homem, podendo causar diferentes patologias respiratórias como asma e rinite, além de irritação na pele e nas mucosas, infecções fúngicas e manifestações gastrintestinais devido a ocorrências tóxico-alimentares [1]. Entre estes fungos que habitam o ar atmosférico, podemos destacar os gêneros *Alternaria*, *Aspergillus, Cladosporium*, *Curvularia*, *Fusarium* e *Penicillium* [3]. Entretanto, dependendo do grau de exposição, outras espécies também podem colonizar o ar [4].

A presença de infecções causadas por fungos anemófilos em ambientes hospitalares é relatada na literatura, devido à pouca circulação de ar, muitas vezes por esses ambientes serem parcial ou completamente isolados do ambiente externo. Muitas vezes são locais com uso de ar-condicionado, o que contribui ainda mais para a presença desses organismos, tornando a qualidade do ar interior mais crítica, o que reflete na vulnerabilidade dos pacientes que apresentam uma baixa imunidade [3, 5]. Desta forma, esses fungos agem de forma oportunista, causando infecções principalmente em pacientes debilitados e imunodeprimidos, como idosos, crianças, pacientes com doenças crônicas que fazem uso de cateter, fistula, diálise, pacientes com COVID-19, entre outros [6-10].

No Brasil existe um regulamento estipulado pela Agência Nacional de Vigilância Sanitária (ANVISA), a qual visa estabelecer padrões de qualidade do ar interior em ambientes climatizados artificialmente de uso público e coletivo. A resolução Nº 09 de 16 de janeiro de 2003 tem o objetivo de garantir a qualidade do ar nestes ambientes climatizados, através da manutenção destes equipamentos [11].

Nesse contexto, os pacientes acometidos por doenças renais como insuficiência renal aguda, nos quais os rins podem recuperar-se ou parar de funcionar subitamente e insuficiência renal crônica, em que os néfrons perdem sua funcionalidade ao longo do tempo, ao se contaminarem em um hospital pode acarretar a perda das funções renais [12].

Levando em consideração a gravidade do problema renal, o tratamento inicial pode ser feito à base de dietas e terapia medicamentosa e controle da pressão arterial, porém se esses tratamentos não surtem efeito, o paciente é submetido à hemodiálise pelo resto da vida ou até que seu quadro possa ser revertido ou ainda que possa ser submetido a um transplante renal [13].

A hemodiálise é uma operação para a filtração sanguínea que ocorre fora do corpo, realizado por máquinas. É um processo artificial que pode durar em média, de três a quatro horas, três dias por semana, onde o sangue é retirado do corpo do paciente e

encaminhado a um filtro, em seguida, é devolvido ao corpo [14, 15]. Um estudo de caso apontou que os gêneros que mais acometem pacientes renais são *Aspergillus, Rhizopus, Candida* e *Scedosporium,* os quais acometem tecido pulmonar e tecido ósseo; mucosas oral e vaginal, além de outros órgãos se disseminada; e infecções localizadas, respectivamente [16].

Desta forma, levando em consideração o potencial patogênico de alguns fungos e a vulnerabilidade desses pacientes com comprometimento renal, este trabalho teve como objetivo isolar, quantificar e identificar a variedade taxonômica de fungos filamentosos e as leveduras presentes no ambiente de uma clínica de hemodiálise, no município de Caicó, Rio Grande do Norte.

Metodologia

Local de Estudo

As amostras foram coletadas de cinco ambientes (sala de hemodiálise, sala de reprocessamento, sala de tratamento de água, recepção e cozinha), da Clínica do Rim, localizada no município de Caicó, Rio Grande do Norte e foram analisadas no laboratório de Microbiologia Clínica da Universidade Federal de Campina Grande (UFCG), Centro de Educação e Saúde (CES), Campus Cuité. O trabalho possui registro no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN), sob o código: A803E78.

Coleta de Amostras

A coleta foi feita pelo método de sedimentação espontânea em placas de Petri com meio de cultura específico, utilizando um conjunto de três placas para cada ambiente, sendo uma delas o controle, a qual não foi aberta. Cada placa de Petri contendo o meio Ágar Sabouraud-Dextrose da marca ION acrescido de Ceftriaxona (permitindo o isolamento seletivo de fungos) [17] foi exposta em um suporte com aproximadamente 1 metro do chão, no centro de cada ambiente por aproximadamente 15 minutos. Seguindo a Norma Técnica 001 da Resolução nº176 [18]. As placas foram identificadas como placa 1 e 2 para cada setor de coleta.

Após a realização da coleta, as placas foram mantidas em estufa por até 14 dias a temperatura de 28ºC. Durante esse período de incubação, as placas estavam sendo observadas diariamente quanto ao crescimento fúngico [3, 19].

Análise Microbiológica do ar

Finalizado o período de incubação, realizou-se a contagem das Unidades Formadoras de Colônias (UFC) crescidas nas placas de cada ambiente estudado, calculando a sua média. Depois calculou-se a relação entre o ar externo e interno para a avaliação da qualidade do ar interno. De acordo com a ANVISA, o valor máximo de contaminação microbiológica permitida para um ambiente fechado é de ≤ 750 UFC/m^3, e de ≤ 1,5 para a relação entre o ar interior e o exterior [11].

Em seguida, realizou-se a amostragem da sedimentação do ar de cada ambiente, através da equação de Frieberg *et al*., 1999 [20] utilizada por Sobral *et al*., (2017) [21] (Equação 1), na qual faz a relação numérica entre o número de Unidades Formadoras de Colônias (UFC) depositadas na placa pela exposição da mesma sobre a área da placa que foi exposta, multiplicada pela razão entre o número de microrganismos no ar e na superfície do meio de cultura (SAR). A área da placa utilizada corresponde a 90 × 15 mm, a qual foi convertida em m^2 e a proporção 1:23 foi atribuída, devido ser um processo de sedimentação espontânea, onde representa a razão entre o ar e a superfície do meio de cultura [21, 22], a qual está de acordo com a Resolução nº 9 de 16 de janeiro de 2003.

Equação 1. Equação utilizada para calcular a qualidade do ar:

$$Nº UFC/\ m^3 = \frac{Nº\ de\ UFC\ na\ placa}{Área\ da\ placa\ de\ Petri(m^2)} * \frac{1}{23}(SAR)$$

Identificação Fúngica

A identificação macroscópica das colônias foi desenvolvida em conjunto com a análise microscópica dos fragmentos destas colônias em lâminas, com a adição do corante azul de metileno. Quando a identificação não era possível dessa forma citada, realizava-se a técnica de microcultivo em lâmina, na qual são semeados fragmentos dos fungos filamentosos em Agar Batata Dextrose (ABD) DIFCOTM® [3, 19, 23, 24].

Resultados e discussão

Análise e identificação das amostras

Após o período de incubação das placas, observou-se crescimento fúngico na maioria delas, conforme pode ser visualizado na tabela 1 e 2. Destaca-se que não houve crescimento fúngico nas placas tidas como controle, ratificando a esterilidade dos meios de cultura utilizados.

Tabela 1. Fungos isolados em cada ambiente no interior da instituição (+) presença, (-) ausência.

Fungos isolados	Recepção	Sala de Hemodiálise	Sala de reprocessamento	Cozinha	Sala de tratamento de água
Cladosporium spp.	+	-	+	-	-
Aspergillus spp.	+	-	-	-	-
Mycelia sterilia	-	+	-	-	-
Bipolaris sp.	-	+	-	-	-
Leveduras	+	+	-	+	+

Fonte: autores.

Tabela 2. Quantidade total de UFC (%) dos microrganismos.

Fungos identificados	Nº UFC (%)
Leveduras	12 (42,85)
Cladosporium spp.	12 (42,85)
Aspergillus spp.	2 (7,14)
Mycelia Sterilia	1 (3,57)
Bipolaris sp.	1 (3,57)
UFC totais	**28 (100)**

Fonte: autores.

De acordo com Cunha, Souza e Gazola (2021) [25], os gêneros *Aspergillus, Cladosporium, Penicillium* e *Candida* são os de maior importância devido aos altos índices de casos relatados na literatura médica, sendo destes os gêneros *Aspergillus* e *Cladosporium* identificados nesta pesquisa.

Na recepção foram identificadas nove UFC de *Cladosporium* spp., duas de *Aspergillus* spp., e duas UFC de leveduras. O gênero *Cladosporium* (Figura 1) é amplamente associado às feo-hifomicoses, que são infecções fúngicas causadas por hifas ou pseudo-hifas melanizadas, que dão uma coloração negra ao tecido infectado, podendo ser cutâneas ou sistêmicas, podendo causar lesão nos olhos, a oculomicose, bolor fúngico nos pulmões e ainda lesões no cérebro [25].

Aspergillus spp. geralmente está associado as infecções pulmonares oportunistas, mas podem também causar infeções nos seios nasais, canais auditivos e as chamadas onicomicoses [26].

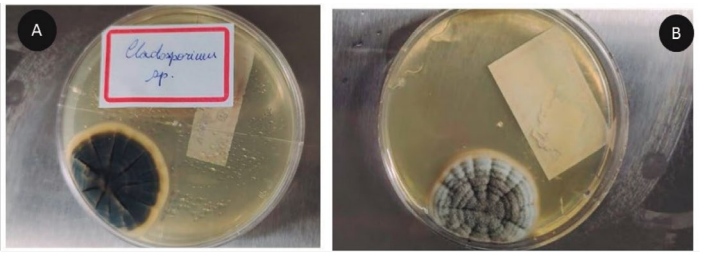

Figura 1. Colônia de *Cladosporium* spp, isolada na recepção da clínica. **Fonte:** autores.

Na sala onde os pacientes realizam hemodiálise, foi identificada apenas uma UFC do gênero *Bipolaris* sp., além de uma outra espécie que não apresentou estrutura reprodutiva, e assim não pode ser identificada, sendo classificada como *Mycelia Sterilia*, e três UFC de leveduras. O gênero *Bipolaris* está presente em plantas, sendo patogênico em várias espécies vegetais. Nos seres humanos pode causar alergias como rinite e sinusite, e ainda infecções pulmonares e cutâneas [27]. Sendo o principal ambiente da instituição, a sala de hemodiálise é um espaço em constante higienização, visivelmente limpo, não apresentou um grande crescimento de colônias.

Os demais ambientes de coleta (cozinha, sala de reprocessamento e sala de tratamento de água) apresentaram contaminação com a presença de um microrganismo cada. São locais que possuem uma limpeza constante, mas uma frequente mudança de temperatura, além de uma baixa circulação de ar e presença de fluidos biológicos, deixando assim o ambiente propício ao crescimento e proliferação fúngica.

A segurança do tratamento hemodialítico tem como um de seus principais determinantes a qualidade da água empregada no processo de diálise. O tratamento desta água utilizada para a hemodiálise deve ser feito em um sistema específico garantindo que essa água esteja livre de qualquer interferente, seja ele químico, físico ou biológico, uma vez que essa é utilizada para um procedimento invasivo por meio da corrente sanguínea do paciente. Esse tratamento deve ser feito em um ambiente de uso exclusivo para este fim [28].

Análise Microbiológica do Ar

No Brasil, a ANVISA estabeleceu os fungos como marcadores epidemiológicos da qualidade do ar de um ambiente climatizado artificialmente na norma reguladora nº 09, de 16 de janeiro de 2003. O órgão recomenda um padrão de qualidade que estabelece um

valor máximo para a contaminação biológica do ar interior de ≤ 750 UFC/m³ de fungos. Além disso, é estabelecida a relação I/E = ≤ 1,5, tendo como "I" a quantidade de fungos no interior do ambiente e "E" a quantidade de fungos no exterior do ambiente. Essa relação é reivindicada como uma forma de avaliar a ideia de normalidade representado pelo ambiente exterior e a predisposição epidemiológica de aumento dos poluentes nos ambientes fechados [11].

A análise do ar foi feita através da equação utilizada por Sobral *et al.* (2017) [21]. A tabela 3 representa a quantidade total de Unidades Formadoras de Colônias em cada placa obtida na pesquisa e a média calculada de UFC que foram utilizadas na execução da equação.

Tabela 3. Quantidade de UFC coletadas em cada ambiente.

Ambiente	UFC		Total UFC	Média UFC
	Placa 1	Placa 2		
Ambiente Externo	5	16	21	10,5
Recepção	6	7	13	6,5
Sala de Hemodiálise	4	4	8	4
Sala de Reprocessamento	2	1	3	1,5
Cozinha	1	1	2	1
Sala de tratamento de água	1	1	2	1

Após os dados necessários serem obtidos, estes foram aplicados na equação e foi possível alcançar os resultados desejados. Como determinado pela ANVISA, a quantidade máxima permitida de UFC/m³ em um ambiente é ≤ 750, e nos ambientes analisados nenhum chegou a esta quantidade. A relação entre o ar interior e exterior deve ser ≤ 1,5. Essa relação é feita através da soma da média de cada placa e a média da placa de coleta do ambiente externo a qual foi coletada apenas para que essa avaliação fosse feita e não foi atingida em nenhum ambiente estudado, como pode-se observar na tabela 4.

Tabela 4. UFC/m³ e a relação I/E dos ambientes analisados.

Ambiente	UFC/m³	I/E ≤ 1,5
Recepção	209,3	0,62

(*Continue*)

Ambiente	UFC/m³	I/E ≤ 1,5
Sala de Hemodiálise	128,8	0,38
Sala de Reprocessamento	48,3	0,14
Cozinha	32,2	0,10
Sala de tratamento de água	32,2	0,10

Fonte: autores.

A partir da tabela 4 é possível identificar que todos os ambientes da clínica analisados estão de acordo com a norma reguladora (NR 09 de 16 de janeiro de 2003), tanto as UFC/m³ quanto a relação que analisa o ar dos ambientes internos e externos, não apresentando risco aos seus usuários. O valor de UFC total do ambiente externo calculado utilizando a equação de Frieberg que na Legislação diz que deve ser menor ou igual a 750 UFC por ambiente, foi de 338,2 UFC/m³ neste trabalho.

A recepção foi o ambiente que apresentou o maior crescimento de UFC (Tabela 3), e isso pode estar associado a vários fatores, como o grande fluxo de pessoas entrando e saindo, uma manutenção inadequada do ar-condicionado que gerou uma devolução de umidade ao ambiente, o deixando propício ao crescimento de fungos, visto que a temperatura do ar externo era bem maior e a porta estava sempre aberta, esse constante atrito de temperaturas, a movimentação de carros, dentre outros.

Ainda na mesma Norma Reguladora, é determinado que em um ambiente fechado e climatizado artificialmente é inaceitável a presença de fungos patogênicos e toxigênicos, porém, nesta pesquisa, apesar dos fungos identificados apresentarem potencial patogênico como descrito na literatura, como algumas espécies de *Aspergillus*, ensaios mais específicos para detectar o potencial patogênico e toxigênico não foram realizados neste estudo, se fazendo necessário futuras pesquisas sobre o tema.

Apesar de não haver tantos estudos sobre qualidade do ar interior em clínicas de hemodiálise para que possa ser feitas comparações de dados, em outros estudos em ambientes hospitalares é possível observar que nem sempre esse resultado vai está dentro dos limites aceitáveis, como pode ser observado no estudo feito por Sodré, Tórtora e Corrêa (2014) [29], o qual analisou o ar interior do Hospital Universitário Pedro Ernesto (UERJ-RJ) e foi constatado em diversos ambientes valores acima de 1000 UFC/m³, podendo chegar a mais de 3000 UFC/m³ e a relação do ar I/E chegando a mais de 6.

No estudo de Zenaide e Nascimento (2020) [30] essa análise foi feita em um hospital na Paraíba e 12 de 23 amostras foi possível identificar um número de UFC/m^3 e uma razão I/E acima do estabelecido. Nas duas pesquisas, o gênero que teve maior prevalência foi o *Aspergillus* e os autores frisaram a limpeza como principal ponto de mudança para reverter essa situação, além de uma fiscalização adequada por parte dos órgãos competentes.

Algumas medidas podem ser adotadas para evitar a propagação dos esporos e diminuir a contaminação dos ambientes, tais quais como: intensificar a manutenção dos aparelhos de ar-condicionado, adotar uma frequência maior de limpeza dos ambientes, adotar medidas de higiene para os colaboradores e pacientes, como a limpeza de mãos e calçados e providenciar uma limpeza nas superfícies que recebem contato frequentemente, como maçanetas, cadeiras e poltronas, torneiras, entre outros.

Conclusão

Os microrganismos anemófilos, apesar de terem sido encontrados em diversos ambientes da clínica em estudo, variando em concentração, estavam dentro dos limites preconizados pela legislação brasileira. E isso pode estar relacionado com a limpeza realizada nas dependências da clínica e com a manutenção dos sistemas de ar-condicionado.

Mesmo estando dentro do permitido pelas normas, após o isolamento e identificação, foi possível observar a presença dos gêneros *Cladosporium*, *Aspergillus*, *Bipolaris, a espécie Mycelia sterilia* e algumas leveduras não identificadas, havendo uma maior prevalência dessas leveduras seguido do gênero *Cladosporium*.

Com relação a qualidade do ar, foi possível observar que o método de sedimentação espontânea se demonstrou eficaz ao que se propôs e que todos os locais analisados estão dentro do que a ANVISA determina, tanto para o número máximo de UFC/m^3, quanto para a relação do ar interior/exterior. Para que haja a manutenção desses parâmetros adequados, sugere-se apenas que os cuidados com higiene continuem. Como perspectiva, sugere-se que estudos sazonais possam ser realizados na referida clínica de hemodiálise e que ensaios para avaliar o verdadeiro potencial patogênico e/ou toxigênico dos fungos presentes na microbiota anemófila possam ser realizados.

Conflitos de Interesses

Os autores declaram não haver conflitos de interesses.

Referências

1. R.C. Lobato, V.S. Vargas, E.S. Silveira, Sazonalidade e prevalência de fungos anemófilos em ambiente hospitalar no sul do Rio Grande do Sul, Brasil, *Revista da Faculdade de Ciências Médicas de Sorocaba*, **11**(2), 21–28 (2009).

2. A.M.L. Moraes, R.A. Paes, V.L. Holanda, Micologia, en: E.M. Molinaro, L.F.G. Caputo, M.R.R. Amendoeira (editores), *Conceitos e Métodos para a Formação de Profissionais em Laboratórios de Saúde*, EPSJV, IOC, Rio de Janeiro, 2009, pp. 400–496.

3. R.J.N. Calumby, J.A. Silva, D.P. Silva, R.T.F. Moreira, M.A.S. Araujo, L.M. Almeida, L.A.M. Grillo, V. Alvino, Isolamento e identificação da microbiota fúngica anemófila em Unidade de Terapia Intensiva / Isolation and identification of anemophilic fungal microbiota in an intensive care unit, *Brazilian Journal of Development*, **5**(10), 19708–19722 (2019). https://doi.org/10.34117/bjdv5n10-186

4. R. Ferro, C. Nunes, I. Camacho, M. Paiva, M. Morais-Almeida, Monitorização de esporos de fungos em Lisboa, 2014-2016, *Revista Portuguesa de Imunoalergologia*, **27**, 29–39 (2019).

5. B.P. Teixeira, R.A. Junior, J.H.P.A. Pinheiro, D.I. Kozusny-Andreani, Avaliação de fungos em bioaerossois em ambiente de um hospital de médio porte do noroeste paulista, *Revista Saúde e Meio Ambiente*, **11**(2), 200–216 (2020).

6. Z. Zhan, M. Lao, F. Su, D. Chen, L. Liang, X. Yan, Hospital-acquired infection in patients with systemic lupus erythematosus: a case-control study in a southern Chinese population, *Clinical Rheumatology*, **37**(3), 709–717 (2018). https://doi.org/10.1007/s10067-017-3919-8

7. D.Y. Thomaz, G.M.B.D. Negro, L.B. Ribeiro, M. Silva, G.O.M.H. Carvalho, C.H. Camargo, J.N.A. Júnior, A.L. Motta, *et al.*, A Brazilian inter-hospital candidemia outbreak caused by fluconazole-resistant *Candida parapsilosis* in the COVID-19 era, *Journal of Fungi*, **8**(2), 100 (2022). https://doi.org/10.3390/jof8020100

8. H. Sun, F. Liu, L. Zhang, L. Zhao, L. Xu, Establishment of scoring system for risk factors of invasive fungal infection in patients with hematologic diseases, *China Pharmacy*, **12**, 1270–1273 (2018).

9. E. Stohs, A. Zimmer, An approach to suspected invasive fungal infection in patients with hematologic malignancy and HCT recipients with persistent neutropenic fever despite mold-active prophylaxis, *Current Fungal Infection Reports*, **14**(1), 89–98 (2020). https://doi.org/10.1007/s12281-020-00375-6

10. S. Kaluarachchi, M. Abeykoon, A case of endogenous *Candida* endophthalmitis with incidental cytomegalovirus infection and optic neuropathy in a patient recovered from severe COVID-19, *Indian Journal of Ophthalmology*, **70**(1), 323–326 (2022). https://doi.org/10.4103/ijo.IJO_2454_21

11. Brasil, Ministério da Saúde, Agência Nacional de Vigilância Sanitária, Resolução – RE nº 9, de 16 de janeiro de 2003, Determina a publicação de Orientação Técnica elaborada por Grupo Técnico Assessor, sobre Padrões Referenciais de Qualidade do Ar Interior, em ambientes climatizados artificialmente de uso público e coletivo, ANVISA, Brasília, 2003. URL: http://antigo.anvisa.gov.br/documents/10181/2718376/RE_09_2003_.pdf/8ccafc91-1437-4695-8e3a--2a97deca4e10

12. S.C. Smeltzer, B.G. Bare, *Brunner & Suddarth: tratado de enfermagem médico-cirúrgica*, 9. ed., Guanabara Koogan, Rio de Janeiro, 2002.

13. R.d.C.H. Mendonza-Ribeiro, G.A.S. Alves de Oliveira, D. Fávaro-Ribeiro, D.C. Bertolin, C. Bernardi-Cesarino, L.C.E. Quintino de Lima, S.M. de Oliveira, Caracterização e etiologia da insuficiência renal crônica em unidade de nefrologia do interior do Estado de São Paulo, *Acta Paulista de Enfermagem*, **21**, 207–211 (2008).

14. S.C. Miranzi, C.D.L. Cravo, H.H. Iwamoto, J.L.S. Júnior, Perfil epidemiológico dos pacientes em hemodiálise de um hospital universitário, *Ciência, Cuidado e Saúde*, **10**(1), 110–115 (2011). https://doi.org/10.4025/ciencuidsaude.v10i1.10720

15. E.C. Dias, N.A. Silva, S.F. Maia, F.F. Morais, R.S.S. Silva, L.S. Oliveira, Avaliação dos índices de infecção relacionados ao cateter duplo lúmen para hemodiálise antes e após orientação para o autocuidado, *Revista Uningá*, **53**(2), 18–25 (2017).

16. T.A. Guimarães, W.B. Rodrigues, A.M.M. Fontenele, Infecções fúngicas em transplantados renais: uma revisão integrativa / Fungal infection in renal transplantation: a integrative review, *Revista de Pesquisa em Saúde*, **18**(2), 119–123 (2018).

17. M.M.D. Ferreira, J.B.P. Souza, E.S. Carmo, Avaliação microbiológica de máscaras de cílios utilizadas em salões de beleza, *Journal of Medicine and Health Promotion*, **5**(3), 120–127 (2020).

18. Brasil, Ministério da Saúde, Agência Nacional de Vigilância Sanitária (ANVISA), Resolução – RE nº 176, de 24 de outubro de 2000, Determina a publicação de Orientação Técnica elaborada por Grupo Técnico Assessor, sobre Padrões Referenciais de Qualidade do Ar Interior, em ambientes climatizados artificialmente de uso público e coletivo, ANVISA, Brasília, 2000. URL: http://www.pncq.org.br/uploads/2015/qualinews/RE%20176%202000.pdf

19. E.S. Carmo, L.d.F. Belém, R.M.R. Catão, E.d.O. Lima, I.L.d. Silveira, L.H.M. Soares, Microbiota fúngica presente em diversos setores deum hospital público em Campina Grande – PB, *Revista Brasileira de Análises Clínicas*, **39**(3), 213–216 (2007).

20. B. Friberg, S. Friberg, L.G. Burman, Correlation between surface and air counts of particles carrying aerobic bacteria in operating rooms with turbulent ventilation: an experimental study, *Journal of Hospital Infection*, **42**(1), 61–68 (1999). https://doi.org/10.1053/jhin.1998.0542

21. L.V. Sobral, K.N. Melo, C.M. Souza, S.F. Silva, G.L.R. Silva, A.L.F. Silva, K.A.A. Wanderley, I.S. Oliveira, R. Cruz, Antimicrobial and enzymatic activity of anemophilous fungi of a public university in Brazil, *Anais da Academia Brasileira de Ciências*, **89**(3), 2327–2340 (2017). https://doi.org/10.1590/0001-3765201720160903

22. G.R. Morais, M.A. Silva, M.V. Carvalho, J.S. Santos, E.L.O.V. Dolinger, D.V.D. Brito, Qualidade do ar interno de uma instituição de ensino superior, *Bioscience Journal*, **26**(2), 305–310 (2010).

23. Brasil, Agência Nacional de Vigilância Sanitária, *Microbiologia Clínica para o Controle de Infecção Relacionada à Assistência à Saúde, Módulo 8: Detecção e identificação de fungos de importância médica*, ANVISA, Brasília, 2013. URL: https://www.anvisa.gov.br/servicosaude/microbiologia/mod_7_2004.pdf

24. A.D.C. Sobreira, A.D.R. Sousa, E.S. Carmo, D.A. Costa, Research of antifungal potential of *Sida planicaulis* Cav. (Malvaceae) fungus filamentosos front, *Periódico Tchê Química*, **16**(31), 607–615 (2019).

25. R.M.A. Cunha, E.B.A. Souza, H.Q.G.B. Gazola, Qualidade microbiológica do ar em ambiente de um Instituto de Oncologia e Radioterapia do município de Porto Velho, *Revista Saber Científico*, **6**(2), 54–63 (2021).

26. V.B. Duo Filho, J.P.Z. Siqueira, T.E. Colombo, Monitoramento de fungos anemófilos no ambiente de uma biblioteca no Município de São José do Rio Preto-SP, Brasil, *Arquivos de Ciências da Saúde da UNIPAR*, **24**(2), 75–80 (2020).

27. L.D.C. Oliveira, L.R. Borges-Palucha, Alergias respiratórias: uma revisão dos principais fungos anemófilos e fatores desencadeantes, *Revista Baiana de Saúde Pública*, **39**(2), 426–441 (2015). https://doi.org/10.22278/2318-2660.2015.v39.n2.a1279

28. Brasil, Ministério da Saúde, Portaria nº 82, de 03 janeiro de 2000, Estabelece Regulamento técnico para o funcionamento dos serviços de diálise e as normas para cadastramento destes junto ao Sistema Único de Saúde. Ministério da Saúde, ANVISA, Brasília, 2000. URL: https://bvsms.saude.gov.br/bvs/saudelegis/gm/2000/prt0082_03_01_2000.html

29. E.D. Sodré, J.C.O. Tórtora, S.M. Corrêa, Avaliação da qualidade do ar interior do Hospital Universitário Pedro Ernesto, *Revista Sustinere*, **2**(2), 36–56 (2014). https://doi.org/10.12957/sustinere.2014.14126

30. H. Zenaide-Neto, J.D. Nascimento, Air quality and microbiological control in a hospital in Paraíba, Brazil, *International Journal of Advanced Research in Science*, **7**, 99–108 (2020). https://doi.org/10.22161/ijaers.79.13

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TRAIL receptors as prognostic markers and survival predictors in ovarian cancer: A systematic review of clinical studies and meta-analysis

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Summary

Introduction: TRAIL cytokine (TNF-Related Apoptosis-Inducing Ligand) interacts with five receptors, four of which are expressed at the plasma membrane (DR4, DR5, DcR1, DcR2), and the fifth is a soluble osteoprotegerin receptor (OPG). Only the death receptors DR4 and DR5 contain the cytoplasmic death domain (DD), which is involved in triggering the apoptotic cascade. These receptors are found in tumor cells of various types, including ovarian cancer cells. **Purpose:** The aim of this article is to describe in a systematic review the presence of death receptors in cancer cells of patients and to discuss the clinical implications of this approach on various signs and clinical mechanisms of cancer. **Method:** The systematic review was performed on June 1, 2022, using PubMed Central - PMC, SCOPUS (Elsevier), Web of Science, Cochrane Library, and Biblioteca Virtual em Saúde - BVS (BIREME). The data were summarized in tables and critically analyzed. After the database search, five relevant studies were identified for review. **Results:** Analysis of these studies revealed evidence of increased survival in patients with ovarian cancer who detected these receptors in cancer tissue. In addition, we seek to understand the biological mechanisms involved in the resistance of cancer cells to TRAIL-induced apoptosis.

Keywords: Ovarian cancer, Apoptosis-Inducing, TRAIL, DR4, DR5, Caspase.

Resumen

Receptores TRAIL como marcador de pronóstico y predictores de la supervivencia en el cáncer de ovario: una revisión sistemática de estudios clínicos y metanálisis

Introducción: La citocina TRAIL (Ligando inductor de apoptosis relacionado con TNF) interactúa con cinco receptores, cuatro de los cuales se expresan en la membrana plasmática (DR4, DR5, DcR1, DcR2), y el quinto es un receptor de osteoprotegerina soluble (OPG). Solo los receptores de muerte DR4 y DR5 contienen el dominio de muerte citoplasmático (DD), que está involucrado en el desencadenamiento de la cascada apoptótica. Estos receptores se encuentran en células tumorales de varios tipos, incluidas las células de cáncer de ovario. **Propósito:** El objetivo de este artículo es describir en una revisión sistemática la presencia de receptores de muerte en células cancerosas de pacientes y discutir las implicaciones clínicas de este enfoque en varios signos y mecanismos clínicos del cáncer. **Método:** La revisión sistemática se realizó el 1 de junio de 2022, utilizando PubMed Central - PMC, SCOPUS (Elsevier), Web of Science, Cochrane Library y Biblioteca Virtual em Saúde - BVS (BIREME). Los datos se resumieron en tablas y se analizaron críticamente. Después de la búsqueda en la base de datos, se identificaron 5 estudios relevantes para su revisión. **Resultados:** El análisis de estos estudios reveló evidencia de una mayor supervivencia en pacientes con cáncer de ovario en quienes se detectaron estos receptores en el tejido canceroso. Además, buscamos comprender los mecanismos biológicos involucrados en la resistencia de las células cancerosas a la apoptosis inducida por TRAIL.

Palabras clave: Cáncer de ovario, Inductor de apoptosis, TRAIL, DR4, DR5, Caspasa.

Resumo

Receptores TRAIL como marcadores de prognóstico e preditores da sobrevivência no câncer de ovário: uma revisão sistemática de estudos clínicos e metanálises

Introdução: A citocina TRAIL (TNF-*Related Apoptosis-Inducing Ligand*) interage com cinco receptores, quatro dos quais são expressos na membrana plasmática (DR4, DR5, DcR1, DcR2) e o quinto é um receptor solúvel de osteoprotegerina (OPG). Apenas os receptores de morte DR4 e DR5 contêm o domínio citoplasmático de

morte (DD), que está envolvido no desencadeamento da cascata apoptótica. Esses receptores são encontrados em células tumorais de vários tipos, incluindo células de câncer de ovário. **Propósito:** O objetivo deste artigo é descrever em uma revisão sistemática, a presença de receptores de morte em células cancerígenas de pacientes e discutir as implicações clínicas desta abordagem em vários sinais e mecanismos clínicos do câncer. **Método:** A revisão sistemática foi realizada em 1º de junho de 2022, utilizando PubMed Central - PMC, SCOPUS (Elsevier), *Web of Science*, *Cochrane Library* e Biblioteca Virtual em Saúde - BVS (BIREME). Os dados foram resumidos em tabelas e analisados criticamente. Após a busca nos bancos de dados, cinco estudos relevantes foram identificados para revisão. **Resultados:** A análise desses estudos revelou evidências de aumento da sobrevida em pacientes com câncer de ovário nos quais esses receptores foram detectados no tecido canceroso. Além disso, buscamos entender os mecanismos biológicos envolvidos na resistência das células cancerígenas à apoptose induzida por TRAIL.

Palavras chaves: Câncer de ovário, Indutor de apoptoses, TRAIL, DR4, DR5, Caspase.

Introduction

Despite remarkable progress in understanding the biology of cancer and the development of new diagnostic and therapeutic strategies, cancer remains one of the leading causes of death. Worldwide, 225,500 new cases of ovarian cancer are diagnosed each year, and 140,200 people die from this cancer [1, 2]. Although ovarian cancer was considered a single entity, it can be divided into different histological subtypes with different identifiable risk factors, cells of origin, molecular compositions, clinical features, and treatments [3].

Effective screening strategies for early detection of ovarian cancer do not exist, but individuals at high risk for developing ovarian cancer, such as those with germline mutations in BRCA1 or BRCA2 or other genes associated with high risk for developing ovarian cancer, can be identified [3, 4]. The discovery of this unique feature among members of the TNF superfamily has laid the foundation for testing the clinical potential of TRAIL-R targeted therapies in cancer clinics [5]. However, the efficacy of TRAIL-based cancer therapies has yet to be demonstrated, as most cancer cells are TRAIL-resistant or develop resistance after multiple treatments [6, 7].

TRAIL is a multifunctional cytokine produced and secreted by most normal tissue cells. It acts in the process of cellular apoptosis, mainly in tumor cells [8]. Targeting the

apoptosis death receptor pathway represents a promising approach for the development of new cancer therapies, as cell surface death receptors are directly linked to the apoptotic machinery, preferentially in cancer cells, while sparing non-malignant cells [9].

Therefore, in this study, we aim to summarize the currently available evidence on whether TRAIL receptors serve as prognostic markers and predict survival in ovarian cancer through a systematic review and meta-analysis, and to present the extent of clinical relevance of these genes.

This systematic review summarizes the mechanisms of TRAIL-induced apoptosis via extrinsic and intrinsic apoptotic signaling pathways. It also reviews the mechanisms of cancer cell resistance to TRAIL-induced apoptosis in ovarian cancer.

Methods

The presence of death receptors significantly correlated with cancer patient survival in experimental models was assessed by a systematic review and meta-analysis performed according to the principles described in the Cochrane Handbook [10]. The steps related to the search and selection of articles and the extraction, analysis, and interpretation of data of interest, were performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [11]. To identify studies of interest, we applied the **PEOS** strategy to the articles from the electronic search as follows: "population," patients with ovarian cancer; "exposure," presence or absence of death receptors; "outcomes," survival; "study design," clinical, observational, and epidemiologic hospital surveillance studies [12].

Search strategy

First, a systematic search was performed in five databases (PubMed Central, SCOPUS, Web of Science, Cochrane Library, and *Biblioteca Virtual em Saúde*) using the Medical Subject Heading (MeSH) term "TNFRSF10A" in conjunction with at least two of each of the following descriptors: "track R1," "TNFRSF10A," "death receptors," "cancer," "survival," "mortality." For the search, these descriptors were combined with the connector "AND" between them, as in the following example: "cancer" AND "track R1". The search was conducted through June 1, 2022, and was limited to studies written in English, with no date limit. A search for gray literature in theses and dissertations was also conducted through the Thesis and Dissertation Catalog of the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and the Digital Library of

Theses and Dissertations of the Universidade Federal de Minas Gerais (UFMG) and the Universidade de São Paulo (USP). In addition, the reference lists of all included articles and relevant narrative reviews in the field were reviewed for relevant articles.

Study selection

In addition to the electronic search, studies identified as review articles, notes, correspondence, editorials, and letters were excluded. Other studies were excluded based on the following criteria: (i) in silico and in vitro studies; (ii) studies in which the death receptor was not identified; (iii) studies in which ovarian cancer was not identified; (iv) studies in which none of the main outcomes (survival) were examined. In cases where the article met the inclusion criteria but the full text was not available, the corresponding author was contacted three times by e-mail (14 days apart), and articles were included if received by the last contact. The details of the search strategy for each database are provided in the supplementary files.

In the first phase of selection, two independent researchers (J.C.M.B. and P.C.) searched the databases. Duplicate records were deleted using Rayyan (a web and mobile app for systematic reviews) [13] and the titles and abstracts of the selected studies were reviewed according to PEOS eligibility criteria. Studies that examined cancer patient survival in the presence of death receptors were selected and subsequently assessed by full-text review.

Any disagreements were resolved through discussion with the other investigator (L.M.S), and the kappa coefficient (with a 95% confidence interval) was used to analyze the level of agreement between assessors [14].

Data extraction and statistical analysis

After a complete analytical reading, all data of interest were summarized in a table for further critical analysis and interpretation. Ovarian cancer patient mortality rates with death were analyzed by meta-analysis using RStudio® software, using the meta package and metaprop command. Heterogeneity of the primary data was determined using the I-squared index (I^2), were $I^2 > 50\%$ was considered to be substantial heterogeneity [15]. To calculate the frequency of death receptors between ovarian cancer patients, either the fixed effects model low heterogeneity or the random effects model with high heterogeneity was used to pool the data [16]. For all methods, the significance level was 5%.

Results

Study inclusion

Bibliographic searches of the databases yielded a review of 1,527 articles, including 82 from PubMed/MEDLINE, 756 from Scopus, 545 from BVS, 55 from Web of Science, and 89 from Cochrane. No additional study was identified in the gray literature search or by screening the reference list of included studies, Figure 1.

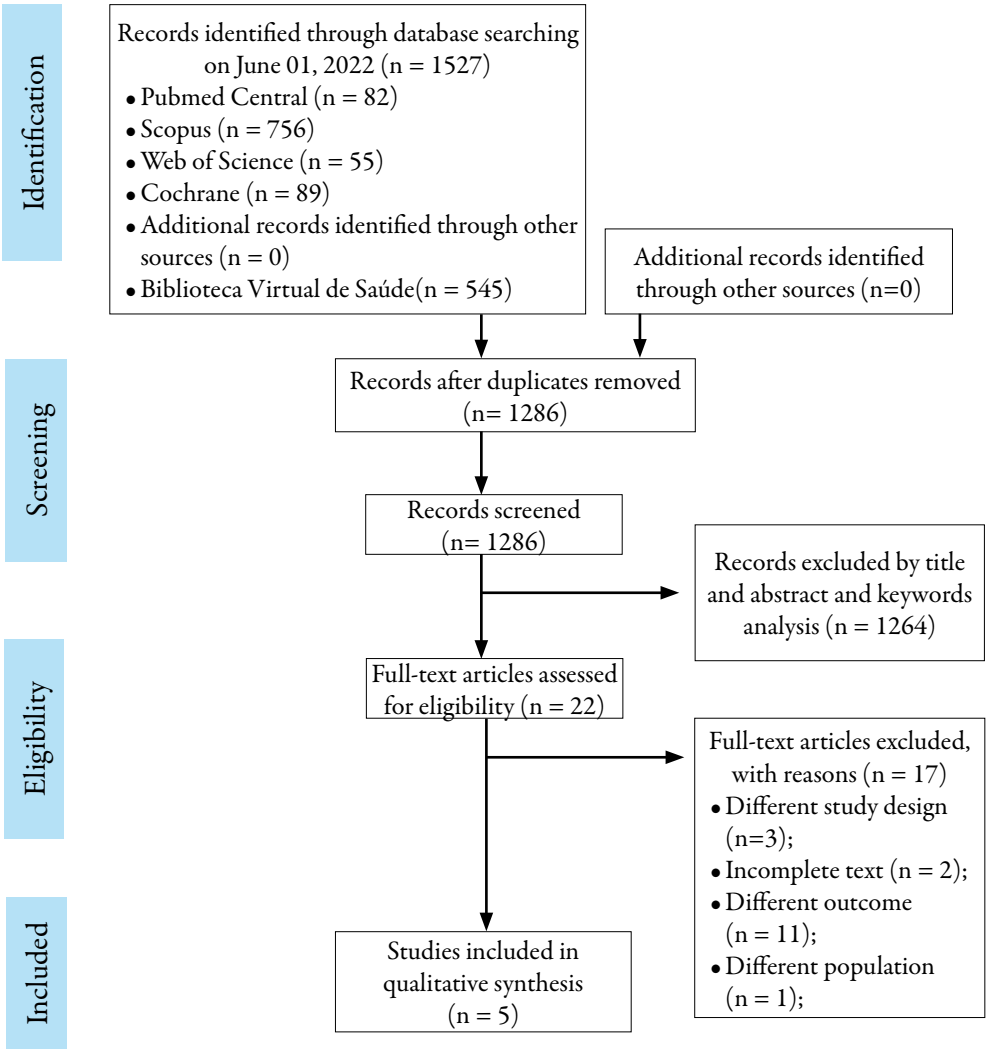

Figure 1. Flowchart of the selected articles for systematic review according to PRISMA criteria [10].

After exclusion of duplicate studies, 1,286 records were screened by title and abstract, leaving 22 studies that met the inclusion criteria. Therefore, 22 full-text articles were screened for eligibility and 17 were excluded for the following reasons: Different study design (n=2); or incomplete text (n=2); different outcomes (n=11); and different population (n=1) (Figure 1). Finally, 5 studies were selected for qualitative analysis [17-21]. Of these articles, 2 were also included in the quantitative studies and used to perform the meta-analysis [17, 19], Figure 2. The degree of agreement between the two researchers was found to be substantial, as indicated by the kappa coefficient of 0.7963.

Characteristics of the studies

According to Table 1, the studies included 809 patients with ovarian cancer.

Table 1. Main characteristics of included studies

OCP (n)	Method	Receptors/ Gene	Outcomes	Ref.
68	Immunohistochemical analysis	55 OCP with DR4 49 OCP with DR 5 54 OCP with TRAIL	• Favorable overall survival for low grade (grade 1 versus grade 2-3; *p-value* 0.047) and low stage (FIGO I/II versus III/IV; *p-value* 0.0008) in OCP with TRAIL. • DR4 and DR5 expression levels in OCP were predictors for survival benefit.	[17]
359	Immunohistochemical analysis	245 OCP with DR4 330 OCP with DR 5 49 OCP with TRAIL	• TRAIL was associated with low tumor grade and better progression-free survival (HR 0.63, *p-value* 0.018).	[19]
120	QRT-PCR	Mean TRAIL gene expression	• OCP-10-fold higher mean TRAIL expression than normal ovarian epithelial samples (*p-value* <0.001); • OCP with high TRAIL expression prolonged survival 2.2-fold higher (*p-value* 0.03); • TRAIL expression was 2.3-fold higher in cancers from women who lived >5 years than in cancers from those who died in <1 year (*p-value* 0.03);	[20]

(*Continued*)

OCP (n)	Method	Receptors/ Gene	Outcomes	Ref.
120	QRT-PCR	Mean TRAIL gene expression	• Advanced-stage (III/IV) cases, relative TRAIL expression was 2.2-fold higher (1.6 versus 0.9) in cancers of women who lived >5 years than in cancers from those who lived <1 year (*p-value* 0.18); • Association between high TRAIL expression and favorable survival (*p-valu*e 0.14).	[20]
27	qRT-PCR	19 OCP with TRAIL-R3	• No significant associations were observed between the TRAIL-3 expression level in primary ovarian cancer and the cumulative progression-free survival or the overall survival.	[18]
235	Immunohistochemical analysis	------	• DR5 and DcR1 had higher expression levels in grade 2 and 3 tumors compared with the levels in grade 0 tumors (*p-value* 0.01); • Trail and DR4 had lower expression levels in higher grade tumors (*p-valu*e 0.01) • Lower expression levels of Trail were observed for grade 3 tumors; • Grade2 tumors expressed significantly higher levels of Trail and DR4 compared with grade 3 tumors.	[21]

OCP: ovarian cancer patients.

The death receptor was identified by immunohistochemical analysis (427/809; 52.78%), 300 ovarian cancer patients (OCP) with DR4 (300/427; 70.26%), and 379 OCP with DR5 (379/427; 88.75%) by [17, 19]. The OCP with TRAIL (103/427; 24.12%) [17, 19], TRAIL-R3 (19/27; 70.37%) [18] and mean TRAIL gene expression were also quantified [20].

High expression of DR4 and DR5 in OCP were predictors of survival benefit [17]. DR5 and DcR1 had higher expression levels in grade 2 and 3 tumors compared with levels in grade 0 tumors (*p-value* 0.01). TRAIL and DR4 had lower expression levels in higher grade tumors (*p-value* 0.01). Lower expression of TRAIL was observed in grade 3 tumors, and grade2 tumors expressed significantly more TRAIL and DR4 compared with grade 3 tumors [21].

TRAIL was associated with low tumor grade and better progression-free survival (HR 0.63, p=0.018) [19], favorable overall survival in low grade (grade 1 versus grade 2-3; *p-value*) and low stage (FIGO I/II versus III/IV; *p-value* 0.0008) OCP with TRAIL [17]. Association between high TRAIL expression and favorable survival (*p-value* 0.14). Tenfold higher mean TRAIL expression than normal ovarian epithelial samples (*p-value* 0.001) with high TRAIL expression prolonged survival 2.2-fold (*p-value* 0.03). TRAIL expression was 2.3-fold higher in cancers from women who lived more than 5 years than in cancers from women who died in less than1 year (*p-value* 0.03), and in advanced stage cases (III/IV), relative TRAIL expression was 2.2-fold higher in cancers from women who lived more than 5 years than in cancers from women who lived less than 1 year (1.6 versus 0.9, *p-value* 0.18) [20]. In addition, no significant association was found between the TRAIL-3 expression level in primary ovarian cancer and cumulative progression-free survival or overall survival [18].

All five included studies examined the association between patient survival and the presence of death or TRAIL receptors using Kaplan-Meier curves and log-rank tests. They found a significant difference in patient survival (*p-value* of log-rank test<0.05) when DR5, DR4 and Flip, DcR1 and DR5, Flip and DR5, DcR2 and Cyc, TRAIL and DR5, Flip and DcR2 were detected in patients [17, 19, 21]. When TRAIL was detected, the survival rate of patients was significantly increased [19, 20].

DR4, DR5, and TRAIL incidence in patients with ovarian cancer

Two studies [17, 19] with 427 confirmed DR4 and DR5 receptors were combined in this meta-analysis. Consistent with the presence of the death receptor in OCP, the analysis revealed an incidence of DR4 in OCP of 73% (95% CI: 62% to 81%; *p-value* 0.04), DR5 in OCP of 85% (95% CI: 67% to 94%; *p-value* 0.01), and TRAIL in OCP of 44% (95% CI: 8% to 88%; *p-value* 0.01), as shown in Figure 2.

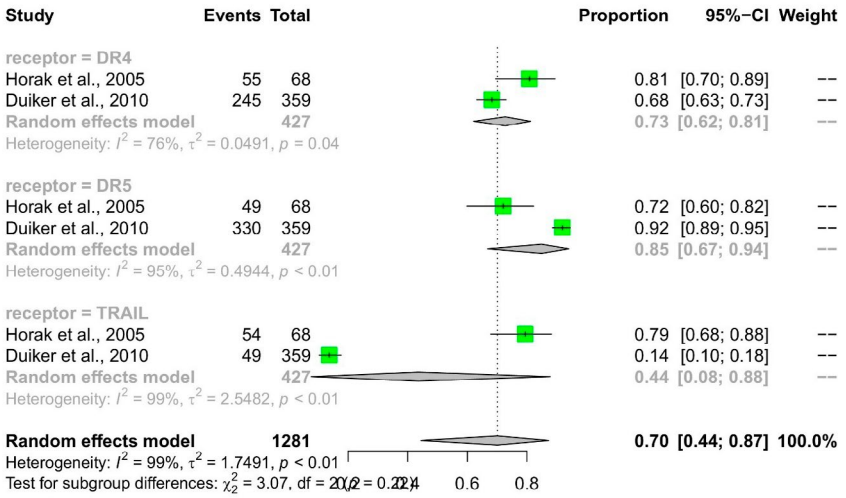

Figure 2. Forest plot of the incidence of death receptors in ovarian cancer. CI, confidence interval; heterogeneity (I^2); heterogeneity's *p-value* (*p*).

Discussion

The most effective therapeutic agents for newly diagnosed ovarian cancer are platinum analogs (either cisplatin or carboplatin) to which taxane (paclitaxel or docetaxel) is added. Recurrence of cancer after initial platinum-based chemotherapy is very common in women with advanced cancer, but the problem with treating cancer in these women is the possible development of platinum resistance [3].

Current therapeutic strategies for the treatment of cancer patients aim to overcome two essential features of cancer, namely excessive proliferation and resistance to apoptosis. Apoptosis is an essential process in several basic physiological processes and is controlled by numerous intracellular and extracellular signals [22].

Endogenous biochemical signaling pathways, which include apoptotic and non-apoptotic signaling pathways, are activated in cancer cells by TRAIL. Alterations in these signaling pathways are major hallmarks of ovarian cancer [23]. TRAIL binds to several receptors, including TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (DcR1), TRAIL-R4 (DcR2), and OPG (Figure 3). When TRAIL interacts with DR4 and DR5 receptors containing a conserved death domain motif (DD), formation of the

death-inducing signaling complex (DISC) occurs with recruitment of Fas-associated protein with death domain (FADD). FADD recruits pro-caspase-8 or -10 via its death effector domain (DED), and the activated caspases-8 or -10 then cleave effector caspases-3, -6, and -7 and directly stimulate the activation of a protease cascade via the extrinsic pathway of apoptosis, ultimately leading to cell death [24, 25].

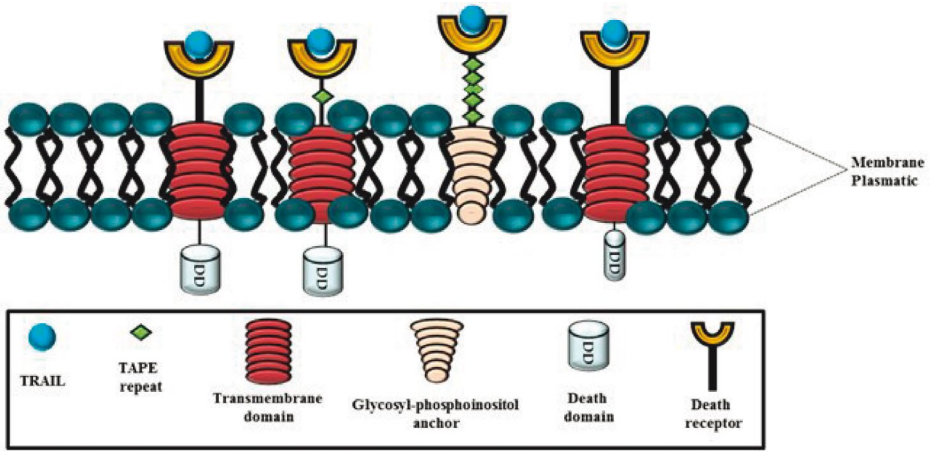

Figure 3. TRAIL-R system. TRAIL Cytokine interacts with death receptors expressed on the plasma membrane (TNFRSF10A/DR4, TNFRSF10B/DR5, TMFRSF10C/DcR1, TNFRSF10D/DcR2). Death receptors DR4 and DR5 contain the cytoplasmic death domain (DD) involved in triggering the apoptotic cascade, and decoy receptors DcR1 and DcR2 inhibit TRAIL-mediated apoptosis.

The caspase cascade can also be activated by the intrinsic pathway of apoptosis, through the formation of a complex called the apoptosome. The apoptosome is formed in the cytosol when cytochrome c is released from mitochondria in response to a cell death stimulus and binds the monomer of apoptotic protease activation factor 1 (APAF-1), promoting the formation of a heptameric APAF-1 complex accompanied by synchronized recruitment of procaspase-9. Activation of apoptosis-initiating caspase-9 also promotes activation of effector caspases [26].

Members of the Bcl-2 family such as Bid, Bax and Bak provide a link between intrinsic and extrinsic apoptosis pathways and control the life or death decision of a cell. Smac/ DIABLO, a proapoptogenic mitochondrial protein, is also released into the cytosol when the TRAIL-caspase-8-tBid-Bax cascade is required to reverse the inhibitory effect of X-linked inhibitor of apoptosis (XIAP). XIAP consists of three BIR domains, BIR1, BIR2 and BIR3, with Smac/ DIABLO interacting with the BIR2 and BIR3 domains and allowing the release of caspase-3 and caspase-9, respectively [27, 28].

In humans, the gene encoding TRAIL is located on chromosome 3q26 and is directly regulated by the p53 protein. However, the tumor suppressor gene p53 is often inactivated in ovarian cancer cells that develop resistance to TRAIL. Resistance to TRAIL can be caused by several mechanisms. For example, inactivation of Bax in mismatch repair (MMR)-deficient tumors may cause resistance to TRAIL because Bax is required for the release of Smac/ DIABLO and consequently exerts an antagonistic effect on the IAP protein family. Increased expression of caspase inhibitors such as XIAP or overexpression of Bcl-2 are mechanisms that mediate resistance to TRAIL-induced apoptosis [27, 29].

TRAIL death receptors can induce pro-inflammatory, invasion- and metastasis-promoting non-apoptotic signaling pathways under certain conditions [25], as shown in Figure 4. Indeed, anti-apoptotic signaling pathways such as NF-kB, MAPKs, p38 and other signaling pathways such as ERK1/2, PI3K/AKT and JAK-STAT repair TRAIL-induced apoptosis [27]. These survival signaling cascades have also been associated with resistance to TRAIL therapy in most primary cancer cells [30]. Akt activation upregulates the level of the c- FLIP protein, which has sequence homology to caspase-8 and -10 but lacks protease activity. Recruitment of FLIP to DISC instead of caspase-8 or -10 results in its inhibition, leading to resistance of TRAIL in ovarian cancer. Silencing of BID suggests that BID is important for TRAIL-induced cell death, and as Akt expression increases, BID transcription is downregulated. Activation of the ERK -signaling pathway leads to upregulation of the anti-apoptotic Mcl-1 protein and thus also contributes to TRAIL-resistance [31, 32].

Nowadays it has been described that TRAIL-R2 can act as a negative regulator of p53. TRAIL-R2 facilitates the degradation of p53 and regulates the stability of p53 protein by interacting with promyelocytic leukemia (PML) protein, which has elucidated an oncogenic role of TRAIL-R2 in tumorigenesis [33].

The tumor microenvironment (TME) plays an important role in modulating TRAIL signaling through cellular compounds (e.g., neutrophils, macrophages, NK cells, cytotoxic T cells, and stromal cells), hypoxia, increased acidity, mechanical stress, glucose availability, and changes in the extracellular matrix (ECM). All factors have a direct effect on tumor cell apoptosis induced by TRAIL [34, 35].

Dynamin-1, a protein involved in clathrin-mediated endocytosis (CME), regulates the endocytosis of TRAIL-DR in several cancer cells, attenuating apoptotic signals and increasing cell survival [36].

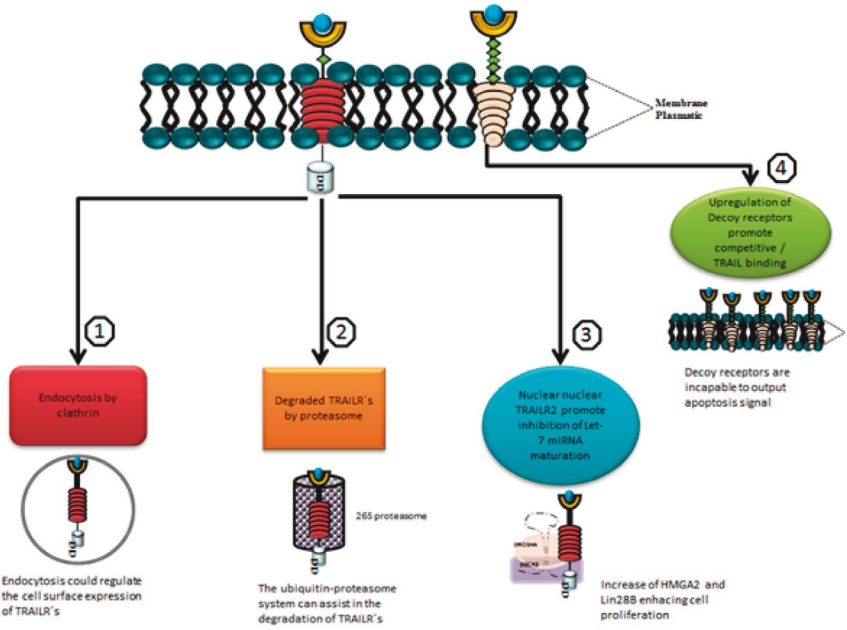

Figure 4. Mechanisms that may play a role in TRAIL-R resistance. 1) Autophagy and endocytosis might be involved in the regulation of TRAIL death receptors DR4 and DR5 [37]. 2) The TRAIL-receptors may be subject to regulation by the UPS (ubiquitin-proteasome system). The E3 ligase c-Cbl has been shown to directly regulate ubiquitination of TRAIL-R after receptor activation. Stimulation with the ligand TRAIL-induced c-Cbl-mediated mono-ubiquitination of TRAIL-R1 and TRAIL-R2, resulting in the degradation of the internalized receptors by the proteasome [38]. 3) TRAIL-R2 may be associated with the process of miRNA maturation when it interacts with the central microprocessor complex Drosha/DGCR8. Through this interaction, TRAIL-R2 inhibits the maturation of miRNA let-7 and consequently increases the levels of let-7 targets Lin28B and HMGA2. In this mechanism, inhibition of let-7 maturation by TRAIL-R2 promotes tumor cell malignancy by upregulating the Lin28B and HMGA2 genes, which increases cell proliferation [39]. 4) TRAIL-R3 effectively acts as a "decoy" receptor when expressed at much higher concentrations or has significantly higher affinity than the two "lethal" receptors (TRAIL-R4 and -R2). In this mechanism, the higher concentration in ovarian cancer could be due to the increased proliferation and apoptosis resistance inherent in this tumor type due to the competitive binding of the ligand at the cell surface [18].

Recently, it was also reported that TRAIL can induce necroptosis, a programmed form of necrosis or inflammatory cell death, by recruiting RIPK1 and RIPK3. When RIPK1 is activated, it recruits and phosphorylates RIPK3, which phosphorylates a mixed lin-

eage kinase domain like pseudokinase (MLKL), resulting in the formation of pores in the cell membrane [23, 29, 35]

As an example of treatments based on TRAIL-resistance, several reports have shown that treatment with proteasome inhibitors can upregulate the expression of TRAIL-R receptors on the cell surface, leading to increased sensitivity to TRAIL-induced apoptosis. The clinically used proteasome inhibitor bortezomib (Velcade, Millennium Pharmaceuticals, Inc., Cambridge, MA, and Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, NJ) has been shown to increase sensitivity to TRAIL by upregulating TRAIL-R2 expression in NSLCC cells [40]. Inhibition of the proteasome triggers TRAIL-R2-dependent apoptosis in cells unable to activate the mitochondrial apoptosis pathway. In response to proteasome inhibition, TRAIL-R2 eventually accumulates in cytosolic complexes containing FADD and RIPK1, which serve to activate caspase-8 [41]. The importance of extrinsic apoptosis triggered by caspase-8 activation has been demonstrated in various scenarios, and RIPK1 is a key component of caspase-8 activating complexes (ripoptosomes) [41].

Similar to other human cancers, ovarian cancer is thought to arise from an accumulation of mutations in genes that regulate cellular proliferation and apoptosis [20]. Characteristic of all cancers is the deregulation of the apoptotic machinery, in which the extrinsic apoptotic pathway is activated by the binding of death receptors from the tumor necrosis factor (TNF) family to their corresponding cell surface receptors [19]. The authors also showed that the majority of ovarian carcinomas in their study of 382 patients expressed at least one death receptor as well as caspase 8 and its anti-apoptotic homolog c- FLIP. TRAIL expression was associated with lower tumor grade and better progression-free survival when all tumors were analyzed. These results suggest that loss of expression of TRAIL confers a survival advantage to tumor cells, possibly because they evade apoptosis induction by para- or autocrine-released TRAIL.

A study using real-time quantitative PCR in 120 epithelial ovarian cancer (11 stages I/II, 109 stages III/IV) and 8 normal ovarian surface epithelial samples found that high expression of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene was associated with prolonged survival in advanced ovarian cancer [20]. TRAIL has attracted particular interest because of its apparent tumor cell-specific apoptosis-inducing ability.

Bertsch and coworkers found intracellular localization of the receptors, suggesting that the intracellular TRAIL-R1/DR4 and TRAIL-R2/DR5 may provide a growth advantage or other benefits to tumor cells [39]. Correlation of expression status with clinical parameters revealed the predominant prognostic significance of high expression of

death receptors, with TRAIL-R2 and occasionally TRAIL-R1 in particular identified as negative prognostic markers. The evaluation of their value as prognostic markers for cancer mainly took into account the overall expression of TRAIL-receptors in the tissue and occasionally their presence at the cell surface [39].

Immunohistochemical analyzes of a tissue sample from 235 serous tumors of varying grade and stage to determine if there is differential protein expression for these candidates and Trail's 4 death cell receptors: Dr4, Dr5, DcR1 and DcR2 [21]. This study showed that TRAIL (Tnfsf10, Apo2L) was overexpressed in LMP tumors (low malignant potential) compared to all invasive tumors and was underexpressed in grade 3 tumors TOV (borderline and invasive) compared to grade 1 and 2 tumors TOV and overexpressed in early stages. It was observed that high expression of Dr5 was associated with poorer patient prognosis either when TOV tumors of all grades were included or when only the subset of grade 3 tumors TOV were included, compared to advanced stages when all tumors were included in the analysis [21]. Ouellet and coworkers also observed that high expression of Dr5 was associated with poorer patient prognosis either when TOV tumors of all grades or when only the subset of grade 3 tumors were included TOV [21].

The patient cohort studied by Horak and coworkers included 68 women with epithelial ovarian cancer. TRAIL was expressed by epithelial ovarian cancer cells in 40.4% at different levels. In stromal cells, TRAIL was stained in 43.9% [17]. In both cases, the staining was cytoplasmic. They found statistically significant higher expression of DR5, but not DR4, in the epithelium of ovarian cancer cells, which also had increased expression of TRAIL ($p = 0.041$).

However, there was also statistically significant expression of DR4 and DR5 in ovarian cancer cells ($p = 0.021$). They also investigated the influence of TRAIL and its receptors on the survival of ovarian cancer patients [17].

In a univariate regression analysis, neither epithelial DR4 and DR5 expression nor the levels of TRAIL or FLIPL in the epithelium and/or stroma of ovarian cancer were statistically significant predictors of survival benefit in our study population, but TRAIL showed a survival benefit at the mRNA level in advanced ovarian cancer [17]. They observed a loss of DR4 and/or DR5 expression in a corresponding number of cases, a factor that may contribute to tumor cell tolerance to TRAIL. This loss of DR4 and/or DR5 was significantly more frequent in tumors that did not overexpress FLIPL, suggesting two independent mechanisms of resistance in the TRAIL-induced apoptotic pathway in ovarian cancer. These data demonstrate that in addition to downregulation of DR4/DR5, the sensitivity of TRAIL can also be significantly altered by overexpres-

sion of FLIPL, and that increased stromal TRAIL expression confers a survival advantage for patients with advanced-stage ovarian cancer [17].

The resulting heterogeneity of ovarian cancer and the factors underlying clinical response make it difficult to define prognostic and predictive factors for individualized treatment. The absence of TRAIL-R2/R1 could be a marker for TRAIL resistance.

This meta-analysis has some limitations. The heterogeneity of our meta-analysis was high because of the small number of studies. Nevertheless, these results are consistent with the Kaplan-Meier results, in which the presence of these same death receptors (DR4 and DR5) were the only variables significantly associated with patient survival [17, 19]. Finally, it should be emphasized that the correlation found in the meta-analysis does not imply a causal relationship and that there is always the possibility of residual confounding in the included studies.

Conclusion

Our findings unveil that the presence of DR4, DR5 and TRAIL was predominantly identified in OCP using immunohistochemical analysis and qRT-PCR. Furthermore, we emphasize the crucial role played by elevated levels of DR4 and DR5 expression in OCP, predicting a survival advantage. Notably, in cases where TRAIL was detected, the survival rate of patients was significantly increased. The outcomes of meta-analytic investigations also validate the prevalence of DR4, DR5, and TRAIL in OCP, as well as the correlation between patient survival and the presence of death or TRAIL receptors. This underscores the importance of these signaling pathways as pivotal markers in the context of ovarian cancer. Finally, this study accentuates the necessity for ongoing research aimed at harnessing the potential of TRAIL in modulating cellular responses for enhanced health outcomes.

Compliance with ethical standards

Financial support: None.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Author contributions: All authors contributed to the development, analysis and drafting of this article.

References

1. J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, *et al.*, Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012, *Int. J. Cancer*, **136**, E359–E386 (2015). https://doi.org/10.1002/ijc.29210

2. R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2020, *CA Cancer J. Clin.*, **70**, 7–30 (2020). https://doi.org/10.3322/caac.21590

3. U.A. Matulonis, A.K. Sood, L. Fallowfield, B.E. Howitt, J. Sehouli, B.Y. Karlan, Ovarian cancer, *Nat. Rev. Dis. Prim.*, **2**, 16061 (2016). https://doi.org/10.1038/nrdp.2016.61

4. W.-F. Li, Z. Hu, N.-Y. Rao, C.-G. Song, B. Zhang, M.-Z. Cao, e*t al.*, The prevalence of BRCA1 and BRCA2 germline mutations in high-risk breast cancer patients of Chinese Han nationality: two recurrent mutations were identified, *Breast Cancer Res. Treat.*, **110**, 99–109 (2008). https://doi.org/10.1007/s10549-007-9708-3

5. D. de Miguel, J. Lemke, A. Anel, H. Walczak, L. Martinez-Lostao, Onto better TRAILs for cancer treatment, *Cell Death Differ.*, **23**, 733–747 (2016). https://doi.org/10.1038/cdd.2015.174

6. S.H.M. Wong, W.Y. Kong, C.-M. Fang, H.-S. Loh, L.-H. Chuah, S. Abdullah, *et al.*, The TRAIL to cancer therapy: Hindrances and potential solutions, *Crit. Rev. Oncol. Hematol.*, **143**, 81–94 (2019). https://doi.org/10.1016/j.critrevonc.2019.08.008

7. Y. Huang, X. Yang, T. Xu, Q. Kong, Y. Zhang, Y. Shen, *et al.*, Overcoming resistance to TRAIL-induced apoptosis in solid tumor cells by simultaneously targeting death receptors, c-FLIP and IAPs, *Int. J. Oncol.*, **49**, 153–163 (2016). https://doi.org/10.3892/ijo.2016.3525

8. U. Schaefer, TRAIL: a multifunctional cytokine, *Front. Biosci.*, **12**, 3813 (2007). https://doi.org/10.2741/2354

9. S. Fulda, Safety and tolerability of TRAIL receptor agonists in cancer treatment, *Eur. J. Clin. Pharmacol.*, **71**, 525–527 (2015). https://doi.org/10.1007/s00228-015-1823-1

10. J.P.T. Higgins, J. Thomas, J. Chandler, M. Cumpston, T. Li, M.J. Page, *et al.* (editors), *Cochrane Handbook for Systematic Reviews of Interventions*, Cochrane, 2021.

11. A. Liberati, D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gøtzsche, J.P.A. Ioannidis, *et al.*, The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration, *PLoS Med.*, **6**(7), e1000100 (2009). https://doi.org/10.1371/journal.pmed.1000100

12. M.B. Eriksen, T.F. Frandsen, The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: a systematic review, *J. Med. Libr. Assoc.*, **106**(4), 420–431 (2018). https://doi.org/10.5195/JMLA.2018.345

13. M. Ouzzani, H. Hammady, Z. Fedorowicz, A. Elmagarmid, Rayyan—a web and mobile app for systematic reviews, *Syst. Rev.*, **5**, 210 (2016). https://doi.org/10.1186/s13643-016-0384-4

14. J.R. Landis, G.G. Koch, The measurement of observer agreement for categorical data, *Biometrics*, **33**, 159 (1977). https://doi.org/10.2307/2529310

15. W.G. Lima, J.C.M. Brito, B.G. Cardoso, V.N. Cardoso, M.C. de Paiva, M.E. de Lima, *et al.*, Rate of polymyxin resistance among *Acinetobacter baumannii* recovered from hospitalized patients: a systematic review and meta-analysis, *Eur. J. Clin. Microbiol. Infect. Dis.*, **39**(8), 1427-1438 (2020). https://doi.org/10.1007/s10096-020-03876-x

16. H. Vaez, A. Sahebkar, F. Khademi, Carbapenem-resistant *Klebsiella Pneumoniae* in Iran: a systematic review and meta-Analysis, *J. Chemother.*, **31**, 1–8 (2019). https://doi.org/10.1080/1120009X.2018.1533266

17. P. Horak, D. Pils, M. Roessler, S. Tomek, K. Elandt, R. Zeillinger, *et al.*, Common death receptor 4 (DR4) polymorphisms do not predispose to ovarian cancer, *Gynecol. Oncol.*, **97**, 514–518 (2005). https://doi.org/10.1016/j.ygyno.2005.01.021

18. L.D.C. Braga, A.P. Álvares da Silva-Ramos, P. Traiman, L.M. Silva, A. Lopes da Silva-Filho, TRAIL-R3-related apoptosis: Epigenetic and expression analyses in women with ovarian neoplasia, *Gynecol. Oncol.*, **126**, 268–273 (2012). https://doi.org/10.1016/j.ygyno.2012.04.038

19. E.W. Duiker, A.G.J. van der Zee, P. de Graeff, W. Boersma-van Ek, H. Hollema, G.H. de Bock, *et al.*, The extrinsic apoptosis pathway and its prognostic impact in ovarian câncer, *Gynecol. Oncol.*, **116**, 549–555 (2010). https://doi.org/10.1016/j.ygyno.2009.09.014

20. J.M. Lancaster, R. Sayer, C. Blanchette, B. Calingaert, R. Whitaker, J. Schildkraut, *et al.*, High expression of tumor necrosis factor-related apoptosis-inducing ligand is associated with favorable ovarian cancer survival, *Clin. Cancer Res.*, **9**(2), 762-766 (2003).

21. V. Ouellet, C. Le Page, J. Madore, M.-C. Guyot, V. Barrès, C. Lussier, *et al.*, An apoptotic molecular network identified by microarray: On the TRAIL to new insights in epithelial ovarian cancer, *Cancer*, **110**, 297–308 (2007). https://doi.org/10.1002/cncr.22812

22. T. Griffith, B. James, Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptotic pathways in cancer immunosurveillance: molecular mechanisms and prospects for therapy, *Res. Reports Biochem.*, **5**, 1–10 (2015). https://doi.org/10.2147/RRBC.S59123

23. R. Liang, Y. Yao, G. Wang, E. Yue, G. Yang, X. Qi, *et al.*, Repositioning quinacrine toward treatment of ovarian cancer by rational combination with TRAIL, *Front. Oncol.*, **10**, 1118 (2020). https://doi.org/10.3389/fonc.2020.01118

24. S. Wang, W.S. El-Deiry, TRAIL and apoptosis induction by TNF-family death receptors, *Oncogene*, **22**, 8628–8633 (2003). https://doi.org/10.1038/sj.onc.1207232

25. M. Snajdauf, K. Havlova, J. Vachtenheim, A. Ozaniak, R. Lischke, J. Bartunkova, *et al.*, The TRAIL in the treatment of human cancer: An update on clinical trials, *Front. Mol. Biosci.*, **8**, 628332 (2021). https://doi.org/10.3389/fmolb.2021.628332

26. M.S. D'Arcy, Cell death: a review of the major forms of apoptosis, necrosis and autophagy, *Cell. Biol. Int.*, **43**, 582–592 (2019). https://doi.org/10.1002/cbin.11137

27. C. Falschlehner, C.H. Emmerich, B. Gerlach, H. Walczak, TRAIL signalling: Decisions between life and death, *Int. J. Biochem. Cell. Biol.*, **39**, 1462–1475 (2007). https://doi.org/10.1016/j.biocel.2007.02.007

28. N. Pathoulas, *Investigating TRAIL sensitivity in platinum-resistant ovarian cancer*, College Thesis, The College of Saint Benedict and Saint John's University, St. Joseph, Minnesota, 2019.

29. N.G. Khaider, D. Lane, I. Matte, C. Rancourt, A. Piché, Targeted ovarian cancer treatment: the TRAILs of resistance, *Am. J. Cancer Res.*, **2**, 75–92 (2012).

30. X. Dai, J. Zhang, F. Arfuso, A. Chinnathambi, M. Zayed, S.A. Alharbi, *et al.*, Targeting TNF-related apoptosis-inducing ligand (TRAIL) receptor by natural products as a potential therapeutic approach for cancer therapy, *Exp. Biol. Med.*, **240**, 760–773 (2015). https://doi.org/10.1177/1535370215579167

31. E. Razeghian, W. Suksatan, H. Sulaiman-Rahman, D.O. Bokov, W.K. Abdelbasset, A. Hassanzadeh, *et al.*, Harnessing TRAIL-induced apoptosis pathway for cancer immunotherapy and associated challenges, *Front. Immunol.*, **12**, 699746 (2021). https://doi.org/10.3389/fimmu.2021.699746

32. J. Abdulghani, W.S. El-Deiry, TRAIL receptor signaling and therapeutics, *Expert Opin. Ther. Targets*, **14**, 1091–1108 (2010). https://doi.org/10.1517/14728222.2010.519701

33. A. Willms, H. Schupp, M. Poelker, A. Adawy, J.F. Debus, T. Hartwig, et al., TRAIL-receptor 2—a novel negative regulator of p53, *Cell Death Dis.*, **12**, 757 (2021). https://doi.org/10.1038/s41419-021-04048-1

34. M. de Looff, S. de Jong, F.A.E. Kruyt, Multiple interactions between cancer cells and the tumor microenvironment modulate TRAIL signaling: Implications for TRAIL receptor targeted therapy, *Front. Immunol.*, **10**, 1530 (2019). https://doi.org/10.3389/fimmu.2019.01530

35. Z.-X. Zhang, I. Gan, A. Pavlosky, X. Huang, B. Fuhrmann, A.M. Jevnikar, Intracellular pH regulates TRAIL-induced apoptosis and necroptosis in endothelial cells, *J. Immunol. Res.*, **2017**, 1503960 (2017). https://doi.org/10.1155/2017/1503960

36. C.R. Reis, P.-H. Chen, N. Bendris, S.L. Schmid, TRAIL-death receptor endocytosis and apoptosis are selectively regulated by dynamin-1 activation, *Proc. Natl. Acad. Sci.*, **114**, 504–509 (2017). https://doi.org/10.1073/pnas.1615072114

37. J. Zhu, Q. Zhou, S. Tan, Targeting miRNAs associated with surface expression of death receptors to modulate TRAIL resistance in breast cancer, *Cancer Lett.*, **383**, 154–160 (2016). https://doi.org/10.1016/j.canlet.2016.09.021

38. D. Sarhan, P. D'Arcy, A. Lundqvist, Regulation of TRAIL-receptor expression by the ubiquitin-proteasome system, *Int. J. Mol. Sci.*, **15**, 18557–18573 (2014). https://doi.org/10.3390/ijms151018557

39. U. Bertsch, C. Röder, H. Kalthoff, A. Trauzold, Compartmentalization of TNF-related apoptosis-inducing ligand (TRAIL) death receptor functions: emerging role of nuclear TRAIL-R2, *Cell Death Dis.*, **5**, e1390 (2014). https://doi.org/10.1038/cddis.2014.351

40. D. Chen, M. Frezza, S. Schmitt, J. Kanwar, P.Q. Dou, Bortezomib as the first proteasome inhibitor anticancer drug: Current status and future perspectives, *Curr. Cancer Drug Targets*, **11**(3), 239–253 (2011). https://doi.org/10.2174/156800911794519752

41. C.T. Hellwig, M.E. Delgado, J. Skoko, L. Dyck, C. Hanna, A. Wentges, *et al.*, Proteasome inhibition triggers the formation of TRAIL receptor 2 platforms for caspase-8 activation that accumulate in the cytosol, *Cell Death Differ.*, **29**, 147–155 (2022). https://doi.org/10.1038/s41418-021-00843-7

Supplementary file: Details of the search strategy

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OR (receptor AND 4, AND trail) OR (receptors, AND tumor AND necrosis AND factor, AND member AND 10a) OR (tumor AND necrosis AND factor AND receptor AND superfamily, AND member AND 10a) OR (apo2 AND receptor) OR (receptor, AND apo2) OR (receptor, AND trail-1) OR (receptor, AND trail 1) OR (trail-1 AND receptor) OR (trail AND receptor 1) OR (dr4 AND receptor) OR (receptor, AND dr4) OR (death AND receptor-4) OR (death AND receptor 4) OR (receptor-4, AND death) OR (receptors, AND tumor AND necrosis AND factor, AND member AND 10b) OR (trail AND receptor 2) OR (receptor, AND trail-2) OR (receptor, AND trail 2) OR (trail-2 AND receptor) OR (dr5 AND receptor) OR (receptor, AND dr5) OR (tumor AND necrosis AND factor AND receptor AND superfamily, AND member AND 10b) OR (tnf-related AND apoptosis-inducing AND ligand AND receptor 2) OR (tnf AND related AND apoptosis AND inducing AND ligand AND receptor 2) OR (death AND receptor-5) OR (death AND receptor 5) OR (trail) OR (tnfrsf10) OR (tnfrsf10c) OR (trailr3)) AND TITLE-ABS-KEY (cancer OR cancers OR neoplasm OR neoplasms) AND TITLE-ABS-KEY (ovary OR ovarian) AND TITLE-ABS-KEY (survive OR mortality OR survivorship OR (disease AND free AND survival) OR (overall AND survival))

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(((TI=(trail OR TNFRSF10 OR Death Receptor)) OR AB=(trail OR TNFRSF10 OR Death Receptor)) AND (TI=(cancer OR cancers OR neoplasm OR neoplasms) OR AB=(cancer OR cancers OR neoplasm OR neoplasms)) AND (TI=(ovary OR ovarian) OR AB=(ovary OR ovarian)) AND ((TI=(survive OR mortality OR survivorship)) OR AB=(survive OR mortality OR survivorship)))

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(receptor, AND trail-1) OR (receptor, AND trail 1) OR (trail-1 AND receptor) OR (trail AND receptor 1) OR (dr4 AND receptor) OR (receptor, AND dr4) OR (death AND receptor-4) OR (death AND receptor 4) OR (receptor-4, AND death) OR (receptors, AND tumor AND necrosis AND factor, AND member AND 10b) OR (trail AND receptor 2) OR (receptor, AND trail-2) OR (receptor, AND trail 2) OR (trail-2 AND receptor) OR (dr5 AND receptor) OR (receptor, AND dr5) OR (tumor AND necrosis AND factor AND receptor AND superfamily, AND member AND 10b) OR (tnf-related AND apoptosis-inducing AND ligand AND receptor 2) OR (tnf AND related AND apoptosis AND inducing AND ligand AND receptor 2) OR (death AND receptor-5) OR (death AND receptor 5) OR (trail) OR (tnfrsf10) OR (tnfrsf10c) OR (trailr3))

#2 - (cancer OR cancers OR neoplasm OR neoplasms)

#3 - (ovary OR ovarian)

#4 - (survive OR mortality OR survivorship OR (disease AND free AND survival) OR (overall AND survival))

#5 - #1 AND 2# AND 3# AND 4#

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(trail-2 AND receptor) OR (dr5 AND receptor) OR (receptor, AND dr5) OR (tumor AND necrosis AND factor AND receptor AND superfamily, AND member AND 10b) OR (tnf-related AND apoptosis-inducing AND ligand AND receptor 2) OR (tnf AND related AND apoptosis AND inducing AND ligand AND receptor 2) OR (death AND receptor-5) OR (death AND receptor 5) OR (trail) OR (tnfrsf10) OR (tnfrsf10c) OR (trailr3)) AND (cancer OR cancers OR neoplasm OR neoplasms) AND (ovary OR ovarian) AND (survive OR mortality OR survivorship OR (disease AND free AND survival) OR (overall AND survival)))

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Identificação e avaliação da susceptibilidade antimicrobiana de *Serratia marcescens* recuperadas de um rio urbano

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Resumo

Introdução: *Serratia marcescens* é considerada causa de infecções em pacientes imunocomprometidos e recém-nascidos e o tratamento é desafiador, devido a sua resistência intrínseca a vários antimicrobianos. É encontrada no solo, plantas e água, nesta última a resistência antimicrobiana é menos estudada. Neste trabalho foi investigada a presença e a susceptibilidade antimicrobiana de *S. marcescens* em água de um rio urbano. **Material e Métodos:** Para tal objetivo foi conduzida uma cultura enriquecida da água sob pressão seletiva da colistina. Os isolados foram identificados por métodos bioquímicos-fisiológicos e os testes de perfil de susceptibilidade aos antimicrobianos e investigação da produção de betalactamases de espectro estendido (ESBL) e ampicilinase tipo C (AmpC) seguiram o BrCAST 2017 e 2022. **Resultados:** $1{,}9 \times 10^3$ UFC/mL (aproximadamente 9%) das bactérias recuperadas eram *S. marcescens*. Alta sensibilidade aos betalactâmicos (73,7%) foi observada, mas dois isolados (10,5%) foram ertapenem-resistentes. Todos os isolados foram amicacina-sensíveis e três isolados (15,8%) apresentaram resistência a gentamicina. Também resistência a fosfomicina (52,6%) e sulfametoxazol-trimetoprima (57,9%)

foi observada. De particular preocupação foi o achado de *S. marcescens* multirresistente (31,5 %), mais frequentemente a sulfametoxazol-trimetoprima, cloranfenicol e fosfomicina. De acordo com os testes fenotípicos, foi sugerido que nenhum isolado era produtor de ESBL e AmpC, porém é provável a produção de carbapenemase por dois isolados. **Conclusão:** Rios urbanos são um importante reservatório de *S. marcescens* resistentes a múltiplos antimicrobianos e políticas de vigilância ambiental nestes ambientes devem ser estimuladas para minimizar o impacto de achados como esses sobre a saúde da comunidade local.

Palavras-chave: Serratia marcescens, Resistência, Antimicrobianos, Meio ambiente.

Summary

Identification and antimicrobial susceptibility assessment of *Serratia marcescens* recovered from an urban river

Introduction: *Serratia marcescens* is considered a cause of infections in immunocompromised patients and newborns and treatment is challenging due to its intrinsic resistance to several antimicrobials. It is found in soil, plants and water, in the latter antimicrobial resistance is less studied. In this work, the presence and antimicrobial susceptibility of *S. marcescens* in water from an urban river were investigated. **Material and Methods:** For this purpose, an enriched water culture was carried out under selective colistin pressure. The isolates were identified by biochemical-physiological methods and the antimicrobial susceptibility profile tests and investigation of the production of extended spectrum beta-lactamase (ESBL) and ampicillinase type C (AmpC) followed the BrCAST 2017 and 2022. **Results:** 1.9×10^3 CFU/mL (approximately 9%) of the recovered bacteria were *S. marcescens.* High sensitivity to beta-lactams (73.7%) was observed, but two isolates (10.5%) were ertapenem-resistant. All isolates were amikacin-sensitive and three isolates (15.8%) were resistant to gentamicin. Also, resistance to fosfomycin (52.6%) and trimethoprim-sulfamethoxazole (57.9%) were observed. Of particular concern was the finding of multidrug-resistant *S. marcescens* (31.5%), most frequently to trimethoprim-sulfamethoxazole, chloramphenicol, and fosfomycin. According to the phenotypic tests, it was suggested that none of the isolates produced ESBL and AmpC, but it is probable that two isolates produced carbapenemase. **Conclusion:** Urban Rivers are an important reservoir of *S. marcescens* resistant to multiple antimicrobials and environmental surveillance policies in these environments should be encouraged to minimize the impact of findings like these on the health of the local community.

Keywords: *Serratia marcescens*, Resistance, Antimicrobials, Environment.

Resumen

Identificación y evaluación de la susceptibilidad antimicrobiana de *Serratia marcescens* recuperada de un río urbano

Introducción: *Serratia marcescens* se considera una causa de infecciones en pacientes inmunocomprometidos y recién nacidos y el tratamiento es un desafío debido a su resistencia intrínseca a varios antimicrobianos. Se encuentra en suelo, plantas y agua, en esta última está menos estudiada la resistencia antimicrobiana. En este trabajo se investigó la presencia y susceptibilidad antimicrobiana de *S. marcescens* en agua de un río urbano. **Material y Métodos:** Se realizó un cultivo enriquecido de agua bajo presión selectiva de colistina. Los aislamientos fueron identificados por métodos bioquímicos-fisiológicos y las pruebas de perfil de susceptibilidad antimicrobiana y la investigación de la producción de betalactamasa de espectro extendido (BLEE) y ampicilinasa tipo C (AmpC) siguieron el BrCAST 2017 y 2022. **Resultados:** $1{,}9 \times 10^3$ CFU/ml (aproximadamente el 9%) de las bacterias recuperadas eran *S. marcescens*. Se observó una alta sensibilidad a los betalactámicos (73,7%), pero dos aislados (10,5%) fueron resistentes a ertapenem. Todos los aislamientos fueron sensibles a la amikacina y tres (15,8%) fueron resistentes a la gentamicina. También se observó resistencia a fosfomicina (52,6%) y trimetoprim-sulfametoxazol (57,9%). De particular preocupación fue el hallazgo de *S. marcescens* multirresistente (31,5%), con mayor frecuencia a trimetoprima-sulfametoxazol, cloranfenicol y fosfomicina. De acuerdo con las pruebas fenotípicas, se sugirió que ninguno de los aislamientos produjo BLEE y AmpC, pero es probable que dos aislamientos produjeron carbapenemasa. **Conclusión**: Los ríos urbanos son un reservorio importante de *S. marcescens* resistente a múltiples antimicrobianos y se deben alentar políticas de vigilancia ambiental en estos entornos para minimizar el impacto de hallazgos como estos en la salud de la comunidad local.

Palabras clave: Serratia marcescens, Resistencia, Antimicrobianos, Medio ambiente.

Introdução

Serratia marcescens, um bacilo gram-negativo da família *Yersiniacea*, ordem Enterobacteriales, por adaptar-se facilmente a variações de temperatura e pH, é encontrada em habitats naturais como água, solo, plantas, além de compor a microbiota intestinal de animais homeotérmicos e humanos [1, 2]. Apresenta como característica particular, a

produção de pigmento vermelho (prodigiosina), para o qual têm sido apontadas atividades biológicas, incluindo ação pro-apoptótica contra bactérias, fungos e linhagens celulares de câncer [3].

Apesar de ser associada à baixa virulência, *S. marcescens* tem sido considerada um importante patógeno para pacientes gravemente comprometidos e recém-nascidos, causando principalmente infecções do trato urinário e da corrente sanguínea [4]. No Brasil, surtos dessa espécie em Unidades de Terapia Intensiva têm sido relatados no Rio de Janeiro [5] e Distrito Federal [6], com óbitos confirmados.

O tratamento de infecções por *S. marcescens* é desafiador, uma vez que apresenta resistência intrínseca a vários antimicrobianos (cefalosporinas de primeira geração, tetraciclinas, polimixinas e nitrofuranos), além da crescente resistência adquirida a outros fármacos [7, 8].

Dentre os mais frequentes mecanismos de resistência identificados em *S. marcescens* destaca-se a produção de enzimas betalactamases de espectro estendido (ESBL), superexpressão de ampicilinase tipo C (AmpC), produção de carbapenemases, bomba de efluxo e modificações das proteínas da parede celular bacteriana [9]. Tornando esse cenário mais crítico, a maioria desses mecanismos é veiculada por elementos genéticos móveis, o que confere alto potencial de disseminação [10]. Além disso, espécies de *S. marcescens* multirresistente apresentando associação de determinantes de resistência têm sido encontradas, inclusive no Brasil [11].

Deve ser ressaltado que, ao contrário do cenário clínico, estudos que descrevam o contexto da resistência antimicrobiana de *S. marcescens* de origem ambiental são escassos. De acordo com Joutey *et al.* (2013) [12], bactérias ambientais geralmente são resistentes e conseguem suportar condições extremas, incluindo a presença de metais pesados e outros resíduos antrópicos. De fato, resistência a aminoglicosídeos, carbapenêmicos e quinolonas já foram descritas em *S. marcescens* de rio e efluentes não tratados [13, 14], além do achado de cepas multirresistentes e de maior virulência em peixes [15].

Considerando a perspectiva *One Health*, reconceitualizado e adotado pela Organização Mundial da Saúde [16] e os relatos de resistência antimicrobiana em *S. marcescens* recuperadas de ambientes não clínicos, mais pesquisas são necessárias a fim de ampliar o conhecimento do perfil de susceptibilidade a antimicrobianos clinicamente relevantes nesta espécie, sobretudo em águas de rio urbano, o qual tem grande impacto na vida da população que vive à sua margem. Dessa forma, o objetivo deste trabalho foi investigar a possível presença de *S. marcescens* em água de rio, bem como determinar o perfil de susceptibilidade a antimicrobianos são considerados opções importantes na terapêutica das infecções humanas.

Material e métodos

Amostra de água e cultura enriquecida

Amostra de água (um litro) foi coletada em um ponto do Rio Itapecerica próximo a uma área residencial, hospitalar e comercial da cidade de Divinópolis-MG (Latitude: 20° 28' 24'' Sul, Longitude: 45° 7' 36'' Oeste) em garrafa de polipropileno esterilizada e levada para o laboratório por transporte refrigerado e processada nas duas horas seguintes à coleta, no Laboratório de Microscopia/ Diagnóstico Laboratorial e Microbiologia Clínica da Universidade Federal de São João Del-Rei (Divinópolis-MG/Brasil).

Para a seleção de *S. marcescens*, a amostra de água do rio foi inoculada em dois balões (20 mL em cada), contendo 100 mL de caldo *Brain Heart Infusion* (BHI) (TM Media, Índia), sem e com colistina (COL) (ABL/Brasil) na concentração final de 4 μg/mL, considerando sua resistência intrínseca a este fármaco, e incubados a 35±2 °C por 24 horas. A concentração da COL foi definida de acordo com os breakpoints do *Brazilian Committee on Antimicrobial Susceptibility Testing* (BrCAST, 2022) [17] que considera como "resistente" Enterobacteriales, cuja concentração inibitória mínima (CIM) seja superior a 2 μg/mL.

Quantificação, isolamento e identificação de *S. marcescens*

Após o período de incubação, os balões foram observados para a inspeção macroscópica da turbidez, um indício do crescimento bacteriano, foi realizada uma diluição seriada e 100, 10 e 1 μL de cada diluição foram transferidos para placas de ágar MacConkey (Isofar, Brasil), distribuídos na superfície das placas por meio da técnica de *spread plate*. O experimento foi realizado em duplicata e as placas incubadas a 35±2 °C por até 48 horas.

Após o crescimento bacteriano, foi realizada a quantificação total de bactérias gram-negativas resistentes a colistina nas placas de ágar MacConkey onde houve crescimento de 20 a 200 colônias. O número de Unidades Formadores de Colônias (UFC/mL) foi calculado usando a equação:

UFC = Número de colônias × Fator de diluição / Volume de inóculo

Adicionalmente, colônias com características macroscópicas de *S. marcescens* crescidas em ágar MacConkey (colônias grandes, não fermentadoras de lactose e produtoras de pigmento vermelho) foram também quantificadas como citado anteriormente. Após quantificação, os morfotipos foram selecionados, inoculados em 1 mL de caldo BHI

(TM Media, Índia) e incubados em estufa a 35±2 ºC por 24 horas. Após a incubação, foram inoculadas em ágar MacConkey e incubadas a 35±2 ºC por até 48 horas para verificação da pureza e confirmação da produção de pigmentos específicos (prodigiosina).

A seguir, todas as colônias foram submetidas à coloração de Gram para confirmação da morfologia e identificadas utilizando provas de identificação bioquímico-fisiológicas clássicas, incluindo provas da produção da enzima oxidase, da fermentação de carboidratos (glicose, lactose e sacarose), da produção de pigmentos, da motilidade, da descarboxilação de lisina, da produção de indol, da produção de H_2S, da utilização de citrato e malonato como fonte de carbono e da produção urease [18]. Após finalização da identificação da espécie, os isolados obtidos foram transferidos para microtubos contendo 500 µl de BHI acrescido de 20% de glicerol, sendo então rotulados e armazenados em freezer à -20°C.

Determinação do perfil de susceptibilidade aos antimicrobianos

O perfil de susceptibilidade dos isolados de *S. marcescens* frente à fosfomicina (FOS), aztreonam (ATM), ciprofloxacina (CIP), amicacina (AMI), gentamicina (GEN), ceftazidima (CAZ), cefepime (CPM), ceftriaxona (CRO), meropenem (MEM), ertapenem (ETP), imipenem (IPM), cefotaxima (CTX), sulfametoxazol-trimetoprima (SUT) e clorafenicol (CIO) (CECON®, Brasil) foi determinado pelo método de disco difusão de acordo com o BrCAST (2022) [17], considerando os *breakpoints* estabelecidos para cada antimicrobiano. *S. marcescens* resistentes a compostos de pelo menos três classes diferentes foram consideradas multirresistentes (MDR) [19].

Inóculos em caldo BHI (TM Media, Índia) compatíveis com a escala 0,5 de MacFarland (10^8 UFC/mL) foram realizados a partir das placas de ágar MacConkey. Posteriormente, foi realizado o inóculo em placas de ágar Mueller Hinton (MH) (Himedia, Índia), aplicados os discos de antimicrobianos e as placas incubadas a 35±2 ºC, por 24 h.

Detecção da produção da enzima betalactamase do tipo ESBL e AmpC

Todos os isolados foram submetidos ao teste fenotípico para investigação da presença de enzimas betalactamases do tipo ESBL usando o método dupla difusão seguindo orientações do BrCAST (2017) [20]. Após inoculação de cada isolado de *S. marcescens* (10^8 UFC/mL) em ágar MH (Himedia, Índia), um disco de amoxicilina com ácido clavulânico (30 µg) foi colocado no meio de placa e, a uma distância de 20 mm entre os discos, foram posicionados os substratos CTX, CAZ e CPM. As placas foram incubadas a 35±2 ºC por 24h e os resultado avaliados por meio de uma distorção de halo de inibição observada em qualquer uma das cefalosporinas testadas, o qual indica a produção de ESBL pelo isolado.

A detecção da produção da enzima AmpC foi conduzida de acordo com Elsayed *et al.* (2015) [21], utilizando a abordagem do teste de disco com imipenem, cefoxitina e amoxicilina/ácido clavulânico como indutores e ceftazidima como substrato. Um achatamento do halo da ceftazidima indica resultado positivo, ou seja, produção da enzima AmpC. Além disso, foi utilizado o algoritmo para triagem de produção de AmpC padronizado pelo BrCAST (2017) [20] com a avaliação do perfil de susceptibilidade a cefoxitina, ceftriaxona e ceftazidima.

Adicionalmente, triagem da produção de carbapenemase pelos isolados foi avaliada de acordo com o BrCAST (2017) [20] utilizando os pontos de corte definidos para meropenem e ertapenem.

Resultados e discussão

Rios urbanos frequentemente recebem efluentes domésticos, industriais e hospitalares, o que resulta na composição de uma microbiota altamente diversificada. Além disso, neste microbioma, bactérias resistentes e genes de resistência podem estar presentes, fato pelo qual esses ambientes são considerados *hotspot* para o desenvolvimento e disseminação da resistência antimicrobiana [22].

Neste trabalho, após análise da amostra coletada, a carga total de bactérias resistentes a colistina foi superior a $2{,}0 \times 10^5$ UFC/mL (202 colônias), sendo que $1{,}9 \times 10^3$ UFC/mL (aproximadamente 9,0%) eram de *S. marcescens*, a qual é intrinsecamente resistente a este fármaco. De fato, espécies de Enterobacteriales estão presentes em ambientes aquáticos, tais como estação de tratamento de esgoto, e *S. marcescens* é relatada em estudos dessas amostras em proporções variadas, tais como 16,0% [23] e 6,2% [24].

No Brasil, a maioria dos estudos aponta para uma baixa proporção ou mesmo ausência de *S. marcescens* em ambientes aquáticos. Por exemplo, essa espécie não foi encontrada em águas de uma estação de tratamento de esgoto [25], nem em rios da Bacia do Rio Doce [26]. Além disso, um estudo na mesma região deste trabalho (centro-oeste de Minas Gerais) mostrou ausência de *S. marcescens* no Rio Pará [27].

A partir da cultura em placas, um total de 19 colônias produtoras de prodigiosina foi identificado como *S. marcescens*. Estes isolados foram submetidos ao teste de susceptibilidade aos antimicrobianos que pode ser observado na Tabela 1.

Tabela 1. Perfil de susceptibilidade aos antimicrobianos dos isolados de *S. marcescens* recuperados do rio Itapecerica, Divinópolis-MG.

Isolados de Serratia marcescens	Antimicrobianos													
	CTX	CAZ	CRO	CPM	ATM	IPM	MEM	ERT	GEN	AMI	CIP	FOS	SUT	CLO
SRI1	S	S	S	S	S	S	S	S	**R**	S	**R**	**R**	**R**	**R**
SRI2	S	S	S	S	S	S	S	S	S	S	S	S	**R**	**R**
SRI3	S	S	S	S	S	S	S	S	S	S	S	R	S	S
SRI4	S	S	S	S	**R**	S	S	S	S	S	S	S	**R**	**R**
SRI5	S	S	S	S	S	S	S	S	**R**	S	S	S	**R**	**R**
SRI6	S	S	S	S	S	S	S	S	S	S	S	S	**R**	S
SRI7	S	S	S	S	S	S	S	S	S	S	S	**R**	**R**	S
SRI8	S	S	S	S	S	S	S	S	S	S	S	**R**	**R**	**R**
SRI9	S	S	S	S	**R**	S	S	S	S	S	S	**R**	S	S
SRI11	S	S	S	S	S	S	S	S	S	S	S	**R**	**R**	S
SRI12	S	S	S	S	S	S	S	S	S	S	S	S	S	S
SRI13	S	S	S	S	S	S	S	S	S	S	S	**R**	**R**	S
SRI14	S	S	S	S	S	S	S	S	S	S	S	S	S	S
SRI15	S	S	S	S	S	S	S	S	S	S	S	S	S	S
SRI16	S	S	S	S	S	S	S	S	S	S	S	S	S	S
SRI17	S	**R**	S	**R**	S	S	S	**R**	S	S	S	S	S	**R**
SRI18	S	S	S	S	S	S	S	S	S	S	S	**R**	**R**	**R**
SRI 19	S	S	S	**R**	**R**	S	S	**R**	S	S	S	**R**	S	S
SRI 20	S	**R**	S	S	S	S	S	S	**R**	S	S	**R**	**R**	**R**

SRI- *Serratia marcescens*, Rio Itapecerica. **FOS**: Fosfomicina, **ATM**: Aztreonam,**CIP**: Ciprofloxacina, **AMI**: Amicacina, **GEN**: Gentamicina, **CAZ**: Ceftazidima, **CPM**: Cefepime, **CRO**: Ceftriaxona, **MEM**: Meropenem, **ERT**: Ertapenem, **IPM**: Imipenem, **CTX**: Cefotaxima, **SUT**: Sulfametoxazol-trimetoprima, CLO: Clorafenicol, S: sensível, R: resistente.

Aqui, além da resistência intrínseca a colistina, a nitrofuranos e a betalactâmicos (ampicilina, amoxicilina/ácido clavulânico, ticarcilina, cefalotina, cefazolina, cefoxitina, cefotetan e cefuroxima) CLSI, 2022) [28], alguns isolados de *S. marcescens* também exibiram resistência a outros compostos antimicrobianos. No entanto, quatro isolados foram sensíveis a todos os antimicrobianos testados (Tabela 1).

Com relação aos betalactâmicos, uma taxa geral de sensibilidade de 73,7% (14/19) foi observada, enquanto dois isolados mostraram resistência só ao aztreonam e um só a ceftazidima. Curiosamente, resistência a cefepime, uma cefalosporina de quarta geração, também foi observada em dois isolados (SRI 17 e SRI 19), no primeiro a resistência foi associada à baixa susceptibilidade a ceftazidima. Resistência a três compostos betalactâmicos (CAZ, CPM, ERT ou CPM, ATM, ERT), apesar de baixa (2/19, 10,5%), foi observada entre os isolados, um perfil relevante no contexto clínico uma vez que inutiliza antimicrobianos de gerações mais recentes dos betalactâmicos. De fato, tem sido relatado aumento da resistência a betalactâmicos em isolados extra-clínicos, como por exemplo, em *S. marcescens* recuperadas de animais, na qual altas taxas de resistência a CPM e CAZ, além de isolados com perfil de resistência multirresistência [29] são observadas.

Aqui, a taxa geral de sensibilidade aos três carbapenêmicos simultaneamente foi alta (17/19, 89,5%), mas dois isolados devem ser destacados, ambos apresentando resistência ao ertapenem. Enterobacteriales, incluindo *S. marcescens* carbapenem-resistentes estão na lista de prioridade crítica da *World Health Organization* (WHO, 2017) [30] uma vez que tem aumentado o relato de espécies com esse fenótipo no cenário clínico humano [31] e, apesar de taxas ainda baixas, também em isolados de origem veterinária [32]. Ao contrário do cenário clínico, estudos que investigam *S. marcescens* carbapenem-resistentes em ambientes como rio são escassos. Dessa forma, o achado deste estudo é preocupante, uma vez que se trata de antimicrobianos de última geração, utilizados para tratar infecções por bactérias gram-negativas multirresistentes, além da possibilidade de disseminação da resistência com limitação das opções terapêuticas [33].

Outra classe de antimicrobiano investigada neste estudo foi a dos aminoglicosídeos, especificamente gentamicina e amicacina. Todos os isolados foram sensíveis a amicacina, mas três isolados (15,8%) apresentaram resistência à gentamicina. Em um contexto de saúde animal, *S. marcescens* causando mastite em bovinos foram sensíveis a gentamicina e, provavelmente pela sua menor utilização para o tratamento dessas infecções, apresentou maior eficácia [34]. A sensibilidade dos isolados a amicacina nesse estudo, de acordo com Gad *et al.* (2011) [35], pode se dar pelo fato dos antimicrobianos aminoglicosídeos serem mais antigos e menos utilizados na atualidade. Dessa forma, a sensibilidade pode estar relacionada a pouca exposição da *S. marcescens* a esses antimicrobianos.

Ciprofloxacina, fosfomicina e sulfametoxazol-trimetoprima são antimicrobianos amplamente utilizados para o tratamento de infecções do trato urinário causadas por bactérias gram-negativas [36, 37]. Apesar dos resíduos de quinolonas poderem permanecer ativos em ambientes aquáticos e com isso favorecer a seleção de bactérias resistentes [38], resistência a ciprofloxacina foi observada apenas em um isolado de *S. marcescens* (SRI1). No entanto, apesar do perfil de susceptibilidade a esta classe de antimicrobianos ser pouco estudada em isolados ambientais, é relatada a presença de mecanismos de resistência a quinolonas nesta espécie, mais frequentemente bomba de efluxo (*superfamily* RND) [39], mas mutações em *gyrA* também têm sido descritas [40].

Por outro lado, resistência maior a fosfomicina (10/19, 52,6%) e sulfametoxazol-trimetoprima (11/19, 57,9%) alerta para um risco iminente de impossibilidade de utilização terapêutica desses antimicrobianos. Dados na literatura sobre o contexto da resistência de *S. marcescens* de ambiente aquático a esses fármacos são escassos, porém Li *et al.* (2022) [41] relataram que fosfomicina é pobremente degradada em águas residuais e exerce pressão seletiva na comunidade bacteriana, o que provavelmente resulta em resistência antimicrobiana. Da mesma forma, de acordo com Oharisi *et al*. 2023 [42], sulfametoxazol foi o antimicrobiano encontrado em maior concentração em estações de tratamento de esgoto e corpos d'água receptores do efluente na África do Sul, o que impõe risco ao ecossistema aquático, inclusive com a possibilidade de aumento da resistência antimicrobiana.

Resistência ao cloranfenicol também tem sido relatada, relacionada à produção de aceltiltransferase, que pode ser disseminada entre as espécies e, neste estudo, 42,1% dos isolados foram resistentes a esse fármaco. Em 1982, Bartlett *et al. [43]* já relatavam taxas de resistência elevadas (85,0%) a este antimicrobiano em isolados de *S.* marcescens, assim como Tomanic´ *et al*. (2022) [34], que detectaram resistência ao cloranfenicol em todos os isolados de origem animal estudados.

De acordo com Magiorakos *et al.* (2012) [19], bactérias multirresistentes (MDR) apresentam resistência a pelo menos um antimicrobiano de três ou mais classes diferentes. A resistência simultânea a várias classes de antimicrobianos tem aumentado entre isolados bacterianos, desafiando a comunidade científica e causando grande preocupação na comunidade médica no tocante às opções terapêuticas.

No presente estudo, a análise dos dados mostrou que 31,5 % dos isolados de *S. marcescens* exibiram fenótipo MDR, mais frequentemente resistentes a SUT, FOS e CLO. Destacam-se nesse contexto os isolados SRI1 e SRI20, para os quais resistência a compostos de cinco classes distintas de antimicrobianos foi observada (**Tabela 1**). Assim, nossos achados revelam que *S. marcescens* MDR estão circulando no rio estudado, o

que leva a uma grande preocupação uma vez que a resistência microbiana é um dos mais graves e emergentes problemas de saúde pública [44]. Em paralelo, Iguchi *et al.* (2014) [45] relataram *S. marcescens* MDR no cenário clínico, com a resistência simultânea relacionada às cefalosporinas, como aqui observado, além de aminoglicosídeos. Ainda, um estudo em isolados de *S. marcescens* de origem animal relatou o achado de espécies extensivamente resistentes (XDR) [29], o que aponta para um cenário ainda mais crítico considerando que bactérias XDR são susceptíveis a apenas uma ou duas categorias de antimicrobianos atualmente disponíveis para a terapêutica das infecções [19].

Considerando a resistência antimicrobiana em ambiente aquático, deve ser discutida a presença de resíduos de fármacos como fator preponderante para o surgimento de bactérias resistentes. A maioria dos antimicrobianos usados é excretada diretamente ou indiretamente nos rios, através de esgoto hospitalar ou domiciliar e pelas indústrias farmacêuticas. O tempo de biodegradação desses antimicrobianos varia de meses a anos dependendo das suas condições físico-químicas, sendo que a capacidade de resistir aos processos de degradação aumenta seu tempo no ambiente e, por consequência, a pressão seletiva exercida [46]. Além disso, é frequente o descarte de antimicrobianos na sua forma original ou metabólitos ativos no ambiente, ocasionando acúmulo nos rios, maior exposição microbiana e resistência antimicrobiana [47]. Diante do exposto, além do uso racional de antimicrobianos, políticas de proteção ambiental deveriam ser implementadas para minimizar o aporte de antimicrobianos e o surgimento da resistência microbiana.

Vale ressaltar que o rio Itapecerica, objeto desse estudo, recebe esgoto em drenagem espontânea, sem qualquer tratamento e que a amostra de água incluída foi coletada próxima a pontos de descarga de esgoto doméstico e hospitalar. Assim, uma hipótese para a resistência observada nas cepas de *S. marcescens* seria a alta exposição a resíduos de antimicrobianos, os quais impactam no desenvolvimento de resistência aos fármacos por exercerem pressão seletiva sobre a comunidade bacteriana [48].

Tão importante quanto conhecer o perfil de susceptibilidade aos antimicrobianos dos isolados ambientais é a investigação dos mecanismos de resistência bacteriana, sobretudo aqueles que são veiculados por elementos genéticos móveis, com o intuito de conter a disseminação dessa resistência. Todos os isolados de *S. marcescens* foram submetidos a testes investigativos da produção das enzimas ESBL e AmpC por abordagem fenotípica.

Curiosamente, nenhum isolado foi considerado produtor de ESBL, um importante mecanismo de resistência comum entre bactérias gram-negativas MDR no cenário clínico. De fato, *S. marcescens* ESBL-positivas de ambientes aquáticos não tem sido um relato frequente, apesar de ser considerada a espécie origem de uma classe de ESBL, a

enzima BES-1 descrita no Brasil [49] em um isolado clínico. Lu *et al.* (2010) [50] relatam o achado desta espécie com este fenótipo em um rio da China e Rybak *et al.* (2021) [51] encontraram *Serratia fonticola* produtora de ESBL em reservatório de água na Polônia, no entanto, no Brasil estudos são escassos e assim como neste trabalho, Chagas *et al.* (2011) [52] não detectaram *S. marcescens* ESBL-positiva em amostras de esgoto hospitalar no Rio de Janeiro.

Produção de AmpC não foi detectada entre os isolados considerando as abordagens fenotípicas propostas por Elsayed *et al.* (2015) [21] e BrCAST (2017) [20]. *S. marcescens*, assim como *Citrobacter freundii*, *Enterobacter* spp, *Providência rettgeri*, *Providência stuartii* e *Morganella morganii* que compõem o grupo CESP, são caracterizadas por albergarem cromossomicamente o gene *ampC*, que em uma condição de indução ou superexpressão, resulta em perda da atividade e do espectro de ação das cefalosporinas da terceira geração, monobactâmicos e penicilinas [53, 54].

Por outro lado, o BrCAST (2017) [20] recomenda a triagem da produção de carbapenemase por análise dos halos de inibição obtidos para meropenem e ertapenem. Considerando o proposto (halos de inibição < 25 mm), a resistência ao ertapenem dos isolados SRI17 e SRI19, pode ser devido a produção de carbapenemase. É conhecido que a triagem com ertapenem tem sensibilidade alta, mas especificidade baixa, porém para descartar a produção de carbapenemase em isolados ertapenem-resistentes, testes para ESBL e AmpC fenotípicos devem ser positivos [20]. Valiatti *et al.* (2023) [29] mostraram que *S. marcescens* carbapenemases-positiva estão presentes em fezes de animais criados para consumo humano, fato esse que pode favorecer a disseminação desta bactéria para o ambiente aquático.

A não detecção de ESBL e AmpC sugere que a resistência aos betalactâmicos exibida pelos isolados estudados não está relacionada com mecanismo enzimático. Porém, apenas a abordagem molecular, considerada padrão-ouro, pode descartar a ausência dos genes produtores destas enzimas nos isolados [55]. Apesar de no ambiente clínico *S. marcescens* produtora dessas enzimas ser frequentemente relatada, na literatura pesquisada, dados sobre a produção dessas enzimas em isolados ambientais não são encontrados.

Finalmente, com relação a resistência antimicrobiana de *S. marcescens*, deve ser lembrado que isolados dessa espécie de origem clínica exibem maior resistência que aqueles de origem ambiental, apesar deste ser considerado reservatório de determinantes de resistência antimicrobiana [40]. Estudos que abordam a resistência antimicrobiana em *S. marcescens* de origem aquática são escassos na literatura, e assim, possivelmente a distribuição da resistência aos antimicrobianos nesta espécie pode estar subestimada.

Conclusão

Esses resultados mostram que os rios urbanos são um importante reservatório de *S. marcescens* resistentes a múltiplos antimicrobianos. Assim, considerando a perspectiva *One Health,* onde a saúde está diretamente associada ao ambiente em que as pessoas estão inseridas, a presença de *S. marcescens* MDR deve estimular políticas de vigilância ambiental na bacia hidrográfica do rio Itapecerica para assim minimizar o impacto desses achados sobre a saúde da comunidade local.

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Os autores não têm conflitos de interesse a declarar.

Referências

1. Y. Kim, K. Kim, J. Seo, S. Shrestha, H.H. Kim, M. Nalini, Y. Yi, Identification of an entomopathogenic bacterium, *Serratia* sp. anu101, and its hemolytic activity, *Journal of Microbiology and Biotechnology*, **19**(3), 314–322 (2009).

2. M. Adeolu, S. Alnajar, S. Naushad, R.S. Gupta, Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for enterobacterales ord. nov. divided into the families enterobacteriaceae, erwiniaceae fam. nov., pectobacteriaceae fam. nov., yersiniaceae fam. nov., hafniaceae fam. nov., morganellaceae fam. nov., and budviciaceae fam. nov, *International Journal of Systematic and Evolutionary Microbiology,* **66**(12), 5575-5599 (2016). https://doi.org/10.1099/ijsem.0.001485

3. R.G. Araújo, N.R. Zavala, C. Castillo-Zacarias, M.E. Barocio, E. Hidalgo-Vasquez, L. Parra-Arroyo, J. Rodriguez-Hernandez, M.A. Martinez-Prado, J.E. Sosa-Hernandez, M. Martinez-Ruiz, W.N. Chen, D. Barcelo, H.M.N. Igbal, R. Parra-Saldivia, Recent advances in prodigiosin as a bioactive compound in nanocomposite applications, *Molecules*, **27**(15), 4982 (2022). https://doi.org/10.3390/molecules27154982

4. M. Cristina, M. Sartini, A. Spagnolo, *Serratia marcescens* infections in neonatal intensive care units (NICUs), *International Journal of Environmental Research and Public Health,* **16**(4), 610 (2019). https://doi.org/10.3390/ijerph16040610

5. I.C. Ribeiro, B.G.C. Aguiar, Strategies adopted for the hospital infections´s prevention and control caused by *Serratia marcescens* in the neonatal intensive unit-the nursing participating of the process, *Revista de Enfermagem UFPE On Line*, **4**(4), 1761 (2010). https://doi.org/10.5205/reuol.1114-9555-1-le.0401201024

6. A.R. Tavares, *Infecções por Serratia spp. em ambientes de terapia intensiva: uma revisão integrativa***,** TCC (Graduação) - Curso de Enfermagem, Universidade de Brasília, Ceilândia, DF, 2015, 33 f.

7. R. Manfredi, A. Nanetti, M. Ferri, F. Chiodo, Clinical and microbiological survey of *Serratia marcescens* infection during HIV disease, *European Journal of Clinical Microbiolology Infectious Diseases*, **19**(4), 248–253 (2000).

8. F. Tavares-Carreon, K.d. Anda-Mora, I.C. Rojas-Barrera, A. Andrade, *Serratia marcescens* antibiotic resistance mechanisms of an opportunistic pathogen: a literature review, *PeerJ*, **11**, e14399 (2023). https://doi.org/10.7717/peerj.14399

9. V.K. Phadke, J.T. Jacob, Marvelous but morbid: Infective endocarditis due to *Serratia marcescens*, *Infectious Diseases in Clinical Practice*, **24**(3), 143-150 (2016). https://doi.org/10.1097/ipc.0000000000000360

10. B. Suh, I.L.K. Bae, J. Kim, S.H. Jeong, D. Yong, K. Lee, Outbreak of meropenem-resistant *Serratia marcescens* comediated by chromosomal AmpC β-lactamase overproduction and outer membrane protein loss, *Antimicrobial Agents and Chemotherapy,* **54**(12), 5057–5061 (2010). https://doi.org/10.1128/aac.00768-10

11. G.S. Rezende, *Caracterização fenotípica e molecular da susceptibilidade antimicrobiana em Serratia marcescens isoladas de pacientes em um hospital terciário do estado de Tocantis***,** Dissertação (Mestrado), Curso de Genética Evolutiva e Biologia Molecular, Universidade Federal de São Carlos, São Carlos, 2019, 69 f.

12. N.T. Joutey, W. Bahafid, H. Sayel, S. Ananou, N. El Ghachtouli, Hexavalent chromium removal by a novel *Serratia proteamaculans* isolated from the bank of Sebou River (Morocco), *Environmental Science and Pollution Research*, **21**(4), 3060–3072 (2013). https://doi.org/10.1007/s11356-013-2249-x

13. J. Dong, J. Ruan, N. Xu, Y. Yang, X. Ai, *In vitro* synergistic effects of fisetin and norfloxacin against aquatic isolates of *Serratia marcescens, FEMS Microbiology Letters,* **363**(1), fnv220 (2015). https://doi.org/10.1093/femsle/fnv220

14. A.F.G. Rave, A.V. Kuss, G.H.S. Peil, S.R. Ladeira, J.P.V. Villarreal, P.S. Nascente, Biochemical identification techniques and antibiotic susceptibility profile of lipolytic ambiental bacteria from effluents, *Brazilian Journal of Biology,* **79**(4), 555–565 (2019). https://doi.org/10.1590/1519-6984.05616

15. P.G. Preena, A. Dharmaratnam, N.S. Raj, S.A. Raja, R.R. Nair, T.R. Swaminathan, Antibiotic-resistant Enterobacteriaceae from diseased freshwater goldfish, *Archives of Microbiology,* **203**(1), 219–231 (2020). https://doi.org/10.1007/s00203-020-02021-8

16. B.R. Evans, F.A. Leighton, A history of One Health, *Revue Scientifique et Technique*, **33**(2), 413–420 (2014). https://doi.org/10.20506/rst.33.2.2298

17. Brazilian Committee on Antimicrobial Susceptibily Testing (BrCAST), *Orientações do EUCast/BRCast para a detecção de mecanismos de resistência e resistências específicas de importância clínica e/ou epidemiológica*, URL: https://brcast.org.br/wp-content/uploads/2022/07/Orientacoes-do-EUCAST-para-a-detecccao-de-mecanismos-de-resistencia-e-resistencias-especificas-de-importancia-clinica-e-ou-epidemiologica.pdf, acessado Junho de 2022.

18. E.W. Koneman, G.W. Procop, G.L. Woods, W.M. Janda, D.L. Church, P.C. Schreckenberger, D.S. Hall, *Diagnóstico Microbiológico: Texto e Atlas Colorido*, 6ª ed., Guanabara-Koogan, São Paulo, 2008.

19. A.P. Magiorakos, A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, S. Harbarth, J.F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance, *Clinical Microbiology and Infection,* **18**(3), 268–281 (2012). https://doi.org/10.1111/j.1469-0691.2011. 03570.x

20. Brazilian Committee on Antimicrobial Susceptibily Testing (BrCAST), *Teste de sensibilidade aos antimicrobianos: Método de disco-difusão EUCAST*, versão 6, janeiro de 2017, URL: https://brcast.org.br/wp-content/uploads/2022/07/Manual-Disco-Difusao-BrCAST-21092018.pdf.

21. N.Y. Elsayed, A.M.R. Awad, M.M. Omar, D.G. Desouki, Rapid simultaneous detection of ampC and ESBLs among enterobacteriaceae using mastd68c detection set and possible therapeutic options, *The Egyptian Journal of Medical Microbiology*, **24**(3), 1–12 (2015).

22. R.P. Pandey, A.F. Yousef, H. Alsafar, S.W. Hasan, Surveillance, distribution, and treatment methods of antimicrobial resistance in water: A review, *Science of the Total Environment*, **890**, 164360 (2023). https://doi.org/10.1016/j.scitotenv.2023.164360

23. S. Riedel, N. Boire, K.A. Carson, A. Vadlamudi, J. Khuvis, V. Vadlamudi, V. Atukorale, V.A.A. Riedel, N. Parrish, A survey of antimicrobial resistance in Enterobacteriaceae isolated from the Chesapeake Bay and adjacent upper tributaries, *Microbiologyopen*, **8**(9), e00839 (2019). https://doi.org/10.1002/mbo3.839

24. L.C. Corrales-Ramírez, L.C. Sánchez-Leal, M.E. Qimbayo-Salamanca, Microorganismos potencialmente fitopatógenos en aguas de riego proveniente de la cuenca media del rio Bogotá, *Nova*, **16**(29), 71–89 (2018). https://doi.org/10.22490/24629448.2691

25. M.C. Paiva, M.P. Reis, P.S. Costa, M.F. Dias, L. Bleicher, L.L.S. Scholte, R.M.D. Nardi, A.M.A. Nascimento, Identification of new bacteria harboring qnrS and aac(6′)-Ib/cr and mutations possibly involved in fluoroquinolone resistance in raw sewage and activated sludge samples from a full-scale WWTP, *Water Research*, **110**, 27–37 (2016). https://doi.org/10.1016/j.watres.2016.11.056

26. M.L.S. Suhadolnik, P.S. Costa, M.C. Paiva, A.C.d.M. Salim, F.A.R. Barbosa, F.P. Lobo, A.M.A. Nascimento, Spatiotemporal dynamics of the resistome and virulome of riverine microbiomes disturbed by a mining mud tsunami, *Science of the Total Environment,* **806**, 150936 (2022). https://doi.org/10.1016/j.scitotenv.2021.150936

27. D.C. Simião, F.P. Andrade, W.G. Lima, M.L. Jesus, P.H.G. Dorim, M.C. Paiva, Determination of mercury concentration by a new spectrophotometric method and evaluation of bacterial diversity in river water samples from Brazil, *Water Supply,* **22**(5), 5535–5548 (2022). https://doi.org/10.2166/ws.2022.173

28. Clinical and Laboratory Standards Institute (CLSI), *Performance standards for antimicrobial susceptibility testing*, CLSI supplement M100-S31, 31th ed., Wayne (PA), 2022.

29. T.B. Valiatti, F.O. Bessa-Neto, F.F. Santos, R.G.B. Silva, R. Veiga, D. Cassu-Corsi, T.C.F. Moura, A.R.F. Lobato, C.C. Pigntari, C.O. Souza, D.M. Brasiliense, R. Cauo, A.G. Gales, Clonal dissemination of highly virulent *Serratia marcescens* strains producing KPC-2 in food-producing animals, *One Health*, **17**, 100591 (2023). https://doi.org/10.1016/j.onehlt.2023.100591

30. World Health Organization (WHO), *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*, URL: https://revive.gardp.org/wp-content/uploads/2023/01/WHO-global-PPL.pdf, acessado em 29 mai 2023.

31. A.J. Overmeyer, E. Prentice, A. Brink, K. Lennard, C. Moodley, The genomic characterization of carbapenem-resistant *Serratia marcescens* at a tertiary hospital in South Africa, *JAC Antimicrobial Resistance*, **5**(4), dlad089 (2023). https://doi.org/10.1093/jacamr/dlad089

32. Z. Liang, J. Shen, J. Liu, X. Sun, Y. Yang, Y. Lv, J. Zheng, X. Mou, H. Li, X. Ding, F. Yang, F. Prevalence and characterization of *Serratia marcescens* isolated from clinical bovine mastitis cases in Ningxia Hui autonomous region of China, *Infection and Drug Resistance*, **4**(16), 2727–2735 (2023). https://doi.org/10.2147/IDR.S408632

33. K.E. Silva, L. Rossato, S. Jorge, N.R. Oliveira, F.S. Kremer, V.F. Campos, L.S. Pinto, O.A. Dellagostin, S. Simionatto, Three challenging cases of infections by multidrug-resistant *Serratia marcescens* in patients admitted to intensive care units, *Brazilian Journal of Microbiology,* **52**(3), 1341–1345 (2021). https://doi.org/10.1007/s42770-021-00477-4

34. D. Tomanić, B. Božin, N. Kladar, J. Stanojević, I. Čabarkapa, N. Stilinović, J. Apić, D.D. Božić, Z. Kovačević, Environmental bovine mastitis pathogens: Prevalence, antimicrobial susceptibility, and sensitivity to *Thymus vulgaris* L., *Thymus serpyllum* L., and *Origanum vulgare* L. essential oils, *Antibiotics* (Basel), **11**(8), 1077 (2022). https://doi.org/10.3390/antibiotics11081077

35. G.F. Gad, H.A. Mohamed, H.M. Ashour, Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients in Upper Egypt, *Plos One*, **6**(2), e17224 (2011). https://doi.org/10.1371/journal.pone.0017224

36. G.M. Keating, Fosfomycin Trometamol: A review of its use as a single-dose oral treatment for patients with acute lower urinary tract infections and pregnant women with asymptomatic bacteriuria, *Drugs*, **73**(17), 1951–1966 (2013). https://doi.org/10.1007/s40265-013-0143-y

37. S.G.P. Souza, I.C. Santos, M.A.D. Bondezan, L.F.M. Corsatto, I.C.S. Caetano, M.M. Zaniolo, R. Matta, L.S. Merlini, L.N. Barbosa, D.D. Gonçalves, Bacteria with a potential for multidrug resistance in hospital material, *Microbial Drug Resistance*, **27**(6), 835–842 (2021). https://doi.org/10.1089/mdr.2019.0305

38. L. Robles-Jimenez, E. Aranda-Aguirre, O.A. Castelan-Ortega, B.S. Schettino-Bermudez, R. Ortiz-Salinas, M. Miranda, X. Li, J. Angeles-Hernandez, E. Vargas-Bello-Pérez, M. Gonzalez-Ronquillo, Worldwide traceability of antibiotic residues from livestock in wastewater and soil: A systematic review, *Animals* (Basel), **12**(1), 60 (2021). https://doi.org/10.3390/ani12010060

39. S. Toba, Y. Minato, Y. Kondo, K. Hoshikawa, S. Minagawa, S. Komaki, T. Kumagai, Y. Matoba, D. Morita, W. Ogawa, N. Gotoh, T. Tsuchiya, T. Kuroda, Comprehensive analysis of resistance-nodulation-cell division superfamily (RND) efflux pumps from *Serratia marcescens*, Db10, *Scientific Reports*, **9**(1), 4854 (2019). https://doi.org/10.1038/s41598-019-41237-7

40. L. Sander-Miranda, P. Vinuesa, A. Cravioto, R. Morales-Espinosa, The genomic basis of intrinsic and acquired antibiotic resistance in the genus *Serratia*, *Frontiers in Microbiology*, **9**, 828 (2018). https://doi.org/10.3389/fmicb.2018.00828

41. S. Li, Z. Liu, C. Suring, L. Chen, S. Muller, P. Zeng, The impact of the antibiotic fosfomycin on wastewater communities measured by flow cytometry, *Frontiers in Microbiology*, **12**, 737831 (2022). https://doi.org/10.3389/fmicb.2021.737831

42. O.L. Oharisi, S. Ncube, H. Nyoni, M.L. Madikizela, O.J. Olowoyo, B.R. Maseko, Occurrence and prevalence of antibiotics in wastewater treatment plants and effluent receiving rivers in South Africa using UHPLC-MS determination, *Journal of Environmental Management*, **345**, 118621 (2023). https://doi.org/10.1016/j.jenvman.2023.118621

43. J.G. Bartlett, Chloramphenicol, *Medical Clinics of North America*, **66**(1), 91–102 (1982). https://doi.org/10.1016/s0025-7125(16)31444-4

44. L. Maragakis, E.N. Perencevich, S.E. Cosgrove, Clinical and economic burden of antimicrobial resistance, *Expert Review of Anti-Infective Therapy*, **6**, 751–763 (2008). https://doi.org/10.1586/14787210.6.5.751

45. A. Iguchi, Y. Nagaya, E. Pradel, T. Ooka, Y. Ogura, K. Katsura, K. Kurokawa, K. Oshima, M. Hattori, J. Parkhill, M. Sebaihia, S.J. Couthurst, N. Gotoh, N.R. Thomson, J.J. Ewbank, T. Hayashi, Genome evolution and plasticity of *Serratia marcescens*, an important multidrug-resistant nosocomial pathogen, *Genome Biology and Evolution*, **6**(8), 2096–2110 (2014). https://doi.org/10.1093/gbe/evu160

46. C.I. Alvarez-Mendoza, A. Teodoro, L. Ramirez-Cando, Improving NDVI by removing cirrus clouds with optical remote sensing data from Landsat-8 – A case study in Quito, Ecuador, *Remote Sensing Applications: Society and Environment*, **13**, 257-274 (2019). https://doi.org/10.1016/j.rsase.2018.11.008

47. C.R. Costa, P. Olivi, C.M.R. Botta, E.L.G. Espindola, A toxicidade em ambientes aquáticos: discussão e métodos de avaliação, *Química Nova*, **31**(7), 1820-1830 (2008). https://doi.org/10.1590/s0100-40422008000700038

48. M.A. Pavez-Aguilar, *Análise molecular da expressão do fenótipo multidroga resistente (MDR) em Enterobactérias isoladas de amostras clínicas após exposição in vitro ao imipenem*, Tese de Doutorado em Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2013.

49. R. Bonnet, J.L.M. Sampaio, C. Chanal, D. Sirot, C. De Champs, J.L. Viallard, R. Labia, J. Sirot, A novel Class A Extended-Spectrum β-Lactamase (BES-1) in *Serratia marcescens* isolated in Brazil, *Antimicrobial Agents Chemoterapy*, **44**(11), 3061-3068 (20009. https://doi.org/10.1128/aac.44.11.3061-3068.2000

50. S.Y. Lu, Y.L. Zhang, S.N. Gen, T.Y. Li, Z.M. Ye, D.S. Zhang, F. Zou, H.W. Zhou, High diversity of extended-spectrum beta-lactamase-producing bacteria in an urban river sediment habitat, *Applied and Environmental Microbiology*, **76**, 5972–5976 (2010). https://doi.org/10.1128/AEM.00711-10

51. B. Rybac, N. Wawrzyniak, L. Wolska, M. Potrykus, *Escherichia coli* and *Serratia fonticola* ESBLs as a potential source of antibiotics resistance dissemination in the Tricity water reservoirs, *Acta Biochimica Polonica*, **68**(3), 437–448 (2021). https://doi.org/10.18388/abp.2020_5740

52. T.P.G. Chagas, L.M. Seki, J.C. Cury, J.A.L. Oliveir, A.M.R. Dávila, D.M. Silva, M.D. Asensi, Multiresistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro, Brazil, *Journal of Applied Microbiology*, **111**(3), 572–581 (2011). https://doi.org/10.1111/j.1365-2672.2011.05072.x

53. M.A. Ramadan, A.F. Tawfik, A.M. Shibl, Effect of beta-lactamase expression on susceptibility of local isolates of *Enterobacter cloacae*, *Serratia marcescens* and *Pseudomona aeruginosa* to beta-lactam antibiotics, *Chemotherapy*, **41**(3), 193–199 (1995). https://doi.org/10.1159/000239343

54. K. Bush, G.A. Jacoby, Updated functional classification of β-lactamases, *Antimicrobial Agents and Chemotherapy*, **54**(3), 969–976 (2010). https://doi.org/10.1128/aac.01009-09

55. G.A. March-Rosselló, Métodos rápidos para la detección de la resistencia bacteriana a antibióticos, *Enfermedades Infecciosas y Microbiología Clínica*, **35**(3), 182–188 (2017). https://doi.org/10.1016/j.eimc.2016.12.005

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The epidemiology of self-medication in Colombia: A systematic literature review and meta-analysis

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Summary

Objectives: The reported frequency of self-medication in Colombia ranges from 32.3% to 84.7%, depending on the study sample and time frame. This study aimed to estimate a pooled prevalence of self-medication and its associated factors in Colombian youth and adults. Method: A comprehensive systematic literature review and meta-analysis of the studies on self-medication in Colombia published from January 2000 - June 2022 was conducted. **Results:** Twelve studies (n=5,668) from urban areas were included, and a pooled prevalence of self-medication of 64.2% (95% confidence interval [CI] 50.8%-77.5%) was found. However, the prevalence was lower when self-medication was assessed during the last 30 days (32.3%; 95%CI 25.4%-39.3%) than when longer time frames were used. Female university students had a higher frequency of self-medication (OR= 1.72; 95% CI 1.17-2.53) than males. The most common medications were analgesics (37.7%), anti-inflammatories (33.2%), antihistamines (14.8%), and antibiotics/antiparasitics (12.1%). Lack of time and delays in medical care were reported in 35.2% (95% CI 25.6%-44.7%) of the cases. **Conclusions:** The reported frequency of self-medication in urban areas

of Colombia changed across the studies depending on the time frame used; therefore, this should be considered when conducting and comparing studies on self-medication prevalence. Although over-the-counter drugs were the most frequently involved (85.7%), the reported use of prescription drugs such as antibiotics/antiparasitics was 12.1%. Considering that lacking time and delays in medical care were reported in a third of cases of self-medication, shortening the long waiting times for healthcare services might contribute to the proper use of medications.

Keywords: Self-medication, prevalence, Colombia, systematic review, meta-analysis.

Resumen

Epidemiología de la automedicación en Colombia: revisión sistemática de la literatura y metanálisis

Objetivos: La frecuencia reportada de automedicación en Colombia oscila entre 32,3% y 84,7%, según la muestra de estudio y el marco temporal utilizado. Este estudio tuvo como objetivo estimar la prevalencia agrupada de la automedicación y sus factores asociados en jóvenes y adultos colombianos. **Método**: Se realizó una revisión sistemática de la literatura y un metanálisis de los estudios sobre automedicación en Colombia publicados entre enero de 2000 y junio de 2022. **Resultados:** Se incluyeron 12 estudios (n=5.668) realizados en áreas urbanas y se encontró una prevalencia combinada de automedicación de 64,2 % (intervalo de confianza [IC] del 95 % 50,8 %-77,5 %). Sin embargo, la prevalencia fue menor cuando se evaluó la automedicación durante los últimos 30 días (32,3%; IC95% 25,4%-39,3%) que cuando se utilizaron marcos de tiempo más prolongados. Las mujeres universitarias presentaron mayor frecuencia de automedicación (OR= 1,72; IC 95% 1,17-2,53) que los hombres. Los medicamentos más comunes fueron analgésicos (37,7%), antiinflamatorios (33,2%), antihistamínicos (14,8%) y antibióticos/antiparasitarios (12,1%). La falta de tiempo y las demoras en la atención médica se reportaron en el 35,2% (IC 95% 25,6%-44,7%) de los casos. **Conclusiones:** La frecuencia reportada de automedicación en áreas urbanas de Colombia cambió entre los estudios dependiendo del marco de tiempo utilizado; por lo tanto, esto debe tenerse en cuenta al realizar y comparar estudios sobre la prevalencia de la automedicación. Aunque los medicamentos de venta libre fueron los más frecuentemente involucrados (85,7%), el uso informado de medicamentos de prescripción como antibióticos/antiparasitarios fue 12,1%. Además, considerando que la falta de tiempo y las demoras en la atención médica fueron informadas en un tercio de los casos de automedicación, la

reducción de los largos tiempos de espera en los servicios de salud podría contribuir al uso adecuado de los medicamentos.

Palabras clave: Automedicación, prevalencia, Colombia, revisión sistemática, metanálisis.

Resumo

A epidemiologia da automedicação na Colômbia: revisão sistemática da literatura e metanálise

Objetivos: A frequência relatada de automedicação na Colômbia varia de 32,3% a 84,7%, dependendo da amostra do estudo e do período de tempo utilizado. Este estudo teve como objetivo estimar a prevalência da automedicação e seus fatores associados em jovens e adultos colombianos. **Método:** Foi realizada uma revisão sistemática e exaustiva da literatura e uma metanálise dos estudos sobre automedicação na Colômbia publicados entre janeiro de 2000 e junho de 2022. **Resultados:** Um total de 12 estudos (n=5668) realizados em áreas urbanas foram incluídos e encontraram uma prevalência agrupada de automedicação de 64,2% (intervalo de confiança [IC] de 95%: 50,8-77,5%). No entanto, a prevalência foi menor quando se avaliou a automedicação nos últimos 30 dias (32,3%; IC 95% 25,4%-39,3%) do que quando foram utilizados intervalos de tempo mais longos. As mulheres universitárias apresentaram maior frequência de automedicação (OR= 1,72; IC 95% 1,17-2,53) do que os homens. Os medicamentos mais comuns foram analgésicos (37,7%), anti-inflamatórios (33,2%), anti-histamínicos (14,8%) e antibióticos/antiparasitários (12,1%). Falta de tempo e demora no atendimento médico foram relatados em 35,2% (IC 95% 25,6%-44,7%) dos casos. **Conclusões:** A frequência relatada de automedicação em áreas urbanas da Colômbia variou entre os estudos, dependendo do período de tempo usado; portanto, isso deve ser levado em consideração na condução e comparação de estudos sobre a prevalência da automedicação. Embora os medicamentos de venda livre tenham sido os mais frequentemente envolvidos (85,7%), o uso relatado de medicamentos prescritos como antibióticos/antiparasitários foi de 12,1%. Além disso, considerando que a falta de tempo e a demora no atendimento médico foram relatadas em um terço dos casos de automedicação, a redução do longo tempo de espera nos serviços de saúde pode contribuir para o uso adequado dos medicamentos.

Palavras-chave: Automedicação, prevalência, Colômbia, revisão sistemática, metanálise.

Introduction

Self-medication involves using medicinal products to treat self-recognized disorders or symptoms or the intermittent or continued use of a medication prescribed by a physician for chronic or recurring diseases or symptoms [1]. According to the World Health Organization, responsible self-medication is carried out with over-the-counter (OTC) drugs, using medications for the intended indications, and reading and following the manufacturer's instructions on the label and package insert [2]. Unresponsible self-medication can result in health risks, such as delayed diagnosis and treatment of disease, adverse drug reactions, drug-drug interactions, and antimicrobial resistance [3, 4].

Although most of the world population self-medicates or has done so at some point, the frequency of self-medication and its associated factors varies depending on the population studied. Indeed, a recent global meta-analysis of 69 articles published between 2000 and 2018, including a total of 27,890 individuals, estimated a prevalence of self-medication of 55.9% (95% CI 42.4%-68.5%) in Africa; 71.2% (95% CI 63.0%-78.3%) in Asia; 74.0% (95% CI 56.2%-86.4%) in Europe; and 60.0% (95% CI 40.2%-77.0%) in South America [5]. In Colombia, different studies about self-medication have been conducted and reported frequencies ranging from 32.3% [6] to 84.7% [7], depending on the study sample and time frame used. However, there are no systematic reviews, nor is there a global quantitative estimate (meta-analysis) of its prevalence in the Colombian population. Therefore, this systematic review and meta-analysis aimed to estimate the pooled prevalence of self-medication and its associated factors in young people and adults in Colombia.

Methods

Comprehensive systematic literature search

Search strategy. A comprehensive systematic literature review was conducted and reported according to the standards set out in the *PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses* [8] and guided by the overview of Munn and colleagues [9]. The search was performed to identify studies published from January 2000 – June 2022 in English, Spanish, and Portuguese. The search was conducted in multiple electronic bibliographic databases: MEDLINE, PubMed, Academic Search, Web of Science (including Science Citation Index, Social Sciences Citation Index, and Arts and Humanities Citation Index), LILACS, the catalog of the Universidad Nacional de Colombia (UNAL), BIBLAT, and Google Scholar. Multiple combinations of the following keywords were used: cross-sectional study, statistics and numerical data,

prevalence, multivariate analysis, bivariate analysis, and Colombia. In addition, the citations in the relevant articles were manually screened.

Study selection. The inclusion criteria were: (i) primary and secondary sources studies; (ii) descriptive studies (cross-sectional, cohort, case-control, and prospective), carried out in Colombia and reporting the prevalence of self-medication in young people and adults, as well as its associated factors; and (iii) articles, theses, and book chapters. Studies were excluded if: (i) they were review articles, letters to the editor, editorials, comments, expert opinions, case studies, case series, or conference abstracts, and (ii) they were published in languages other than English, Spanish, or Portuguese. The selection of the studies was carried out by two investigators independently, and discrepancies were resolved by consensus.

Quality assessment of studies. Using a standardized form, each study's bias risk was assessed using Hoy et al.'s criteria [10]. Each criterion was assessed as 0 if the risk of bias was low; 0.5 if the risk was intermediate or unclear; and 1 if the risk was high. The sum of the scores for the ten criteria indicated the overall risk of bias of the study, was defined as criterion 11, and was used to determine whether a study was included in the analysis. Studies with scores of 0-3 were considered low risk and were included in the quantitative analysis; studies with scores of 4-6 had moderate risk and were excluded, as well as those with scores of 7-10, which had a high risk of bias. This process of assessing the quality and risk of bias of the studies was carried out independently by two investigators, and when discrepancies were found, the final scoring and the decision to include or exclude the study was made by consensus. The summary figure of the studies' risk of bias was prepared using the RevMan v5.0 package.

Data extraction. Using a standardized format in Microsoft Excel, two authors independently extracted data on study characteristics (first author, publication date, characteristics) and outcomes of interest (sample size, self-medication time frame, prevalence of self-medication, women who self-medicated, men who self-medicated, lack of time and delays in medical care as causes of self-medication, and use of analgesics, anti-inflammatories, antihistamines, antibiotics and antiparasitics). When the prevalence of self-medication was evaluated in several time frames in the same study, the data were extracted separately.

Data processing and meta-analysis. Relevant data extracted from the included studies in the Microsoft Excel format were exported to the statistical package Stata v17. A random-effects meta-analysis model was used to estimate DerSimonian and Laird's pooled effect to show heterogeneity. Subgroup analysis was conducted to adjust random variation between point estimates of the original studies and investigate how the prevalence of self-medication fluctuates across subgroups depending on the self-medication time

frame. The outliers within the included articles were checked using sensitivity analysis. Publication bias across studies was assessed using a funnel plot, Egger's regression test, and Begg's test (Z statistic) at 0.05. A forest plot format presented the point prevalence (percent) and 95% confidence intervals (CIs). In this plot, each crossed line referred to a 95% CI. The odds ratio (OR) was used to determine the association between self-medication and sex, and point prevalence (percent) was used for causes of self-medication and medications groups.

Results

Search results

Initially, the search yielded 31 studies. After removing one duplicate, 30 studies were screened using titles and abstracts, 24 of which (16 articles and eight theses) underwent methodological evaluation. Eleven studies with moderate and high risk of bias were excluded, and the reasons for their exclusion are presented in Supplementary Table 1. Ultimately, 13 were included for the qualitative and quantitative synthesis: 12 about general self-medication with 5,668 participants were used for the pooled prevalence estimation, and one about self-medication with antibiotics and 140 participants was used only when analyzing medication groups (Figure 1).

Study characteristics

Table 1 shows that 12 studies with 5,668 participants were included in the systematic review and meta-analysis of self-medication prevalence. One additional study with 140 participants was included to analyze antibiotic and antiparasitic use through self-medication [11]. All the included studies had a cross-sectional design and were published between 2002 and 2021. All the included studies were conducted in urban areas and used randomized sampling, except one, whose sample selection was by convenience [12]. The sample sizes ranged from 140 in Cali [11] to 1,127 in Pereira [6]. The prevalence of self-medication varied from 32.3% in Pereira [6] to 84.7% in Neiva [7]. Regarding geographical distribution, five studies were conducted in the Caribbean region [12-16], three in the Andean región [6, 7, 17], four in the capital city Bogotá [18-21], and one in the Pacific region [11]. There were no studies from the Amazonia and Orinoco regions. Nine studies included adults from the general population [6, 11-15, 17-19], and four included university students [7, 16, 20, 21]. Six studies used instruments with no known validity or reliability [7, 16-19, 21], and seven used instruments with partially documented validity and reliability [6, 11-15, 20]. The overall risk of bias score of the included studies was between 0.5/10 and 2.5/10 (Supplementary Figure 1).

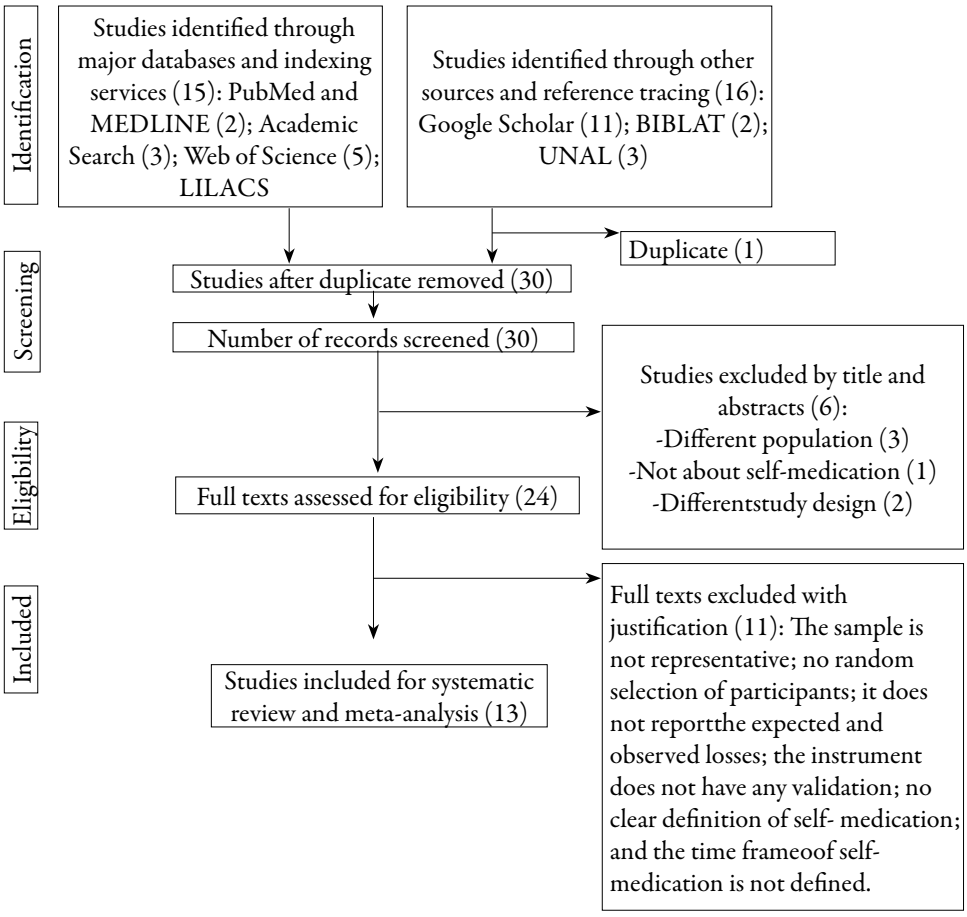


Figure 1. Flowchart of study selection.

Each study used a different self-medication time frame (Table 1), so they were categorized into three subgroups. Five studies with 2,344 participants were included in the subgroup that evaluated current self-medication in the last 30 days. Five studies with 2,355 participants were included in the subgroup that evaluated self-medication in the previous year and throughout life. Four studies with 1,383 participants were included in the subgroup that evaluated self-medication in university students during the last six months and throughout life. Two studies assessing self-medication in various time frames [12, 17] were simultaneously included in subgroups 1 and 2, and their data were extracted separately for meta-analysis. One study evaluated self-medication with antibiotics and was included only in the analysis by medication groups [11].

Table 1. Summary of included studies evaluating the frequency of self-medication and associated factors in young people and adults in Colombia 2000-2022.

First author	Year	Source	Study Area and Population	Sample size	Sampling	Self-medication time frame	Setting	Ref.
Feria-Mejía	2021	Thesis	Adults and their families in the city of Sincelejo, department of Sucre	150	Covenience	Self-medication in the last 30 days and throughout life	Neighborhood/ home	[12]
Peñuela	2002	Journal article	Inhabitants of the city of Barranquilla, department of Atlántico	350	Simple randomized	Self-medication in the last 30 days	Pharmacy	[13]
Machado-Alba	2014	Journal article	Adults from the city of Pereira, department of Risaralda	414	Simple randomized	Self-medication in the last 30 days and throughout life	Home	[17]
López	2009	Journal article	Adult heads of household in the locality of Suba, Bogotá	453	Simple randomized	Self-medication in the last 30 days	Home	[18]
Villegas-Cardona	2014	Journal article	Homes in the urban area of the city of Pereira; people belonging to the family nucleus (over 18 years of age)	1,127	Random, stratified by socio-economic stratum	Self-medication in the last 30 days	Home	[6]
Del Toro-Rubio	2017	Journal article	Inhabitants between 20 and 59 years old, from Locality 2 of the city of Cartagena	428	Simple randomized	Self-medication throughout life	Neighborhood/ home	[14]
Fajardo-Zapata	2013	Journal article	People older than 20 years, living in 20 localities in Bogotá	588	Multistage probabilistic	Self-medication throughout life	Neighborhood/ home	[19]
Macías-Vidal	2015	Thesis	Adults who go to grocery stores located in four municipalities of the department of Atlántico	775	Pobabilistic stratified	Self-medication throughout life	Grocery stores	[15]

(*Continued*)

First author	Year	Source	Study Area and Population	Sample size	Sampling	Self-medication time frame	Setting	Ref.
Bravo	2017	Thesis	First to fifth-semester medical students, UDCA University, Bogotá	201	Simple randomized	Self-medication in the last six months and throughout life	University	[20]
Lopez-Cabra	2016	Journal article	First to sixth-year medical students, Universidad del Rosario, Bogotá.	270	Randomized, stratified by semester	Self-medication throughout life	University	[21]
Oviedo-Córdoba	2021	Thesis	Undergraduate students enrolled in the second semester of 2019, Universidad del Magdalena, Santa Marta	312	Cluster randomized	Self-medication throughout life	University	[16]
Ortiz	2019	Journal article	University students, Universidad Cooperativa de Colombia, Neiva	600	Simple randomized	Self-medication throughout life	University	[7]
Castro-Espinosa	2014	Journal article	Adults who buy antibiotics in selected pharmacies in Commune 5 of the city of Cali	140	Simple randomized	Self-medication throughout life	Pharmacy	[11]

Meta-analysis

Self-medication prevalence. The pooled prevalence of self-medication among the Colombian youth and adult population was 62.4%, with a 95% CI from 47.2 to 77.7%. As the I^2 statistic revealed, there was a high degree of heterogeneity across studies (I^2= 99.6%, p<0.001). The random effects model was assumed for this meta-analysis.

Subgroup and sensitivity analysis. No significant change in heterogeneity was observed when trying to exclude outliers, or one or more studies. For this reason, all the studies were included in the meta-analysis, and a subgroup analysis was performed according to the self-medication time frame and the type of population (general population or university students). The subgroup analysis revealed a lower frequency of self-medication among the general population when evaluated during the last 30 days (32.3%; 95% CI 25.4%-39.3%). In contrast, the prevalence of self-medication was significantly higher when assessed in the general population during the last year or lifetime (74.6%; 95% CI 61.3%-87.9%) and in university students in the previous six months or lifetime (84.7%; 95% CI 75.1%-94.3%), with no significant difference between them (Figure 2). Therefore, the effect of the time frame during which the self-medication behavior is evaluated seems more important than the effect of age or the university student status. We did not find evidence of publication bias (Egger's regression test, one-tailed, p= 0.2924; Begg's correlation test, one-tailed, p= 0.3811).

Prevalence of self-medication according to sex. Data on the frequency of self-medication in women and men were extracted from 10 studies. In the meta-analysis, no significant association was observed between sex and self-medication, with a pooled OR of 1.12 (95% CI 0.87–1.44). Moreover, the subgroup analysis showed no association between self-medication and sex among the general population in the last 30 days (OR= 0.87; 95% CI 0.57-1.32); nor in the previous year or at some point in life (OR= 1.11; 95% CI 0.61-2.03). In contrast, female university students had a greater probability of self-medication in the last six months or throughout their lives than males (OR= 1.72; 95% CI 1.17-2.53) (Figure 3). We did not find evidence of publication bias (Egger's regression test, one-tailed, p= 0.7983; Begg's correlation test, one-tailed, p= 1.00).

Determinants of self-medication. Due to the lack of a standardized and validated instrument to evaluate self-medication, each study addressed different determinants for this phenomenon. However, among these, the most recurring determinants reported were the lack of time and delays in medical care. Data on these causes could only be extracted from eight studies. In the meta-analysis, the lack of time and delays in medical care were reported with an overall frequency of 35.2% (95% CI 25.6%-44.7%). Moreover, the subgroup meta-analysis showed that the frequency with which these

causes were reported by university students who self-medicated in the last six months or throughout their lives was significantly lower (19.7%; 95% CI 10.3-29.2) than that of the general population who self-medicated in the last 30 days (47.8%; 95% CI 31.5-64.2) (Figure 4).

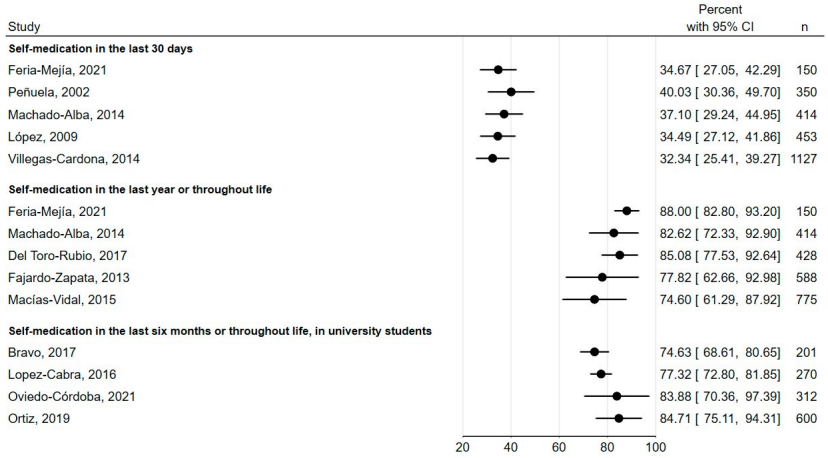


Figure 2. Subgroup analysis of studies describing the prevalence of self-medication disaggregated by time frame and the type of population (general population or university students).

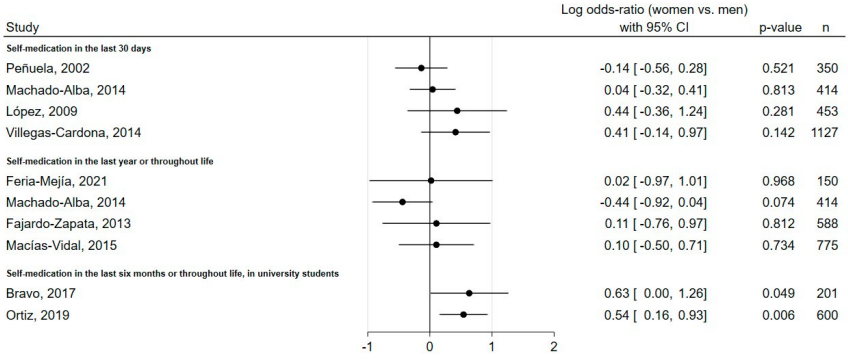


Figure 3. The association between self-medication and sex, disaggregated by time frame and the type of population (general population or university students), with results presented as the neperian logarithm of the OR.

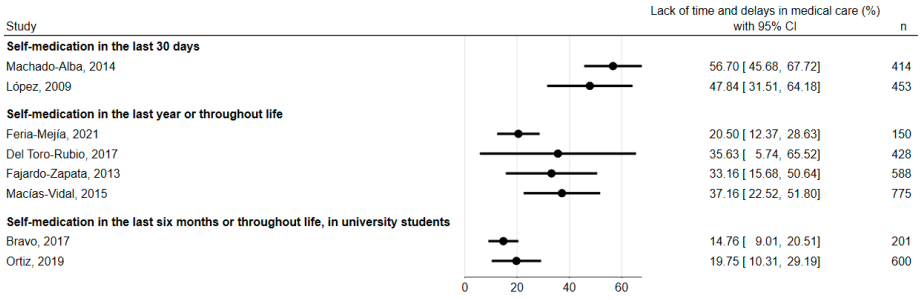


Figure 4. The frequency of reporting the lack of time and delays in care as causes of self-medication.

Medication groups used for self-medication. Data on the medication groups used for self-medication could be extracted from nine studies. The meta-analysis showed that the pooled frequency of self-medication with analgesics was 37.7% (95% CI 15.8%-47.6%), anti-inflammatories 33.2% (95% CI 15.4%-51.0%), and antihistamines 14.8% (95% CI 6.8%-23.0%), while the pooled frequency for antibiotics and antiparasitics was 12.1% (95% CI 7.4%-16.9%) (Table 2).

Table 2. Medication groups used for self-medication.

Subgroup	Studies	Effect size (95% CI)			
		Analgesics	Anti-inflammatories	Antihistamines	Antibiotics and antiparasitics
Subgroup 1: Self-medication in the last 30 days	Machado-Alba, 2014	44.3% (39.5 - 49.1)	36.4% (31.8 - 41.0)	8.5% (5.8 - 11.2)	6.3% (4.0- 8.6)
	López, 2009	15.0% (11.7 - 18.3)	18.0% (14.5- 21.5)	8.0% (5.5 - 10.5)	1.0% (0.1 - 1.9)
	Villegas-Cardona, 2014	58.0% (55.1 - 60.9)	12.0% (10.1 - 13.9)	12.0% (10.1 - 13.9)	4.0% (2.9 - 5.1)
	Peñuela, 2002	19.8% (15.6 - 24.0)	30.1% (25.3 - 34.9)	5.1% (2.8 - 7.4)	16.6% (12.7 - 20.5)
	Castro-Espinosa, 2014 *	-	-	-	7.0% (2.8 - 11.2)
	Subgroup 1	34.3% (11.9 - 56.7)	24.0% (12.9 - 35.0)	8.4% (5.3 - 11.5)	6.6% (3.0- 10.1)

(*Continued*)

Subgroup	Studies	Effect size (95% CI)			
		Analgesics	Anti-inflammatories	Antihistamines	Antibiotics and antiparasitics
Subgroup 2: Self-medication in the last year or throughout life	Macías-Vidal, 2015	46.1% (42.6 - 49.6)	74.4% (71.3 - 77.5)	53.7% (50.2 - 57.2)	10.2% (8.1 - 12.3)
Subgroup 3: Self-medication in the last six months or throughout life, in university students	Bravo, 2017	1.6% (0.2 - 3.3)	14.5% (9.6 -19.4)	11.2% (6.8 - 15.6)	13.5% (8.8 - 18.2)
	Ortiz, 2019	44.0% (40.0 - 48.0)	67.0% (63.2 - 70.8)	16.0% (13.1 - 18.9)	5.0% (3.2- 6.7)
	Lopez-Cabra, 2016	14.4% (10.2 - 18.6)	3.9% (1.6 - 6.2)	3.6% (1.4 - 5.8)	4.4% (2.0 - 6.8)
	Oviedo-Córdoba, 2021	42.4% (36.9 - 47.9)	42.4% (36.9 - 47.8)	15.8% (11.8 - 19.9)	58.4% (52.9 -63.9)
	Subgroup 3	25.5% (15.8 - 47.6)	31.9% (0.3 - 64.1)	11.6% (4.6 - 18.5)	20.1% (3.9 - 36.4)
Overall		37.7% (15.8 - 47.6)	33.2 (15.4 - 51.0)	14.8% (6.8- 23.0)	12.1% (7.4 - 16.9)

*This study only evaluated self-medication with antibiotics.

Discussion

A systematic review and meta-analysis were carried out to estimate the prevalence of self-medication in Colombia and its associated factors in the population over 16. The pooled prevalence of self-medication found in young people and adults from urban areas of Colombia (64.2%; 95% CI 50.8%-77.5%) was similar to that recently found in a global meta-analysis, according to which the prevalence of self-medication was 60.0% (95% CI 40.2%-77.0%) in South America [5]. All the studies included in this meta-analysis were conducted in the urban population, which, according to the 2018 Colombian population census, constitutes 77% of the total Colombian population [22]. Additionally, 11 of the 12 included studies used randomized sampling. Only one study used a convenience sample, which had a low weight on the pooled calculations because of its small sample size (n= 150 subjects) [12]. Therefore, the pooled prevalence could be considered representative of the Colombian population, specifically those living in municipal capitals.

On the other hand, since there is no universal and standardized definition of self-medication, most studies use different time frames to measure its frequency [23]. This has led most of the systematic literature reviews to establish comparisons of the prevalence of self-medication between diverse populations without analyzing in depth the effect that these different time frames could have on the reported frequency. To address the problem of the different time frames used for measuring self-medication, which can undoubtedly contribute to the high heterogeneity observed, we performed a meta-analysis by subgroups, according to the time frame and the study population (General population vs. University students). Our results showed that when self-medication was evaluated for long periods, such as the previous 6-12 months or throughout life, the reported frequency was significantly higher, both in the general population (74.6%; 95% CI 61.3%- 87.9%) and in university students (84.7%; 95% CI 75.1%-94.3%). In contrast, when self-medication was assessed during the previous 30 days, a significantly lower prevalence of self-medication was found (32.3%; 95% CI 25.4%-39.3%). This suggests that, at least in the urban population of Colombia, the time frame during which the self-medication behavior is evaluated could be more important than the effect of age or the university student status. However, the impact that the variables, time frame, age, socioeconomic status, and educational level have on the frequency of self-medication is complex and requires in-depth study. Previous studies have already reported an effect of time frame on the reported frequency of self-medication. This is the case of a systematic review from Brazil that reported a pooled prevalence of self-medication in the last 15 days of 35.0% (95% CI 39.0%-40.0%) [24]; meanwhile, a study evaluating self-medication during lifetime found a frequency of 66.6% (95% CI 61.3%-71.4%) [25]. In another case, a community-based cross-sectional study conducted in southwestern Ethiopia reported that the prevalence of self-medication changed depending on the time frame (58.2% for lifetime vs. 31.2% for current use) [26]. Indeed, when a behavior is evaluated by self-reporting, a long time frame does not allow recent changes to be detected and is associated with recall bias. For this reason, to assess the frequency of self-medication through questionnaires, surveys, and interviews, it is essential to select the recall period appropriately. Generally, a short recording period is preferable to a long one, especially when asking about recurring or frequent events [27].

Although the second objective of this study was to evaluate the factors associated with self-medication in Colombia, only some of the included studies assessed the same characteristics. However, it was possible to extract and analyze the data related to sex, groups of medications used for self-medication, and some causes associated with self-medication behavior. Regarding self-medication in men and women, we did not find differences within the general population, but we did find a higher frequency of self-

medication in female university students compared to males (OR= 1.72; 95% CI 1.17-2.53), a finding that other authors have previously reported, and that can be explained as a strategy for relieving headaches, colds, and dysmenorrhea [28].

This meta-analysis found that, within the population that self-medicated, 37.7% used analgesics, 33.2% anti-inflammatories, and 14.8% antihistamines. Other studies have also shown these drugs to be the most used for self-medication. For example, in Ethiopia, the frequency of analgesics and anti-inflammatories was 46.1% [29], and in Iran, the frequency of analgesic use was 53.2%, with 39.3% for anti-inflammatories and 24.3% for antihistamines [30]. The non-responsible use of OTC medicines is an essential cause of adverse effects and severe drug interactions [31-33]. It is favored by selling in large-area stores without mediating the dispensing process and providing the user with the required information rather than by the insert that needs to be read.

The use of antimicrobials without a medical prescription is a cause for concern due to its implications for developing antimicrobial resistance, to which 1.27 million deaths were attributed in 2019 [34]. We found that antibiotics and antiparasitics were used for self-medication in 12.1% (95% CI 7.4-16.9%) of the cases, a lower frequency than that reported in other studies and systematic reviews. Within low- and middle-income countries, the pooled prevalence of self-medication with antimicrobials has been found to range from 39 to 78%, depending on the countries included in each analysis [35-37]. Therefore, given this high heterogeneity, it is essential to conduct investigations by countries within the same income level group [38]. In Colombia, in particular, it was observed that by 2011, up to 80% of pharmacies did not comply with the rule of dispensing antibiotics only with a medical prescription, and the pharmacy staff did not provide the required information to the user [39]. This phenomenon contributes substantially to using antibiotics through self-medication and is added to other associated factors, such as those discussed below.

Finally, some of the determinants of self-medication were explored; however, despite these causes being so varied and complex, it was only possible to extract information about two: lack of time and delays in care. These two situations were reported by those who self-medicated in 35.2% (95% CI 25.6%-44.7%) of the cases, although their frequency in university students was significantly lower (19.7%; 95% CI 10.3%-29.2%). Another systematic review also reported that the lack of time to see a doctor is reported recurrently by those who self-medicate [40], reaching a frequency of up to 68% in a high-income country such as Saudi Arabia [41]. Therefore, shortening the long waiting times for healthcare services and improving pharmaceutical services might encourage the proper use of medications [42]. However, these strategies must be accompanied by others that involve pharmacy staff, who must have the appropriate formation and

training, comply with the rules for dispensing prescription drugs, and provide the user with the required information on the adequate use of drugs [43, 44].As limitations of the present study, it should be considered that some studies included in the meta-analysis evaluated self-medication by self-report (memory bias). In contrast, others did so by direct pharmacy observation (information bias), which may contribute to the observed heterogeneity. Additionally, the studies analyzed did not use a standardized time frame. These limitations are common to all studies on self-medication and point to the need for standardized definitions and tools. Therefore, within the recommendations and prospects for future studies, the need to develop and validate instruments to assess the occurrence of self-medication, especially responsible self-medication, as defined by the World Health Organization, is emphasized. These instruments should set a specified time frame, ideally short, as this minimizes recall bias, allows for population assessments, and can detect recent changes in behavior, for example, after an intervention. Additionally, given the impact of self-medication globally, these instruments must be developed and validated in different languages and cultural contexts, and they must evaluate the medications used.

Conclusion

A systematic review and meta-analysis were carried out to estimate the prevalence of self-medication in Colombia and its associated factors in the population over 16. A total of 12 studies with 5,668 were included, and a pooled prevalence of self-medication in young people and adults in Colombia (64.2%; 95% CI 50.8%-77.5%) was calculated. However, the prevalence was significantly lower when self-medication was evaluated during the last 30 days (32.3%) compared to more extended time frames (six months, one year, or throughout life). The self-medication frequency was higher in female university students than in males. The most frequently used medications were analgesics (37.7%), anti-inflammatories (33.2%), antihistamines (14.8%), and antibiotics/antiparasitics (12.1%). As determinants of self-medication, lack of time and delays in medical care were reported in 35.2% (95% CI 25.6%-44.7%) of the cases. The reported frequency of self-medication in Colombia changed across the studies depending on the time frame used; therefore, this should be considered when conducting and comparing studies on self-medication prevalence. Although over-the-counter drugs were the most frequently involved (85.7%), the reported use of prescription drugs such as antibiotics/antiparasitics was 12.1%. Moreover, considering that lacking time and delays in medical care were reported in a third of cases of self-medication, shortening the long waiting times for healthcare services might contribute to the proper use of medications.

Conflict of interests statement

The authors have no relevant financial or non-financial interests to disclose.

Author contribution

All authors contributed to the study conception and design, data collection and analysis, prepared and approved the final version of this manuscript.

References

1. World Health Organization, *WHO guidelines for the regulatory assessment of medicinal products for use in self-medication: general information*, WHO Drug Inf 2000 141 18-26, 2000. URL: https://apps.who.int/iris/handle/10665/57624

2. World Health Organization, *The Role of the pharmacist in self-care and self-medication: report of the 4th WHO Consultative Group on the Role of the Pharmacist*, 1998. URL: https://apps.who.int/iris/handle/10665/65860, accessed: Oct 28, 2022.

3. A.M. Ruiz-Sternberg, A.M. Pérez-Acosta, Automedicación y términos relacionados: una reflexión conceptual, *Rev. Cienc. Salud*, **9**(1), 83–97 (2011).

4. I.A. Rather, B.-C. Kim, V.K. Bajpai, Y.-H. Park, Self-medication and antibiotic resistance: Crisis, current challenges, and prevention, *Saudi J. Biol. Sci.*, **24**(4), 808–812 (2017).

5. S. Ghasemyani, M.R. Benis, H. Hosseinifard, R. Jahangiri, A. Aryankhesal, H. Shabaninejad, *et al.*, Global, WHO regional, and continental prevalence of self-medication from 2000 to 2018: A systematic review and meta-analysis, *Ann. Public Health*, 1, 585 (2022).

6. F. Villegas-Cardona, K.M. Nasner-Posso, D.P. Buitrago-Gómez, S. Cruz-Calderón, S.R. Restrepo, J.E. Bedoya-Arias, *et al.*, Patrón de automedicación en la zona urbana de la ciudad de Pereira (Colombia) en el trimestre marzo-mayo 2013, *Investig. Andina*, **16**(29), 1073–1085 (2014).

7. C.P. Ortiz, O.D. Fúnez-David, H. Rubiano-Daza, C.L. García-Rojas, C.A. Calderón-Ospina, D.R. Delgado, Automedicación en estudiantes de la Sede Neiva de la Universidad Cooperativa de Colombia, *Rev. Colomb. Cienc. Quím. Farm.*, **48**(1), 128–144 (2019).

8. A. Liberati, D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gøtzsche, J.P.A. Ioannidis, *et al.*, The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration, *PLoS Med.*, **6**(7), e1000100 (2009).

9. Z. Munn, S. Moola, K. Lisy, D. Riitano, C. Tufanaru, Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data, *Int. J. Evid. Based Healthc.*, **13**(3), 147–153 (2015).

10. D. Hoy, P. Brooks, A. Woolf, F. Blyth, L. March, C. Bain, *et al.*, Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement, *J. Clin. Epidemiol.*, **65**(9), 934–939 (2012).

11. J. Castro-Espinosa, J.F.A. Arboleda-Geovo, P. Samboni-Novoa, Prevalencia y determinantes de automedicación con antibióticos en una comuna de Santiago de Cali, Colombia, *Rev. Cubana Farm.*, **48**(1), 43–54 (2014).

12. D.M. Feria-Mejía, G.A. Romero-Martínez, K.J. Carrascal-García, *Caracterización de la práctica de la automedicación en adultos beneficiarios de un centro de desarrollo integral de la Comuna Ocho de la Cuidad de Sincelejo, Sucre en 2020*, College Thesis, Universidad de Sucre, Sincelejo, Colombia, 2021. URL:: https://repositorio.unisucre.edu.co/bitstream/handle/001/1136/T615.856%20F356.pdf?sequence=1&isAllowed=y

13. M. Peñuela, A. De la Espriella, E. Escobar, M.V. Velásquez, J. Sánchez, A. Arango, *et al.*, Factores socioeconómicos y culturales asociados a la autoformulación en expendios de medicamentos en la ciudad de Barranquilla, *Salud Uninorte*, **16**, 30–38 (2002).

14. M. Del Toro-Rubio, A. Díaz-Pérez, Z. Barrios-Puerta, I.Y. Castillo-Avila, Automedicación y creencias en torno a su práctica en Cartagena, Colombia, *Revista Cuidarte*, **8**(1), 1509 (2017).

15. M. Macías-Vidal, *Factores asociados a la automedicación con productos farmacéuticos de venta libre, adquiridos en tiendas, por personas mayores de 18 años de cuatro municipios del departamento del Atlántico en el 2014*, Master Thesis, Universidad del Norte, Barranquilla, Colombia, 2015. URL: https://manglar.uninorte.edu.co/bitstream/handle/10584/10023/Mac%c3%adas%20Vidal.pdf?sequence=1&isAllowed=y

16. H. Oviedo-Córdoba, C.E. Cortina-Navarro, J.A. Osorio-Coronel, S.M. Romero-Torres, Realidades de la práctica de la automedicación en estudiantes de la Universidad del Magdalena, *Enfermería Global*, **62**, 531–543 (2021).

17. J.E. Machado-Alba, L.F. Echeverri-Cataño, M.J. Londoño-Builes, P.A. Moreno-Gutiérrez, S.A. Ochoa-Orozco, J.O. Ruiz-Villa, Social, cultural and economic factors associated with self-medication, *Biomedica*, **34**(4), 580–588 (2014).

18. J.J. López, R. Dennis, S.M. Moscoso, Estudio sobre la Automedicación en una Localidad de Bogotá, *Rev. Salud Pública*, **11**(3), 432–442 (2009).

19. A.L. Fajardo-Zapata, F.J. Méndez-Casallas, J.F. Hernández-Niño, L.H. Molina, A.M. Tarazona, C. Nossa, *et al.*, La automedicación de antibióticos: un problema de salud pública, *Salud Uninorte*, **29**(2), 226–235 (2013).

20. K. Bravo, E Espinel, A. Pardo, A. Robles, *Automedicación en estudiantes de medicina de la Universidad de Ciencias Aplicadas y Ambientales UDCA en el segundo semestre de 2017*, College Thesis, Universidad de Ciencias Aplicadas y Ambientales, Bogotá, 2017. URL: https://repository.udca.edu.co/bitstream/handle/11158/830/28-10-2017%20AUTOMEDICACION%20final.pdf?sequence=1&isAllowed=y

21. C.A. López-Cabra, J.M. Gálvez-Bermúdez, C. Domínguez-Domínguez, A. Urbina-Bonilla, C.A. Calderón-Ospina, A. Vallejos-Narváez, Automedicación en estudiantes de medicina de la Universidad del Rosario en Bogotá D. C., Colombia, *Rev. Colomb. Cienc. Quím. Farm.*, **45**(3), 374–384 (2016).

22. Departamento Administrativo Nacional de Estadística (DANE), *DANE - ¿Dónde estamos?*, 2023. URL: https://www.dane.gov.co/index.php/estadisticas-por-tema/demografia-y-poblacion/censo-nacional-de-poblacion-y-vivenda-2018/donde-estamos, accessed: Aug 28, 2023.

23. D. Baracaldo-Santamaría, M.J. Trujillo-Moreno, A.M. Pérez-Acosta, J.E. Feliciano-Alfonso, C.A. Calderon-Ospina, F. Soler, Definition of self-medication: A scoping review, *Ther. Adv. Drug Saf.*, **13**, 20420986221127501 (2022).

24. P.H.F. Domingues, T.F. Galvão, K.R.C. de Andrade, P.T.T. de Sá, M.T. Silva, M.G. Pereira, Prevalence of self-medication in the adult population of Brazil: A systematic review, *Rev. Saude Publica*, **49**, 36 (2015).

25. M.C.X. Pinto, F. Ferré, M.L.P. Pinheiro, Potentially inappropriate medication use in a city of Southeast Brazil, *Braz. J. Pharm. Sci.*, **48**(1), 79–86 (2012).

26. W.A. Adedeji, M.D. Dairo, P.M. Nguku, A. Oyemakinde, F.A. Fehintola, Pattern and predictors of medication use among adults in southwestern Nigeria: A community-based cross-sectional study, *Pharmacol. Res. Perspect.*, **11**(1), e01017 (2023).

27. A. Althubaiti, Information bias in health research: Definition, pitfalls, and adjustment methods, *J. Multidiscip. Healthc.*, **9**, 211–217 (2016).

28. Z. Klemenc-Ketis, Z. Hladnik, J. Kersnik, A cross sectional study of sex differences in self-medication practices among university students in Slovenia, *Coll. Antropol.*, **35**(2), 329–334 (2011).

29. M. Sisay, G. Mengistu, D. Edessa, Epidemiology of self-medication in Ethiopia: A systematic review and meta-analysis of observational studies, *BMC Pharmacol. Toxicol.*, **19**, 56 (2018).

30. S. Azami-Aghdash, M. Mohseni, M. Etemadi, S. Royani, A. Moosavi, M. Nakhaee, Prevalence and cause of self-medication in Iran: A systematic review and meta-analysis article, *Iran. J. Public Health*, **44**(12), 1580–1593 (2015).

31. Q. Zhang, C. Bal-dit-Sollier, L. Drouet, G. Simoneau, J.-C. Alvarez, S. Pruvot, *et al.*, Interaction between acetaminophen and warfarin in adults receiving long-term oral anticoagulants: A randomized controlled trial, *Eur. J. Clin. Pharmacol.*, **67**(3), 309–314 (2011).

32. R. Gualtierotti, A. Zoppi, A. Mugellini, G. Derosa, A. D'Angelo, R. Fogari, Effect of naproxen and acetaminophen on blood pressure lowering by ramipril, valsartan and aliskiren in hypertensive patients, *Expert Opin. Pharmacother.*, **14**(14), 1875–1884 (2013).

33. I.M. MacIntyre, E.J. Turtle, T.E. Farrah, C. Graham, J.W. Dear, D.J. Webb, *et al.*, Regular acetaminophen use and blood pressure in people with hypertension: The PATH-BP trial, *Circulation*, **145**(6), 416–423 (2022).

34. Antimicrobial Resistance Collaborators, Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis, *Lancet* (Lond Engl.), **399**(10325), 629–655 (2022).

35. M. Ocan, E.A. Obuku, F. Bwanga, D. Akena, S. Richard, J. Ogwal-Okeng, *et al.*, Household antimicrobial self-medication: A systematic review and meta-analysis of the burden, risk factors and outcomes in developing countries, *BMC Public Health*, **15**(1), 742 (2015).

36. G. Nepal, S. Bhatta, Self-medication with antibiotics in WHO Southeast Asian Region: A systematic review, *Cureus*, **10**(4), e2428 (2018).

37. G. Sulis, P. Adam, V. Nafade, G. Gore, B. Daniels, A. Daftary, *et al.*, Antibiotic prescription practices in primary care in low- and middle-income countries: A systematic review and meta-analysis, *PLoS Med.*, **17**(6), e1003139 (2020).

38. N. Mallah, N. Orsini, A. Figueiras, B. Takkouche, Income level and antibiotic misuse: A systematic review and dose–response meta-analysis, *Eur. J. Health Econ.*, **23**(6), 1015–1035 (2022).

39. C.P. Vacca, C.Y. Niño, L. Reveiz, Restricción de la venta de antibióticos en farmacias de Bogotá, Colombia: estudio descriptivo, *Rev. Panam. Salud Publica*, **30**(6), 586–591 (2011).

40. A. Shaghaghi, M. Asadi, H. Allahverdipour, Predictors of self-medication behavior: A systematic review, *Iran. J. Public Health*, **43**(2), 136–146 (2014).

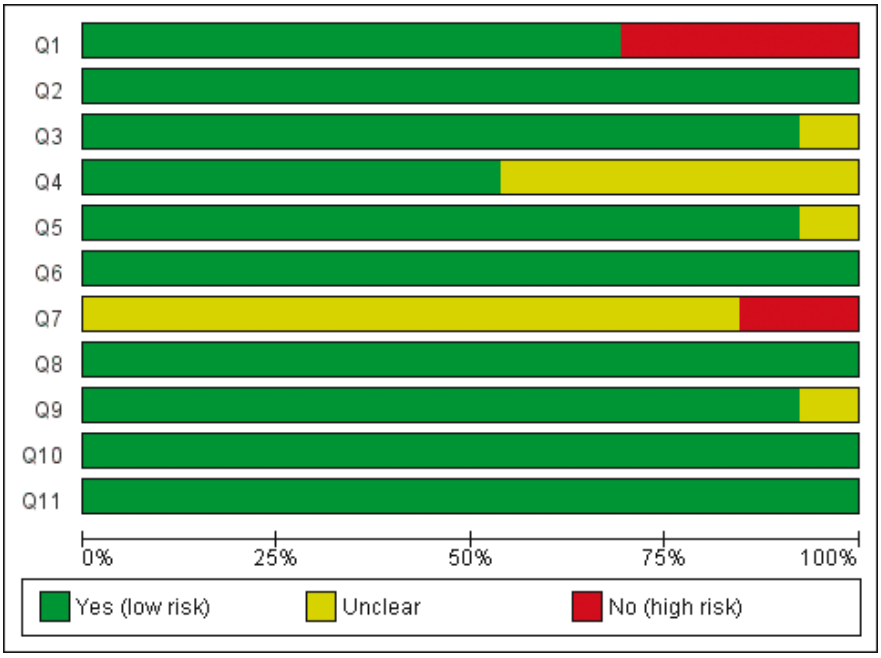
41. A.H.M.A. Faqihi, S.F. Sayed, Self-medication practice with analgesics (NSAIDs and acetaminophen), and antibiotics among nursing undergraduates in University College Farasan Campus, Jazan University, KSA, *Ann. Pharm. Fr.*, **79**(3), 275–285 (2021).

42. K. Tripković, A. Nešković, J. Janković, M. Odalović, Predictors of self-medication in Serbian adult population: cross-sectional study, *Int. J. Clin. Pharm.*, **40**(3), 627–634 (2018).

43. J. Aponte-González, A. González-Acuña, J. Lopez, P. Brown, J. Eslava-Schmalbach, Perceptions in the community about the use of antibiotics without a prescription: Exploring ideas behind this practice, *Pharm. Pract.*, **17**(1), 1394 (2019).

44. J. Aponte-González, P. Brown, J. Eslava-Schmalbach, Preferences based interventions to address the use of antibiotics without prescription: A discrete choice experiment, *Pharm. Pract.*, **19**(3), 2401 (2021).

Supplementary Material



Supplementary Figure 1. Risk of bias graph.

Supplementary Table 1. Excluded studies.

First author	Year	Reference	Reasons for exclusion
Tobón-Marulanda	2002	[*1*]	The sample does not represent the Colombian population; it only partially reports the obtained and expected losses; the instrument needs to be validated; and it does not specify the self-medication time frame.
Mejía	2017	[*2*]	The sample does not represent the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; it does not establish a clear definition of self-medication; the instrument does not have any validation; and it only partially specifies the self-medication time frame.

First author	Year	Reference	Reasons for exclusion
Mejía	2018	[3]	The sample is not representative of the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; it does not establish a clear definition of self-medication; the instrument does not have any validation; it only partially specifies the self-medication time frame.
Martínez-Domínguez	2013	[4]	The sample is not representative of the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; and the instrument does not have any validation.
Gallardo	2011	[5]	The sample does not represent the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; the instrument does not have any validation; and it only partially specifies the self-medication time frame.
Suárez	2019	[6]	The sample is not representative of the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; and the instrument does not have any validation.
Calderón	2009	[7]	The sample is not representative of the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; it does not establish a clear definition of self-medication; the instrument does not have any validation; and it only partially specifies the self-medication time frame.
Luna	2021	[8]	The sample does not represent the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; the instrument does not have any type of validation; and it only partially specifies the self-medication time frame.
Atehortúa	2011	[9]	The sample does not represent the Colombian population; there is no random selection of participants; it does not report the expected and observed losses; the instrument does not have any validation; and it only partially specifies the self-medication time frame.

First author	Year	Reference	Reasons for exclusion
Fajardo-Zapata	2018	[*10*]	The sample is not representative of the Colombian population; it does not establish a clear definition of self-medication; the instrument does not have any validation; and it only partially specifies the self-medication time frame.
Flórez-Molina	2020	[*11*]	The sample is not representative of the Colombian population; convenience sampling; expected and observed losses are not reported; and the time frame of self-medication is not defined.

Supplementary References

1. F.A. Tobón-Marulanda, Estudio sobre automedicación en la Universidad de Antioquia, Medellín, Colombia, *Iatreia*, **15**, 242–247 (2002).

2. M.C. Berruet-Mejía, M. Lince-Restrepo, D. Restrepo-Bernal, Automedicación de analgésicos y antibióticos en estudiantes de pregrado de medicina, *Medicina U.P.B.*, **36**, 115–122 (2017).

3. M.C. Berruet-Mejía, M. Lince-Restrepo, D. Restrepo-Bernal, Actitudes, conocimientos y prácticas frente a la automedicación con productos herbales y psicofármacos en estudiantes de medicina de Medellín-Colombia, *Medicina U.P.B.*, **37**, 17–24 (2018).

4. M.D.A. Martínez-Domínguez, L.M. Martínez-Sánchez, G.I. Martínez-Domínguez, Características del consumo de medicamentos de venta libre en una población de adultos de la ciudad de Medellín (Colombia), *Salud Uninorte*, **29**, 360–367 (2013).

5. E.T. Gallardo, G.A. Ortiz-Ochoa, J.N. Muñoz-Restrepo, J.O. Higuita-Restrepo, K.C. Ceballos-Borja, *Análisis sobre la automedicación en Colombia: un problema de todos*, College Report, Universidad Nacional Abierta y a Distancia, Palmira, Valle, Colombia, 2011. URL: https://repository.unad.edu.co/bitstream/handle/10596/26382/TRABAJO%20CURSO%20DE%20PROFUNDIZACION%20EVELYN.pdf?sequence=1&isAllowed=y#:~:text=Las%20razones%20por%20las%20que,recibir%20el%20mismo%20analg%C3%A9sico%20y

6. A.J. Suárez-López, Automedicación con antibióticos en habitantes de la comuna tres del municipio de San Juan de Pasto, *Revista Criterios*, **26**, 37–55 (2019).

7. A.A. Calderón-Murillo, L.M. Castaño-Mejia, M.M. Gómez-Samper, D.N. Rojas-Ortiz, G.E. Rendón-Restrepo, D. Pineda-Álvarez, *La automedicación: una costumbre sin control adecuado, que atenta contra la salud y calidad de vida de los ciudadanos*, College Report, Universidad Nacional Abierta y a Distancia, Medellín, Colombia, 2009. URL: https://repository.unad.edu.co/bitstream/handle/10596/1701/2009-09T-05.pdf?sequence=1

8. M.A. Luna-Narvaéz, L.Y. Sierra-López, L.M. Solar-Jimenez, J.P. Ruíz-Castro, *Automedicación en estudiantes de una universidad pública de la ciudad de Montería*, College Report, Universidad de Córdoba, Montería, Córdoba, Colombia, 2021. URL: https://repositorio.unicordoba.edu.co/bitstream/handle/ucordoba/4469/lunanarvaezmaira-sierralopezlaura-solarjimenezluis-ruizcastrojessica.pdf?sequence=1&isAllowed=y

9. J. Atehortua, L. Castaño-Díaz, L. Hernández-Posso, A. Monje-Trujillo, O. Ordoñez-Galindo, *Prevalencia de la automedicación en veinticinco droguerías de los municipios de Pradera, Neiva, Itahui, Otanche y Dos Quebradas*, College Report, Universidad Nacional Abierta y a Distancia, Palmira, Valle, Colombia, 2011. URL: https://repository.unad.edu.co/bitstream/handle/10596/26413/LEYNER%20HERNANDEZ.pdf?sequence=1&isAllowed=y

10. A.L. Fajardo-Zapata, Consumo de psicofármacos en la ciudad de Bogotá D.C. (Colombia): una nueva realidad, *Archivos de Medicina*, **18**, 404–412 (2018).

11. J. Flórez-Molina, P.E. Chaparro-Narváez, *Automedicación y factores asociados en docentes de dos instituciones educativas en Bogotá y Cali (Colombia): un estudio transversal*, Master Thesis, Universidad Santo Tomás, Bogotá D.C., Colombia, 2020. URL: https://repository.usta.edu.co/bitstream/handle/11634/22816/2020jhonflorez.pdf?sequence=5&isAllowed=y

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A descrição teórica da detecção eletroanalítica do ácido ibotênico e da muscazona, assistida pelo compósito de oxihidróxido de vanádio com o polímero condutor

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Resumo

Introdução: Neste trabalho, pela primeira vez foi analisada, do ponto de vista teórico, a possibilidade da determinação eletroanalítica das micotoxinas muscazona e ácido ibotênico, assistida pelo compósito de oxihidróxido de vanádio com polímero condutor. **Desenvolvimento**: O processo eletroanalítico realiza-se mediante a redução catódica, e a reação eletroquímica se realiza de maneira diferente para o ácido ibotênico e para a muscazona. Em virtude disto, a realização do comporta-

mento oscilatório em parâmetro eletroquímico é muito mais provável que em casos mais simples. **Resultado**: Malgrado o supracitado, o compósito polímero condutor – VO(OH) pode ser um modificador eficiente para a detecção eletroanalítica do ácido ibotênico com a muscazona.

Palavras-chave: ácido ibotênico, muscazona, sensor eletroquímico, polímero condutor, oxihidróxido de vanádio trivalente, estado estacionário estável

Summary

The theoretical description for ibotenic acid and muscazone electrochemical determination, assisted by the vanadium oxyhydroxide composite with conducting polymer.

Introduction: In this work, the possibility for the electroanalytical determination of ibotenic acid and muscazone mycotoxin, assisted by a conducting polymer/vanadium oxyhydroxide composite has been analyzed for the first time. **Proposal**: The electroanalytical process is carried out by cathodic reduction by different manner for ibotenic acid and for muscazone. For this reason, the oscillatory behavior in electrochemical parameter is far more probable than in the simplest case. **Result**: the conducting polymer – VO(OH) composite may be an efficient electrode modifier for electroanalytical detection of ibotenic acid and muscazone.

Keywords: ibotenic acid, muscazone, electrochemical sensor, conducting polymer, trivalente vanadium oxyhydroxide, stable steady-state

Resumen

Descripción teórica de la detección electroanalítica del ácido ibotênico y de la muscazona, asistida por el compuesto de oxihidróxido de vanadio con el polímero conductor

Introducción: En este trabajo, por la primera vez, ha sido analizada, del punto de vista teórico, la posibilidad de la determinación electroanalítica de las micotoxinas muscazona y ácido ibotênico, asistida por el compuesto de oxihidróxido de vanadio con el polímero conductor. **Propuesta**: El proceso electroanalítico se realiza mediante la reducción catódica, y la reacción electroquímica se realiza de manera

diferente para el ácido iboténico y la muscazona. Por esta razón, la realización del comportamiento oscilatorio en parámetro electroquímico es mucho más probable que en casos más simples. **Resultado**: A pesar de lo arriba dicho, el compuesto polímero conductor – VO(OH) puede ser un modificador eficiente para la detección electroanalítica del ácido iboténico con la muscazona.

Palabras clave: ácido iboténico, muscazona, sensor electroquímico, polímero conductor, oxihidróxido de vanadio trivalente, estado estacionario estable

Introdução

O cogumelo *Amamita muscaria,* conhecido popularmente como mata-moscas, é um dos cogumelos venenosos mais abundantes na Europa e na América do Norte, principalmente nas zonas climáticas moderada e subtropical [1-4]. Existem também relatos da expansão do seu habitat para países tropicais e até equatoriais [4]. Assim como a maior parte dos cogumelos do género *Amamita,* o mata-moscas é considerado tóxico e, por conseguinte, não se usa na alimentação, mesmo que seja nas formas cozida e assada.

Os sintomas principais da intoxicação pelo cogumelo [5-8] são euforia, sentimento de felicidade falsa, vivacidade, alucinações, seguidos pela depressão repentina, ansiedade, sonolência, entre outros. Mesmo com as doses relativamente pequenas, a desintoxicação realiza-se lentamente, não passando pela "ressaca". As doses altas podem causar danos cerebrais irreparáveis e mudanças comportamentais permanentes. Os efeitos tóxicos do mata-moscas são associados à presença do ácido ibotênico e derivados (Fig. 1):

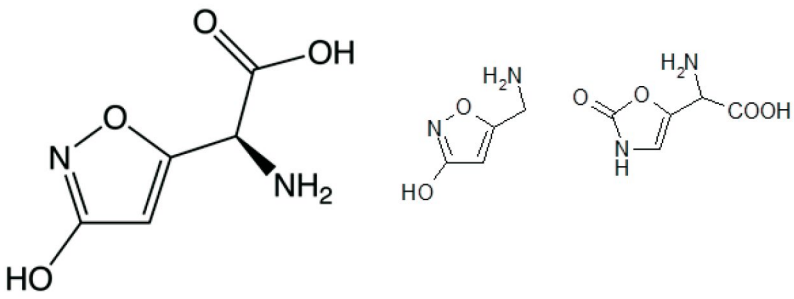

Fig. 1. Da esquerda à direita: acido ibotênico, muscimol e muscazona

O ácido ibotênico (Fig. 1, à esquerda) é um aminoácido principal do mata-moscas. Do ponto de vista químico, é um aminoácido, substituído pelo anel isoxazólico, que o faz um composto com propriedades básicas predominantes. A sua toxicidade baseia-se na sua facilidade de trespassar a barreira hematoencefálica, intervindo na síntese de proteínas cerebrais, modificando a sua composição [9-11]. Entretanto, em alguns países ele ainda é usado na medicina popular para alcançar a estimulação cerebral e espiritual [12]. Outrossim, este ácido é usado em testes bioquímicos de resposta neurofisiológica [13]. A sua decarboxilação transforma-o numa amina, conhecida como muscimol, um composto ainda mais tóxico [14].

Por outro lado, a muscazona (Fig. 1. à direita), é um dos isômeros do ácido ibotênico, que é produzido dentro do cogumelo a partir deste sob irradiação ultravioleta. Em relação ao ácido ibotênico e, ainda mais, ao seu isômero, a muscazona é menos tóxica [5, 14-16], mas possui um efeito mais duradouro, além de reforçar o efeito deles dois, provocando perdas de memória, atenção, orientação e visão.

Haja vista o supracitado, o desenvolvimento de um método de detecção e quantificação precisa e exata da concentração de ambos os ácidos é realmente atual, e os métodos eletroanalíticos poderiam servir de boa solução a este problema [17, 18].

Alguns métodos eletroanalíticos e eletroforéticos têm sido desenvolvidos para a quantificação do ácido ibotênico [18, 19]. Quanto à muscazona, nenhum método eletroanalítico para a detecção deste aminoácido tem sido desenvolvido até agora. Haja vista o anel isoxazólico, é preferível usar o processo catódico ao anódico, pois o composto é difícil de oxidar, mas que facilmente se reduz. Um dos modificadores de elétrodo, que poderiam providenciar esse processo, poderia ser o oxihidróxido de vanádio [20]. É um composto com comportamento eletroquímico flexível, mas com maior tendência de ser oxidado [21].

Na pesquisa bibliográfica, realizada pelo grupo dos autores do presente trabalho, não se encontraram citações, referentes a trabalhos experimentais acerca da modificação do elétrodo por VO (OH) para fins eletroanalíticos, apesar de este mesmo grupo ter publicado alguns teóricos [22-24], que comprovam essa possibilidade. O principal é que a introdução de um novo modificador de elétrodo pode acarretar os problemas como:

- Possível incompatibilidade do modificador com o meio da eletroanálise;
- Possibilidade da aparição das instabilidades eletroquímicas, características para o comportamento do composto semelhante, que é o oxihidróxido de cobalto [25-28], durante o desempenho mediador de VO(OH). A sua aparição pode não estar aquém do desiderato de um eficiente processo eletroanalítico.

Destarte, o objetivo geral deste trabalho é avaliar, do ponto de vista teórico, o comportamento do sistema com a determinação do ácido ibotênico e muscazona no cátodo, modificado pelo oxihidróxido de vanádio. A sua realização incluirá o alcance dos objetivos específicos:

- Sugerir um mecanismo do desempenho do sensor, incluindo as etapas químicas e eletroquímicas, com a expressão clara do papel que o modificador (no caso, o oxihidróxido de vanádio) desempenha no processo;
- Elaborar e analisar um modelo matemático, capaz de descrever de maneira adequada o comportamento do sistema;
- Da análise do modelo, derivar os requisitos do melhor desempenho eletroanalítico do elétrodo com o ácido inotênico, bem como as condições, sob cuja satisfação são realizadas as instabilidades oscilatória e monotônica;
- Comparar o desempenho do sistema com o dos semelhantes [22-24].

O sistema e o seu modelo

A redução do ácido ibotênico e da muscazona dá-se de mecanismos diferentes. Em ambos os casos, porém, ocorre a ruptura do anel. No caso do ácido ibotênico, a redução realizar-se-á de duas maneiras. Assim, esquematicamente, o comportamento do sistema será descrito conforme na Fig. 2:

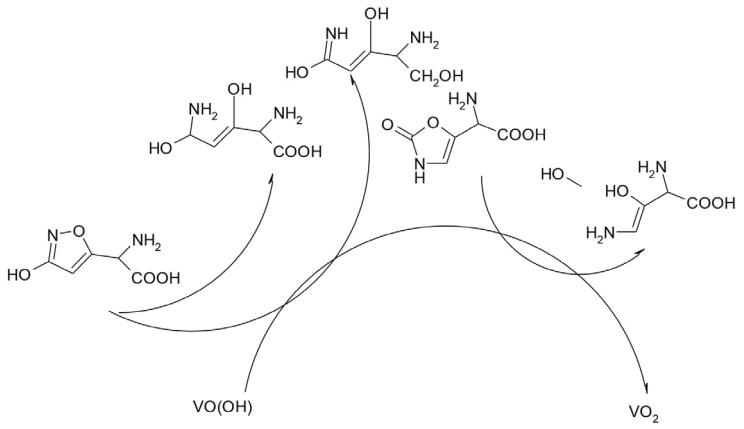

Fig. 2. Representação esquemática do sistema eletroanalítico

Vale a pena mencionar que a redução de muscazona no processo reproduz o seu metabolismo no organismo humano. Um dos produtos da sua redução é o metanol, responsável pela cegueira parcial ou total – um dos efeitos da intoxicação pelo mata-moscas.

Havendo vista o supracitado, para investigar o comportamento do sistema, introduzimos três variáveis:

m – concentração do ácido ibotênico na camada pré-superficial;

m^* - concentração da muscazona na camada pré-superficial;

v – grau de recobrimento da matriz polimérica pelo vanádio tetravalente.

Para simplificar a modelagem, supomos que o reator esteja agitando-se intensamente, de modo que possamos menosprezar o fluxo de convecção, que o eletrólito de suporte esteja em excesso, para menosprezar o fluxo de migração. Também supomos que a camada pré-superficial esteja de espessura constante, igual a δ, e que o perfil concentracional dos dois analitos seja linear.

É possível mostrar que o comportamento do sistema será descrito pelo conjunto de equações diferenciais (1):

$$\begin{cases} \frac{dm}{dt} = \frac{2}{\delta}\left(\frac{M}{\delta}(m_0 - m) - r_{iso} - r_{11} - r_{12}\right) \\ \frac{dm*}{dt} = \frac{2}{\delta}\left(\frac{M*}{\delta}(m*_0 - m*) + r_{iso} - r_2\right) \\ \frac{dv}{dt} = \frac{1}{V}(r_{11} + r_{12} + r_2 - r_V) \end{cases} \quad (1)$$

Sendo M e M^* os coeficientes de difusão de cada um dos aminoácidos m_0 e m^*_0 são as concentrações de cada um dos aminoácidos, V é a concentração superficial máxima e os parâmetros r são as velocidades das reações correspondentes, que se podem calcular conforme (2 – 6):

$$r_{iso} = k_{iso} m \exp(-am) \quad (2)$$

$$r_{11} = k_{11} m(1 - v)^6 \exp(-am) \quad (3)$$

$$r_{12} = k_{12}\, m(1 - v)^8 \exp(-am) \tag{4}$$

$$r_2 = k_2\, m * (1 - v)^4 \exp(-bm) \tag{5}$$

$$r_v = k_v v \exp\left(-\frac{F\varphi_0}{RT}\right) \tag{6}$$

Em que os parâmetros k são as constantes das respectivas reações, a e b são parâmetros, que relacionam a concentração do ácido ibotênico e da muscazona com a DCE, F é o número de Faraday, φ_0 é o salto do potencial, relativo ao potencial da carga zero, R é a constante universal de gases, T é a temperatura absoluta.

Como todas as etapas químicas e eletroquímicas do processo influem a DCE, o comportamento deste sistema é mais dinâmico que nos casos semelhantes [22-24]. Nada obstante, o sistema eletroanalítico é eficiente, o que será representado abaixo.

Resultados e discussão

Para investigar o comportamento do sistema com a detecção eletroanalítica do ácido ibotênico na presença da muscazona, assistida pelo compósito do oxihidróxido de vanádio com polímero condutor, analisamos o conjunto de equações diferenciais (1), além das relações algébricas (2 – 6) mediante a teoria de estabilidade linear. Os valores estacionários da matriz funcional Jacobiana podem ser expressos conforme (7):

$$\begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} \tag{8}$$

Sendo:

$$a_{11} = \frac{2}{\delta}\left(-\frac{M}{\delta} - k_{iso}\exp(-am) + ak_{iso}m\exp(-am) - k_{11}(1 - v)^6\exp(-am) + ak_{11}m(1 - v)^6\exp(-am) - k_{12}(1 - v)^8\exp(-am) + ak_{12}(1 - v)^8\exp(-am)\right) \tag{9}$$

$$a_{12} = 0 \tag{10}$$

$$a_{13} = \frac{2}{\delta}(6k_{11}m(1 - v)^5 \exp(-am) + 8k_{12}m(1 - v)^7 \exp(-am)) \tag{11}$$

$$a_{21} = \frac{2}{\delta}(k_{iso} \exp(-am)) \tag{12}$$

$$a_{22} = \frac{2}{\delta}(-\frac{M*}{\delta}(m*_0 - m*) - k_2(1 - v)^4 \exp(-bm) + bk_2m*(1 - v)^4 \exp(-bm)) \tag{13}$$

$$a_{23} = \frac{2}{\delta}(4k_2m*(1 - v)^3 \exp(-bm)) \tag{14}$$

$$a_{31} = \frac{1}{V}(k_{11}(1 - v)^6 \exp(-am) - ak_{11}m(1 - v)^6 \exp(-am) + k_{12}(1 - v)^8 \exp(-am) - ak_{12}(1 - v)^8 \exp(-am)) \tag{15}$$

$$a_{32} = \frac{1}{V}(k_2(1 - v)^4 \exp(-bm) - bk_2m*(1 - v)^4 \exp(-bm)) \tag{16}$$

$$a_{33} = \frac{1}{V}(-6k_{11}m(1 - v)^5 \exp(-am) - 8k_{12}m(1 - v)^7 \exp(-am) - 4k_2m*(1 - v)^3 \exp(-bm) - k_v \exp(-\frac{F\varphi_0}{RT}) + jk_v v \exp(-\frac{F\varphi_0}{RT})) \tag{17}$$

O comportamento oscilatório é passível de realizar, desde que sejam satisfeitas as condições da bifurcação de Hopf. A sua condição necessária é a presença dos elementos positivos na diagonal principal da matriz Jacobiana. Como se vê, o comportamento oscilatório é ainda mais provável neste sistema que nos semelhantes [22-24], por haver mais de um elemento, que pode ter valores positivos.

Neste caso, a estrutura da DCE está alterada não só durante a etapa eletroquímica, mas durante a isomerização, que leva à transformação de uma forma iônica noutra. Essas alterações estruturais provocam, também, as alterações nos valores da capacitância da DCE, o que pode causar o comportamento oscilatório.

Este comportamento se descreve pela positividade dos elementos, $ak_{iso}m\,exp(-am) > 0$, $ak_{11}m(1 - v)^6 \exp(-am) > 0$ e $ak_{12}(1 - v)^8 \exp(-am) > 0$ se $a > 0$, $bk_2m \cdot (1 - v)^4 \exp(-bm) > 0$, se $b > 0$ e $jk_v v \exp(-(F\varphi_0)/RT) > 0$ se $j > 0$. Estes elementos descrevem as influências

das etapas químicas nas capacitâncias da DCE, que também podem ser responsáveis pelo comportamento oscilatório. No entretanto, a realização de todas essas influências desestabilizadoras na dupla camada dar-se-á nos valores paramétricos, afastados do limite de detecção, o que se mostrará abaixo.

Para investigar a estabilidade do estado estacionário, aplicamos ao conjunto de equações diferenciais (1) o critério Routh-Hurwitz. Evitando as expressões grandes, introduzimos as novas variáveis, reescrevendo o determinante conforme (18):

$$\frac{4}{\delta^2 V}\begin{vmatrix} -\kappa_1 - \Xi - \Lambda & 0 & \Sigma \\ \Xi & -\kappa_2 - X & T \\ \Lambda & X & -\Sigma - T - \Omega \end{vmatrix} \tag{18}$$

Abrindo os parênteses e aplicando o requisito Det J < 0, saliente do critério, obtemos a condição de estabilidade do estado estacionário, exposta conforme (19):

$$-\kappa_1(\kappa_2\Sigma + X\Sigma + \kappa_2 T + \kappa_2\Omega + X\Omega) - \Xi(\kappa_2\Sigma + \kappa_2 T + \kappa_2\Omega + X\Omega) - \Lambda(\kappa_2 T + \kappa_2\Omega + X\Omega) + \Sigma\kappa_2\Sigma < 0 \tag{19}$$

Ela obtém uma forma mais complexa que nos casos semelhantes [22-24] e descreve um sistema eletroanalítico eficiente, controlado pela difusão e cinética do processo ao mesmo tempo.

Haja vista o serem usadas as condições de pH, correspondentes ao ponto isoelétrico do ácido ibotênico, a estabilidade dele e do oxihidróxido de vanádio não é comprometida. Assim, o estado estacionário estável é eletroanaliticamente eficiente, o que corresponde à linearidade da dependência entre a concentração do analito e o parâmetro eletroquímico (neste caso, corrente).

A condição da instabilidade monotônica, relativa ao limite de detecção, para este sistema descreve-se conforme:

$$\kappa_1(\kappa_2\Sigma + X\Sigma + \kappa_2 T + \kappa_2\Omega + X\Omega) - \Xi(\kappa_2\Sigma + \kappa_2 T + \kappa_2\Omega + X\Omega) - \Lambda(\kappa_2 T + \kappa_2\Omega + X\Omega) + \Sigma\kappa_2\Sigma = 0 \tag{20}$$

Caso, em vez do ácido ibotênico, seja usado um analito cíclico, mas que é difícil de ionizar, os parâmetros α e β anular-se-ão, e o comportamento do sistema far-se-á menos dinâmico. O estado estacionário, porém, far-se-á mais estabilizado. Assim, todas as reações de redução podem ser apresentadas em suma, e, destarte, o conjunto de equações difereiciais (1) será análogo ao já descrito em [22-24].

Conclusões

Da análise do comportamento do sistema eletroanalítico, baseado em oxihidróxido de vanádio na detecção eletroquímica do ácido ibotênico na presença da muscazona, pode-se concluir que:

- O oxihidróxido de vanádio pode ser um modificador eficiente na determinação de ambos os ácidos no cátodo. Realiza-se a abertura do anel izoxazólico, com a redução subsequente do seu produto acíclico. A reversibilidade de VO(OH) obtém-se na etapa eletroquímica;
- O processo eletroanalítico é eficiente e controlado pela difusão e cinética dos processos;
- O comportamento oscilatório neste sistema é mais provável que nos semelhantes, haja vista o existirem mais processos a influenciarem a dupla camada elétrica e as suas capacitâncias. A realização deste tipo de comportamento obtém-se quando os valores dos parâmetros estão além do limite de detecção.

Conflitos de interesse

Os autores não têm conflitos de interesse a declarar.

Referências

1. Q. Carboue, M. Lopez, *Amanita muscaria*: Ecology, chemistry, myths, *Encyclopedia*, **1**(3) 905–914 (2021).
2. F.I. Rampoll, P. Kamler, C.C. Carlino, F. Bedussi, The deceptive mushroom: Accidental *Amanita muscaria* poisoning, *Eur. J. Case Rep. Int. Med.*, **8**(2), 002212 (2021).
3. C.E. Housecroft, The fungus *Amanita Muscaria*: From neutoroxin to vanadium accumulation, *Chimia*, **73**, 96–97 (2019).
4. N. Vargas, S.C. Gonçalves, A.E. Franco-Molano *et al.*, In Colombia the Eurasian fungus *Amanita muscaria* is expanding its range into native, tropical, *Quercus humboldtii* forests, *Mycologia*, **111**(5), 758–771 (2019).

5. M. Voynova, A. Shkondrov, M. Kondeva-Burdina, I. Krasteva, Toxicological and pharmacological profile of *Amanita muscaria* (L.) Lam. – A new rising opportunity for biomedicine, *Pharmacia*, **67**(4), 317–323 (2020).

6. S. Modaberi, M. Shahbazi, N. Naghdi, F. Bagherzadeh, Effects of bilateral microinjections of ibotenic acid into neostriatal region and forced exercise on spatial learning and memory and anthropometric characteristics of male rats, *Koomesh J.*, **23**(5), 654–664 (2021).

7. M.A. Mikaszewska-Sokolewicz, S. Pankowska, M. Janiak, *et al.*, Coma in the course of severe poisoning after consumption of red fly agaric (*Amanita muscaria*), *Acta Biochim. Pol.*, **63**(1), 181–182 (2016).

8. H. Li, H. Zhang, Y. Zhang, *et al.*, Mushroom Poisoning Outbreaks — China, 2019, *China CDC Wkly.*, **2**(2), 19–24 (2020)

9. M.R. Lee, E. Dukan, I. Milne, *Amanita Muscaria* (Fly Agaric): From a shamanistic hallucinogen to the search for acetylcholine, *J. R. Coll. Physicians Edinb.*, **48**(1), 85–91 (2018).

10. ChemSpider: Search and share chemistry, Ibotecnic acid. URL: http://www.chemspider.com/Chemical-Structure.5036193.html, acesso aos 8 de abril de 2022.

11. K. Stebelska, Psychoactive drugs of fungal origin, psilocin, ibotenic acid, and muscimol, *Ther. Drug. Monit.*, **35**, 420–422 (2013).

12. M. Lumpert, S. Kreft, Catching flies with *Amanita muscaria*: traditional recipes from Slovenia and their efficacy in the extraction of ibotenic acid, *J. Ethnopharmacol.*, **187**, 1–8 (2016).

13. S. Lee, Y.S. Yu, S.R. Lee, K.H. Kim, Non-peptide secondary metabolites from poisonous mushrooms: overview of chemistry, bioactivity, and biosynthesis, *Nat. Prod. Rep.*, **39**, 512–559 (2022).

14. M. Voynova, A. Shkondrov, M. Kondeva-Burdina, I. Krasteva, *In vitro* effects of synthetic muscimol and an extract from *Amanita muscaria* on human recombinant MAOB enzyme, *Pharmacia*, **68**(1), 147–150 (2021).

15. A. Sawangjit, M. Harkotte, C. Oyanedel, *et al.*, Two distinct ways to form long-term object recognition memory during sleep and wakefulness, *Proc. Natl. Acad. Sci. U. S. A.,* **119**(34), e2203165119 (2022).

16. S. Obermaier, M. Müller, Ibotenic acid biosynthesis in the fly agaric is initiated by glutamate hydroxylation, *Angew. Chem.*, *Int. Ed.*, **59**, 12432–12435 (2020).

17. A. Poliwoda, K. Zielinska, M. Halama, P.P. Wieczorek, Determination of muscimol and ibotenic acid in mushrooms of Amanitaceae by capillary electrophoresis, *Electrophoresis*, **35**(18), 2593–2599 (2014)

18. P. Ginterova, B. Sokolova, P. Ondra, *et al.*, Determination of mushroom toxins ibotenic acid, muscimol and muscarine by capillary electrophoresis coupled with electrospray tandem mass spectrometry, *Talanta*, **125**, 242–247 (2014).

19. S. Deja, E. Jawien, I. Jasicka-Mysiak, Rapid determination of ibotenic acid and muscimol in human urine, *Magn. Reson. Chem.*, **52**(11), 711–714 82014).

20. T. Sasaki, S. Hirokatsu, Yu. Hiradate, *et al*., Behavioural effects in mice orally exposed to domoic acid or ibotenic acid are influenced by developmental stages and sex differences, *Biochem. Biophys Res. Commun.*, **558**, 175–182 (2021).

21. Y. Xu, X. Han, L. Zheng, S. Wei, Y. Xie, First investigation on charge-discharge reaction mechanism of aqueous lithium ion batteries: A new anode material of $Ag_2V_4O_{11}$ nanobelts, *Dalton Trans.*, **40**, 10751–10757 (2011).

22. V.V. Tkach, M.V. Kushnir, V.V. Kopiika, *et al.*, Theoretical description for ibotenic acid and muscazone determination in mushroom pulp and biological liquids over conducting polymer-modified electrode, *Biointerface Res. Appl. Chem.*, **13**, **275** (2023).

23. V.V. Tkach, M.V. Kushnir, L.V. Romaniv, *et al.*, The Theoretical description for ibotenic acid and muscimol electrochemical determinbation in mushroom pulp and mushroom-based alcoholic beverages on nano-CuS composite with conducting polymer, *Lett. Appl. NanoBioSci.*, **13**, 37 (2024).

24. V.V. Tkach, M.V. Kushnir, V.V. Kopiika, *et al.*, The theoretical description for amavadin ion electrochemical determination in *Amanita muscaria* mushroom pulp and extract by galvanostatic conducting polymer doping, *Biointerface Res. Appl. Chem.*, **13**, 400 (2023).

25. I. Das, N.R. Agrawal, S.A. Ansari, S.K. Gupta, Pattern formation and oscillatory electropolymerization of thiophene, *Ind. J. Chem. A*, **47**(12), 1798–1803 (2008).

26. K. Aoki, I. Mukoyama, J. Chen, Competition between polymerization and dissolution of poly(3-methylthiophene) films, *Russ. J. Electrochem.*, **40**, 280–285 (2004).

27. I. Das, N. Goel, N.R. Agrawal, S.K. Gupta, Growth patterns of dendrimers and electric potential oscillations during electropolymerization of pyrrole using mono-and mixed surfactants, *J. Phys. Chem.*, **114**, 12888–12896 (2010).

28. I. Das, N. Goel, S.K. Gupta, N.R. Agrawal, Electropolymerization of pyrrole: Dendrimers, nano-sized patterns and oscillations in potential in presence of aromatic and aliphatic surfactants, *J. Electroanal. Chem.*, **670**, 1–10 (2012).

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Modelagem matemática da detecção eletroanalítica da nereistoxina, assistida pelo compósito de oxihidróxido de cobalto com polímeros condutores

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Resumo

Introdução: Pela primeira vez, a possibilidade da detecção eletroanalítica da nereistoxina no elétrodo, modificado pelo compósito de oxihidróxido de cobalto e polímeros condutores. **Desenvolvimento**: A eletrooxidação dá-se gradualmente pelos átomos do enxofre levando à rutura do anel e formação do ácido dissulfênico, seguida pela formação do ácido dissulfônico. **Resultado**: A análise do modelo correspondente, mediante a teoria de estabilidade lineal e de bifurcações confirma a eficiência

do oxihidróxido de cobalto e polímero condutor como modificador de ânodo para a determinação eletroquímica da nereistoxina. Por outro lado, a probabilidade do comportamento oscilatório neste sistema aumenta, haja vista a formação de novas formas iônicas.

Palavras-chave: nereistoxina, sensor eletroquímico, oxihidróxido de cobalto, polímero condutor, estado estacionário estável

Summary

The mathematical modeling for nereistoxin electrochemical determination, assisted by cobalt oxyhydroxide/conducting polymer composite

Introduction: For the first time, the possibility of nereistoxin electrochemical determination on a CoO(OH)/CP-modified electrode has been given. **Proposal:** The electrooxidation is carried out gradually by the sulfur atoms, leading to the ring cleavage and formation of a disulfenic acid, followed by a disulfonic acid formation. **Result:** The analysis of the correspondente model by means of the linear stability theory and bifurcation analysis confirms the efficiency of cobalto oxyhydroxide and conducting polymer for nereistoxin determination. On the other hand, the probability of the oscillatory behavior in this system is enhanced, due to the formation of the new ionic forms.

Keywords: nereistoxine, electrochemical sensor cobalt oxyhydroxide, conducting polymer, stable steady-state

Resumen

Modelado matemático de la detección electroanalítica de la nereistoxina, asistida por el compósito de oxihidróxido de cobalto con polímeros conductores

Introducción: Por primera vez, ha sido analizada teóricamente la posibilidad de la detección electroanalítica de la nereistoxina en el electrodo, modificado por el compuesto de oxihidróxido de cobalto y polímeros conductores. **Propuesta**: La electrooxidación se hace gradualmente por los átomos do azufre, llevando a la

ruptura del anillo heterocíclico y formación del ácido disulfénico, seguida por la formación del ácido disulfónico. **Resultado**: El análisis del modelo correspondiente, mediante la teoría de la estabilidad lineal y de bifurcaciones confirma la eficiencia del oxihidróxido de cobalto y polímero conductor como modificador de ánodo para la determinación electroquímica de la nereistoxina. Por outro lado, la probabilidad del comportamiento oscilatorio en este sistema aumenta, puesto que ocurre la formación de nuevas formas iónicas.

Palavras clave: nereistoxina, sensor electroquímico, oxihidróxido de cobalto, polímero conductor, estado estacionario estable

Introdução

Nereistoxina (4-*N*,*N*-dimetil-1,2-ditiolano (IUPAC, Hantzsch – Widmann, CAS: 1631-58-9, *M*=149,27 g/mol, Fig. 1.) [1-4] é um composto tóxico natural, isolado pela primeira vez dos vermes anelares *Lumbriconereis heteropoda* em 1934. Os vermes introduzem a toxina, mordendo a vítima ou picando-a pelas espinhas, como dragões-do-mar.

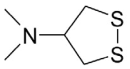

Fig. 1. Nereistoxina

A nereistoxina possui dois grupos funcionais – ambos, responsáveis pela ação tóxica [5-9]. A amina ternária irrita e bloqueia os receptores nicotínicos, e a "ponte" dos heteroátomos do enxofre facilmente se irrompe, rendendo os derivados de 2-dimetilamino-1,3-propanoditiol (isto é, 2-dimetilaminotrimetilenoditioglicol ou 2-dimetilamino-1,3-propilodimercaptano) e seus derivados. A nereistoxina deu origem a vários fármacos e pesticidas, como a inseticida cartap (Fig. 2):

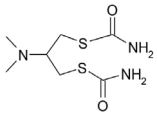

Fig. 2. Cartap

que é de fato o éster do 2-dimetilamino-1,3-propanoditiol e o ácido aminofôrmico, que é instável a temperaturas superiores a -23^0. Destarte, o desenvolvimento de um método eficaz da determinação do cartap é realmente atual [10-12].

Possuindo uma "ponte" dissulfídica, em que os átomos do enxofre têm número de oxidação como no pireto, o analito pode ser tanto oxidado, como reduzido. Portanto, tanto os métodos anódicos, como os catódicos são aplicáveis à sua determinação eletroanalítica. Um dos modificadores de elétrodo, passíveis de aplicar à determinação da nereistoxina, pode ser o oxihidróxido de cobalto [13-15], material semicondutor, frequentemente usado nos aparelhos semicondutores como substituição para o dióxido de titânio, porém, ao contrário deste, é eletroquimicamente ativo. Ele, sozinho ou em compósito com polímeros condutores [16-20], podia ter um desempenho eficiente como modificador de elétrodo para a detecção da nereistoxina.

No entretanto, o seu uso poderia acarretar uma influência comportamental, nociva à estabilidade do sistema e à sua eficiência eletroanalítica [21-23]. Destarte, o uso prático deste sistema eletroanalítico não pode ser realizado sem uma descrição teórica prévia do comportamento do sistema, o que se faz neste trabalho.

Assim, neste trabalho, pela primeira vez, faz-se uma análise comportamental do sistema eletroanalítico da detecção eletroquímica da nereistoxina sobre o oxihidróxido de cobalto, estabilizado pelo polímero condutor. A análise comportamental do sistema eletroanalítico faz-se mediante o desenvolvimento e a análise do modelo correspondente. Com isto também se faz a comparação do comportamento do sistema eletroanalítico com o dos semelhantes [24-28].

O sistema e o seu modelo

O oxihidróxido de cobalto oxida a nereistoxina gradualmente. Na primeira etapa, ocorre a rutura do anel, com a formação do ácido dissulfênico. Depois, este se oxida até ao ácido dissulfônico (Fig. 3):

O pH do sistema decresce levemente na primeira etapa e bruscamente na segunda, o que pode prejudicar a estabilidade do oxihidróxido de cobalto depositado na matriz polimérica. Portanto, é aconselhável usar o pH moderadamente alcalino da solução, para equilibrar a atividade dos prótons nos produtos de oxidação.

Haja vista o supracitado, para descrever a detecção eletroanalítica da nereistoxina, introduzimos três variáveis:

n – a concentração da nereistoxina na camada pré-superficial;

n^* – a concentração do ácido dissulfênico na camada pré-superficial;

c – o grau do recobrimento da matriz polimérica pelo óxido de cobalto (II).

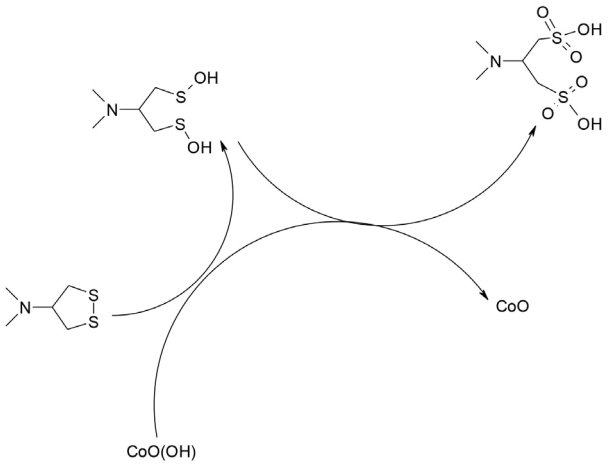

Fig. 3. A sequência das reações basilares do processo eletroanalítico

Para simplificar a modelagem, supomos que o reator esteja sob agitação intensa (destarte, podemos menosprezar o fluxo de convecção), que o eletrólito de suporte esteja em excesso (destarte, podemos menosprezar o fluxo de migração). Outrossim, supomos que o perfil concentracional das substâncias na camada pré-superficial seja lineal, e a sua espessura, constante, igual a δ.

É possível provar que o comportamento do sistema pode ser descrito pelo conjunto de equações diferenciais de balanço (1):

$$\begin{cases} \frac{dn}{dt} = \frac{2}{\delta}\left(\frac{N}{\delta}(n_0 - n) - r_1\right) \\ \frac{dn*}{dt} = \frac{2}{\delta}(r_1 - r_2) \\ \frac{dc}{dt} = \frac{1}{C}(r_1 + r_2 - r_3) \end{cases} \quad (1)$$

Sendo N o coeficiente de difusão, n_0 é a concentração da nereistoxina no interior da solução, C é a concentração superficial máxima do óxido de cobalto (II) e os parâmetros r são as velocidades das reações correspondentes, que se podem calcular como:

$$r_1 = k_1 n(1 - c)^2 \exp(-an) \quad (2)$$

$$r_2 = k_2 n * (1 - c)^8 \exp(- bn *) \quad (3)$$

$$r_3 = k_3 c \exp\left(\frac{F\varphi_0}{RT}\right) \quad (4)$$

Em que os parâmetros *k* são constantes de velocidades das respectivas reações, *a* e *b* são variáveis, que relacionam as capacitâncias da DCE com as transformações de formas iônicas, *F* é o número de Faraday, φ_0 é o salto do potencial, relativo ao potencial da carga zero, *R* é a constante universal de gases e *T* é temperatura absoluta do vaso.

Haja vista a formação com a seguinte transformação de novas formas iônicas, o comportamento oscilatório far-se-á mais provável que nos casos mais simples do uso do oxihidróxido de cobalto [24-28]. Malgrado o supracitado, o compósito do oxihidróxido de cobalto com o polímero condutor pode ser um modificador eficiente para a detecção da nereistoxina por um processo anódico, conforme exposto abaixo.

Resultados e discussão

Para investigar o comportamento do sistema com a determinação eletroanalítica da nereistoxina, assistida pelo ânodo, modificado pelo compósito CoO(OH) – polímero condutor, analisamos o conjunto de equações diferenciais (1), considerando as relações algébricas (2 – 4), mediante a teoria de estabilidade linear. Os elementos estacionários da matriz funcional de Jacobi podem ser expostos como:

$$\begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} \quad (5)$$

Sendo:

$$a_{11} = \frac{2}{\delta}\left(- \frac{N}{\delta} - k_1(1 - c)^2 \exp(- an) + ak_1 n(1 - c)^2 \exp(- an)\right) \quad (6)$$

$$a_{12} = 0 \quad (7)$$

$$a_{13} = \frac{2}{\delta}(2k_1 n(1 - c) \exp(- an)) \quad (8)$$

$$a_{21} = \frac{2}{\delta}(k_1(1 - c)^2 \exp(- an) - ak_1 n(1 - c)^2 \exp(- an)) \quad (9)$$

$$a_{22} = \frac{2}{\delta}(-k_2(1-c)^8 \exp(-bn*) + bk_2 n*(1-c)^8 \exp(-bn*)) \quad (10)$$

$$a_{23} = \frac{2}{\delta}(8k_2 n*(1-c)^7 \exp(-bn*) - 2k_1 n(1-c)\exp(-an)) \quad (11)$$

$$a_{31} = \frac{1}{C}(k_1(1-c)^2 \exp(-an) - ak_1 n(1-c)^2 \exp(-an)) \quad (12)$$

$$a_{32} = \frac{1}{C}(k_2(1-c)^8 \exp(-bn*) - bk_2 n*(1-c)^8 \exp(-bn*)) \quad (13)$$

$$a_{33} = \frac{1}{C}\left(-2k_1 n(1-c)\exp(-an) - 8k_2 n*(1-c)^7 \exp(-bn*) - k_3 \exp\left(\frac{F\varphi_0}{RT}\right) + jk_3 c \exp\left(\frac{F\varphi_0}{RT}\right)\right) \quad (14)$$

Observando os elementos da diagonal principal da matriz Jacobiana (6), (10) e (14), podemos concluir que o *comportamento oscilatório* neste sistema é passível de realizar. Ademais, ele é mais provável que nos sistemas mais simples [24-28], já que além do fator das influências da etapa eletroquímica na DCE, descritas pela positividade do elemento $jk_3c \exp\left(\frac{F\varphi_0}{RT}\right) > 0$, se $j > 0$, típico para os sistemas mais simples, neste caso existem também as influências dos câmbios na estrutura da DCE, causados pela formação de uma nova forma iônica (sulfenato) na primeira etapa química, descritas pela positividade do elemento $ak_1 n(1 - c)^2 \exp(-an) > 0$, se $a > 0$, e dos câmbios na estrutura da camada, causados pela transformação do íon de um ácido menos forte (sulfenato) para o íon de um ácido mais forte (sulfonato) na segunda etapa química, descritos pela positividade do elemento $bk_2 n * (1 - c)^8 \exp(-bn*) > 0$, se $b > 0$.

Assim como em [22-28], a manifestação do comportamento oscilatório (frequência, amplitude e tipo de oscilações), será dependente da composição do eletrólito de suporte, usado para o processo eletroanalítico. De qualquer maneira, esta manifestação dar-se-á nos valores de parâmetros afastados do limite de detecção, como será exposto abaixo.

A *estabilidade do estado estacionário,* por sua vez, tem uma ampla zona topológica de parâmetros, correspondente à sua realização, embora seja mais estreita que nos casos mais simples. Para investigá-la, aplicamos ao conjunto de equações diferenciais (1) o critério Routh-Hurwitz e, para facilitar a análise do determinante, introduzimos as novas variáveis e reescrevemo-lo conforme (15):

$$\frac{4}{\delta^2 C}\begin{vmatrix} -\kappa - \Xi & 0 & P \\ \Xi & -\Lambda & T - P \\ \Xi & \Lambda & -T - P - \Omega \end{vmatrix} \quad (15),$$

O que, conforme as propriedades do determinante, pode ser reescrito como (16)

$$\frac{4}{\delta^2 C}\begin{vmatrix} -\kappa - \Xi & 0 & P \\ 2\Xi & 0 & -2P - \Omega \\ \Xi & \Lambda & -T - P - \Omega \end{vmatrix} \quad (16)$$

Abrindo os parênteses e aplicando a condição Det J < 0, saliente do critério, obtemos o requisito de estabilidade do estado estacionário expresso como (17):

$$\Lambda(2P\kappa - \Omega P - \Omega\Xi) > 0 \quad (18),$$

que se satisfaz de forma garantida, sendo positivos os valores dos parâmetros Λ, Ξ e Ω, o que acontece na esmagadora maioria dos casos, definindo um processo eletroanalítico eficiente cineticamente controlado. A zona topológica da satisfação do requisito (18) far-se-á mais estreita que nos sistemas mais simples. Ainda assim, ela permanecerá vasta.

Não havendo reações laterais, capazes de comprometer a estabilidade do analito e(ou) modificador do ânodo, a estabilidade do estado estacionário far-se-á correspondente à linearidade da dependência entre o parâmetro eletroquímico (no caso, corrente) e a concentração da toxina, o que é relacionado com o melhor desempenho do sensor, até que seja alcançado o limite de detecção.

Este limite é descrito pela *instabilidade monotônica,* descrevendo a margem entre os estados estacionários estáveis e instáveis. A sua realização é condicionada ao requisito de Det J=0, ou

$$\Lambda(2P\kappa - \Omega P - \Omega\Xi) = 0 \quad (19),$$

Que é satisfeito, caso se satisfaça pelo menos um dos seguintes requisitos:

$$\Lambda = 0 \quad (20)$$

e(ou):

$$2P\kappa = \Omega(P + \Xi) \tag{21}$$

Neste ponto, para o sistema podem coexistir vários estados instáveis, dos quais o sistema escolhe um. Este se destrói facilmente, ao se mudarem as condições e, geralmente, não se regenera, se as condições iniciais forem restauradas (o sistema passa para um estado instável diferente).

Caso o pH inicial seja neutro, o comportamento do sistema será complicado por conta da redução do pH, causada pela formação dos ácidos. No caso, o conjunto de equações diferenciais (1) sofrerá alterações, e este conjunto será descrito e analisado num dos nossos próximos trabalhos.

Conclusões

A análise teórica do processo eletroanalítico da detecção da perilartina, assistida pelo compósito CoO(OH) – polímero condutor, deixou concluir que:

- se trata de um processo eletroanalítico eficiente, em que o composto de cobalto funciona como substância ativa, e o corante desempenha o papel de mediador;
- o processo eletroanalítico é controlado pela tanto pela cinética do processo;
- a realização do comportamento oscilatório é mas provável que no caso mais simples e comum, haja vista os câmbios cíclicos da força iónica da dupla camada elétrica, causados pela formação das formas iônicas dos ácidos sulfênico e sulfônico.

Conflitos de interesse

Os autores não têm conflitos de interesse a declarar.

Referências

1. W.R. Kem, K. Andrud, G. Bruno, *et al.*, Interactions of nereistoxin and its analogs with vertebrate nicotinic acetylcholine receptors and molluscan ACh binding proteins, *Marine Drugs*, **20**, 49 (2022).

2. R. López-Ruiz, E. Belmonte-Sánchez, R. Romero-González, *et al.*, A laboratory study on dissipation and risk assessment of the proinsecticide thiocyclam and its metabolite nereistoxin in tomato using liquid chromatography high resolution mass spectrometry, *Food Chem.*, **344**, 128729 (2021).

3. J. Dai, Ch. Jiang, Y. Chai, *et al.*, Photolysis kinetics of cartap and nereistoxin in water and tea beverages under irradiation of simulated sunlight and ultraviolet under laboratory conditions, *Food Chem.*, **355**, 129595 (2021).

4. J. Zhang, L. Pang, J. Jing, *et al.*, Development, optimization, and validation of a method for detection of cartap, thiocyclam, thiosultap-monosodium, and thiosultap-disodium residues in plant foods by GC-ECD, *Food Chem.*, **371**, 131198 (2022).

5. E. Kurisaki, N. Kato, T. Ishida, *et al.*, Fatal human poisoning with Padan™: A cartap-containing pesticide, *Clin. Toxicol.*, **48**, 153–155 (2010).

6. Y. Nagawa, Y. Saji, S. Chiba, T. Yui, Neuromuscular blocking action of nereistoxin and its derivatives and antagonism by sulfhydryl compounds, *Japan J. Pharmacol.*, **21**, 185–197 (1971).

7. Makoto Ihara, Steven D. Buckingham, Kazuhiko Matsuda, David B Sattelle, Modes of action, resistance and toxicity of insecticides targeting nicotinic acetylcholine receptors, *Curr. Med. Chem.*, **24**(27), 2925–2934 (2017).

8. N. Suganthan, R. Manmathan, T. Kumanan, Rhabdomyolysis and acute kidney injury associated with thiocyclam hydrogen oxalate (Evisect) poisoning, *SAGE Open Med. Case Rep.*, **8**, 2050313X20954942 (2020).

9. G. Preetha, Toxicity of different insecticides against rice stem borer, *Pesticide Res. J.*, **32**(2), 311–315 (2020).

10. M. Senthilvelan, B. Nageswaran, K. Baburaj, A. Elaiaraja, Cartap hydrochloride poisoning - A case report, *J. Med. Sci. Clin. Res.*, **7**, 701–704 (2019).

11. V.V. Oberemok, K.V. Laikova, Yu. I. Gninenko, *et al.*, A short history of insecticides, *J. Plant Prot. Res.*, **55**(3), 221–225 (2015)

12. M. Awad, S.I. El-Desoky, E. Osman, *et al.*, Nano-insecticides against the black cutworm Agrotis ipsilon (Lepidoptera: Noctuidae): Toxicity, development, enzyme activity, and DNA mutagenicity, *PLoS One*, **17**, 0254285 (2022).

13. A. Stadnik, E.M. Caldas, A. Galli, F.J. Anaissi, Eletrodo modificado com [CoO(OH)] coloidal aplicado na detecção de ácido oxálico, *Orbital. Elec. J. Chem.*, **7**, 122–128 (2015).

14. J.S. Bonini, F.Q. Mariani, E. Guimarães Castro, *et al.*, Partículas de CoO(OH) dispersas em pasta de carbono aplicado na eletrooxidação de compostos fenólicos, *Orbital Elec. J. Chem.*, **7**, 318–326 (2015).

15. O. Stadnik, *Synthesis, electrochemical and photoelectrochemical properties of the oxide-hydroxide compounds of cobalt*, Diss. Kand. Chim. N., Kyiv, 2011.

16. S. Sadki, P. Schottland, N. Brodie, G. Saboraud, The mechanisms of pyrrole electropolymerization, *Chem. Soc. Rev.*, **29**, 283–293 (2000).

17. F. Ağın, Electrochemical determination of amoxicillin on a poly (acridine orange) modified glassy carbon electrode, *Anal. Lett.*, **49**(9), 1366–1378 (2016).

18. T.D. Martins, M.L. Pacheco, R. Boto, *et al.*, Synthesis, characterization and protein-association of dicyanomethylene squaraine dyes, *Dyes Pigm.*, **147**, 120–129 (2017).

19. S. Sreejith, P. Carol, P. Chithra, A. Ajayaghosh, Squaraine dyes: A mine of molecular materials, *J. Mater. Chem.*, **18**, 264–274 (2008).

20. D.E. Lynch, Pyrrolyl-squaraines – Fifty golden years, *Metals*, **5**(3), 1349–1370 (2015).

21. M.S. Ba-Shammakh, *Electropolymerization of pyrrole on mild steel for corrosion protection*, M.Sc. Thesis, King Fahd University of Petroleum and Minerals, Dharan, Saudi Arabia, 2002.

22. I. Das, N.R. Agrawal, S.A. Ansari, S.K. Gupta, Pattern formation and oscillatory electropolymerization of thiophene, *Ind. J. Chem. A*, **47**(12), 1798–1803 (2008).

23. K. Aoki, I. Mukoyama, J. Chen., Competition between polymerization and dissolution of poly(3-methylthiophene) films, *Russ. J. Electrochem.*, **40**, 280–285 (2004).

24. I. Das, N. Goel, N.R. Agrawal, S.K. Gupta, Electropolymerization of pyrrole: Dendrimers, nano-sized patterns and oscillations in potential in presence of aromatic and aliphatic surfactants, *J. Phys. Chem.*, **114**, 12888–12896 (2010).

25. M. Bazzaoui, E.A. Bazzaoui, L. Martins, J.I. Martins, Electropolymerization of pyrrole on zinc-lead-silver alloys' electrodes in neutral and acid organic media, *Synth. Metals*, **130**, 73–83 (2002).

26. I. Das, N. Goel, N.R. Agrawal, S.K. Gupta, Growth patterns of dendrimers and electric potential oscillations during electropolymerization of pyrrole using mono- and mixed surfactants, J. Electroanal. Chem, *J. Phys. Chem. B*, **114**, 12888–128961 (2010)

27. V.V. Tkach, M.V. Kushnir, S.C. de Oliveira, *et al.*, Theoretical description for anti-COVID-19 drug molnupiravir electrochemical determination over the poly(1,2,4-triazole)-co-squaraine dye composite with Cobalt (III) oxyhydroxide, *Biointerface Res. Appl. Chem.*, **13**, 74 (2023).

28. V.V. Tkach, M.M. Kucher, N. Slyvka, *et al.*, The modeling for anti-Covid-19 drug molnupiravir electrochemical sensing on C_3N_4, *Biointerface Res. Appl. Chem.*, **13,** 446 (2023).

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Diversity of bioactive compounds from *Tropaeolum tuberosum* (mashua)

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Summary

Introduction: *Tropaeolum tuberosum* (mashua), is a member of the *Tropaeolaceae* family, is considered an ecological, millenary crop native in Peru, which began to be consumed in The Andes for having nutritional and medicinal properties. The **objective** of the present literature review is to highlight the importance of mashua as a source of diverse bioactives and to summarize its pharmacological activities. The **method** of the present study is a literature review, where original scientific articles on pharmacological activity and bioactive compounds of mashua were searched in the following electronic databases: PubMed, ScienceDirect and Google Scholar, published during the period 2019 to 2023. **Result**: After the bibliographic search, a total of 22 articles were obtained. It is **concluded** that despite the fact that mashua is attributed several medicinal uses, there are no studies to date that support the efficacy and safety of its use in a pathology. The information presented in this document will be useful to increase the interest and use in the future of the biological compounds of mashua, as well as to give it a new pharmacological or clinical use,

thus, becoming useful for the creation of a new drug formulation in the future, or through the application of the bioactive compounds of mashua in combination with existing drugs to optimize the treatment of pathologies such as cancer, anemia, lithiasis, among others.

Keywords: *Tropaeolum tuberosum*, bioactive compounds, biological activity.

Resumen

Diversidad de compuestos bioactivos de *Tropaeolum tuberosum* (mashua)

Introducción: *Tropaeolum tuberosum* (mashua), es un miembro de la familia Tropaeolaceae, es considerado un cultivo ecológico, milenario, originario del Perú, que comenzó a consumirse en Los Andes por tener propiedades nutricionales y medicinales. El **objetivo** de la presente revisión de la literatura es resaltar la importancia de la mashua como fuente de diversos bioactivos y resumir sus actividades farmacológicas. El **método** del presente estudio es una revisión de la literatura, donde se buscaron artículos científicos originales sobre la actividad farmacológica y compuestos bioactivos de la mashua en las siguientes bases de datos electrónicas: PubMed, ScienceDirect y Google Scholar, publicados durante el periodo 2019 al 2023. **Resultado**: Después de la búsqueda bibliográfica se obtuvo un total de 22 artículos. Se **concluye** que a pesar de que a la mashua se le atribuyen varios usos medicinales, no existen hasta la fecha estudios que avalen la eficacia y seguridad de su uso en alguna patología. La información presentada en este documento será útil para incrementar el interés y uso en el futuro de los compuestos biológicos de mashua, así como para darle un nuevo uso farmacológico o clínico, siendo así de utilidad para la creación de una nueva formulación del fármaco en el futuro, o mediante la aplicación de los compuestos bioactivos de la mashua en combinación con fármacos existentes para optimizar el tratamiento de patologías como cáncer, anemia, litiasis, entre otras.

Palabras clave: Tropaeolum tuberosum, compuestos bioactivos, actividad biológica.

Resumo

Diversidade de compostos bioativos de *Tropaeolum tuberosum* (mashua)

Introdução: *Tropaeolum tuberosum* (mashua), membro da família Tropaeolaceae, é considerada uma cultura ecológica, milenar, nativa do Peru, que começou a ser consumida na Cordilheira dos Andes por possuir propriedades nutricionais e medicinais. **O objetivo** da presente revisão de literatura é destacar a importância do mashua como fonte de diversos bioativos e resumir suas atividades farmacológicas. O método do presente estudo é uma revisão de literatura, onde foram pesquisados artigos científicos originais sobre atividade farmacológica e compostos bioativos do mashua nas seguintes bases de dados eletrônicas: PubMed, ScienceDirect e Google Scholar, publicados durante o período de 2019 a 2023. **Resultado:** Após a pesquisa bibliográfica, obteve-se um total de 22 artigos. **Conclui-se** que apesar de serem atribuídos vários usos medicinais ao mashua, não existem até o momento estudos que sustentem a eficácia e segurança do seu uso em uma patologia. As informações apresentadas neste documento serão úteis para aumentar o interesse e utilização futura dos compostos biológicos do mashua, bem como para dar-lhe um novo uso farmacológico ou clínico, tornando-se assim útil para a criação de uma nova formulação de medicamento no futuro, ou através da aplicação dos compostos bioativos do mashua em combinação com medicamentos existentes para otimizar o tratamento de patologias como câncer, anemia, litíase, entre outras.

Palavras-chave: Tropaeolum tuberosum, compostos bioativos, atividade biológica.

Introduction

Medicinal plants are important resources when it comes to receiving treatment for different pathologies, since they can be used as an alternative or complementary treatment [1]. Conventional treatments are usually good alternatives; however, in the case of certain pathologies or symptomatology, it is recommended to first exhaust some type of non-drug treatment [2]. In addition, pharmacological treatments can lead to dependence, resistance and loss of the sensation of relief when taken for a prolonged period of time [3]. Approximately for the last two decades, there has been a peculiar interest in the use and research of medicinal plants as an alternative to conventional treatments [4, 5]. Despite the strongest criticism of natural therapies such as herbal

medicine is the lack of scientific evidence of efficacy, despite which the use of natural products is increasing in the West, so that herbal medicine has become important worldwide [6, 7].

Mashua (*Tropaeolum tuberosum*) is a tuber considered one of the main food sources worldwide, cultivated in the central zone of the Andes, in countries such as Ecuador, Venezuela, Argentina, Bolivia, Colombia and Peru, where up to 200 morphotypes of this species can be found [8]. This tuber is part of the daily diet of the Andean population and is cooked after being left to rest in the sun in order to obtain a more pleasant flavor [9]. On one side, in Peru, mashua is widely used in the pharmaceutical industry [10]; on the other side, a decrease in the commercialization of mashua has been observed, resulting in low consumer acceptance, especially abroad [11].

An example of an attractive and valuable medicinal plant is mashua (*Tropaeolum tuberosum*), a member of the Tropaeolaceae family. Consequently, researchers have been encouraged to choose plants with medicinal use, to study bioactive compounds and pharmacological activities. The mashua tuber, presents a wide variety of biological properties such as antibacterial, antioxidant, antifungal, anti-inflammatory, etc. In traditional medicine it is used for the treatment of skin diseases, urinary disorders and as diuretic [12, 13]. It has been reported that the mashua tuber has been used until today as a treatment for diseases related to the lungs, kidneys, bladder, skin and even venereal diseases [14]. In fact, the bioactive compounds of mashua have been constantly investigated for their biological activity and chemical compounds, however, the most important data are fragmented and discontinuous. Therefore, the objective of this review is to highlight the importance of mashua as a source of various bioactives and to summarize its pharmacological activities.

Methodology

A literature review was performed by electronic search of original articles in databases such as: PubMed, ScienceDirect and Google Scholar, published during the period 2019 to 2023. The search was performed with the following combined terms: "*Tropaeolum tuberosum*" "medicinal plant", "bioactive compounds" and "biological activities". Studies detailing the chemical composition and biological activity of *Tropaeolum tuberosum* were included, such as: antimicrobial, antioxidant, diuretic, antineoplastic, hypoglycemic, analgesic, and anti-inflammatory, among others.

Results

Table 1 summarizes the main pharmacological indications as well as the chemical components and analytical methods employed.

Table 1. Main chemical components and pharmacological activity

Chemical components under study	Pharmacological activity under study/Instrument
Glucosinolates were identified and quantified in ten mashua cultures.	It was determined whether postharvest cooking and storage techniques affect the stability of glucosinolates and myrosinase activity of Andean mashua tubers. Instrument: UPLC method, UV-visible spectrophotometer, one-way ANOVA and Tukey statistical analysis [11].
Total anthocyanins, total flavonoids, total phenolics, tannin content.	Determination of antioxidant activity. Instrument: AOAC method, Spectrophotometer, ANOVA and Tukey statistical analysis [12].
Horsetail aqueous extract: Flavonoids. Isaño aqueous extract: Tannins, flavonoids and anthocyanins.	Evaluation of diuretic and saliuretic activity. Instrument: Phytochemical screening test, one-way ANOVA statistical analysis and the Bonferroni post-test [13].
Alkamides: → *N*–oleoyldopamine → *N*–(2–Hydroxyethyl)–7Z,10Z,13Z,16Z–docosatetraenamide	Anti-inflammatory activity (Inhibition of TNF-α and NF-kB production in THP-1 monocyte cells). Instrument: TLC technique, column chromatography, FAB ionization technique, cytotoxicity assay, MTT cytotoxicity assay, LDH cytotoxicity assay, TNF-α inhibition assay, NF-kB inhibition assay, ANOVA and Tukey statistical analysis [14] .
Analogues of *N*-benzyl linoleamide, Polyphenols.	Cell viability and anti-inflammatory activity (migraine treatment). Instrument: Nuclear magnetic resonance techniques and mass spectrometry [15].
Glucosinolates, alkamides	Analgesic activity mediated by the transient receptor potential of the vanilloid 1 receptor. Instrument: TLC technique, Spectrophotometry, cytotoxicity assay, MTT viability assay, ANOVA and Tukey statistical analysis [16].

(*Continued*)

Chemical components under study	Pharmacological activity under study/Instrument
2 alkaloid components: → 2-Benzyl-3-thioxohexahydropyrrolo[1,2-c] imidazole-1-one → *N*-(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide	Cytotoxic activity and capacity for apoptosis. Instrument: Column chromatography, spectroscopy method, ANOVA and Tukey statistical analysis [17]
Carbohydrates, phenolic compounds, anthocyanins, carotenoids, starch and minerals.	Potential sources of bioactive compounds. Instrument: Soxhlet method, Tests to determine pH, Gas Chromatography, HPLC technique, ANOVA and Tukey statistical analysis [18].
Crude fiber, Protein, Vitamin C, Phenolic compounds.	Antioxidant capacity. Instrument: Sensory evaluation through a survey using a 5-point hedonic scale [19].
Macamides isolated from yellow mashua tubers.	Determination of anti-inflammatory activity Instrument: NF-kB inhibition assays, viability and cytotoxicity assays, STAT3 inhibition assays [20].
Phenols, flavonoids, phytosterols, triterpenoids, phytosterols and triterpenoids.	Determination of anti-inflammatory activity Instrument: Gas chromatography-mass spectrometry technique. antioxidant capacity Instrument: Titration technique, Spectrometric method, Pearson correlation coefficient test [21-23].
Anthocyanins, glucosinolates and phenolic compounds.	Anti-inflammatory activity. Instrument: Gas chromatography technique, mass spectrometry, ANOVA and Tukey statistical analysis [24, 25].
High content of polyphenols.	Antioxidant activity, ability to induce detoxifying enzymes and antiproliferative activity. Instrument: Folin-Ciocalteu method, antioxidant capacity assay, glutathione S-transferase assay, quinone oxidoreductase assay, UPLC technique coupled to mass spectrometer, statistical analysis by homogeneity of variances, t-test-, F-test [26-28].
Presence of phenols and flavonoids in three varieties of mashua (pink, yellow and black).	The antioxidant activity was evaluated. Instrument: Phytochemical screening, Folin-Ciocalteu method, aluminum trichloride colorimetric method, mass-coupled gas chromatography method, DPPH method, UV-visible spectrophotometer, Duncan statistical analysis, one-way ANOVA [29].

(*Continued*)

Chemical components under study	Pharmacological activity under study/Instrument
Show significant variation in the basic components of food in the form of energy sources (starch and sugars) or functional elements (essential amino acids, vitamins, FOS, glucosinolates and antioxidants).	Functional foods with healthy properties. Instrument: HPLC technique, ANOVA statistical analysis, LSD test [30].
Presence of monomeric anthocyanins, phenolic compounds in mashua.	Antioxidant capacity. Instrument: Folin-Ciocalteu colorimetric method, DPPH method, Statistical analysis and Least Significant Difference (LMD) test [31].
Non-flavonoid compounds, such as hydroxycinnamic derivatives that represent 100, 26 and 15% of the total phenolic compounds, respectively. Presence of flavan-3-ol monomers. The presence of flavonols was found.	The presence of the compounds and the consideration of the total phenolic content suggest the use of these Andean tubers as promising sources of natural antioxidants widely used in the food industry. Instrument: HPLC-DAD-ESI/MS, Tukey statistical analysis [32].

Use in traditional medicine

Tropaelum tuberosum, also popularly known as mashua, is a tuber widely used as a culinary input by gastronomic professionals worldwide. Likewise, it is also used by farmers and consumed by the high Andean population [15] owing the medicinal properties attributed to it, which can be because of its healing, anti-inflammatory, analgesic, and antioxidant properties [16, 29]. In Andean medicine it was used for the relief of kidney problems [17-19]. Moreover, the high Andean population also used it for liver and skin problems [20] and prostatic affections [31]. Regarding the traditional use of mashua compresses in decoction, it was only for local use to heal skin wounds [16-18, 31]. Finally, it was in pre-Columbian times that the indigenous population used mashua as a treatment for pulmonary, digestive and venereal diseases [16, 17].

Bioactive compounds

The mashua is formed by phytoconstituents such as phenolic compounds [24, 25], fatty acids, alkamides, glucosinolates [2, 21], as well as flavonoids, tannins, anthocyanins and isothiocyanates [18, 21, 24]. The anti-inflammatory property of mashua is due to the presence of macamides, specifically *N*-benzyl linoleamide, which are able to treat soroche also known as altitude sickness [20]. Additionally, there are the alkamides N-oleoyldopamine and *N*-(2-hydroxyethyl)-7Z,10Z,13Z,13Z,16Z-docosatetraenamide, which are also associated with anti-inflammatory effect [14]. On the

other hand, depending on the genotype of the mashua varies the content of phenolic compounds such as anthocyanins, as well as tannins, flavonoids, also contains a large amount of fiber, proteins, carbohydrates, among others [22]. In addition, there are chemical substances such as antioxidant compounds and ascorbic acid [23]. As well as glucosinolates, carotenoids [24].

Several authors indicate that mashua shows the presence of triterpenoids, phytosterols and fatty acids [25]. A study conducted in the province of Cotopaxi in Ecuador, confirmed the amount of total phenolic compounds was 1000 mg gallic acid equivalent (GAE)/100 g dry weight, the total carotenoids was 0.14±0.02 mg/100 g dry weight and anthocyanins 14.0±1.1 mg/100 g dry weight [26]. A second study was also conducted in Ecuador, where phenolic compounds were quantified according to the high-performance liquid chromatography with diode array detection and mass spectrometry detection (HPLC-DAD-ESI/MS^n) technique, finding compounds such as Quercetin-3-O-rutinoside (40.6 µg/g dry matter), kaempferol-O-dirhamnosyl-hexoside (29.5 µg/g dry material (DM)) and kaempferol-O-dihexoside (46.22 µg/g DM), mashua is one of the high Andean plant species, which has been shown to exhibit antineoplastic properties, since in one study the isolated alkaloids produced apoptosis of tumor cells under laboratory conditions [27]. There is also a study that found that total phenolic compounds were found in an amount of 18.27±0.83 ìg GAE/mg of extract by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) technique [28, 29].

A study performed in Spain found that Mashua cultivated in Peru had 28.6 ìg/g dry matter of carotenoids, 0.01 mg/g dry matter of anthocyanins and 7.9 mg chlorogenic acid/g dry matter of phenols; while samples from Bolivia had 21.9 ìg/g dry matter of carotenoids, 3.63 mg/g dry matter of anthocyanins and 22.3 mg chlorogenic acid/g dry matter of phenols [30]. Likewise, mashua contain. Another study quantified the glucosinolates (Gls) present in the mashua tuber by means of UPLC coupled to MS, finding amounts of Gls in the range of 4.9-54.2 ìmol/g of dry matter [32].

Pharmacological activities

It has been demonstrated that mashua has biological properties, which can be antibacterial, anti-inflammatory [18], antioxidant, anticancer, including protection of the immune system, due to the presence of isothiocyanate, as well as carotenoids [26], antimicrobial, and even antitumor [14]. In addition, the local population empirically uses this product against skin, pulmonary, venereal and analgesic conditions, among others. In folk medicine, mashua is widely used for its aphrodisiac properties, as well as to alleviate kidney and diuretic problems [18].

It is also used for the treatment of hepatic and metabolic pathologies [22]. It has been reported that the consumption of mashua protects cells from the carcinogenic activity of certain substances, as well as preventing the onset of cardiovascular pathologies [23], in addition to its already known effects against prostate disorders [25].

In the last 5 years, interest in the therapeutic properties of mashua has increased, this is reflected in the variety of in vivo and in vitro scientific studies that have been carried out to confirm its effects, such as its antioxidant and anti-inflammatory activity [22, 25, 28, 31]. As well as publications where they carry out quantification and purification studies of their secondary metabolites, responsible for their pharmacological properties [14, 17, 20, 21]. Many of these studies have raised a molecular basis for these properties, which would justify the consumption of this plant species by the general population. But it has also been shown that it can be consumed in the form of a drink, in which it has been shown that it continues to maintain its most important organic chemical components despite undergoing various manufacturing processes [24]. With this series of evidence, different sectors of the population can be encouraged to consume this tuber, but they must also be educated so that they do not affect the availability of this plant in nature, as well as updated and concise information on its effects on health to avoid inappropriate use. Finally, the different varieties of this species should continue to be investigated because the phytoconstituents may vary depending on the phenotype, as well as their respective therapeutic properties.

Conclusions

This literature review is a brief summary of articles on bioactive compounds and pharmacological activities of mashua, where the therapeutic potential of this medicinal plant is evidenced. The historical aspects of mashua have not fully been addressed because its long history as a traditional medicine is known.

At present, there are no known studies that cover the efficacy of mashua in some ailments. However, there is new research on medicinal properties such as antineoplastic. Due to the diverse spectrum of pharmacological effects that "mashua" presents, studies have been carried out to demonstrate its bioactivity. Each of the constituents have numerous biological properties including anti-inflammatory, analgesic, antioxidant, diuretic and antibacterial.

Several studies have shown that mashua has antimicrobial activity from which a potent antibiotic can be developed, but the biggest problem with mashua is that it is food as such for the culinary and nutraceutical industry, but it is not suitable as an individual component. Research should also be conducted on optimal dosage, efficacy and toxicity

levels. Ultimately, toxicological and clinical trials need to be developed, as mashua is a potent candidate for its main pharmacological activities, which are neoplastic and antibacterial. Likewise, mashua should be used as a complementary treatment, to treat diseases such as cancer, unfortunately there are no studies available that describe its effective use. The present information reported on mashua with respect to its biological activities and chemical compounds will be useful to generate more interest towards mashua and new clinical pharmacological applications can be investigated. Therefore, the present compilation may be useful in formulating new drugs in the future.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

References

1. J. Silva-Alarcón, J. Cabrera-Melendez, O. Trujillo-Villarroel, I. Reyes-Mandujano, Características de las plantas medicinales comercializadas en diferentes mercados de Lima Metropolitana y sus efectos sobre el medio ambiente y la salud pública, *Horizonte Médico*, **19**(4), 63–69 (2019). http://dx.doi.org/10.24265/horizmed.2019.v19n4.09

2. N. Díez-Alejandre, *Probióticos en el tratamiento del climaterio*, College Thesis, Universidad del País Vasco, Vizcaya, Spain, 2023. URL: https://addi.ehu.es/handle/10810/60178

3. L.d.l.C. Benítez-Rojas, R.A. Tamayo-Velazquez, R.A. Ramos-Valverde, Bioethical considerations in the diagnosis and therapeutic treatment of drug dependence, *Revista Electrónica Dr. Zoilo E. Marinello Vidaurreta*, **44**(2), 1–10 (2019). URL: https://revzoilomarinello.sld.cu/index.php/zmv/article/view/1690/pdf_579

4. K. Cuenca-León, E.-M. Pacheco-Quito, Y. Granda-Granda, E. Vélez-León, A. Zarzuelo-Castañeda, Phytotherapy: A solution to decrease antifungal resistance in the dental field, *Biomolecules*, **12**(6), 189 (2022). https://doi.org/10.3390/biom12060789

5. A. Ore, O. Adeboye-Akinloye, Phytotherapy as a multi-hit therapy to address multiple pathophysiology in non-alcoholic fatty liver disease: a systematic review of experimental interventions, *Medicine* (Kaunas), **57**(8), 822 (2021). https://doi.org/10.3390/medicina57080822

6. C. Grigoriu, V. Varlas, G. Călinescu, A. Magdalena, N. Bacalbașa, C. Mădălina, T. Salmen, C. Zugravu, R. Bohîlțea, Phytotherapy in obstetrics: therapeutic indications, limits and dangers, *Journal of Medicine and Life*, **14**(6), 748–755 (2021). https://doi.org/10.25122/jml-2021-0353

7. A. Zimmermann, J. Reinhardt, M. Winker, C. Gründemann, Phytotherapy in integrative oncology: An update on promising treatment options, *Molecules*, **27**(10), 3209 (2022). https://doi.org/10.3390/molecules27103209

8. D.A. Jacobo-Velázquez, G. Peña-Rojas, L.E. Paredes-Avila, V. Andía-Ayme, A.M. Torres-Contreras, O. Herrera-Calderon, Phytochemical characterization of twenty-seven morphotypes of Peruvian mashua (*Tropaeolum tuberosum* Ruíz & Pavón) and the effect of postharvest application of methyl jasmonate on antioxidant accumulation, *Horticulturae*, **8**(6), 471 (2020). https://doi.org/10.3390/horticulturae806047

9. A. Coloma, E. Flores-Mamani, G. Quille-Calizaya, A. Zaira-Churata, J. Apaza-Ticona, W.C. Calsina-Ponce, P. Huata-Panca, J. Inquilla-Mamani, F. Huanca-Rojas, Characterization of nutritional and bioactive compounds in three genotypes of mashua (*Tropaeolum tuberosum* Ruiz y Pavón) from different agroecological zones of Puno, *International Journal of Food Science*, **2022**, ID 75509872022 (2022). https://doi.org/10.1155/2022/7550987

10. G. Peña-Rojas, R. Carhuaz-Condori, V. Andía-Ayme, V.A. Leon, O. Herrera-Calderon, Improved production of micro tubers of mashua (*Tropaeolum tuberosum*) morphotype MAC-3 in liquid medium using temporary immersion system (TIS-RITA®), *Agriculture*, **12**(7), 943 (2022). https://doi.org/10.3390/agricultura12070943

11. P.N. Farro-Carillo, J.J. Flores-Depaz, *Aplicación del marketing estratégico para el diseño y desarrollo de productos a base de Mashua (Tropaeolum tuberosum) – Nuevo Chimbote – 2020*, College Thesis, Universidad César Vallejo, Chimbote, Peru, 2020. URL: https://repositorio.ucv.edu.pe/handle/20.500.12692/67709

12. A. Aguilar-Galvis, R. Pedreschi, S. Carpentier, R. Chirinos, D. García-Ríos, D. Campos, Proteomic analysis of mashua (*Tropaeolum tuberosum*) tubers subjected to postharvest treatments, *Food Chemistry*, **305**, 125485 (2020). https://doi.org/10.1016/j.foodchem.2019.125485

13. L. Apaza-Ticona, Á. Rumbero-Sánchez, Ó. Orozco-Gonzáles, M. Ortega-Doménech, Antimicrobial compounds isolated from *Tropaeolum tuberosum*, *Natural Products Research*, **35**, 4698–4702 (2020). https://doi.org/10.1080/14786419.2019.1710700

14. L. Apaza-T., V. Tena-Pérez, A.M. Serban, M.J. Alonso-Navarro, A. Rumbero, Alkamides from *Tropaeolum tuberosum* inhibit inflammatory response induced by TNF-α and NF-κB, *Journal of Ethnopharmacology*, **235**, 199–205 (2019). https://doi.org/10.1016/j.jep.2019.02.015

15. J.L. Fajardo-Escoffié, Size, color and freshness: Standards and heritage of native potatoes in Peru, *Food and Foodways*, **30**(3), 165–184 (2022). https://doi.org/10.1080/07409710.2022.2089826

16. L. Apaza-Ticona, Á. Rumbero-Sánchez, C. Thiebaut-Estrada, O.M. Palomino, Identification of TRPV1 ion channel agonists from *Tropaeolum tuberosum* in human skin keratinocytes, *Planta Medica*, **87**(5), 383–394 (2021). https://doi.org/10.1055/a-1264-0572

17. L. Apaza-Ticona, J. Arnanz-Sebastián, A.M. Serban, Á. Rumbero-Sánchez, Alkaloids isolated from *Tropaeolum tuberosum* with cytotoxic activity and apoptotic capacity in tumor cell lines, *Phytochemistry*, **177**, 112435 (2021). https://doi.org/10.1016/j.phytochem.2020.112435

18. M. Castro-Rua, E. Gutiérrez-Patzi, G. Pacheco-Felipes, E. Tola-Guarachi, A. Callisaya-Mamani, M. Flores-Quispe, H. Gonzales-Beltrán, A. Villca-Condori, L. Apaza-Chambi, E. Terán-Ventura, Determinación del efecto diurético del isaño (*Tropaeolum tuberosum*) y cola de caballo (*Equisetum arvense*) en ratones, *Revista Científica de Ciencias de la Salud*, **14**(2), 80–90 (2022). https://doi.org/10.17162/rccs.v14i2.1658

19. J. Ticsihua-Huaman, R. Leon-Gomez, L.L. Aguirre-Huayhua, F. Ore-Areche. Aceptabilidad, determinación de las propiedades fisicoquímicas y microbiológicas de una bebida funcional a partir aguaymanto (*Physalis peruviana L.*) y mashua (*Tropaeolum tuberosum Ruíz & Pav.*), *Polo del Conocimiento*, **6**(12), 1014–1028 (2021). URL: https://dialnet.unirioja.es/servlet/articulo?codigo=8219305

20. L. Apaza-Ticona, V. Tena-Pérez, A.M. Serban, J. Sánchez-Corral, Á. Rumbero-Sánchez, Synthesis and pharmacological evaluation of *N*-Benzyl Linoleamide analogues from *Tropaeolum tuberosum* as NF-κB inhibitors and Nrf2 activators, *ChemistrySelect*, **5**(38), 11825–11836 (2020). https://doi.org/10.1002/slct.202003062

21. L. Apaza-Ticona, M. Rodríguez-Coballes, G. Potente, Á. Rumbero-Sánchez, Anti-inflammatory potential of macamides isolated from yellow tubers of Mashua (*Tropaeolum Tuberosum*), *Planta Medica*, *Int Open*, **7**(3), e88-e99 (2020). https://doi.org/10.1055/a-1159-4242

22. M.E. Jiménez-Heinert, Y.I. Gutiérrez-Gaitén, I. Chóez-Guaranda, M. Miranda-Martínez, Pharmacognostic, chemical and anti-inflammatory activity study of two varieties of *Tropaeolum tuberosum* (Ruiz & Pav.) Kuntze (Tropaeolaceae), *International Journal of Pharmacy and Chemistry*, **6**(4), 41–53 (2020). https://doi.org/10.11648/j.ijpc.20200604.12

23. R. Gómez-Mamani, J. Machaca-Yana, J. Arias-Escobar, Efecto del almacenamiento poscosecha sobre compuestos funcionales del *Tropaeolum tuberosum* variedad yana añu, *Revista de Investigaciones de la Universidad Nacional del Altiplano de Puno*, **10**(2), 84–98 (2021). URL: https://dialnet.unirioja.es/servlet/articulo?codigo=8023408

24. O. Feliciano-Muñoz, R. Robles-Calderón, J.H. Chirre-Flores, O. Santisteban-Rojas, J.C. Feliciano-Nishikawa, W.D. Florez-Ponde de Leon, Identificación de los principios activos de la mashua negra (*Tropaeolum tuberosum*) y el efecto del proceso de elaboración de una bebida mix de mashua con piña, *Ingenieria Industrial*, **40**, 171–190 (2020). https://doi.org/10.26439/ing.ind2021.n40.5150

25. M.E. Jiménez-Heinert, M. Miranda-Martínez, I.A. Chóez-Guaranda, Y.I. Gutiérrez-Gaitén, R. Scull-Lizama, J. Abreu-Payrol, Caracterización morfológica, química y actividad antiinflamatoria de *Tropaeolum tuberosum* (Ruiz & Pav.) Kuntze. (*Tropaeolaceae*) var. rosa, *Revista Cubana de Farmacia*, **54**(2), e574 (2021). URL: https://revfarmacia.sld.cu/index.php/far/article/view/574

26. M.T. Pacheco, O. Hernández-Hernández, F.J. Moreno, M. Villamiel, Andean tubers grown in Ecuador: New sources of functional ingredients, *Food Bioscience*, **35**, 100601 (2023). https://doi.org/10.1016/j.fbio.2020.100601

27. M.T. Pacheco, M.T. Escribano-Bailón, F.J. Moreno, M. Villamiel, M. Dueñas, Determination by HPLC-DAD-ESI/MSn of phenolic compounds in Andean tubers grown in Ecuador, *Journal of Food Composition and Analysis*, **84**, 103258 (2019). https://doi.org/10.1016/j.jfca.2019.103258

28. M. Fuel, C. Mesas, R. Martínez, R. Ortiz, F. Quiñonero, J. Prados, J.M. Porres, C. Melguizo, Antioxidant and antiproliferative potential of ethanolic extracts from *Moringa oleifera*, *Tropaeolum tuberosum* and *Annona cherimola* in colorrectal cancer cells, *Biomedicine & Pharmacotherapy*, **143**, 112248 (2021). https://doi.org/10.1016/j.biopha.2021.112248

29. H. Gonzáles-Torre, I. Aliaga-Barrera, F.F. Velasquez-Barreto, Efecto del Clorprofam sobre la brotación y comportamiento de compuestos bioactivos en mashua morada (*Tropaeolum tuberosum* Ruiz & Pavón) durante el almacenamiento poscosecha, *Bioagro*, **32**(1), 49-58 (2020). URL: https://revistas.uclave.org/index.php/bioagro/article/view/2688

30. L.A. Choquechambi, I.R. Callisaya, A. Ramos, H. Bosque, A. Mújica, S.-E. Jacobsen, M. Sørensen, E.O. Leidi, Assessing the nutritional value of root and tuber crops from Bolivia and Peru, *Foods*, **8**(11), 526 (2019). https://doi.org/10.3390/foods8110526

31. M.E. Jiménez-Heinert, Y.I. Gutiérrez-Gaitén, I. Yamilet-Guaranda, M. Miranda-Martínez, Chemical study and determination of the antioxidant activity of three varieties *Tropaeolum tuberosum* (Mashua), *American Journal of Plant Sciences*, **10**(12), 2279–2297 (2019). https://doi.org/10.4236/ajps.2019.1012159

32. D. Campos, A. Aguilar-Galvez, D. García-Ríos, R. Chirinos, E. Limaymanta, R. Pedreschi, Postharvest storage and cooking techniques affect the stability of glucosinolates and myrosinase activity of Andean mashua tubers (*Tropaeolum tuberosum*), *International Journal of Food Science & Technology*, **54**(7), 2387-2395 (2019). https://doi.org/10.1111/ijfs.14150

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Development of a surfactant mediated method for direct monitoring of atracurium in exhaled breath condensate

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Summary

Aim: This study aimed to establish an enhanced fluorometric assay for directly monitoring atracurium in exhaled breath condensate (EBC). The principal of the technique is the rigidity induced medium in the presence of the sodium dodecyl sulfate (SDS) surfactant which eliminates quenching results from collisional and vibrational conversions. This process causes an improvement in the emission intensity of atracurium. **Methods:** Fluorescence recordings were made on a spectrofluorometer at 15 ˚C at 310 nm. The crucial factors for reaching to maximum response

were investigated and optimized. **Results:** The technique was validated and the calibration curve was linear in the range of 0.01-2.0 μg·mL^{-1} of atracurium with a limit of detection of 0.007 μg·mL^{-1}. Finally, the proposed technique was used for the atracurium analysis in EBC of patients receiving the drug. **Conclusion:** The validated technique was found to be accurate and reliable for the quantification of atracurium from the repeatability and reproducibility points of view.

Keywords: Atracurium; Sodium dodecyl sulfate; Fluorescence enhancement, Exhaled breath condensate

Resumen

Desarrollo de un método mediado por surfactante para la monitorización directa de atracurio en el condensado del aliento exhalado

Objetivo: Este estudio tuvo como objetivo establecer un ensayo fluorométrico mejorado para monitorear directamente el atracurio en el condensado del aliento exhalado (EBC). El principio de la técnica es el medio inducido por rigidez en presencia del tensioactivo dodecilsulfato de sodio (SDS), que elimina los resultados de enfriamiento de las conversiones por colisión y vibración. Este proceso provoca una mejora en la intensidad de emisión del atracurio. **Métodos:** Los registros de fluorescencia se realizaron en un espectrofluorómetro a 15 °C a 310 nm. Se investigaron y optimizaron los factores cruciales para alcanzar la máxima respuesta. **Resultados:** La técnica fue validada y la curva de calibración fue lineal en el rango de 0,01-2,0 μg·mL^{-1} de atracurio con un límite de detección de 0,007 μg·mL^{-1}. Finalmente, la técnica propuesta se utilizó para el análisis de atracurio en EBC de pacientes que recibieron el fármaco. **Conclusión:** La técnica validada resultó ser precisa y confiable para la cuantificación de atracurio desde los puntos de vista de repetibilidad y reproducibilidad.

Palabras clave: Atracurio; Dodecil sulfato de sodio; Mejora de la fluorescencia, condensado del aliento exhalado

Resumo

Desenvolvimento de um método mediado por surfactante para monitoramento direto de atracúrio no condensado do ar exalado

Objetivo: Este estudo teve como objetivo estabelecer um ensaio fluorométrico aprimorado para monitorar diretamente o atracúrio no condensado do ar exalado (EBC). O principal da técnica é o meio induzido por rigidez na presença do surfactante dodecil sulfato de sódio (SDS) que elimina os resultados de têmpera de conversões colisionais e vibracionais. Este processo provoca uma melhoria na intensidade de emissão de atracúrio. **Métodos:** Os registros de fluorescência foram feitos em espectrofluorômetro a 15 °C a 310 nm. Os fatores cruciais para atingir a resposta máxima foram investigados e otimizados. **Resultados:** A técnica foi validada e a curva de calibração foi linear na faixa de 0,01-2,0 $\mu g \cdot mL^{-1}$ de atracúrio com limite de detecção de 0,007 $\mu g \cdot mL^{-1.}$ Por fim, a técnica proposta foi utilizada para análise de atracúrio em EBC de pacientes que receberam o medicamento. **Conclusão:** A técnica validada mostrou-se precisa e confiável para a quantificação de atracúrio do ponto de vista da repetibilidade e reprodutibilidade.

Palavras-chave: Atracúrio; Dodecilsulfato de sódio; Aumento de fluorescência, condensação da respiração exalada

Introduction

Atracurium besylate, 2,2'- [1,5-pentanediylbis [oxy(3-oxo-3,1-propanediyl)]] bis[1-[(3,4-dimethoxyphenyl) methyl] -1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisoquinolinium] dibenzenesulfonate, is a highly selective and widely used medication in surgical operations. Generally, its action mechanism is competition with acetylcholine receptors of the neuromuscular junction to block the neurons. First of all, the muscles responsible for face fine rapid movements, then muscles of the limbs and torso and finally diaphragm affect by this drug [1]. Disparate surgical anesthesia, the atracurium's pharmacodynamic/pharmacokinetic profile is poorly documented in care unit patients. Its plasma concentration is about 10 $\mu g \cdot mL^{-1}$ with half-life of 2-3.4 min (distribution) and 20 min (terminal). The protein binding of this non-depolarizing skeletal muscle relaxant is 82% and its volume of distribution is approximately 160 mL/kg (range: 120-188 mL/kg). It is metabolized to laudanoside using non-enzymatic cleavage method independent of hepatic/renal function and also ester hydrolysis by nonspecific esterases and Hofmann elimination (retro-Michael addition) [2]. The only dosage form of

this drug is 10 mg·mL^{-1} injectable solution. Overdosage with this drug may escalate the risk of cardiovascular effects and histamine release, especially hypotension. Therefore, the quantification of atracurium is one of the noticeable subjects in clinical and analytical chemistry. Knowing the exact drug amount in the patient's body help the physician to adjust the drug dose more accurately.

Some analytical techniques are revealed in the literature for the determination of atracurium in clinical samples including spectrophotometry [3], high-performance liquid chromatographic (HPLC) with detection system of fluorimetry [4, 5], mass spectroscopy [6, 7], and electrochemiluminescence [8]. Despite the acceptable selectivity reported for the mentioned methods, most of them are time-consuming and need complex apparatus, also have some disadvantages such as difficult running procedures and great and expensive material consumption which is not appropriate for routine clinical applications. It is essential to valid a fast and reliable technique for the analysis of atracurium concentration in biological samples [9]. Usually plasma, blood, or serum are the common biological fluids used for determining drug concentrations. However, exhaled breath condensate (EBC) is an alternative matrix that is accessible, repeatable, easy to collect, and any considerable threat to the patient [10]. For patients under surgical operation and mechanical ventilation, there is an easy way to collect the EBC sampler via expiratory circuit of the ventilator. The literature data demonstrate that many drugs and their metabolites can be exhaled [11] and possibly can be used as diagnostic biomarkers for various diseases [12, 13]. In comparison with more complicated matrices like urine, blood, and other biological samples, EBC samples decrease the number of interfering materials due to its diluted matrix. In the current work, we have tried to improve the inherent atracurium fluorescence employing rigidity-induced substrates and to estimate their performance for direct atracurium analysis in EBC samples. This technique has some benefits over present technique with respect to the facility, analysis range, speed, sensitivity, and less difficulties of operation that has no sample preparation techniques such as separation, extraction, or preconcentration.

Methods And Materials

Reagents and Solutions

The ultrapure deionized water was provided from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran). Polyvinylpyrrolidone (PVP, Daana Pharmaceutical Co., Tabriz, Iran), sodium dodecyl sulfate (SDS, Carlo Erba, Milan, Italy), cetyltrimethylammonium bromide (CTAB, Merck, Darmstadt, Germany), β-cyclodextrin (β-CD, Sigma, St Louis MO, USA), and atracurium besylate (Iran Hormone Pharmaceutical Co., Tehran,

Iran) were employed in the curent study. Standard stock (1000 μg·mL^{-1}) solution of atracurium was used and diluted work solutions were prepared with ultrapure deionized water before each experiment.

Apparatus and Instruments

The UV–Vis absorption were determined on a double-beam UV–Vis spectrophotometer model UV-1800 (Shimadzu, Japan) with 1.0 cm quartz cells. Fluorescence spectra were recorded on a FP-750 spectrofluorometer (JASCO Corp., Japan) at 15 °C with a 10 nm band-pass in emission and excitation paths and the "medium" sensitivity. The pH adjustments were done by employing a digital pH-meter model 744 (Metrohm Ltd., Switzerland).

EBC Collection

EBC samples for development and validation of the method were gathered by a homemade cooling trap method [14]. EBC (drug free) samples utilized for the technique optimization and validation were collected from healthy sample donors. Real EBC samples have gotten from the expiratory circuit of the mechanical ventilator [15] of the patients under ventilation after intravenous administration of atracurium for their routine surgical operation without any more intervention. Sample donors signed an agreement form confirmed by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.903).

General Procedure

An appropriate amount of sample or standard solution containing atracurium in the range of 0.01–2.0 μg·mL^{-1} was inserted into a 2 mL vial containing 200 μL of EBC sample, then 10 μL of phosphate buffer with pH: 9 alongside 36 μL of SDS solution (1.0 mmol·L^{-1}) was added to the mixture. The solution was reached to 400 μL volume and mixed well. Then, the fluorescence intensity was documented at 315 nm with excitation at 280 nm.

Results and discussion

Enhancement of Fluorescence Response

The fluorescence spectra and UV–Vis absorption spectra of the atracurium (1.0 μg·mL^{-1}) in the presence and absence of SDS were shown in Fig. 1. From Figs. 1A and 1B, it can be realized that atracurium has an emission peak about 310 nm with an excitation peak of 280 nm in an aqueous solution. There was not any shift in the absorption or emission peak of atracurium by adding SDS. But their intensity has significantly increased.

In the SDS present, the inherent fluorescence intensity of atracurium is enhanced in concentrations higher than a critical micelle concentration value as a rigidity-induced substance which can form a micelle system. It is a fact that the non-radiative transition pathways cause usually dramatic decrease and compete or completely quench the radiation pathway. The quantum yield of the fluorophore is reduced in the presence of such competition [16, 17] which is a crucial subject, particularly in the fluorophore low concentration and decrease the sensitivity of analyses. Beside, other reason reported for the atracurium fluorescence improvement is removal of water molecules surrounding the atracurium and the reduction of the rotational freedom into the SDS micelle that provides a protective medium for atracurium in the excited state. The results in the presence of SDS and other rigidity-induced substance were investigated for benzene [18], indole [19], 8-hydroxyquinoline [20], dapoxy sodium sulphonate [21], warfarin [22], graphitic carbon nitride quantum dots [23] and verapamil [24].

Signal intensity in the analyses is considered a challenge and the methods which amplified response signals have intensely garnered interest. We recognized that emission intensity of atracurium is enhanced in the SDS present which obtains an improvement in the limit of detection of atracurium ~ 2.2 orders of magnitude. In this research, we employed SDS to the amplify inherent emission of atracurium without employing any preparation or pre-concentration technique which decrease the detection limit (LOD) values for direct monitoring of atracurium from 0.028 $\mu g \cdot mL^{-1}$ in the absence of SDS to 0.0077 $\mu g \cdot mL^{-1}$ in the SDS present, respectively. Therefore, the concentration of atracurium could be determined in the EBC of patients by the current technique.

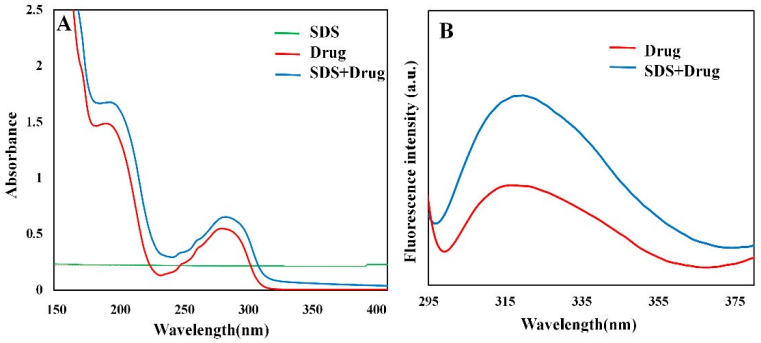

Fig. 1. Absorption (A) and emission (B) spectra of atracurium (1.0 $\mu g \cdot mL^{-1}$), SDS, and SDS+atracurium.

Optimization

To achieve the maximum response, the impact of factors that affect the response such as pH, the type of rigidity-induced substance, concentration of the reagents, incubation time, and temperature were investigated by the one-at-a-time trend. A 1.0 $\mu g \cdot mL^{-1}$ solution of atracurium was used for all records, and each test was replicated three times.

Type of Rigidity-Induced Material

In the current work, several rigidity-induced reagents such as β-CD, SDS, PVP, and CTAB were tested as the protecting materials. As shown in Fig. 2A, SDS shows a good sensitivity in compared to other reagents. It is better to note that, while employing the studied reagents, there was no any change in the wavelength of 310 nm. We studied all reagents in various concentrations including the concentrations higher and lower than the critical micelle concentration for each surfactant. According to the findings and data, SDS with a concentration of 0.09 $mmol \cdot L^{-1}$ was chosen as a suitable rigidity-induced reagent for the next actions.

Effect of pH

The pH impact was examined over a pHs of 3–11, by utilizing sodium hydroxide and hydrochloric acid solutions for pH setting. As seen in Fig. 2B, a maximum response ($\Delta F = F - F_0$, in which F and F_0 are the fluorescence intensity in the presence and absence of SDS) for the atracurium was achieved at pH = 9.0. The pK_a of atracurium with two amino groups is 19.02; hence at pH = 9.0 atracurium is entirely ionized and supplies the positively charged nitrogen required for strong interaction with negatively charged surfactant like SDS.

Effect of Temperature and Incubation Time

The effect of temperature was also examined at 10 to 37 °C. As critical micelle concentration of surfactants can change with the environmental mediums, the temperature impact was also investigated in an SDS concentration of 0.09 $mmol \cdot L^{-1}$. As can be obtained from Fig. 2C, as the temperature increase, the fluorescence response get decrease. So, 15 °C was suggested as a selected temperature. Moreover, the influence of the experiment time on the response was examined. There is no significant difference over time.

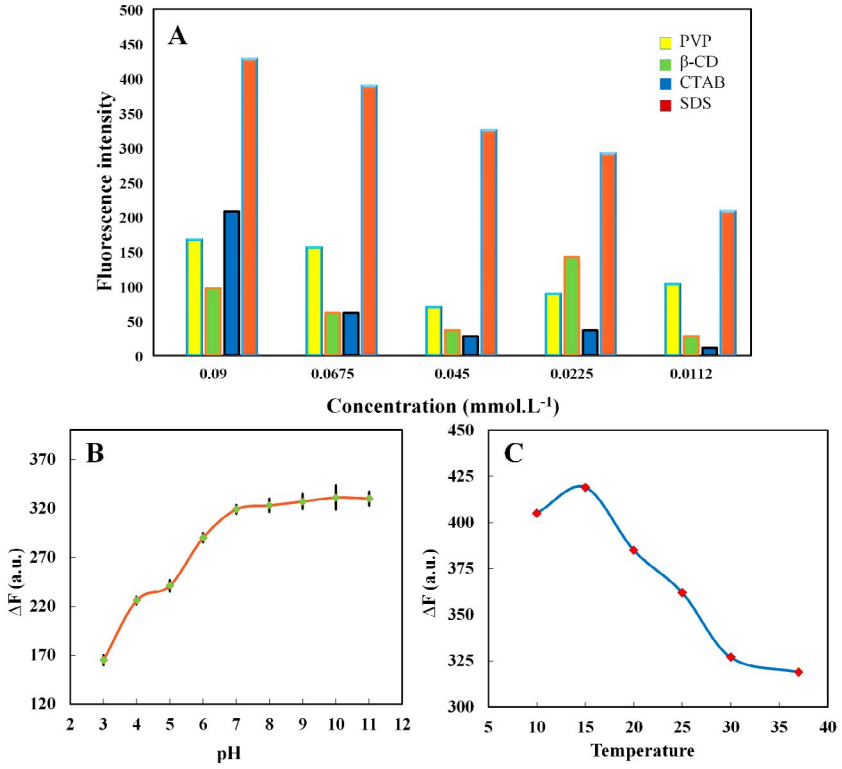

Fig. 2. Effect of the type of rigidity-induced reagent and their concentrations (A), pH (B), and temperature (C) on the fluorescence intensity of atracurium. Condition: 1.0 μg·mL^{-1} of atracurium.

Study of Interferences

With the analytical process set up at optimized condition that mentioned above, the probable interference between atracurium and some other co-administered pharmaceuticals, *i.e.*, acetylsalicylic acid (ASA), losartan, caffeine, nicotinic acid, ibuprofen, ascorbic acid, and diazepam were investigated. Herein, 2.0 μg·mL^{-1} of these species was mixed with the EBC sample spiked with a standard solution of 2.0 μg·mL^{-1} atracurium. The results are shown in Fig. 3. It can be found that ascorbic acid, ibuprofen, nicotinic acid, and losartan had almost no interference in the determination of atracurium. However, among the commonly used drugs investigated for selectivity studies, ASA, diazepam, and caffeine represent significantly decreasing effects demonstrating interference with atracurium determination. It is suggested that this method could be performed for atracurium quantification in the EBC of the patients not receiving ASA, diazepam, and caffeine or an extraction method could be suggested before direct determination.

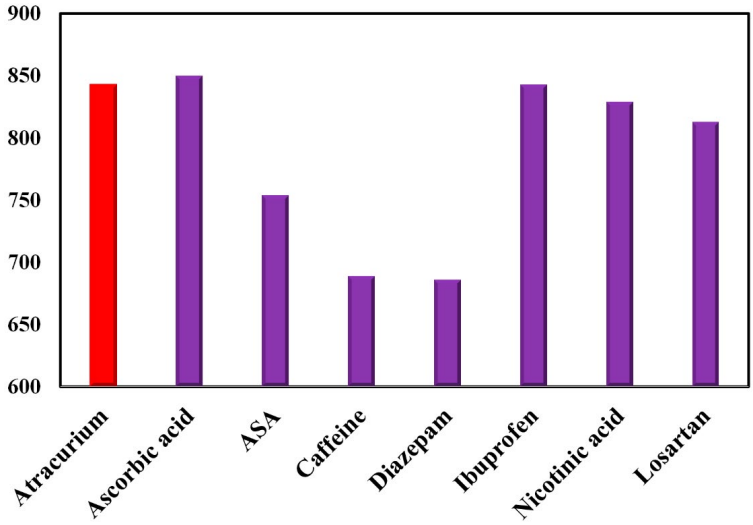

Fig. 3. Selectivity of the probe toward atracurium in the presence of some over-the-counter or some co-administrated drugs in the concentration of 2.0 μg·mL^{-1}

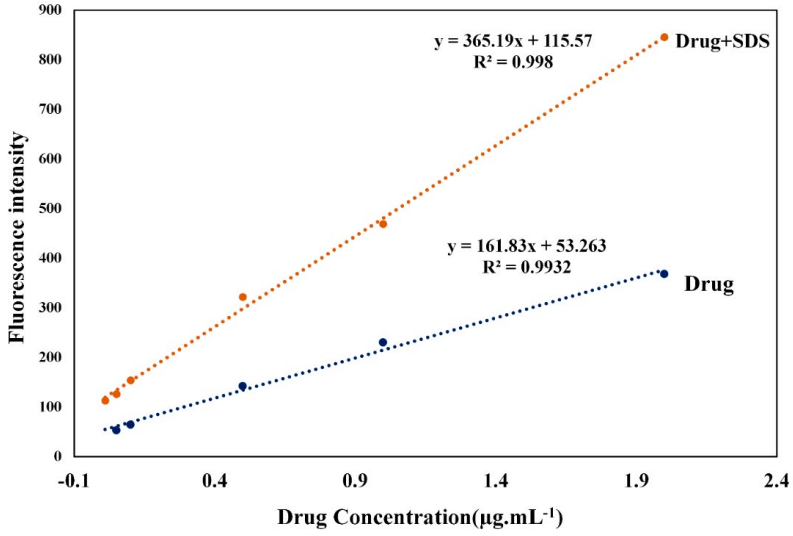

Fig. 4. Calibration curves of atracurium with different concentrations in the absence and presence of SDS

Analytical Figures of Merit

The equation for the regression line of atracurium without SDS adding was $F = 161.83\ C + 53.263$ ($R^2 = 0.9932$), where F is the fluorescence intensity in arbitrary unit, and C is the concentration of atracurium in $\mu g \cdot mL^{-1}$. The calibration chart in direct monitoring of atracurium in the SDS absent was linear at 0.05-2.0 $\mu g \cdot mL^{-1}$ with a LOD and LOQ of 0.028 $\mu g \cdot mL^{-1}$ and 0.093 $\mu g \cdot mL^{-1}$. Whereas, the calibration graph in the SDS present as an enhancer was linear from 0.01 - 2.0 $\mu g \cdot mL^{-1}$ atracurium with the regression equation of $F = 365.19\ C + 115.57$ ($R^2 = 0.998$) and LOD and LOQ of 0.007 $\mu g \cdot mL^{-1}$ and 0.023 $\mu g \cdot mL^{-1}$. With comparing the slope of regression line (Fig. 4), it can find that an enhancement about 2.2 was observed for atracurium florescence intensity in the presence of SDS. This enhancement leads to reach a low LOD for measurements.

Precision

To investigate the method precision, the standard solution having concentration 0.5, 1.0, 2.0 $\mu g \cdot mL^{-1}$ of atracurium were examined during the experiment course on different days and on the same day. For both intra-day and inter-day variations, atracurium solutions were assessed three times and the results are shown in Table 1.

Table 1. Inter-day and intra-day relative standard deviations (%*RSD*) for replicated determinations for different levels of ethanol for redox reaction with dichromate in EBC.

[atracurium] $\mu g \cdot mL^{-1}$	%*RSD*	
	Intra-day	**Inter-day**
0.5	1.87	4.15
1.0	1.28	1.41
2.0	2.53	3.77

Stability

Stability investigations are examined by three concentrations of atracurium. EBC samples containing atracurium are frozen in -20 °C and thawed three cycles with interval of 24 hours (24, 48, 72 hours) and then determined. The variations from initial fluorescence intensity value are summarized in Table 2. According to the results, samples are stable for 2 cycles of freeze and thaw and more than 2 cycles, a relatively high deviation is observed in the results.

Table 2. Stability study for different levels of atracurium.

[atracurium] μg·mL^{-1}	Freeze-thaw stability (%RE)		
	After 24 h	After 48 h	After 72 h
0.5	3.47	7.63	15.03
1.0	6.21	9.48	17.37
2.0	5.16	10.04	20.90

$\%RE = [((I_{Measured}) - (I_{Expected})) / (I_{Expected})] \times 100$.

Robustness

To test the potential variability in the experiment conditions when a technique is done by another analyst or transferred from one laboratory to another, the method robustness was examined and finding are reported in Table 3. As can be achieved, the finding of this examination demonstrate no significant variation on the analytical results representing the method robustness.

Table 3. Robustness study for different levels of atracurium.

Condition	RE% for three level of atracurium (μg·mL^{-1})		
	0.5	1.0	2.0
One laboratory to another	9.23	8.10	2.98
One researcher to another	10.91	9.21	4.97

$\%RE = [((I_{Measured}) - (I_{Expected})) / (I_{Expected})] \times 100$.

Real samples analysis

The method applicability was verified by atracurium analysis in six EBC samples gotten from the patients receiving atracurium. The found concentration varied from 0.10 to 0.53 μg·mL^{-1}. To investigate the model accuracy, the standard addition was performed and recoveries of the spiked concentrations (in two concentration level 0.5 and 1.0 μg·mL^{-1}) were obtained (Table 4). It is better to note that this method is a direct method without any sample preparation technique which can be installed on the mechanical ventilators as a point of care setup. However, as has be reported in the interferences studies, some interferents have negative or positive effect on the analytical response and should be considered in the monitoring procedure. The high variations in the recovery values of sample 2 and 3 could be assigned into such interferents.

Table 4. Determination of atracurium in patient EBC samples.

No.	Added ($\mu g \cdot mL^{-1}$)	Found ($\mu g \cdot mL^{-1}$)	Recovery (%)[a]
1	- 0.5 1.0	0.25 0.54 1.10	- 108.0 110.0
2	- 0.5 1.0	0.53 0.91 1.41	- 76.0 88.0
3	- 0.5 1.0	0.36 0.79 1.46	- 86.0 110.0
4	- 0.5 1.0	0.10 0.61 1.15	- 102.0 105.0
5	- 0.5 1.0	0.10 0.57 1.14	- 94.0 104.0
6	- 0.5 1.0	<LOD 0.48 1.16	- 96.0 116.0

[a] Recovery (%) = [atracurium concentration in samples (after spiking – before spiking)/Added] × 100.

Conclusions

In the current study, a reliable fluorescence-enhancement method was confirmed for the direct monitoring of atracurium in EBC of the patients receiving atracurium. The benefits of the technique are developing a simple fluorometric method including the least time needed for sample analysis without any pre-concentration or sample preparation which makes it a valuable technique for routine clinical utilizations. The procedure is based on an improvement in the emission of atracurium in the SDS present as an enhancer. We ascribe the detected fluorescence improvement of atracurium to the viscosity increasing of the microenvironment into the SDS micelle which limits molecular movements of atracurium and results in a fluorescence improvement. This technique is fast, reproducible, sensitive, simple, and easy to use in routine utilizations. The proposed method could be further developed online monitoring system installed on the mechanical ventilators for providing real time analysis of atracurium.

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Conflict of interests

M. Khoubnasabjafari, V. Jouyban-Gharamaleki and A. Jouyban patented the EBC collection setup in the Iranian Patent Office. The other authors claim that there is no conflict of interest.

References

1. T.A. Torda, The 'new'relaxants: A review of the clinical pharmacology of atracurium and vecuronium, *Anaesth. Intensive Care Med.*, **15**, 72-82 (1987).

2. V. Fodale, L.B. Santamaria, Laudanosine, an atracurium and cisatracurium metabolite, *Eur. J. Anaesthesiol.*, **19**(7), 466-473 (2002).

3. P. Lakshmi, C. Rambabu, Simple and selective spectrophotometric determination of atracurium besylate in pharmaceutical formulations using acidic dyes, *Anal. Chem.*, *An Indian J.*, **15**, 466-473 (2015).

4. C. Farenc, M. Audran, J.Y. Lefrant, I. Mazerm, F. Bressolle, High-performance liquid chromatographic method for the determination of atracurium and laudanosine in human plasma. Application to pharmacokinetics, *J. Chromatogr. B*, *Biomed. Sci. Appl.*, **724**, 117–126 (1999).

5. M.M. Ayad, F. Belal, M.M. Hosney, H. Elmansi, N. Elsayed, Simultaneous HPLC determination of cisatracurium and propofol in human plasma via fluorometric detection, *J. Chromatogr. Sci.*, **56**, 524–530 (2018).

6. A. Błazewicz, Z. Fijałek, M. Warowna-Grześkiewicz, M. Jadach, Determination of atracurium, cisatracurium and mivacurium with their impurities in pharmaceutical preparations by liquid chromatography with charged aerosol detection, *J. Chromatogr. A*, **1217**, 1266–1272 (2010).

7. S.D .Ferrara, A. Nalesso, F. Castagna, M. Montisci, S. Vogliardi, D. Favretto, Simultaneous determination of atracurium and its metabolite laudanosine in

post-mortem fluids by liquid chromatography/multiple-stage mass spectrometry on an ion trap, *Rapid Commun. Mass Spectrom.*, **21**, 2944–2950 (2007).

8. M. Zuo, J. Gao, X. Zhang, Y. Cui, Z. Fan, M. Ding, Capillary electrophoresis with electrochemiluminescence detection for the simultaneous determination of cisatracurium besylate and its degradation products in pharmaceutical preparations, *J. Sep. Sci.*, **38**, 2332–2339 (2015).

9. M.E. El Sharkasy, M. Walash, F. Belal, MM. Salim, Conventional and first derivative synchronous spectrofluorimetric methods for the simultaneous determination of cisatracurium and nalbuphine in biological fluids, *Spectrochim. Acta A, Mol. Biomol. Spectrosc.*, **228**, 117841 (2020).

10. M. Khoubnasabjafari, E. Rahimpour, A. Jouyban, Exhaled breath condensate as an alternative sample for drug monitoring, *Bioanalysis*, **10**, 61–64 (2018).

11. N. Hashemzadeh, E. Rahimpour, A. Jouyban, Applications of exhaled breath condensate analysis for drug monitoring and bioequivalence study of inhaled drugs, *J. Pharm. Pharm. Sci.*, **25**, 391-401 (2022).

12. C. Berchtold, M. Bosilkovska, Y. Daali, B. Walder, R. Zenobi, Real-time monitoring of exhaled drugs by mass spectrometry, *Mass Spectrom. Rev.,* **33**, 394–413 (2014).

13. F. Di Francesco, R. Fuoco, M. Trivella, A. Ceccarini, Breath analysis: trends in techniques and clinical applications, *Microchem. J.*, **79**, 405–410 (2005).

14. A. Jouyban, M. Khoubnasabjafari, K. Ansarin, V. JouybanGharamaleki, *Breath sampling setup*, Iranian patent. 81363, 2013.

15. M. Khoubnasabjafari, H. Pourfathi, A. Mahmoodpoor, A. Jouyban, Waste of mechanical ventilators as a biological sample for follow up of biomarkers and drugs, *Pharm. Sci.*, **26**, 343 (2020).

16. Chapter 2: Instrumentation for Fluorescence Spectroscopy, in: J.R. Lakowicz (editor), *Principles of Fluorescence Spectroscopy*, Springer, Boston, MA, 2006. p. 27–61. https://doi.org/10.1007/978-0-387-46312-4_2

17. Y.C. Tsang, R. Pop, P. Gordon, J. Hems, M. Spino, High variability in drug pharmacokinetics complicates determination of bioequivalence: Experience with verapamil, *Pharm. Res.,* **13**, 846–850 (1996).

18. M. Hoshino, M. Imamura, K. Ikehara, Y. Hama, Fluorescence enhancement of benzene derivatives by forming inclusion complexes with .beta.-cyclodextrin in aqueous solutions, *J. Phys. Chem.*, **85**, 1820–1823 (1981).

19. A. Orstan, J.B.A. Ross, Investigation of the .beta.-cyclodextrin-indole inclusion complex by absorption and fluorescence spectroscopies, *J. Phys. Chem.*, **91**, 2739–2745 (1987).

20. H. Abdolmohammad-Zadeh, E. Rahimpour, $CoFe_2O_4$ nano-particles functionalized with 8-hydroxyquinoline for dispersive solid-phase micro-extraction and direct fluorometric monitoring of aluminum in human serum and water samples, *Anal. Chim. Acta,* **881**, 54–64 (2015).

21. K. Pal, S. Mallick, A.L. Koner, Complexation induced fluorescence and acid–base properties of dapoxyl dye with γ-cyclodextrin: A drug-binding application using displacement assays, *Phys. Chem. Chem. Phys.*, **17**, 16015–16022 (2015).

22. J.M. Vasquez, A. Vu, J.S. Schultz, V.I. Vullev, Fluorescence enhancement of warfarin induced by interaction with beta-cyclodextrin, *Biotechnol. Prog.*, **25**, 906–914 (2009).

23. H. Abdolmohammad-Zadeh, E. Rahimpour, A novel chemosensor based on graphitic carbon nitride quantum dots and potassium ferricyanide chemiluminescence system for Hg(II) ion detection, *Sens. Actuators B Chem.*, **225**, 258–266 (2016).

24. F. Pourkarim, E. Rahimpour, M. Khoubnasabjafari, V. Jouyban-Gharamaleki, A. Jouyban, Direct monitoring of verapamil level in exhaled breath condensate samples, *Pharm. Sci.*, **25**, 50–56 (2019).

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Chemical composition and *in vitro* antibacterial activity of three lichen species (Usnea sp., *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula*) collected from the Jauja and Huaral provinces-Peru

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Summary

Introduction: Lichens are complex organisms conformed by a symbiotic association between algae and fungi; they are haven considerable interest owing to their pharmacological properties. **Objectives:** To determine chemical composition of the fatty acid (FA) and the percentage of usnic acid (UA), and to evaluate the *in vitro* antibacterial activity of the ethanolic extract obtained from the lichens *Usnea* sp.*, Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula*. **Materials and Methods:** Organic extracts from the three lichens were obtained using a rotary evaporator, soxhlet extractor and ultrasound equipment. The chemical composition of FA was determined by gas chromatography-flame ionisation detector, and the percentage of UA was quantified by UV-Vis spectroscopy. The *in vitro* antibacterial activity of the lichen ethanolic extracts was evaluated against *E. coli* ATCC 25922,

Bacillus subtilis ATCC 11774*, Staphylococcus aureus* ATCC 43300 and *S. aureus* ATCC 29213 using the diffusion and microdilution methods. The minimum inhibitory concentration (MIC) was determined. Results: In the three tested lichens, the identified FAs were palmitic, stearic, oleic, linoleic, and linolenic acids; and the obtained percentage of UA was between 0.2 and 1.07 %. All the ethanolic extracts obtained from three lichens showed a high activity against *B. subtilis* (MIC = 0.5 mg/mL), while the ethanolic extracts obtained from *T. vermicularis* subsp. *solida* and *R. asperula* showed moderate activity against *S. aureus* ATCC 43300 (MIC = 3 and 1 mg/mL) and *S. aureus* ATCC29213 (MIC = 4 and 1 mg/mL). **Conclusions:** Our findings found for the three studied lichens confirmed the presence of some FAs and AU. These species provide an acceptable nutritional content and besides they can be considered as potential antibacterial agents.

Keywords: lichens, fatty acids, antimicrobial activity

Resumen

Composición química y actividad antibacteriana *in vitro* de tres especies de líquenes (*Usnea* sp., *Thamnolia vermicularis* subsp. solida and *Ramalina Asperula*) recolectadas en las provincias de Jauja y Huaral-Perú

Introducción: Los líquenes son organismos complejos conformados por una asociación simbiótica entre algas y hongos; tienen un interés considerable debido a sus propiedades farmacológicas. **Objetivos:** Determinar la composición química de los ácidos grasos (AG) y el porcentaje de ácido úsnico (AU), y evaluar la actividad antibacteriana *in vitro* del extracto etanólico obtenido a partir de los líquenes *Usnea sp*., *Thamnolia vermicularis* subsp. *solida* y *Ramalina asperula*. **Materiales y Métodos:** Los extractos orgánicos de los tres líquenes fueron obtenidos usando un rotavapor, extractor soxhlet y un equipo de ultrasonido. La composición química de los AG fue determinada mediante cromatografía de gases con detector de ionización de llama y el porcentaje de AU fue cuantificado mediante espectroscopia UV-Vis. La actividad antibacteriana *in vitro* de los extractos etanólicos de líquenes fue evaluada frente a las especies *E. coli* ATCC 25922, *Bacillus subtilis* ATCC 11774, *Staphylococcus aureus* ATCC 43300 y *S. aureus* ATCC 29213 mediante los métodos de difusión y microdilución. Se determinó la concentración inhibitoria mínima (MIC). **Resultados:** En los tres líquenes ensayados, los ácidos grasos identificados

fueron los ácidos palmítico, esteárico, oleico, linoleico y linolénico; y el porcentaje de AU obtenido estuvo entre 0,2 y 1,07 %. Todos los extractos etanólicos obtenidos mostraron una alta actividad frente a la especie *B. subtilis* (MIC = 0,5 mg/mL), mientras que los extractos etanólicos obtenidos a partir de *T. vermicularis* subsp. *solida* y *R. asperula* mostraron actividad moderada frente a la especie *S. aureus* ATCC 43300 (MIC = 3 y 1 mg/mL) y *S. aureus* ATCC29213 (MIC = 4 y 1 mg/mL). Conclusiones: Nuestros hallazgos encontrados para los tres líquenes estudiados confirmaron la presencia de algunos AG y AU. Estas especies aportan un aceptable contenido nutricional y además, pueden ser consideradas como potenciales agentes antibacterianos.

Palabras clave: líquenes, ácidos grasos, actividad antibacteriana.

Resumo

Composição química e atividade antibacteriana in vitro de três espécies de líquenes (*Usnea* sp., *Thamnolia vermicularis* subsp. *solida* e *Ramalina Asperula*) coletadas nas províncias de Jauja e Huaral-Peru

Introdução: Os líquenes são organismos complexos constituídos por uma associação simbiótica entre algas e fungos; eles são de considerável interesse devido às suas propriedades farmacológicas. **Objetivos:** Determinar a composição química dos ácidos graxos (AG) e a porcentagem de ácido úsnico (AU), e avaliar a atividade antibacteriana *in vitro* do extrato etanólico obtido dos líquenes Usnea sp., *Thamnolia vermicularis* subsp. *solida* e *Ramalina aspérula*. **Materiais e Métodos:** Os extratos orgânicos dos três líquenes foram obtidos utilizando evaporador rotativo, extrator soxhlet e equipamento de ultrassom. A composição química do FA foi determinada por cromatografia gasosa com detector de ionização de chama e a porcentagem de UA foi quantificada por espectroscopia UV-Vis. A atividade antibacteriana *in vitro* de extratos etanólicos de líquen foi avaliada contra as espécies *E. coli* ATCC 25922, *Bacillus subtilis* ATCC 11774, *Staphylococcus aureus* ATCC 43300 e *S. aureus* ATCC 29213 utilizando métodos de difusão e microdiluição. A concentração inibitória mínima (CIM) foi determinada. **Resultados:** Nos três líquenes testados, os ácidos graxos identificados foram os ácidos palmítico, esteárico, oleico, linoléico e linolênico; e o percentual de UA obtido ficou entre 0,2 e 1,07%. Todos os extratos etanólicos obtidos apresentaram alta atividade contra a espécie *B. subtilis*

(CIM = 0,5 mg/mL), enquanto os extratos etanólicos obtidos de *T. vermicularis* subsp. *solida* e R. asperula apresentaram atividade moderada contra as espécies *S. aureus* ATCC 43300 (CIM = 3 e 1 mg/mL) e *S. aureus* ATCC29213 (CIM = 4 e 1 mg/mL). **Conclusões:** Nossos achados para os três líquenes estudados confirmaram a presença de alguns AG e AU. Estas espécies fornecem um conteúdo nutricional aceitável e também podem ser consideradas como potenciais agentes antibacterianos.

Palavras-chave: líquenes, ácidos graxos, atividade antibacteriana.

Introduction

In the treatment of some diseases, the drug resistance of pathogenic microorganisms is attributed to the overuse of drugs by humans [1], generating a public health problem of major global impact [2]. The World Health Organization (WHO) has published a list of bacteria resistant to clinical use antibiotics with the aim of motivating researchers to discover novel drugs against certain types of bacterial strains, such as *E. coli* and *S. aureu*s, and thus avoid the resistance of mentioned strains to drugs [3]. In this sense, the research has been focused on the study of secondary metabolites present in plants in order to provide a natural structural model for the discovery of new drugs [4, 5]. In addition, many primary and secondary plant metabolites constitute a food source due to their glucose contents [5, 6] and other nutritional components such as fatty acids (FA) [7-9]. Lichens consist of a symbiotic association of an algae or cyanobacteria and a fungi [10, 11]. These complex organisms show high adaptability to severe climatic conditions probably due to the presence of lipids and FAs in their compositions, which enable cell membranes to continue to function at low temperatures [11]. FAs, such as linoleic acid, linolenic acid [7, 9] and arachidonic acid [8], are important in the diet of humans, thus making the study of lichens to be relevant in terms of nutrition [12]. Additionally, lichens produce other substances such as usnic acid (UA), nostistic acid and sekiakic acid, among others, which exert a broad spectrum of pharmacological properties such as antifungal, antibacterial [13] and antimycobacterial activities [14]. Recently, 34 lichen species from North American have been shown to be effective against methicillin-sensitive and methicillin-resistant *S. aureus* and *Pseudomonas aeruginosa* with MIC values ranging from 3.9 to 500 μg/mL. Besides, extracts from *Letharia vulpina*, *L. columbiana* and *Vulpicida canadensis* were effective against *E. coli* [15]. On the other hand, *Thamnolia vermicularis* acetonic extracts were used as a functional food in obese mice and the results of these tests demonstrated that these extracts exert anti-obesity activity and they can be considered as a natural resource for controlling obesity [16].

Our research group has previously studied the lichen *Thamnolia vermicularis* subsp. vermicularis and identified decarboxythamnolic acid and thamnolic acid by spectroscopic (FT-IR, ^{1}H NMR, and ^{13}C NMR) and mass techniques [17]. The aim of this work was to determine the chemical composition of FAs and the percentage of UA in *Usnea* sp., *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula*. The antimicrobial activity of ethanolic extracts of these lichen species was also evaluated against *E. coli* ATCC25922, *S. aureus* ATCC29213, *S. aureus* ATCC43300 (methicillin and oxacillin resistant) and *B. subtilis* ATCC11774.

Materials and methods

Microorganisms

Bacterial strains: Gram-negative *E. coli* ATCC 25922 and Gram-positive *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 (methicillin and oxacillin resistant) and *B. subtilis* ATCC 11774 were obtained from the Centro de Investigación de la Biodiversidad y Recursos Genéticos de Ancash, Universidad Nacional Santiago Antúnez de Mayolo, Huaraz, Perú.

Lichens

Lichen species such as *Usnea* sp. and *Thamnolia vermicularis* subsp. *solida* were collected in the province of Jauja, department of Junín, Peru (4100 m above sea level). *Ramalina asperula* Kremp was collected in the province of Huaral, Lima, Peru (2000 m above sea level). The lichens were identified by biologist Daniel Ramos and the samples were deposited in the Herbarium of Southern Peru (Arequipa).

Preparation of the Lichen Extracts

Fresh and clean lichen samples were placed into an oven with circulating air at 40 °C for 3 days. Then, the dried material was ground as fine as 20 mesh and stored at 4 °C in sterilized amber glass bottles. A total of 100 g of each lichen was placed in a beaker containing 250 mL $CHCl_3$-MeOH (1:1 v/v) and left to stand for three days at room temperature. The extracts were separated by filtration and then the filtrate was concentrated under reduced pressure using a Buchi 110 rotary evaporator. Other lichen extracts in chloroform or ethanol were obtained by soxhlet extraction [1 g lichen in chloroform (150 mL); extraction time: 2 h)] and ultrasound extraction [20 g lichen in ethanol (50 mL); extraction time: 1 h]. The obtained extracts were concentrated.

Antimicrobial Activity Tests

The antimicrobial activity of the lichen ethanolic extracts was evaluated using diffusion (M02-A7 protocol, CLSI) and microdilution (M07-A8 protocol, CLSI) methods [18] against *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 and *B. subtilis* ATCC 11774. In a laminar flow cabinet, the samples were prepared by dissolving the dried ethanolic extracts at 200 mg/L in DMSO. The samples were then stored at −20 °C for 20 days.

Diffusion Method

Each ethanolic extract (5 µL) were added to sterile filter paper discs (5 mm diameter) and placed in Petri dishes containing Mueller and Hinton agar previously inoculated with bacteria prepared as previously described [19]. Disks of Ampicillin (25 µg) were used as reference antibiotic, and discs with 5 µL of DMSO were used as the negative control. Four replicates of all samples were prepared and incubated at 35 °C for 24 h. The inhibition halos were measured in those samples showing clear zones around the discs.

Microdilution Method

Samples showing an inhibition halo in the diffusion test were used in the microdilution assay. Serial dilutions of samples were made in Mueller and Hinton broth II to obtain final concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL. Dilutions were transferred into sterile 96-well plates (100 µL per well) and then inoculated with 10 µL of bacterial suspension [19]. Contamination controls with or without extract and growth controls without extract were also prepared. Ampicillin (final concentration of 1 or 5 µg/mL in well) was used as reference antibiotic. Three replicates of all samples and controls were prepared and incubated at 35 °C for 24 h. To assess bacterial growth, 10 µL of 1% sterile tetrazolium violet was added to each plate and turbidity was observed [19]. The MIC value was defined as the minimum concentration of the reference drug or tested compound (in µg/mL) that inhibits bacterial growth. The assay was repeated for 7 days to evaluate the activity.

Fatty Acid Analysis via gas chromatography–flame ionisation detector

In a 100 mL flask, 10 mL of 0.5 N KOH solution were added to 200 mg of $CHCl_3$/MeOH organic extract and incubated for 20 min at 55 °C in a water bath. After adding 5 mL of diluted HCl (1:1) solution, the FAs were extracted with 10 mL of petroleum ether. The extract was washed with 10 mL water, dried with anhydrous sodium sulphate and then concentrated to dryness with gaseous N_2. The residue was dissolved in 10 mL of petroleum ether, and 1 mL of aliquot was then evaporated to dryness in

gaseous N_2. This extract was then dissolved in 10 mL of 5% $HClO_4$ methanolic solution and heated for 5 min at 55 °C. Finally, the esterified FAs of the three samples under study were extracted with 10 mL of petroleum ether.

Esterified FAs dissolved in petroleum ether were analysed in a GC-2010 Shimadzu gas chromatograph equipped with a fused silica column (75 m × 0.18 mm × 0.14 μm) at 140 °C and flame ionisation detector (FID). The injection was performed in split mode with a flow rate of 6.25 mL/min using an injector temperature of 260 °C. The carrier gas was helium with a run time of 45 min, and with an injection volume of 1 μL. The esterified FAs were identified by comparison with the retention times of the FAME standard mixture (Lot: LRAC3241), detected by FID, injected under the same conditions as the samples [18]. Calculations for the identification and quantification of esterified FAs by GC-FID were performed with the GCsolution Software (Shimazdu, ver. 2.44.00) and the results were expressed in mg FA per g of esterified extract according to the following formula:

$$\frac{mg\ FA}{g} = \frac{Asmp}{Astd} \cdot \frac{Wstd}{Wsmp} \cdot \frac{1}{200}$$

The percentage value of each FA in relation to the total concentration was determined using the following formula:

$$\%\ FA = \frac{mg\ FA/g}{mg\ FA\ \ g} \cdot 100$$

Where:

Asmp: Area of the sample recorded on the chromatogram

Astd: Area of the FAME standard mixture recorded on the chromatogram

Wstd: Weight of the FAME standard mixture

Wsmp: Weight of the sample

% FA: Percentage of the fatty acid

mg FA/g: Concentration of the fatty acid (mg/g)

Σ mg FA/g: Total concentration of fatty acids (mg/g)

Quantitative Analysis of Usnic Acid

UA standard (CAS No. 7562-61-0) was quantified using a Shimadzu UV-1601 UV-Vis spectrophotometer. Briefly, 5 mg of the standard UA were dissolved in 100 mL of chloroform; then, an aliquot of 100 µL was diluted with 50 mL of chloroform to obtain a final concentration of 0.1 mg/L. The maximum absorbance was recorded at 284 nm. Dilutions of 1/10, 1/50, 1/100, 1/500 and 1/1000 were made from the chloroformic extract to record absorbance values at 284 nm within the linear range of the calibration curve, which was previously defined from the analytical standard in the range of 0–10 ppm [17].

Results

Antibacterial Activity

From the diffusion method, all samples showed inhibitory activity against *B. subtilis* ATCC 11774. In addition, the *T. vermicularis* subsp. *solida* and *R. asperula* ethanolic extracts showed a moderate activity against *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300. None of the tested extracts were active against *E. coli* ATCC 25922. These results were also confirmed using the microdilution method. All tested samples (MIC= 0.5 mg/mL) inhibited the growth of *B. subtilis* ATCC 11774 while the *T. vermicularis* subsp. *solida* and *R. asperula* ethanolic extracts stopped the growth of *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 when registering MIC values in the range of 1–4 mg/mL, as shown in Table 1 and Figure 1.

Table 1. Antibacterial activity of the lichen ethanolic extracts. The extract discs contained 1 mg of sample; the ampicillin disc contained 25 µg of ampicillin. [a] The values represent the means of 4 replicates ± the standard deviation.

Extracts	*B. subtillis* ATCC 11774		*S. aureus* ATCC 43300		*S. aureus* ATCC 29213	
	Halo (mm)[a]	MIC (mg/mL)	Halo (mm)[a]	MIC (mg/mL)	Halo (mm)[a]	MIC (mg/mL)
Thamnolia vermicularis subsp. *solida*	3.4 ± 0.5	0.5	2.9 ± 0.4	3	1.9 ± 0.2	4
Usnea sp.	6.9 ± 0.6	0.5	-	-	-	-
Ramalina asperula Kremp	8.5 ± 0.6	0.5	4.5 ± 0.6	1	3.8 ± 0.5	1
Ampicillin	33.0 ± 0.8	> 0.005	15.3 ± 0.5	<0.005	24.0 ± 0.0	0.005

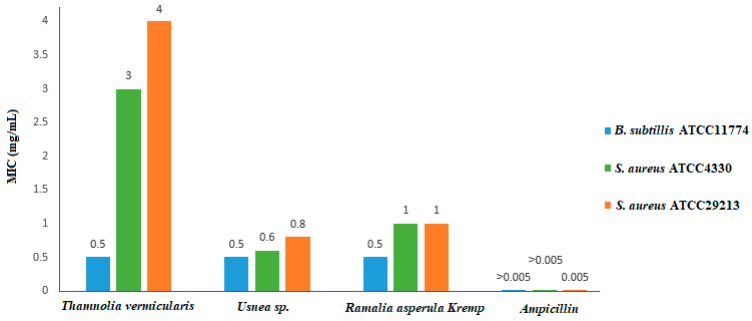

Figure 1. *In vitro* antibacterial activity expressed as MIC value (mg/mL) of lichen extracts and Ampicillin antibiotic tested

Identification and Quantification of FAs

The identification and quantification of the FAs from the three lichen species were carried out by comparing the retention times and areas of the chromatograms obtained for the samples (Figures 2-4) with the chromatograms for the FAME standard mixture using GC-FID. The results are shown in Table 2.

Table 2. Fatty acid (FA) concentration in *Usnea* sp., *Thamnolia vermicularis* subsp. *solida*, and *Ramalina asperula* Kremp. RT: retention time; SFA: saturated fatty acid; UFA: unsaturated fatty acid

Fatty acids	Formula	RT (min)	Concentration [mg FA/g sample], (% FA)		
			Thamnolia vermicularis subsp. *solida*	*Usnea* sp.	*Ramalina asperula* Kremp
Palmitic acid	C16:0	21.189	[18.4], (6.2)	[31.0], (7.2)	[22.7], (8.2)
Stearic acid	C18:0	24.724	[3.8], (1.2)	[12.3], (2.8)	[4.1], (1.5)
Oleic acid	C18:1 n-9	25.773	[21.3], (7.2)	[36.0], (8.4)	[18.8], (6.8)
Linoleic acid	C18:2 n-6	27.344	[162.4], (54.7)	[175.8], (40.8)	[107.6], (38.8)
Linolenic acid	C18:3 n-3	29.116	[48.4], (16.3)	[175.4], (40.9)	[124.1], (44.8)
Arachidonic acid	C20:4 n-6	32.428	[42.7], (14.4)	------	------
Total SFA			[22.3], (7.5)	[43.2], (10.0)	[26.8], (9.7)
Total UFA			[274.8], (92.5)	[388.2], (90.0)	[250.4], (90.3)
Total FA			[297.1], (100)	[431.4], (100)	[277.2], (100)

Quantitative Analysis of Usnic Acid

The calibration curve for the quantification of UA was linear in the range of 0–10 ppm where the obtained linear correlation equation was $Y = 0.0343\ X$. The percentages of UA determined for *Thamnolia vermicularis* subsp. *solida*, *Ramalina asperula Kremp* and *Usnea* sp. were 0.2, 1.01 and 1.07 %, respectively.

Discussion

The chemical characterization of the lichen extracts and the identification of FAs and UA were performed by gas chromatography (GC-FID) and UV-Vis spectroscopy, respectively. For extraction of FA, the volume ratio of the $CHCl_3$:MeOH mixture was 1: 1. This volume ratio was different with respect to that used in the literature (2:1 v/v) [10]. With the $CHCl_3$:MeOH (1:1 v/v) mixture used in this work, the solubility of low polarity compounds and the amount of unwanted metabolites dissolved in the organic extract was decreased. The esterification reaction was carried out using a 5% $HClO_4$ methanolic solution [20] in order to reduce the reaction time [21].

As shown in Table 2, saturated and unsaturated FAs were identified in the three assessed lichens (C16:0, C:18:0, C:18:1, C:18:2 and C:18:3). In all three studied lichens, stearic acid (C18:0)—a saturated FA—was found with the lowest concentration level, 3.8 and 4.1 mg/g sample in *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula Kremp*, respectively. In the present study, the FAs found are similar to those reported for the genus *Cladonia* [7] and *Stereocaulon scutelligerum* [22]. Furthermore, our results (2.8–4.1 mg/g sample) are comparable to the stearic acid concentrations found for others lichens containing the species *Cladonia cornuta* (L.) Hoff and *Cladonia pyxidata* (3.4 and 4.8 mg/g sample, respectively) [7]. In this work, arachidonic acid (C:20:4 n-6) was only detected in *Thamnolia vermicularis* subsp. *solida* and its concentration was 42.7 mg/g sample (Figure 1). This acid is one of the major unsaturated FAs that constitute brain membrane phospholipids and is found in lichens in varying proportions [8]. As shown in Table 2, the ratio of unsaturated FAs to saturated FAs for the studied lichens was approximately 10:1. This result could be related to the environmental conditions under which the lichens were collected [10].

The UA levels found in the chloroformic extracts of the tested lichens were in agreement with those previously reported for other lichens [5, 23].

All three lichen species (MIC = 0.5 mg/mL) showed significant inhibitory activity with respect to ampicillin (reference drug) against *B. subtilis* ATCC 11774. Furthermore, these lichen species were ten times more cytotoxic than the acetonic extracts of *Cladonia furcata* and *Cladonia subulata* against *B. subtilis* [24]. As shown in Table 1, *Thamnolia vermicularis* subsp. *solida* showed a moderate antibacterial activity against the two resistant *S. aureus* strains (MIC = 3–4 mg/mL). Our MIC values are close to those reported for the acetonic extracts of *D. miniatum* tested against *S. aureus* subsp. *aureus* ATCC 25923 (MIC = 3.75 mg/mL) [25]. The ethanol extract of *Ramalina asperula* Kremp showed a MIC value of 1.0 mg/mL which was similar to that found for the acetone extract of *Acarospora fuscata* (MIC = 1.25 mg/mL) tested against *S. aureus* ATCC 25923 [26]. The ethanol extract of *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula* were highly active against *S. aureus* compared to other *C. crispum, P. squamulosum and L. prophetae-eliae* species assayed against *S. aureus* ATCC 1885 (MIC = 1000, 500 and 125 mg/mL, respectively) [27]. The tested ethanolic extracts of the three studied species were inactive against the Gram-negative bacterium *E. coli*. This result is in agreement with previous reports related with the genus *Cladonia*, where none of the 42 studied species showed significant activity against *E. coli* [28]. The low activity of the lichen ethanolic extracts tested against Gram-negative bacteria may be attributed to the lipopolysaccharides and lipoproteins present in the bacterial cell wall, preventing easy permeability of external agents compared to the cell wall of Gram positive bacteria (composed of peptidoglycans (murein) and teichoic acid), which make them more resistant to certain antibiotics [29, 30].

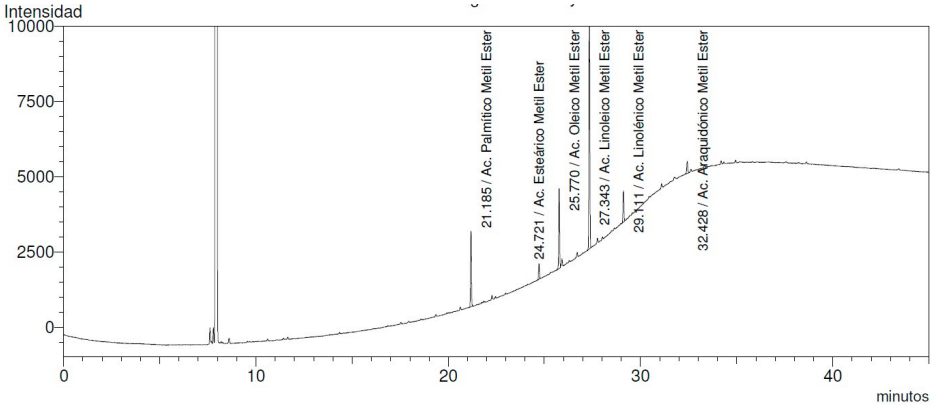

Figure 2. Chromatogram of the lichen *Thamnolia vermicularis subsp. solida*

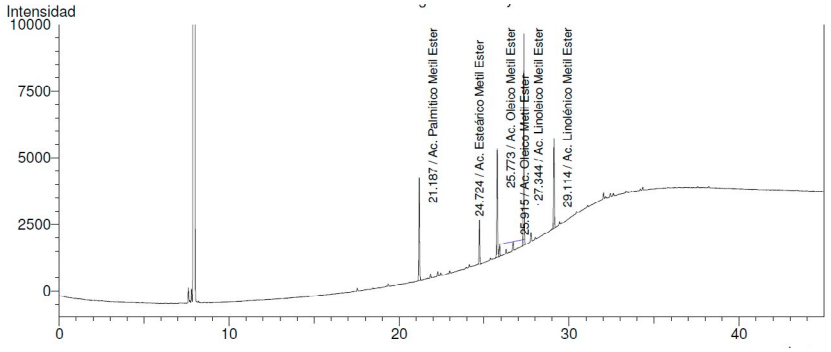

Figure 3. Chromatogram of the lichen *Usnea sp.*

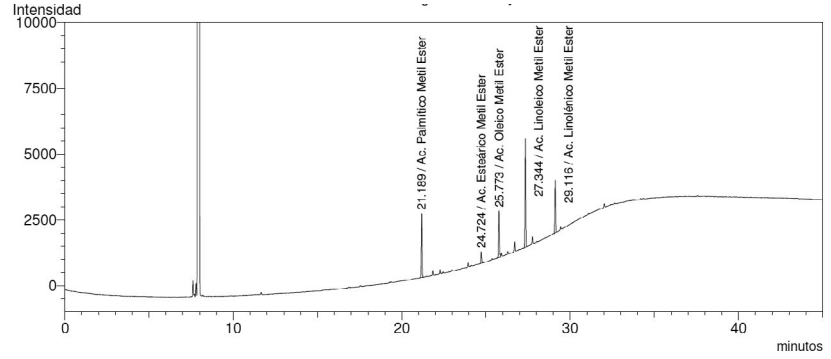

Figure 4. Chromatogram of the lichen *Ramalina asperula Kremp*

Conclusion

In this study, the palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid FAs were identified in the three studied lichens. The percentage of UA found was between 0.2 and 1.07 %. All three ethanolic extracts showed a significant activity against *B. subtilis* ATCC 11774 compared to the ampicillin (drug for clinical use) Furthermore, the ethanolic extracts of *Thamnolia vermicularis* subsp. *solida* and *Ramalina asperula* showed moderate inhibitory activity against *S. aureus* ATCC 43300 and *S. aureus* ATCC 29213. Our obtained results are an important contribution for food and pharmaceutical industries since the studied lichen species can be considered as potential nutritional and antibacterial agents.

Ethical approval

This study is part of a project that has been reviewed and approved by the Ethics Committee of the Universidad de Lima Scientific Research Institute.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

1. C.L. Ventola, The antibiotic resistance crisis: Part 2: Management strategies and new agents, *P. T.*, **40**(5), 344-352 (2015).

2. S.B. Levy, B. Marshall, Antibacterial resistance worldwide: Causes, challenges and responses, *Nat. Med.*, **10**(12), S122-S129 (2004). https://doi.org/10.1038/nm1145

3. World Health Organization, *Global priority list of antibiotic resistant bacteria to guide research, discovery, and development of new antibiotic*, WHO, Geneve, 2017. URL: https://remed.org/wp-content/uploads/2017/03/lobal-priority-list-of-antibiotic-resistant-bacteria-2017.pdf

4. B. Baral, B.L. Maharjan, Assessment of antimicrobial and phytochemical potentials of high altitudinal Nepalese lichens, *J. Microbiol. Biotechnol. Food Sci.*, **1**(2), 98-112 (2011).

5. A.P. Podterob, Chemical composition of lichens and their medical applications, *Pharm. Chem. J.*, **42**(10), 582-588 (2008). https://doi.org/10.1007/s11094-009-0183-5

6. C. Illana, Líquenes comestibles, *Bol. Soc. Micol. Madrid*, **33**, 273-282 (2009).

7. V.M. Dembitsky, Lipids of lichens, *Prog. Lipid Res.*, **31**(4), 373-397 (1992). https://doi.org/10.1016/0163-7827(92)90002-Z

8. S.M.M. Shanab, R.M. Hafez, A.S. Fouad, A review on algae and plants as potential source of arachidonic acid, *J. Adv. Res.*, **11**, 3-13 (2018). https://doi.org/10.1016/j.jare.2018.03.004

9. L.O. Hanus, M. Temina, V. Dembitsky, Biodiversity of the chemical constituents in the epiphytic lichenized ascomycete *Ramalina lacera* grown on difference substrates *Crataegus sinaicus*, *Pinus halepensis*, and *Quercus calliprinos*, *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.*, **152**(2), 203-208 (2008). https://doi.org/10.5507/bp.2008.031

10. R.A. Reis, M. Iacomini, P.A. Gorin, L.M. de Souza, M. Grube, L.M. Cordeiro, *et al.*, Fatty acid composition of the tropical lichen *Teloschistes flavicans* and its cultivated symbionts, *FEMS Microbiol. Lett.*, **247**(1), 1-6 (2005). https://doi.org/10.1016/j.femsle.2005.04.023

11. B.K. Dertien, L.J. De Kok, P.J.C. Kuiper, Lipid and fatty acid composition of tree-growing and terrestrial lichens, *Physiol. Plant.*, **40**(3), 175-180 (1997). https://doi.org/10.1111/j.1399-3054.1977.tb04053.x

12. D. Ivanova, D. Ivanov, Ethnobotanical use of lichens: lichens for food review, *Scrip. Sci. Med.*, **41**(1), 11-16 (2009). https://doi.org/10.14748/ssm.v41i1.456

13. A.S.N. Moreira, R. Braz-Filho, V. Mussi-Dias, I.J.C. Vieira, Chemistry and biological activity of *Ramalina* lichenized fungi, *Molecules*, **20**(5), 8952-8987 (2015). https://doi.org/10.3390/molecules20058952

14. S.N. Yilmaz, C.M. Yilmaz, Antimycobacterial activity of some lichen extracts, *Biol. Divers. Conserv.*, **10**(1), 65-68 (2017).

15. G. Shrestha, J. Raphael, S.D. Leavitt, L.L. St Clair, *In vitro* evaluation of the antibacterial activity of extracts from 34 species of North American lichens, *Pharm. Biol.*, **52**(10), 1262-1266. https://doi.org/10.3109/13880209.2014.889175

16. R.Y. Choi, J.R. Ham, J. Yeo, J.S. Hur, S.K. Park, M.J. Kim, *et al.*, Anti-obesity property of lichen *Thamnolia vermicularis* extract in 3T3-L1 cells and diet-induced obese mice, *Prev. Nutr. Food Sci.*, **22**(4), 285-292 (2017). https://doi.org/10.3746/pnf.2017.22.4.285

17. N. Castro, Contribución al estudio fitoquímico del liquen *Thamnolia vermicularis* Subsp. *vermicularis* S. Str., *Rev. Soc. Quím. Perú*, **76**(4), 322-329 (2010).

18. M. Danish, M. Nizami, Complete fatty acid analysis data of flaxseed oil using GC-FID method, *Data Brief*, **23**, 103845 (2019). https://doi.org/10.1016/j.dib.2019.103845

19. C. Tamariz-Angeles, P. Olivera-Gonzales, M. Santillán-Torres, Antimicrobial, antioxidant and phytochemical assessment of wild medicinal plants from Cordillera Blanca (Ancash, Peru), *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas – BLACPMA*, **17**(3), 270-285 (2018).

20. W.W. Christie, *Gas chromatography and lipids*, The Oily Press, Ayr, Scotland, 1989. p. 66-70.

21. M. Delange, D.M. Delange, Obtención y determinación de ácidos grasos de muy elevada masa molecular, *Rev. CENIC Cienc. Quím.*, **37**(1), 23-33 (2006).

22. T.H. Vu, D. Catheline, D. Delmail, J. Boustie , P. Legrand, F. Lohézic-le Dévéhat, Gas chromatographic analysis to compare the fatty acid composition of fifteen lichen species, with a focus on *Stereocaulon*, *Lichenologist*, **48**(4), 323-337 (2016). https://doi.org/10.1017/S0024282916000141

23. Y. Bazarnova, N. Politaeva, N. Lyskova, Research for the lichen *Usnea barbata* metabolites, *Z. Naturforsch C*, *J. Biosci.*, **73**(7-8), 291-296 (2018). https://doi.org/10.1515/znc-2017-0177

24. M. Kosanić, S. Ristić, T. Stanojković, N. Manojlović, B. Ranković, Extracts of five Cladonia lichens as sources of biologically active compounds, *Farmacia*, **66**(4), 644-651. https://doi.org/10.31925/farmacia.2018.4.13

25. Z. Karaahmet, K. Kinalioğlu, S. Aydin, Antioxidant and antibacterial potencies of *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale and *Dermatocarpon miniatum* (L.) W. Mann. lichens from Black Sea Region in Turkey, *Gümüşhane Üniversitesi Fen Bilimleri Dergisi*, **9**(3), 415-424 (2019). https://doi.org/10.17714/gumusfenbil.462040

26. M. Kosanić, B. Ranković, T. Stanojković, P. Vasiljević, N. Manojlović, Biological activities and chemical composition of lichens from Serbia, *EXCLI J.*, **13**, 1226-1238 (2014).

27. T. Valadbeigi, M. Shaddel, Antibacterial and antifungal activities of gelatinose and non-gelatinose lichen species, *J. Arch. Mil. Med.*, **3**(4), e31610 (2015). https://doi.org/10.5812/jamm.31610

28. P.R. Burkholder, A.W. Evans, I. McVeigh, H.K. Thornton, Antibiotic activity of lichens, *Proc. Natl. Acad. Sci. U. S. A.*, **30**(9), 250-255 (1944). https://doi.org/10.1073/pnas.30.9.250

29. C.M. Plaza, C.P. Salazar, R.E. Plaza, M. Vizcaya, G. Rodriguez-Castillo, G. Medina-Ramirez, *In vitro* analysis of antibacterial and antifungal potential of lichen species of *Everniastrum* cf vexans, *Parmotrema blanquetianum*, *Parmotrema reticulatum* and *Peltigera laciniata*, *Drug. Design Dev. Ther.*, **2**(3), 125-134 (2018). https://doi.org/10.15406/mojddt.2018.02.00038

30. B. Ranković, M. Mišić, S. Sukdolak, Antimicrobial activity of extracts of the lichens *Cladonia furcata*, *Parmelia caperata*, *Parmelia pertusa*, *Hypogymnia physodes* and *Umbilicaria polyphylla*, *Biologia*, **64**(1), 53-58 (2009). https://doi.org/10.2478/s11756-009-0007-9

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Antidote effect of Honey against arsenic induced toxicity in Charles Foster rats

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Summary

Introduction: Arsenic poisoning in the groundwater in recent times has become a major problem in the entire world. The exposed subjects exhibit typical symptoms of arsenicosis such as skin manifestations, gastrointestinal disorders, neurological disorders, hormonal disorders etc. **Objective:** The present study is focused to combat the deleterious effect of arsenic toxicity in animal models using honey. **Materials and Methods:** The animals (Charles Foster rats) were treated with Sodium arsenite at the dose of 8 mg per Kg body weight for 90 days to make arsenic model and upon these arsenic pre-treated rats honey at the dose of 200 mg per Kg body weight (1:1) was administered for 60 days to study the antidote effects. After the entire treatment, rats were sacrificed and their blood samples were obtained and analysed for haematological and biochemical and histopathological study. **Results:** The study shows that arsenic induced toxicity caused severe damage to the rats at the haematological level such as decrease in the RBC counts, WBC counts, haemoglobin percentage, and biochemical level such as increase in the levels of SGPT, SGOT, ALP, bilirubin, urea, uric acid, creatinine, and lipid peroxidation. There was also high magnitude of degeneration observed at the histopathological level in the liver and kidney tissues. But, there was significant normalization in the honey on arsenic pretreated group of rats at all the respective studied parameters. **Conclusion:** The studied parameters denote that honey possesses antidote properties against arsenic induced toxicity and can be used for human purpose after dose titration as antidote.

Keywords: Sodium arsenite, honey, antidote effect, Charles Foster Rats.

Resumen

Efecto antídoto de la miel contra la toxicidad inducida por arsénico en ratas Charles Foster

Introducción: El envenenamiento por arsénico en las aguas subterráneas en los últimos tiempos se ha convertido en un problema importante en todo el mundo. Los sujetos expuestos presentan síntomas típicos de arsenicosis, como manifestaciones cutáneas, trastornos gastrointestinales, trastornos neurológicos, trastornos hormonales, etc. **Objetivo:** El presente estudio se centra en combatir el efecto nocivo de la toxicidad del arsénico en modelos animales que utilizan extracto de plantas medicinales. **Materiales y métodos:** Los animales (ratas Charles Foster) fueron tratados con arsenito de sodio a una dosis de 8 mg por Kg de peso corporal durante 90 días para hacer un modelo de arsénico y, sobre estas ratas pretratadas con arsénico, miel a una dosis de 200 mg por Kg. Se administró peso corporal (1:1) durante 60 días para estudiar los efectos del antídoto. Después de todo el tratamiento, las ratas fueron sacrificadas y se obtuvieron y analizaron muestras de sangre para su estudio hematológico, bioquímico e histopatológico. **Resultados:** El estudio muestra que la toxicidad inducida por arsénico causó daños graves a las ratas a nivel hematológico, como disminución en los recuentos de glóbulos rojos, recuentos de glóbulos blancos, porcentaje de hemoglobina y nivel bioquímico, como aumento en los niveles de SGPT, SGOT, ALP, bilirrubina. , urea, ácido úrico, creatinina y peroxidación lipídica. También se observó una alta magnitud de degeneración a nivel histopatológico en los tejidos del hígado y el riñón. Pero hubo una normalización significativa en el grupo de ratas pretratadas con miel y arsénico en todos los parámetros estudiados respectivos. **Conclusión:** Los parámetros estudiados denotan que la miel posee propiedades antídoto contra la toxicidad inducida por arsénico y puede ser utilizada para fines humanos después de la titulación de dosis como antídoto.

Palabras clave: arsenito de sodio, miel, efecto antídoto, ratas Charles Foster.

Resumo

Efeito antídoto do mel contra a toxicidade induzida por arsênico em ratos Charles Foster

Introdução: O envenenamento por arsênico nas águas subterrâneas tornou-se nos últimos tempos um grande problema em todo o mundo. Os sujeitos expostos apre-

sentam sintomas típicos de arsenicose, como manifestações cutâneas, distúrbios gastrointestinais, distúrbios neurológicos, distúrbios hormonais, etc. **Objetivo:** O presente estudo tem como objetivo combater o efeito deletério da toxicidade do arsênico em modelos animais utilizando extrato de planta medicinal. **Materiais e Métodos:** Os animais (ratos Charles Foster) foram tratados com arsenito de sódio na dose de 8 mg por Kg de peso corporal durante 90 dias para fazer modelo de arsênico e sobre estes ratos pré-tratados com arsênico mel na dose de 200 mg por Kg peso corporal (1:1) foi administrado por 60 dias para estudar os efeitos do antídoto. Após todo o tratamento, os ratos foram sacrificados e suas amostras de sangue foram obtidas e analisadas para estudo hematológico, bioquímico e histopatológico. **Resultados:** O estudo mostra que a toxicidade induzida pelo arsênico causou danos graves aos ratos no nível hematológico, como diminuição na contagem de glóbulos vermelhos, contagem de leucócitos, porcentagem de hemoglobina e nível bioquímico, como aumento nos níveis de SGPT, SGOT, ALP, bilirrubina, uréia, ácido úrico, creatinina e peroxidação lipídica. Houve também alta magnitude de degeneração observada em nível histopatológico nos tecidos do fígado e dos rins. Mas houve uma normalização significativa no mel do grupo de ratos pré-tratados com arsênico em todos os respectivos parâmetros estudados. **Conclusão:** Os parâmetros estudados indicam que o mel possui propriedades antídoto contra a toxicidade induzida pelo arsênico e pode ser utilizado para fins humanos após titulação da dose como antídoto.

Palavras-chave: Arsenito de sódio, mel, efeito antídoto, ratos Charles Foster.

Introduction

Groundwater arsenic poisoning in the recent times has caused serious health hazards worldwide. A wide population of about 300 million are exposed to arsenic through groundwater poisoning. Moreover, in Asia, India and Bangladesh share major river channels Ganga-Meghna-Brahmaputra plains where an estimated 155 million are exposed to groundwater arsenic poisoning. India and Bangladesh have the cases of arsenic poisoning in the different habitations about 70 million and 85 million population respectively. This arsenic poisoning number is contributed as half of the world's arsenic exposed population. In India, the groundwater arsenic poisoning is being majorly reported from states – West Bengal, Assam, Bihar and Uttar Pradesh. Moreover, this is the chunk area where the population of about 510 million reside in the different habitations [1-6].

In Bihar, Bhojpur was the first district in 2002, when arsenic poisoning was reported for the first time and presently, 22 districts of state are affected with groundwater arsenic poisoning [7-9]. The exposed population exhibit typical symptoms of arsenicosis such as skin manifestations, neurological disorders, hormonal imbalance, reproductive disorders, gastrointestinal disorders, cardiovascular disorders, diabetes, and loss of appetite and disease of cancer [10-14]. Moreover, in this arsenic exposed area the magnitude of disease burden has increased many folds in the recent times. Among the cancer types – skin cancer, lung cancer, colorectal cancer etc. have been reported from the arsenic exposed population [15-23]. Hence, it becomes very important to discover natural products which can be recommended to the arsenic exposed population which can reduce the disease burden in them.

Plethora of medicinal plants have been documented which have therapeutic effect on arsenic induced toxicity in animal models [24-26], but their products have not reached to the exposed population till date. The use of honey has been extensively used against various types of poisons [27], anti-inflammation, anti-apoptosis, anti-fibrosis [28], cardiovascular diseases such as anti-arthrosclerosis effects [29], anti-neuroinflammatory effect [30], and other diseases [31, 32]. Hence, the present study is focused on the usage of natural wild supplement such as honey. Honey has been used since many years as source of energy, for wound healing, anti-bacterial, anti-viral and as natural skin rejuvenators [33-43].

Hence, the present study aims to discover the antidote effect of natural honey against the arsenic induced model on Charles Faster rats and to validate the efficacy of the honey against arsenic induced toxicity.

Materials And Methods

Animals

For the entire experimentation, n=24, male Charles Foster rats, 8 weeks old of weight about 150-180 g were provided from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India. The animals were acclimatized in laboratory for 2 weeks before the start of the experiment. All the laboratorial conditions were maintained with 12 hours of light and dark cycles and room temperatures were maintained at 22 ± 2 °C with adjusted humidity. The animals had free access to food and water *ad libitum*.

Chemicals

For the experiment, to induce the toxic models in rats, Sodium arsenite (98.5%) manufactured by Sigma-Aldrich, USA (CAS Number: 7784-46-5; S7400-100G), Lot# SLBH5736V, PCode 1001683292 as the chemical were utilized. The permissible dose of sodium arsenite was used as 8 mg/Kg/body weight per day for 90 days to make the arsenic model as per the previous study [23].

Preparation of honey dose

Raw wild honey was obtained from the local market and its purity was tested in the laboratory (by vinegar method and light microscopy). The dose of the honey was calculated after LD_{50} estimation and was titrated to 200 mg/Kg body weight per day. The dose was prepared by 1:1 ratio mixed in water as its viscosity was very high.

Experimental Design

The experimental animals were majorly disintegrated into 04 groups and each group consisted of six rats and were divided in following groups - **Group I: Normal Control group**; **Group II: Arsenic treated group** – The rats were treated orally with sodium arsenite at a dose of 8 mg/Kg body weight/day for 90 days; **Group III: Honey administered group**- Rats were pretreated with sodium arsenite 8mg/Kg body weight/day for 90 days followed by administration of honey – 200 mg/Kg body weight/day for 60 days. After the completion of the entire experiment, animals from each group were properly anaesthetized with 50 mg/Kg ketamine hydrochloride (intra-peritoneally) and sacrificed. The dissected animals' blood samples were collected through the orbital puncture for serum separation and were stored properly for the biochemical assays such as -Liver function tests, kidney function tests and lipid peroxidation. Furthermore, their tissue samples such as liver and kidney were properly preserved in the 10% formalin fixative and were processed later.

Haematological study

For the haematological study – RBC counts and WBC counts the Neubauer's chamber was utilized, while for the estimation of haemoglobin, hemoglobinometer was utilized using the Sahli's method.

Biochemical analysis

For the biochemical study, standard kit was used (Coral crest) by the Spectrophotometer (UV-Vis) (UV-10, Thermo Fisher, USA) method. The biochemical parameters used in the present study were - Liver Function Tests- Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) estimated

through the method of [44], Alkaline Phosphate (ALP) assay by the method of [45], total bilirubin activity by method of [46]. The Kidney Function Tests (KFT) were assayed as urea by the method of [47, 48], creatinine assay by the method of [49], and uric acid assay by the method of [50].

Histopathological study

The dissected tissues – liver and kidney which were fixed in 10% formalin for 24 hours were then processed through the series of graded alcohol and were embedded into paraffin blocks. Utilizing the digital microtome (Themo-Fisher), fine sections of about 5 μm thickness were cut and were processed for double staining method with hematoxylin and eosin (H&E). For the microscopic observations, the stained slides were viewed under the light microscope [51].

Lipid Peroxidation (LPO)

For the lipid peroxidation method, thiobarbituric acid reactive substances [TBARS] are the best markers for the evaluation. The assay was evaluated through the double heating method [52], based on the principle of spectrophotometric measurement of colour reproduced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this study, 0.5 mL of serum were mixed with 2.5 mL of 100 g/L solution of Trichloroacetic acid (TCA) and then were properly centrifuged at 3000 rpm for 10 minutes, it was then heated in the water bath at 90 °C for 15 minutes. After cooling at the room temperature, the mixture was re-centrifuged at 3000 rpm for 10 minutes and the supernatant were obtained. The 2 ml of the supernatant were measured and were mixed with 1 mL of 6.7 g/L freshly prepared TBA solution in the test tube which was further heated in water bath at 90 °C for 15 minutes and left for cooling at the room temperature. The final absorbance was measured using UV-vis spectrophotometer (Thermo Scientific UV-10, USA) at 532 nm.

Statistical analysis

The results presented in the study were the Mean ± Standard Error (SE) for six rats' individual groups and total variation represented in a set of data which were analyzed through one-way Analysis of Variance (ANOVA). The differences among mean variance were analyzed by applying Dunnett's 't' test at 99.9% ($p < 0.05$) confidence level. The final calculations were performed utilizing the GraphPad Prism Program 5.0 (GraphPad Software, Inc., San Diego, USA).

Results

Haematological Assay

There were significant changes observed in the studied groups in the haematological parameters –

a. **RBC Counts:** In the arsenic treated group there was significant decrease ($p<0.005$) in the RBC counts in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the RBC counts after the administration of honey to the arsenic pre-treated rat group (Figure 1).

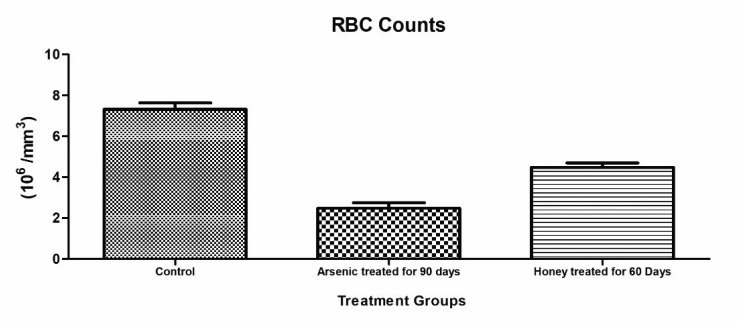

Figure 1. RBC counts of the treated groups (One way ANOVA Test in various group of rats (n=6), values are displayed as Mean ± SE)

b. **WBC Counts:** In the arsenic treated group there was significant decrease ($p<0.005$) in the WBC counts in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) increase in the WBC counts after the administration of honey to the arsenic pre-treated rat group (Figure 2).

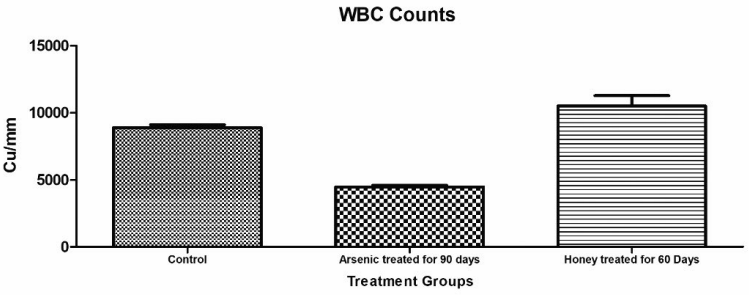

Figure 2. WBC counts of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

c. **Haemoglobin Percentage:** In the arsenic treated group there was significant decrease ($p<0.005$) in the haemoglobin percentage in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) decrease in the haemoglobin percentage after the administration of honey to the arsenic pre-treated rat group (Figure 3).

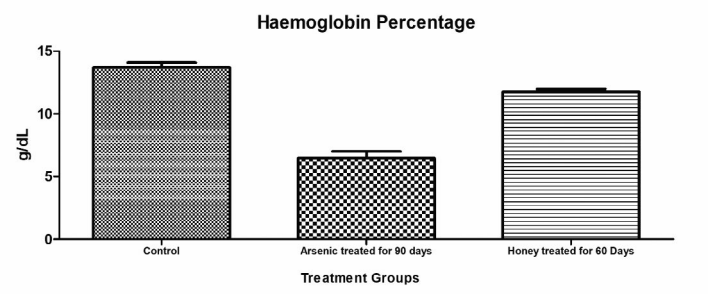

Figure 3. Haemoglobin percentage of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

Biochemical Study

There were significant changes observed in the biochemical parameters of the studied groups –

1. **SGPT Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the SGPT levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the SGPT levels after the administration of honey to the arsenic pre-treated rat group (Figure 4).

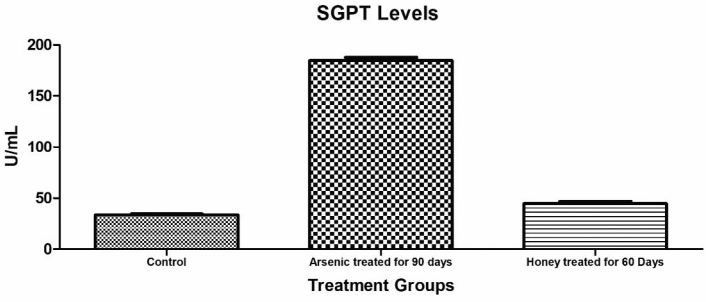

Figure 4. SGPT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

2. **SGOT Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the SGOT levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the SGOT levels after the administration of honey to the arsenic pre-treated rat group (Figure 5).

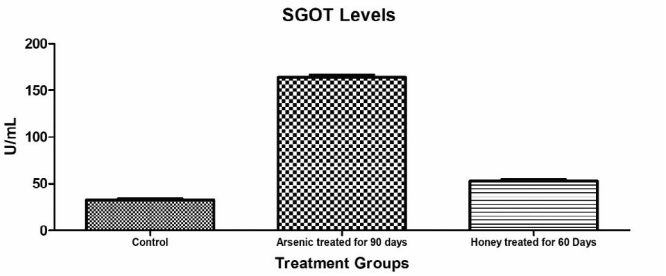

Figure 5. SGOT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6, values displayed as Mean ± SE)

3. **Alkaline Phosphatase (ALP) Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the ALP levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the ALP levels after the administration of honey to the arsenic pre-treated rat group (Figure 6).

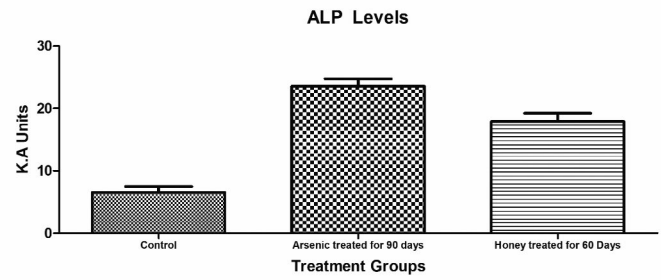

Figure 6. Alkaline phosphatase Levels of the treated groups (One way ANOVA Test in various group of rats (n=6) values displayed as Mean ± SE)

4. **Bilirubin Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the bilirubin levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the bilirubin levels after the administration of honey to the arsenic pre-treated rat group (Figure 7).

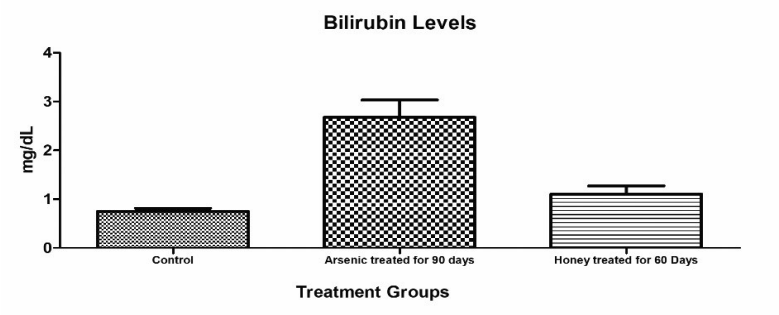

Figure 7. Bilirubin Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

5. **Urea Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the Urea levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the Urea levels after the administration of honey to the arsenic pre-treated rat group (Figure 8).

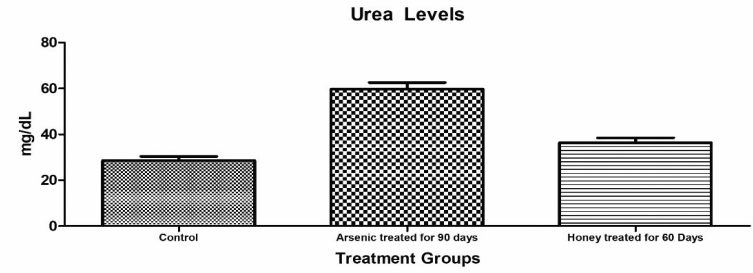

Figure 8. Urea Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

6. **Uric Acid Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the Uric acid levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the uric acid levels after the administration of honey to the arsenic pre-treated rat group (Figure 9).

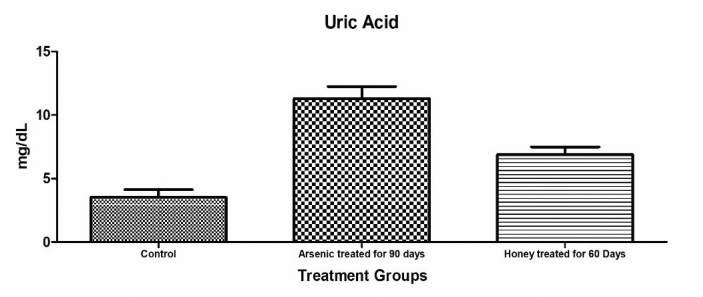

Figure 9. Uric acid Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

7. **Creatinine Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the creatinine levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the creatinine levels after the administration of honey to the arsenic pre-treated rat group (Figure 10).

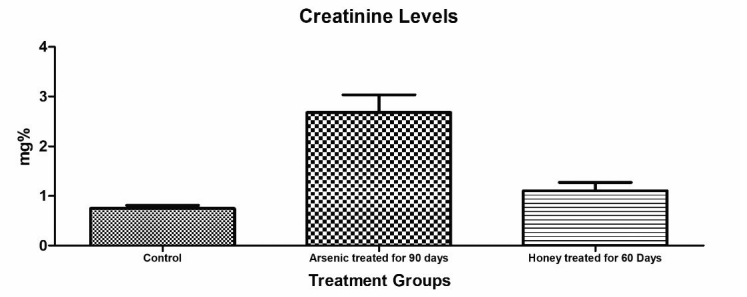

Figure 10. Creatinine levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

8. **Lipid Peroxidation (LPO) Assay:** In the arsenic treated group, there was significant increase ($p<0.005$) in the LPO levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the LPO levels after the administration of honey to the arsenic pre-treated rat group (Figure 11).

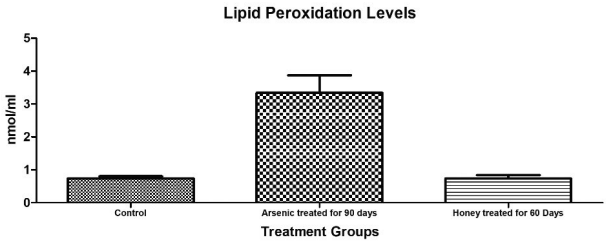

Figure 11. Lipid peroxidation levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

Histopathological Study

There were significant changes observed in the histopathological study in the studied groups – The liver histopathological sections showed normal architecture of hepatocytes with central vein. The hepatocytes were well arranged in the sinusoids denotes the normal functioning of the liver cells (Figure 12A). The arsenic treated rat liver section showed high grade of degeneration in the hepatocytes with pyknotic nuclei. There was manyfolds increase in the number of Kupffer cells denotes the macrophagic activity. Moreover, the endothelial cells of central vein membrane have ruptured severely by which haemorrhage in the sinusoidal spaces can be observed. Vacuolations in the sinusoidal spaces were also observed in the section. (Figure 12A). But after the administration with honey for 60 days, there has been significant restoration observation in the hepatocytes, the central vein and the sinusoids. The hepatocytes were well arranged in the sinusoids with the normal functioning. Moreover, no Kupffer cells were observed denotes the normal functioning of the liver. (Figure 12C & D).

The kidney histopathological sections show normal architecture of glomerulus, Bowman's capsule, the convoluted tubules, and distal tubules (Figure 13A). The arsenic treated section of kidney showed deshaped glomerulus and Bowman's capsule. Morever, severe haemorrhage in the kidney tissue were observed denotes the abnormal filtration process in the kidney due to arsenic induced toxicity. (Figure 13B). But, after administration of honey there was significant amelioration in the nephrocytes, especially in the glomerulus, Bowman's capsule and convoluted tubules, denotes the normal functioning of the nephrocytes (Figure 13C).

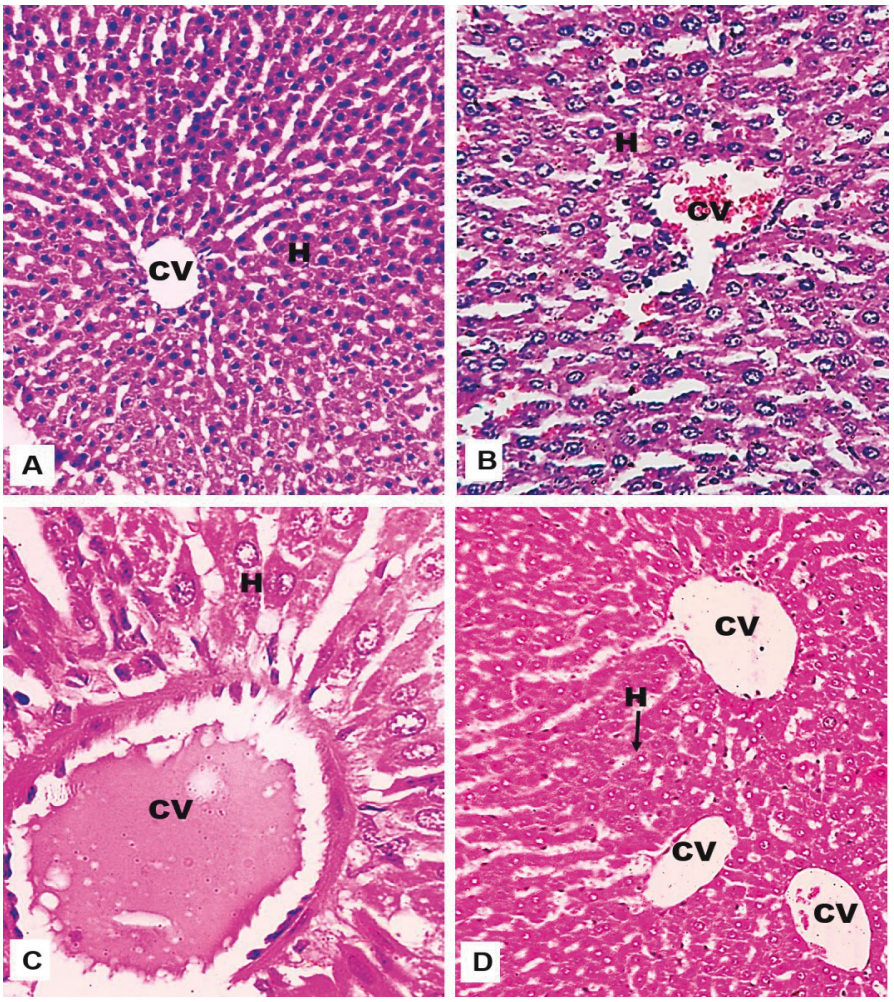

Figure 12. Microphotograph sections of liver of rat stained with haematoxylin and eosin (H&E × 500). **[A]** Showing control rat liver with normal architecture of hepatocytes (H), central vein (CV), with sinusoids. The hepatocytes are well arranged in the sinusoids **[B]**. Showing arsenic treated rat liver sections with degenerated hepatocytes (H) with central vein (CV). Pyknotic nuclei of the hepatocytes are also clearly visualized. The increased number of Kupffer cells (pin shaped) can be seen in the tissue denotes the degree of inflammation in the tissue. Hemorrhage in the sinusoidal spaces can also be observed. **[C&D]** After the administration with honey, the rat liver sections show significant normalization in the hepatocytes (H) with central vein (CV).

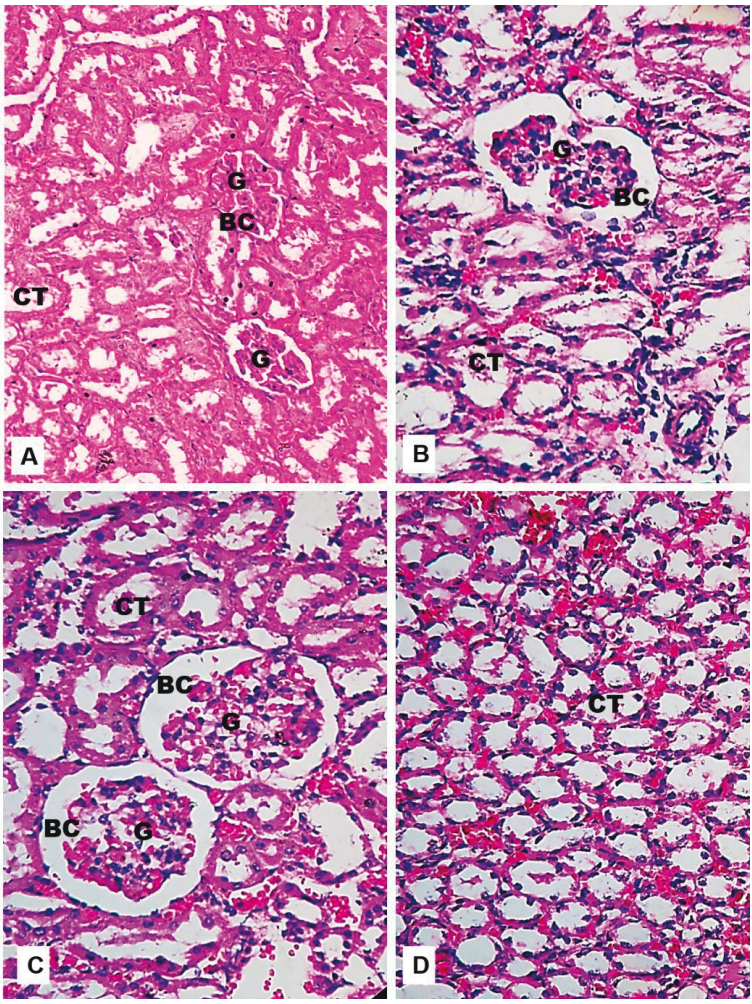

Figure 13. Microphotograph sections of kidney of rat stained with haematoxylin and eosin (H&E × 500). **[A]** The control rat kidney sections show normal architecture of glomerulus (G) with Bowman's capsules (BC). The endothelial cells of convoluted tubules are also in normal architecture. **[B]** The arsenic treated rat kidney sections show significant degeneration in the glomerulus (G) and Bowman's capsule (G) with haemorrhage in the entire kidney tissue. The convoluted tubules (CT) are also severely damaged. **[C&D]** The honey treated rat kidney section shows significant amelioration in the nephrocytes especially the glomerulus (G), Bowman's capsule (BC) and convoluted tubules (CT) denotes the normal functioning of the kidney tissue.

Discussion

Arsenic ingestion by gastrointestinal tract reaches the blood and other vital organs of the body, which in turn reflects its impact on the organ system and to the entire body of the animals. In the present study, in the arsenic treated rats, there was significant ($p<0.005$) decrease in the haematological parameters such as RBC counts, WBC counts and haemoglobin percentage in comparison to the control group of rats. This denotes that the arsenic toxicity has caused damaged the hematopoietic stem cells of the rats by which there is significant changes observed in the haematological parameters of the rats. But, after the administration of honey to the arsenic pre-treated rats, there was significant normalization in the RBC counts, WBC counts and haemoglobin percentage denotes the amelioration at the haematological level. Biochemical parameters are second level of the indicators which reflects the damage at the biochemical level in the tissue level. The liver and the kidney function tests are the important enzyme markers of the body which provide the significant information related to the toxicity in the body. In the present study, there was significant increase ($p<0.005$) in the SGPT, SGOT, alkaline phosphatase, bilirubin, urea, uric acid and creatinine levels, denotes that arsenic toxicity causes significant damage to these vital organs. But, after the administration with honey in arsenic pre-treated rats, there was significant restoration in these liver functions and kidney function tests levels. The third level of toxicity assessment is observed through the histopathological studies. In the present study, the histopathological study of liver and kidney showed severe damage in the liver and kidney tissues. In the arsenic treated liver tissue sections there was significant damage observed in the hepatocytes, central vein, portal vein, and sinusoidal spaces. The increase in the number of Kupffer cells denotes that arsenic toxicity has caused severe inflammation in the liver cells. Apart from this, there was haemorrhage in the sinusoidal spaces. While in kidney tissue the damage was caused to glomerulus, Bowman's capsules, convoluted tubules and ductal tubules. The degeneration caused due to arsenic toxicity has led to haemorrhage in the kidney tissue has hampered the glomerular filtration process. But, after the administration of honey to the arsenic pretreated rats, there was very significant restoration observed in the liver and kidney tissues observed. The hepatocytes, central vein, sinusoidal spaces all have significantly restored in comparison to the arsenic treated rat liver. Similarly, in kidney tissue also there was significant restoration in the nephrocytes especially the glomerulus, Bowman's capsule, convoluted tubules, distal tubules. This denotes that honey has antidote properties, which can control the damage caused by the arsenic induced toxicity. The entire restoration in the organ level observed is due to the honey's rejuvenating properties, antioxidant properties which has combat the damage caused by the arsenic induced toxicity [27-43, 53].

The defense mechanism at the cellular level is maintained by the oxidant- antioxidant system. In the present study, there was significantly very high lipid peroxidation levels observed in the arsenic treated group of rats in comparison to the control group of rats denotes the failure of the defense mechanism in this group. But, after the administration with honey, there was significant restoration in the lipid peroxidation levels, denotes that honey possesses antioxidant properties [24-26, 54-60].

The honey possesses active ingredients such as polyphenols which played the vital role in the normalization in the cellular functions in the arsenic induced toxicity. They rejuvenate the damage caused by arsenic through the antioxidant mechanism normalizing the body functions at the haematological levels, biochemical levels and histopathological level [61-64].

Conclusion

From the entire study it can be concluded that honey possesses antioxidant properties, which has antidote effect against arsenic induced toxicity in rats. The therapeutic effect of honey can be used as antidote drug against the arsenic induced toxicity.

Author contributions

The entire experimental work was conceptualized by M.K., S.S. and A.K. The manuscript's principal author M.K. contributed the majority of writing activities, but support was also provided by S.S, and A.K., Literature search was done by M.K. Figures were developed by M.K. and A.K. The experimentation and data analysis were carried out by M.K. The figures were designed by M.K. and A.K. The statistics and data interpretation were done by M.K. The final manuscript writing was done by M.K. S.S. and A.K. All authors read and approved the final manuscript.

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Conflicts of interests

The authors declare that they have no conflicts of interest.

Ethics approval

Before the start of the experiment, ethical approval of the experimental work was obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, Government of India (CPCSEA Registration no. IAEC No.1129/PO//ReBi/S/07/CPCSA). This research work was approved from the animal house of Institutional Animal Ethics Committee (IAEC) with IAEC No. IAEC No. 2021/1E-06/10/21.

References

1. E. Shaji, M. Santosh, K.V. Sarath, P. Prakash, V. Deepchand, B.V. Divya, Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula, *Geoscience Frontiers*, **12**(3), 101079 (2021).

2. M. Hassan, *Arsenic in groundwater*, CRC Press, Boca Raton, FL, 2018.

3. A. Kumar, M.S. Rahman, M. Ali, P. Salaun, A. Gourain, S. Kumar, *et al.*, Assessment of disease burden in the arsenic exposed population of Chapar village of Samastipur District, Bihar, India, and related mitigation initiative, *Environmental Science and Pollution Research International*, **29**(18), 27443–27459 (2022). https://doi.org/10.1007/s11356-021-18207-6.

4. L.A. Richards, B.G. Fox, M.J. Bowes, K. Khamis, A. Kumar, R. Kumari, *et al.*, A systematic approach to understand hydrogeochemical dynamics in large river systems: Development and application to the River Ganges (Ganga) in India, *Water Research*, **211**, 118054 (2022). https://doi.org/10.1016/j.watres.2022.118054.

5. L.A. Richards, A. Kumar, P. Shankar, A. Gaurav, A. Ghosh, D.A. Polya, Distribution and geochemical controls of arsenic and uranium in groundwater-derived drinking water in Bihar, India, *International Journal of Environmental Research and Public Health*, **17**(7), 2500 (2020). https://doi.org/10.3390/ijerph17072500.

6. L.A. Richards, R. Kumari, D. White, N. Parashar, A. Kumar, A. Ghosh, *et al.*, Emerging organic contaminants in groundwater under a rapidly developing city (Patna) in northern India dominated by high concentrations of lifestyle chemicals, *Environmental Pollution*, **268**(Pt A), 115765 (2021). https://doi.org/10.1016/j.envpol.2020.115765.

7. D. Chakraborti, S.C. Mukherjee, S. Pati, M.K. Sengupta, M.M. Rahman, U.K. Chowdhury, *et al.*, Arsenic groundwater contamination in Middle Ganga Plain, Bihar, India: a future danger?, *Environmental Health Perspectives*, **111**(9), 1194–1201 (2003). https://doi.org/10.1289/ehp.5966.

8. D. Chakraborti, M.M. Rahman, S. Ahamed, R.N. Dutta, S. Pati, S.C. Mukherjee, Arsenic contamination of groundwater and its induced health effects in Shahpur block, Bhojpur district, Bihar state, India: Risk evaluation, *Environmental Science and Pollution Research International*, **23**(10), 9492–9504 (2016). https://doi.org/10.1007/s11356-016-6149-8.

9. A. Kumar, M. Ali, V. Raj, A. Kumari, M. Rachamalla, S. Niyogi, *et al.*, Arsenic causing gallbladder cancer disease in Bihar, *Scientific Reports*, **13**(1), 4259 (2023). https://doi.org/10.1038/s41598-023-30898-0.

10. A. Kumar, R. Kumar, M.S. Rahman, M. Ali, R. Kumar, N. Nupur, *et al.*, Assessment of arsenic exposure in the population of Sabalpur village of Saran District of Bihar with mitigation approach, *Environmental Science and Pollution Research International*, **28**, 43923–43934 (2021). https://doi.org/10.1007/s11356-021-13521-5.

11. A. Kumar, M. Ali, R. Kumar, M. Kumar, P. Sagar, R.K. Pandey, *et al.*, Arsenic exposure in Indo Gangetic plains of Bihar causing increased cancer risk, *Scientific Reports*, **11**(1), 2376 (2021). https://doi.org/10.1038/s41598-021-81579-9.

12. A. Kumar, M.S. Rahman, M. Ali, R. Kumar, P.K. Niraj, V. Akhouri, *et al.*, Assessment of arsenic exposure and its mitigation intervention in severely exposed population of Buxar district of Bihar, India, *Toxicology and Environmental Health Sciences*, **13**, 287–297 (2021). https://doi.org/10.1007/s13530-021-00086-6.

13. A. Kumar, A.K. Ghosh, Assessment of arsenic contamination in groundwater and affected population of Bihar, in: N. Kumar (editor), *Arsenic Toxicity: Challenges and Solutions*, Springer, Singapore, 2021. https://doi.org/10.1007/978-981-33-6068-6_7.

14. A. Kumar, M.S. Rahman, R. Kumar, M. Ali, P.K. Niraj, A. Srivastava, *et al.*, Arsenic contamination in groundwater causing impaired memory and intelligence in school children of Simri village of Buxar District of Bihar, *Journal of Mental Health and Human Behavior*, **24**, 132–138 (2019). https://doi.org/10.4103/jmhhb.jmhhb_31_18.

15. A. Kumar, A.K. Ghosh, Arsenic and cancer, in: P. Papadopoulou, C. Marouli, A. Misseyanni (editors), *Environmental Exposures and Human Health Challenges*, IGI Global, 2019, pp. 106–132. https://doi.org/10.4018/978-1-5225-7635-8.ch005.

16. A. Kumar, C. Ravi, S. Dhingra, M.A. Krishna-Murti, A.K. Ghosh, Arsenic causing gallbladder cancer disease near the Himalayan bound Rivers in Bihar: A case study of gallbladder cancer, *Journal of Cancer Science and Clinical Therapeutics*, **6**, 388–391 (2022). https://doi.org/10.26502/jcsct.5079178.

17. A. Kumar, V. Raj, A. Srivastava, M. Ali, A.K. Ghosh, M. Rachamalla, D. Kumar, Autophagy in arsenic exposed population and cancer patients, in: D. Kumar, S. Asthana (editors), *Autophagy and Metabolism: Potential Target for Cancer Therapy*, Academic Press, 2022, pp. 141–161. https://doi.org/10.1016/B978-0-323-99879-6.00010-9.

18. A. Kumar, M. Ali, R. Kumar, M.S. Rahman, A. Srivastava, N.K. Chayal, *et al.*, High arsenic concentration in blood samples of people of Village Gyaspur Mahaji, Patna, Bihar drinking arsenic-contaminated water, *Exposure and Health*, **12**, 131–140 (2020). https://doi.org/10.1007/s12403-018-00294-5.

19. A. Kumar, R. Kumar, M.S. Rahman, M. Iqubal, M. Ali, P.K. Niraj, *et al.*, Ground water arsenic contamination: A local survey in India, *International Journal of Preventive Medicine*, **7**, 100 (2016). URL: https://journals.lww.com/ijom/fulltext/2016/07000/ground_water_arsenic_contamination__a_local_survey.99.aspx

20. A. Kumar, Md. Ali, S.Md. Rahman, A.Md. Iqubal, G. Anand, P.K. Niraj, P. Shankar, R. Kumar, Ground water arsenic poisoning in "Tilak Rai Ka Hatta" Village of Buxar District, Bihar, India causing severe health hazards and hormonal imbalance, *Journal of Environmental and Analytical Toxicology*, **5**, 290 (2015). https://doi.org/10.4172/2161-0525.1000290.

21. M.S. Rahman, A. Kumar, R. Kumar, M. Ali, A.K. Ghosh, S.K. Singh, Comparative quantification study of arsenic in the groundwater and biological samples of Simri village of Buxar District, Bihar, India, *Indian Journal of Occupational and Environmental Medicine*, **23**, 126–132 (2019).

22. A. Kumar, K. Kumar, M. Ali, V. Raj, A. Srivastava, M. Kumar, *et al.*, Severe disease burden and the mitigation strategy in the arsenic-exposed population of Kaliprasad Village in Bhagalpur District of Bihar, India, *Biological Trace Element Research*, (2023), in press. https://doi.org/10.1007/s12011-023-03822-w.

23. M.S. Rahman, A. Kumar, R. Kumar, M. Ali, A.K. Ghosh, A.K. Singh, Comparative quantification study of arsenic in the groundwater and biological samples of Simri Village of Buxar District, Bihar, India, *Indian Journal of Occupational and Environmental Medicine*, **23**(3), 126–132 (2019). https://doi.org/10.4103/ijoem.IJOEM_240_18.

24. A. Kumar, V. Kumar, V. Akhouri, R. Kumar, M. Ali, T. Rashmi, *et al.*, Protective efficacy of *Coriandrum sativum* seeds against arsenic induced toxicity in Swiss albino mice, *Toxicology Research*, **38**(4), 437–447 (2022). https://doi.org/10.1007/s43188-022-00123-7.

25. V. Kumar, V. Akhouri, S.K. Singh, A. Kumar, Phytoremedial effect of *Tinospora cordifolia* against arsenic induced toxicity in Charles Foster rats, *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine*, **33**(6), 379–396 (2020). https://doi.org/10.1007/s10534-020-00256-y.

26. A. Kumar, R. Kumar, M.S. Rahman, A. Iqubal, G. Anand, P.K. Niraj, M. Ali, Phytoremedial effect of *Withania somnifera* against arsenic-induced testicular toxicity in Charles Foster Rats, *Avicenna Journal of Phytomedicine*, **5**(4), 355–364 (2015).

27. M. Kassim, K.M. Yusoff, G. Ong, S. Sekaran, M.Y. Yusof, M. Mansor, Gelam honey inhibits lipopolysaccharide-induced endotoxemia in rats through the induction of heme oxygenase-1 and the inhibition of cytokines, nitric oxide, and high-mobility group protein B1, *Fitoterapia*, **83**(6), 1054–1059 (2012). https://doi.org/10.1016/j.fitote.2012.05.008.

28. S. Zhang, Y. Liu, Y. Ye, X.R. Wang, L.T. Lin, L.Y. Xiao, *et al.*, Bee venom therapy: Potential mechanisms and therapeutic applications, *Toxicon: Official Journal of the International Society on Toxicology*, **148**, 64–73 (2018). https://doi.org/10.1016/j.toxicon.2018.04.012.

29. B. Olas, Bee products as interesting natural agents for the prevention and treatment of common cardiovascular diseases, *Nutrients*, **14**(11), 2267 (2022). https://doi.org/10.3390/nu14112267.

30. Z. Li, S. Chu, W. He, Z. Zhang, J. Liu, L. Cui, *et al.*, A20 as a novel target for the anti-neuroinflammatory effect of chrysin via inhibition of NF-κB signaling pathway, *Brain*, *Behavior*, *and Immunity*, **79**, 228–235 (2019). https://doi.org/10.1016/j.bbi.2019.02.005.

31. R. Mittal, S. Patel, A. Galor, Alternative therapies for dry eye disease, *Current Opinion in Ophthalmology*, **32**(4), 348–361 (2021). https://doi.org/10.1097/ICU.0000000000000768.

32. A. Weissenstein, E. Luchter, S. Bittmann, Medical honey and its role in paediatric patients, *British Journal of Nursing*, **23**(6), S30–S34 (2014). https://doi.org/10.12968/bjon.2014.23.Sup6.S30.

33. M.D. Mandal, S. Mandal, Honey: Its medicinal property and antibacterial activity, *Asian Pacific Journal of Tropical Biomedicine*, **1**(2), 154–160 (2011). https://doi.org/10.1016/S2221-1691(11)60016-6.

34. J. Boateng, K.N. Diunase, Comparing the antibacterial and functional properties of Cameroonian and Manuka Honeys for potential wound healing-have we come full cycle in dealing with antibiotic resistance?, *Molecules* (Basel), **20**(9), 16068–16084 (2015). https://doi.org/10.3390/molecules200916068.

35. A. Kurek-Górecka, M. Górecki, A. Rzepecka-Stojko, R. Balwierz, J. Stojko, Bee Products in dermatology and skin care, *Molecules* (Basel), **25**(3), 556 (2020). https://doi.org/10.3390/molecules25030556.

36. S. Martinotti, M. Bucekova, J. Majtan, E. Ranzato, Honey: An effective regenerative medicine product in wound management, *Current Medicinal Chemistry*, **26**(27), 5230–5240 (2019). https://doi.org/10.2174/0929867325666180510141824.

37. H. Horn, Honig in der medizin [Honey in medicine], *Deutsche Medizinische Wochenschrift*, **138**(51-52), 2647–2652 (2013). https://doi.org/10.1055/s-0033-1359950.

38. P.C. Molan, The evidence supporting the use of honey as a wound dressing, *The International Journal of Lower Extremity Wounds*, **5**(1), 40–54 (2006). https://doi.org/10.1177/1534734605286014.

39. T. Kontogiannis, T.G. Dimitriou, N.A. Didaras, D. Mossialos, Antiviral activity of bee products, *Current Pharmaceutical Design*, **28**(35), 2867–2878 (2022). https://doi.org/10.2174/1381612828666220928110103.

40. G. Chamani, M.R. Zarei, M. Mehrabani, A. Mehdavinezhad, M. Vahabian, F. Ahmadi-Motamayel, Evaluation of honey as a topical therapy for intraoral wound healing in rats, *Wounds: A Compendium of Clinical Research and Practice*, **29**(3), 80–86 (2017).

41. C. Fox, Honey as a dressing for chronic wounds in adults, *British Journal of Community Nursing*, **7**(10), 530–534 (2002). https://doi.org/10.12968/bjcn.2002.7.10.10667.

42. R. Zubair, N. Aziz, As smooth as honey--The historical use of honey as topical medication, *JAMA Dermatology*, **151**(10), 1102 (2015). https://doi.org/10.1001/jamadermatol.2015.1764.

43. S. Nikhat, M. Fazil, History, phytochemistry, experimental pharmacology and clinical uses of honey: A comprehensive review with special reference to Unani medicine, *Journal of Ethnopharmacology*, **282**, 114614 (2022). https://doi.org/10.1016/j.jep.2021.114614.

44. S. Reitman, S. Frankel, A colorimetric method for determination of serum glutamate oxalacetic and glutamic pyruvate transaminases, *American Journal of Clinical Pathology*, **28**(1), 56–63 (1957).

45. P.R.H. Kind, E.J. King, Determination of alkaline phosphatase activity in serum, *Journal of Clinical Pathology*, **7**, 322 (1954).

46. G.F. Jendrassik, P. Grof, Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubins [Simplified photometric methods for determining blood bilirubin], *Biochemische Zeitschrift*, **297**, 81–89 (1938).

47. J.K. Fawcett, J.E. Scott, A rapid and precise method for the determination of urea, *Journal of Chemical Pathology*, **13**, 156 (1960).

48. G. Toro, P.G. Ackermann, *Practical Clinical Chemistry*, Little Brown & Co, Boston, MA, 1975, p. 154.

49. R.W. Bones, H.H. Tausky, Colorimetric determination of creatinine by the Jaffe reaction, *Journal of Biological Chemistry*, **158**, 581–591 (1945). URL: https://www.jbc.org/article/S0021-9258(19)51334-5/pdf

50. M.P.E. Berthelot, Berthelot's reaction mechanism, *Report de Chimie Applique*, 1, **284** (1859).

51. R.D. Cardiff, C.H. Miller, R.J. Munn, Manual hematoxylin and eosin staining of mouse tissue sections, *Cold Spring Harbor Protocols*, **2014**(6), 655–658 (2014). https://doi.org/10.1101/pdb.prot073411.

52. H.H. Draper, M. Hadley, Malondialdehyde determination as index of lipid peroxidation, *Methods in Enzymology*, **186**, 421–431 (1992). https://doi.org/10.1016/0076-6879(90)86135-i.

53. O.O. Erejuwa, S.A. Sulaiman, M.S. Ab Wahab, Honey: A novel antioxidant, *Molecules* (Basel), **17**(4), 4400–4423 (2012). https://doi.org/10.3390/molecules17044400.

54. A. Iftikhar, R. Nausheen, H. Muzaffar, M.A. Naeem, M. Farooq, M. Khurshid, *et al.*, Potential therapeutic benefits of honey in neurological disorders: The role of polyphenols, *Molecules* (Basel), **27**(10), 3297 (2022). https://doi.org/10.3390/molecules27103297.

55. C.C.F. Pleeging, F.A.D.T.G. Wagener, H. de Rooster, N.A.J. Cremers, Revolutionizing non-conventional wound healing using honey by simultaneously targeting multiple molecular mechanisms, *Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy*, **62**, 100834 (2022). https://doi.org/10.1016/j.drup.2022.100834.

56. K.N. Hashim, K.Y. Chin, F. Ahmad, The mechanism of honey in reversing metabolic syndrome, *Molecules* (Basel), **26**(4), 808 (2021). https://doi.org/10.3390/molecules26040808.

57. H. An, J.S. Heo, P. Kim, Z. Lian, S. Lee, J. Park, *et al.*, Tetraarsenic hexoxide enhances generation of mitochondrial ROS to promote pyroptosis by inducing the activation of caspase-3/GSDME in triple-negative breast cancer cells, *Cell Death & Disease*, **12**(2), 159 (2021). https://doi.org/10.1038/s41419-021-03454-9.

58. C.R. Majhi, S. Khan, M.D. Leo, S. Prawez, A. Kumar, P. Sankar, *et al.*, Acetaminophen increases the risk of arsenic-mediated development of hepatic damage in rats by enhancing redox-signaling mechanism, *Environmental Toxicology*, **29**(2), 187–198 (2014). https://doi.org/10.1002/tox.20785.

59. B.S. Yousefsani, J. Pourahmad, H. Hosseinzadeh, The mechanism of protective effect of crocin against liver mitochondrial toxicity caused by arsenic III, *Toxicology Mechanisms and Methods*, **28**(2), 105–114 (2018). https://doi.org/10.1080/15376516.2017.1368054.

60. X. Duan, J. Li, W. Li, X. Xing, Y. Zhang, W. Li, *et al.*, Antioxidant tert-butylhydroquinone ameliorates arsenic-induced intracellular damages and apoptosis through induction of Nrf2-dependent antioxidant responses as well as stabilization of anti-apoptotic factor Bcl-2 in human keratinocytes, *Free Radical Biology & Medicine*, **94**, 74–87 (2016). https://doi.org/10.1016/j.freeradbiomed.2016.02.009.

61. S. Afrin, S.M. Haneefa, M.J. Fernandez-Cabezudo, F. Giampieri, B.K. Al-Ramadi, M. Battino, Therapeutic and preventive properties of honey and its bioactive compounds in cancer: an evidence-based review, *Nutrition Research Reviews*, **33**(1), 50–76 (2020). https://doi.org/10.1017/S0954422419000192

62. R.U. Khan, S. Naz, A.M. Abudabos, Towards a better understanding of the therapeutic applications and corresponding mechanisms of action of honey, *Environmental Science and Pollution Research International*, **24**(36), 27755–27766 (2017). https://doi.org/10.1007/s11356-017-0567-0.

63. S.G. Bell, The therapeutic use of honey, *Neonatal Network*, **26**(4), 247–251 (2007). https://doi.org/10.1891/0730-0832.26.4.247.

64. M.A.I. Al-Hatamleh, J.C. Boer, K.L. Wilson, M. Plebanski, R. Mohamud, M.Z. Mustafa, Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions, *Biomolecules*, **10**(6), 923 (2020). https://doi.org/10.3390/biom10060923.

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Determinación del contenido de saponina y de proteína en genotipos de quinua (*Chenopodium quinoa* Willd) producidos en la costa central de Ecuador

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Resumen

Objetivo: Evaluar el contenido de proteínas y saponinas en genotipos de quinua es una práctica importante para definir su propósito de producción y poder ser comercializado en mercados que demanden por ejemplo quinuas amargas o dulces. Razón por la cual, este estudio tuvo por objeto determinar el contenido de estos componentes en 30 genotipos de quinua producidos en la costa central del Ecuador. **Metodología:** Para ello, se empleó un diseño experimental completamente al azar con treinta tratamientos y tres repeticiones, evaluándose variables como: altura de la columna de espuma (cm), contenido de saponina (%) y contenido de proteína (%). La metodología empleada para la obtención de datos del contenido de saponina fue mediante el método espuma de Koziol y para proteína se empleó el método Kjeldahl. **Resultados**: En cuanto a los resultados alcanzados, se registró un mayor

contenido de saponina en los tratamientos T19 y T30 con 0,86 y 0,78% respectivamente, mientras que, los tratamientos con menor contenido de saponina T23 y T26, ambos con 0,00%. En cuanto al contenido de proteína, los tratamientos con un mayor registro fueron T26 y T28 con 19,82 y 18,65% respectivamente y en contraparte, tratamientos como presentaron un bajo valor proteico fueron T20 con 12,23 y T5 con 13,54%. **Conclusión**: Finalmente, se concluye que el 76,67% de los genotipos se catalogaron como amargos y el 23,3% como dulces. Mientras que, en relación a la proteína se registró un promedio general de todos los genotipos de 16,19% lo cual en términos de calidad es relativamente aceptable.

Palabras clave: pseudocereal, metabolitos, alimentación, semillas, grano andino.

Summary

Determination of saponin and protein content in quinoa (*Chenopodium quinoa* Willd) genotypes produced on the central coast of Ecuador

Purpose: Evaluating the protein and saponin content in quinoa genotypes is an important practice to define their production purpose and to be able to be marketed in markets that demand, for example, bitter or sweet quinoa. For this reason, this study aimed to determine the content of these components in 30 quinoa genotypes produced in the central coast of Ecuador. **Methodology**: For this purpose, a completely randomized experimental design was used with thirty treatments and three replications, evaluating variables such as: height of the foam column (cm), saponin content (%) and protein content (%). The methodology used to obtain data on saponin content was the Koziol foam method and the Kjeldahl method was used for protein. **Results**: As for the results obtained, the highest saponin content was recorded in treatments T19 and T30 with 0.86 and 0.78%, respectively, while the treatments with the lowest saponin content were T23 and T26, both with 0.00%. In terms of protein content, the treatments with the highest protein content were T26 and T28 with 19.82 and 18.65%, respectively, and on the other hand, the treatments with the lowest protein content were T20 with 12.23 and T5 with 13.54%. **Conclusion**: Finally, it was concluded that 76.67% of the genotypes were classified as bitter and 23.3% as sweet. Meanwhile, in relation to protein, a general average of 16.19% was recorded for all genotypes, which in terms of quality is relatively acceptable.

Keywords: pseudocereal, metabolites, food, seeds, Andean grain.

Resumo

Determinação do teor de saponinas e proteínas em genótipos de quinoa (*Chenopodium quinoa* Willd) produzidos na costa central do Equador

Objetivo: Avaliar o teor de proteínas e saponinas em genótipos de quinoa é uma prática importante para definir sua finalidade de produção e poder ser comercializada em mercados que demandam, por exemplo, quinoas amargas ou doces. Por este motivo, este estudo teve como objetivo determinar o conteúdo destes componentes em 30 genótipos de quinoa produzidos na costa central do Equador. **Metodologia**: Para isso foi utilizado um delineamento experimental inteiramente casualizado com trinta tratamentos e três repetições, avaliando variáveis como: altura da coluna de espuma (cm), teor de saponinas (%) e teor de proteínas (%). A metodologia utilizada para obtenção dos dados sobre o teor de saponinas foi o método da espuma de Koziol e para a proteína foi utilizado o método de Kjeldahl. **Resultados**: Em relação aos resultados alcançados, foi registrado maior teor de saponina nos tratamentos T19 e T30 com 0,86 e 0,78% respectivamente, enquanto os tratamentos com menor teor de saponina T23 e T26, ambos com 0,00%. Em relação ao teor de proteína, os tratamentos com maior registro foram T26 e T28 com 19,82 e 18,65% respectivamente e em contrapartida, os tratamentos que apresentaram baixo valor de proteína foram T20 com 12,23 e T5 com 13,54%. **Conclusão**: Por fim conclui-se que 76,67% dos genótipos foram classificados como amargo e 23,3% como doce. Enquanto, em relação à proteína, foi registrada uma média geral de todos os genótipos de 16,19%, o que em termos de qualidade é relativamente aceitável.

Palavras-chave: pseudocereal, metabólitos, dieta, sementes, grão andino.

Introducción

El cultivo de quinua (*Chenopodium quinoa* Willd) tiene un gran potencial para contribuir con la seguridad alimentaria a nivel mundial. Posee una notable capacidad de adaptación a diferentes regiones agroecológicas y gradiente altitudinal debido a su plasticidad fenotípica. Los principales países productores de quinua en América Latina son: Perú, Bolivia y Ecuador; sin embargo, la producción se está expandiendo a otros continentes y actualmente se cultiva en varios países [1].

La quinua es considerada un súper alimento por sus excelentes propiedades nutricionales, entre las que destacan su alto contenido proteico, proporcionando un 16% de proteína por peso seco de quinua, que es más alta que la mayoría de los cereales [2] y comparable al de la leche [3]. Las proteínas de la quinua tienen niveles adecuados de aminoácidos aromáticos (fenilalanina y tirosina) y de contenidos de histidina, isoleucina, treonina, fenilalanina, tirosina y valina, de acuerdo con los requerimientos sugeridos por la FAO (Organización de las Naciones Unidas para la Agricultura y la Alimentación) – Organización Mundial de la Salud (OMS) para el consumo humano [4], asimismo, posee elementos traza y cantidades significativas de vitaminas [5].

No obstante, la quinua contiene saponinas, mismas que se localizan en la cáscara [6], y responsables de dar una percepción de amargor al gusto [7] si sobrepasan la concentración >0,11 [2]. Por ello, las semillas de quinua se procesan con la finalidad de reducir dicho amargor y ser empleadas para la preparación de diversos platillos gastronómicos y en la fabricación de productos alimenticios procesados [6], razón por la cual son más demandados los genotipos de quinuas dulces (<0,11), cuyo contenido de saponina es mínimo [8].

Por otra parte, es preciso indicar que un mayor contenido de saponinas también puede ser ventajoso para la industria farmacológica [7], debido a que presentan propiedades antibacteriana y antifúngica [9, 10] además de emplearse para la fabricación de productos para el cuidado del cabello como lo es el champú. Por lo cual, es imperativo estudiar genotipos que puedan formar parte de sistemas productivos [11] y que a su vez tengan propósitos definidos, es decir encaminados a la alimentación, con un menor contenido de saponinas y alto contenido de proteínas y para el aprovechamiento industrial y el consumo con un mayor contenido de saponinas.

Se han descrito a nivel mundial cinco ecotipos de quinua, de los cuales el ecotipo de los valles es el predominantemente producido y consumido en Ecuador. Existiendo poca información de la adaptación de quinua a zonas costeras o tropicales de Ecuador [12].

En virtud de lo anterior, este estudio tuvo por objeto evaluar el contenido de saponinas y proteína en 30 genotipos de quinua (*Chenopodium quinoa* Willd) cultivados en el Campus La María, que están siendo introducidas y adaptadas a las condiciones agroclimatológicas de la costa central ecuatoriana.

Metodología

Localización

La determinación del contenido de saponina y proteína de las muestras se realizó en el Laboratorio de Bromatología del Campus La María de la Universidad Técnica Estatal de Quevedo (UTEQ) del cantón Mocache, provincia de Los Ríos, Ecuador, ubicada en el km 7 ½ de la vía Quevedo – El Empalme, empleando 30 genotipos de distintos orígenes (Tabla 1) producidos y cosechados en el Campus entre 90 a 143 días posteriores a la siembra, durante la época lluviosa, específicamente entre los meses de septiembre/2017 y enero/2018. Las condiciones agroclimáticas presentes en el lugar donde se cultivaron los genotipos se muestran en la Tabla 2.

Tabla 1. Genotipos de quinua y sus orígenes

Tratamientos	Genotipos	Origen
T1	26	Chile
T2	36	Chile
T3	41	Chile
T4	42	Chile
T5	48	Chile
T6	49	Chile
T7	52	Chile
T8	54	Chile
T9	0-1	Chile
T10	0-2	Chile
T11	0-3	Chile
T12	0-4	Chile
T13	0-5	Chile
T14	0-6	Chile
T15	0-7	Chile
T16	0-8	Chile
T17	0-9	Chile
T18	0-10	Chile
T19	J4	Argentina
T20	FARO	Chile
T21	REG	Chile
T22	P.V	Ecuador
T23	DULCE	Chile

(*Continúa*)

Tratamientos	Genotipos	Origen
T24	0-10M	Chile
T25	J4-010	Chile
T26	TUNK	Ecuador
T27	X2	Chile
T28	X3	Chile
T29	X4	Chile
T30	X5	Chile

Tabla 2. Condiciones agroclimáticas del cantón Mocache

Parámetros	Promedio
Temperatura promedio °C	25,47
Humedad relativa, %	85,84
Precipitación, anual. mm	2223,85
Heliofanía, horas luz /año	898,66
Zona ecológica	bh – T
Topografía	Ligeramente Ondulada

Fuente: Departamento Agrometeorológico del Instituto Nacional de Investigaciones Agropecuarias (INIAP). Estación Experimental Tropical Pichilingue (EETP).

Diseño experimental y análisis estadístico

Los tratamientos (genotipos) estuvieron dispuestos en un diseño completamente al azar (DCA) con tres repeticiones. Para el análisis de los datos se realizó un análisis de varianza (ANOVA) y para el análisis de las medias entre tratamientos se realizó la prueba de Tukey ($P \leq 0,05$), utilizando el programa estadístico Infostat. Dicho análisis se aplicó a todas las variables evaluadas.

Determinación del contenido de saponina

Para la determinación del contenido de saponina se realizó el método espuma de Koziol establecido en la Norma INEN [13], basada en las propiedades tensoactivas de las saponinas, que al disolverse en agua y agitarse, generan una espuma estable, cuya altura puede correlacionarse con el contenido de saponinas en los granos. Se realizó pesando 0,50 ±0,02 g enteros de quinua y se colocaron en un tubo de ensayo. Se añadió 5,0 mL de agua destilada y tapó el tubo. Se puso en marcha el cronómetro y sacudió vigorosamente el tubo durante 30 segundos. Se dejó el tubo en reposo durante 30 minutos, luego se sacudió otra vez durante 30 segundos. Se repite el proceso. Finalmente se dejó el tubo en reposo 5 minutos, luego se midió la altura de la espuma al 0,1 cm más cercano [14].

Para determinar el porcentaje de saponinas en los distintos genotipos de quinua se empleó la siguiente ecuación:

$$Ps = \frac{(0{,}646 \cdot h) - 0{,}104}{m \cdot 10}$$

Siendo:

Ps = porcentaje de saponina

h = altura de la espuma en cm

m = masa de la muestra, en gramos

Determinación del contenido de proteína por el método Kjeldahl [15]

Preparación de la muestra: Se molió 100 g de muestra en un micro molino que contenga un tamiz de abertura de 1 mm. Se transfirió rápidamente la muestra molida y homogenizada a un recipiente herméticamente cerrado, hasta el momento del análisis. Se homogenizó la muestra interviniendo varias veces el recipiente. El procedimiento a continuación:

Digestión: Se pesó aproximadamente 0,3 g de muestra sobre un papel exento de nitrógeno y colocó sobre un microtubo digestor. Se añadió al microtubo una tableta catalizadora y 5 mL de ácido sulfúrico concentrado. Se colocó los tubos de digestión con las muestras en el *block digest* con el colector de humus funcionando.

La digestión se realizó a una temperatura de 350 a 400 °C y un tiempo de 2 horas. Al finalizar la muestra oscura se transforma en una solución translúcida. Se dejó enfriar la muestra a temperatura ambiente. Se evitó la precipitación agitando de vez en cuando.

Destilación: En cada microtubo adicionar 15 mL de agua destilada. Se colocó el microtubo y el matraz de recepción con 50 mL de ácido bórico al 2% en el sistema de destilación Kjeltec.

Se encendió el sistema y la adición de 30 mL de hidróxido de sodio al 40%. Se recogió aproximadamente 200 mL de destilado, se retiró del sistema los accesorios. Titulación: Del destilado recogido en el matraz se colocó tres gotas de indicador. Se tituló con ácido clorhídrico 0,1 N utilizando un agitador mecánico. Se registró el volumen de ácido consumido.

El porcentaje de proteína se obtuvo mediante el método de Kjeldahl (método directo) bajo la siguiente formula:

$$\%PB = \frac{(VHCl - Vb) \cdot 1{,}401 \cdot NHCl}{g.muestra} \cdot F$$

Siendo:

14,01 = Peso atómico del nitrógeno.

NHCl = Normalidad de Ácido Clorhídrico 0,1 N.

F = Factor de conversión.

VHCl = Volumen del ácido clorhídrico consumido en la titulación.

Vb = Volumen del Blanco (0,01).

Análisis estadístico

Para el análisis de los datos se realizó un análisis de varianza (ANOVA) y para el análisis de las medias entre tratamientos se realizó la prueba de Tukey (P≤0,05), utilizando el programa estadístico Infostat.

Resultados

Altura de la columna de espuma en genotipos de quinua.

Para la altura de la columna de espuma existió una alta significancia estadística (p<0,05). Los genotipos que presentaron los menores promedios fueron T26 y T23 con 0,00 cm; mientras que, el mayor promedio lo obtuvo el T20 con 6,83 cm. (Tabla 3).

Tabla 3. Promedios de la altura de la columna de espuma en genotipos de quinua.

Tratamientos	Genotipos	Altura de la columna de espuma (cm)							
T1	26	0,77	a	b					
T2	36	4,17				d	e		
T3	41	1,93	a	b	c				
T4	42	1,50	a	b	c				
T5	48	2,03		b	c				
T6	49	1,60	a	b	c				
T7	52	1,07	a	b	c				
T8	54	1,50	a	b	c				
T9	0-1	1,37	a	b	c				

(*Continúa*)

Tratamientos	Genotipos	Altura de la columna de espuma (cm)							
T10	0-2	4,80					e	f	
T11	0-3	0,90	a	b	c				
T12	0-4	1,50	a	b	c				
T13	0-5	1,43	a	b	c				
T14	0-6	1,30	a	b	c				
T15	0-7	1,43	a	b	c				
T16	0-8	2,13		b	c				
T17	0-9	0,97	a	b	c				
T18	0-10	1,53	a	b	c				
T19	J4	2,17		b	c				
T20	FARO	6,83							g
T21	REG	5,50					e	f	g
T22	P.V	6,13						f	g
T23	DULCE	0,00	a						
T24	0-10M	6,07			c		e	f	g
T25	J4-010	0,77	a	b					
T26	TUNK	0,00	a						
T27	X2	2,43		b	c	d			
T28	X3	4,30				d	e	f	
T29	X4	2,83			c	d			
T30	X5	6,23						f	g
C.V (%)		24,08							
Probabilidad (p<0,05)		0,0001**							

Letras iguales no difieren estadísticamente Tukey (p≤0,05). C.V: Coeficiente de variación, NS: No significativo; *Significativo, **Alta significancia.

Contenido de saponina (%)

En el contenido de saponina se registró una alta significancia estadística entre tratamientos (p<0,05). Los tratamientos: T1, T7, T11, T17, T18, T23, T25 y T26 presentaron un contenido por debajo del 0,12%. Mientras que T19 obtuvo el contenido más alto de saponina con 0,86% (Tabla 4).

Tabla 4. Promedios de contenido de saponina (%) en genotipos de quinua.

Tratamientos	Genotipos	Contenido de saponina (%)									
T1	26	0,08							g	h	i

(*Continúa*)

Tratamientos	Genotipos	Contenido de saponina (%)									
T2	36	0,52			c	d					
T3	41	0,23					e	f	g		
T4	42	0,17					e	f	g	h	i
T5	48	0,24					e	f	g		
T6	49	0,18					e	f	g	h	
T7	52	0,12						f	g	h	i
T8	54	0,17					e	f	g	h	i
T9	0-1	0,16						f	g	h	i
T10	0-2	0,60		b	c						
T11	0-3	0,10							g	h	i
T12	0-4	0,17					e	f	g	h	i
T13	0-5	0,16						f	g	h	i
T14	0-6	0,15						f	g	h	i
T15	0-7	0,16						f	g	h	i
T16	0-8	0,26					e	f	g		
T17	0-9	0,11							g	h	i
T18	0-10	0,02								h	i
T19	J4	0,86	a								
T20	FARO	0,69	a	b	c						
T21	REG	0,73	a	b							
T22	P.V	0,77	a	b							
T23	DULCE	0,00									i
T24	0-10M	0,76	a	b							
T25	J4-010	0,08							g	h	i
T26	TUNK	0,00									i
T27	X2	0,29					e	f			
T28	X3	0,54			c						
T29	X4	0,35				d	e				
T30	X5	0,78	a								
C.V (%)		17,86									
Probabilidad (p<0,05)		0,0001**									

Letras iguales no difieren estadísticamente Tukey (p≤0,05). C.V: Coeficiente de variación, NS: No significativo; *Significativo, **Alta significancia

Clasificación de los genotipos en base al contenido de saponina

De acuerdo con los resultados de los treinta genotipos evaluados el 23,3% son dulces, es decir estuvieron por debajo del 0,11% de contenido de saponina. Mientras que, en contraparte, el 76,67% correspondió a la categoría de amargo, dado que sobrepasaron el contenido de 0,11% (Tabla 5).

Tabla 5. Clasificación de los genotipos de quinua producidos en el Campus La María.

Tratamientos	Genotipos	Dulce < 0,11%	Amargo > 0,11%
T1	26	0,08	
T2	36		0,52
T3	41		0,23
T4	42		0,17
T5	48		0,24
T6	49		0,18
T7	52		0,12
T8	54		0,17
T9	0-1		0,16
T10	0-2		0,60
T11	0-3	0,10	
T12	0-4		0,17
T13	0-5		0,16
T14	0-6		0,15
T15	0-7		0,16
T16	0-8		0,26
T17	0-9	0,11	
T18	0-10	0,02	
T19	J4		0,86
T20	FARO		0,69
T21	REG		0,73
T22	P.V		0,77
T23	DULCE	0,00	
T24	0-10M		0,76
T25	J4-010	0,08	
T26	TUNK	0,00	
T27	X2		0,29
T28	X3		0,54
T29	X4		0,35
T30	X5		0,78

Contenido de proteína (%)

El porcentaje de presentó alta significancia estadística entre tratamientos ($p<0,05$). El T23 presentó el mayor promedio con 19,82% el cual es estadísticamente igual al T27 con 18,65% de proteína en los granos de quinua. El T20 registró el menor promedio con 12,23% (Tabla 6).

Tabla 6. Promedios de porcentaje de proteína en genotipos de quinua.

Tratamientos	Genotipos	Porcentaje de proteína (%)							
T1	26	15,88		b	c	d	e	f	
T2	36	18,07	a	b	c				
T3	41	15,73		b	c	d	e	f	
T4	42	15,73		b	c	d	e	f	
T5	48	13,54						f	g
T6	49	16,75		b	c	d	e		
T7	52	16,46		b	c	d	e	f	
T8	54	16,61		b	c	d	e		
T9	0-1	16,61		b	c	d	e		
T10	0-2	14,13					e	f	g
T11	0-3	15,44			c	d	e	f	
T12	0-4	16,32		b	c	d	e	f	
T13	0-5	17,48	a	b	c	d			
T14	0-6	15,29			c	d	e	f	
T15	0-7	16,46		b	c	d	e	f	
T16	0-8	14,56				d	e	f	g
T17	0-9	14,71				d	e	f	g
T18	0-10	16,02		b	c	d	e	f	
T19	J4	16,75		b	c	d	e		
T20	FARO	12,23							g
T21	REG	15,29			c	d	e	f	
T22	P.V	17,05	a	b	c	d	e		
T23	DULCE	18,07	a	b	c				
T24	0-10M	15,15			c	d	e	f	g
T25	J4-010	17,48	a	b	c	d			
T26	TUNK	19,82	a	b					
T27	X2	15,44			c	d	e	f	
T28	X3	18,65	a	b					
T29	X4	17,78	a	b					
T30	X5	16,17		b	c	d	e	f	
C.V (%)		5,88							
Probabilidad (p<0,05)		0,0001**							

Letras iguales no difieren estadísticamente Tukey (p≤0,05). C.V: Coeficiente de variación, NS: No significativo; *Significativo, **Alta significancia.

Discusión

Cuando las muestras de quinua se disuelven en agua y se agitan, las saponinas generan una espuma estable, cuya altura puede correlacionarse con el contenido de saponina en los granos [16], demostrando que existe excelente correlación entre la altura de la columna de espuma y el contenido de saponina [2, 17].

El T26 (TUNK), que es una variedad registrada en Ecuador como quinua de bajo contenido de saponina [18], presentó valores de 0,00% empleando el método de Koziol, contenido de saponina al igual a lo reportado en otras investigaciones [12, 19], quienes señalan un 0,03% para contenido de saponinas. El T22 (genotipo P.V) dio como resultado 0,77% (clasificado como quinua amarga), contrastando con lo reportado por el Instituto Nacional de Investigaciones Agropecuarias (INIAP), quienes liberaron esta variedad como una quinua de grano dulce [20]. Este resultado, podría estar influenciado con el manejo inadecuado de la semilla por parte de los agricultores y el flujo de genes existente con quinuas silvestres de grano amargo contiguas a las plantaciones de la variedad P.V. (Eduardo Peralta, investigados del INIAP, comunicación persona).

Mediante el método afrosimétrico, las quinuas que contienen 0,11% de saponinas o menos en base al peso fresco, pueden considerarse dulces [16]. En base a aquello, se pudo definir que la mayoría de los genotipos utilizados en esta investigación se catalogan como amargos (76,67%). El promedio del contenido de saponina de los genotipos de quinua producidos en el Campus La María es de 0,32% por lo cual [21], se clasifican como granos de contenido amargo que van desde 0,12 a 1%. La cáscara de aquellos genotipos considerados amargos suele ser muy útiles en la elaboración de productos y subproductos de la industria farmacéutica [6].

Es preciso indicar que, el hecho de que un genotipo de quinua sea amargo, no significa que no sirva para el consumo humano, ya que para eliminar el amargor de los granos es preciso llevar a cabo un proceso de desaponificación, que consiste en la eliminación de la capa delgada que se encuentra en la periferia del grano mediante el lavado y/o enjuagado constante, de tal manera que el contenido de saponinas en la quinua sin lavar puede ser de 0,24%, mientras que para la quinua desaponificada solo el 0,01% [22].

El porcentaje de proteína en los granos obtenidos por los distintos tratamientos en este estudio fluctuó entre el 12,33 y el 19,82%. Lo cual, podría considerarse dentro del promedio, ya que en rasgos generales el contenido de proteína de los granos de quinua puede a llegar a oscilar entre el 8% al 22%, valores mayores en comparación a cereales comunes como el arroz, el trigo y la cebada [12, 23].

Es preciso indicar que, si la quinua presenta un contenido de proteína entre 17,1 a 18,5% se podría considerar una fuente importante para el desarrollo de productos alimenticios [24, 25]. La capacidad de la quinua para producir proteínas de gran calidad nutricional, bajo condiciones ecológicamente extremas [12, 26], la convierte en una planta importante no sólo para la alimentación de las comunidades andinas, sino también para diversificación de los sistemas agrícolas futuros, siendo un cultivo con alto potencial para ser explotado en el litoral ecuatoriano.

Las condiciones que favorecen los altos porcentajes de proteína y saponina son: la especie y el ecotipo, es decir la genética, y la fertilización nitrogenada que se aplique al suelo [27]. De manera específica, llama la atención los resultados obtenidos tanto para saponina y proteínas en los genotipos TUNK y Dulce, quienes presentan un bajo contenido de saponinas y alto contenido de proteína. Estos resultados podrían estar relacionados con aspectos involucrados con el uso del nitrógeno para generar cada una de dichas estructuras [28, 29]. Un mayor contenido de proteína y saponina está relacionado con un mayor uso de nitrógeno. No obstante, contenido de saponinas [27] está correlacionado con un mayor uso de nitrógeno. No obstante, la genética propia de cada genotipo condiciona la traslocación de nutrientes, haciendo que un mayor porcentaje de nitrógeno se destine para la formación de proteínas que para la formación de saponinas [30, 31]. Razón por la cual podría deberse a que, si un genotipo posee alto contenido de proteínas, poseería un bajo nivel de saponinas, dado que las dos estructuras emplean nitrógeno. Aunque también, un bajo contenido de proteínas está relacionado con una mayor producción de aceites [32], aspecto que no fue evaluado en este trabajo.

Conclusiones

Se logró identificar los genotipos dulces y amargos, correspondiendo siete de ellos a la categoría de dulces lo cual representa el 23,33% del total. Mientras que, en contraparte 23 genotipos se categorizaron como amargos, lo cual representa el 76,67% del total. El contenido proteico de las semillas de quinua en promedio dio como resultado 16,19% lo cual en términos de calidad es relativamente aceptable.

Conflicto de interés

Los autores declaran no presentar ningún conflicto de interés.

Referencias

1. H.A. Castro-Albán, R.d.P. Castro-Gómez, Y. Alvarado-Capó, Variabilidad morfoagronómica de quinua (*Chenopodium quinoa* Willd.) nativa tipo Chimborazo en Ecuador, *Agronomía Mesoamericana*, **34**(3), 53229 (2023).

2. A.C. Morillo, E.H. Manjarres, M.S. Mora, Afrosymetric method for quantifying saponins in *Chenopodium quinoa* Willd. from Colombia, *Brazilian Journal of Biology*, **82**, e262716 (2022).

3. A. Agarwal, Rizwana, A.D. Tripathi, T. Kumar, K.P. Sharma, S.K.S. Patel, Nutritional and functional new perspectives and potential health benefits of quinoa and chia seeds, *Antioxidants* (Basel), **12**(7), 1413 (2023).

4. J. Campos, K. Acosta, L. Paucar, Quinoa (*Chenopodium quinoa*): Nutritional composition and bioactive compounds of grain and leaf, and impact of heat treatment and germination, *Scientia Agropecuaria*, **13**(3), 209–220 (2022).

5. D.E. Jarvis, Y.S. Ho, D.J. Lightfoot, S.M. Schmöckel, B. Li, T.J.A. Borm, *et al.*, The genome of *Chenopodium quinoa*, *Nature*, **542**(7641), 307–312 (2017).

6. A. Ahumada, A. Ortega, D. Chito, R. Benítez, Saponinas de quinua (*Chenopodium quinoa* Willd.): un subproducto con alto potencial biológico, *Revista Colombiana de Ciencias Químico-Farmacéuticas*, **45**(3), 438–469 (2016).

7. K. El Hazzam, J. Hafsa, M. Sobeh, M. Mhada, M. Taourirte, K.E.L. Kacimi, *et al.*, An insight into saponins from Quinoa (*Chenopodium quinoa* Willd): A review, *Molecules*, **25**(5), 1059 (2020).

8. C. Salcedo, E. Peralta, C. Subía, M. Rivera, N. Mazón, Evaluación de 18 líneas de quinua de bajo contenido de saponina en dos localidades de la provincia de Pichincha en Ecuador, en: *Informe Anual de Subproyectos 2003*, Preduza, Quito, 2004, p. 135–143. URL: https://repositorio.iniap.gob.ec/handle/41000/3177

9. R. Apaza, H. Smeltekop, Y. Flores, G.A. Ii, L. Salcedo, Efecto de saponinas de *Chenopodium quinoa* Willd contra el fitopatógeno *Cercospora beticola* Sacc, *Revista de Protección Vegetal*, **31**(1), 63–69 (2016).

10. C. López-Sánchez, C.Y. González-Martínez, M.D. Méndez-López, F.D.J. Palma-Cruz, Las saponinas y su uso farmacéutico, *Revista del Centro de Graduados e Investigación. Instituto Tecnológico de Mérida*, **37**(96), 120–126 (2022).

11. A. Delgado, J. Palacios, C. Betancourt, Evaluación de 16 genotipos de quinua dulce (*Chenopodium quinoa* Willd.) en el municipio de Iles, Nariño (Colombia), *Agronomía Colombiana*, **27**(2), 159–167 (2009).

12. L. Hinojosa, A. Leguizamo, C. Carpio, D. Muñoz, C. Mestanza, J. Ochoa, *et al.*, Quinoa in Ecuador: Recent advances under global expansión, *Plants* (Basel), **10**(2), 298 (2021). URL: https://www.mdpi.com/2223-7747/10/2/298

13. Instituto Ecuatoriano de Normalización, *Quinua. Determinación del contenido de saponinas por medio del método espumoso (método de rutina)*, Norma INEN 1672, Quito, Ecuador, 1988. URL: https://ia801400.us.archive.org/25/items/ec.nte.1672.1988/ec.nte.1672.1988.pdf

14. M.J. Koziol, Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (*Chenopodium quinoa* Willd), *Journal of the Science of Food and Agriculture*, **54**(2), 211–219 (1991).

15. J. Kjeldahl, Neue methode zur bestimmung des stickstoffs in organischen körpern [New method for the determination of nitrogen in organic substances], *Zeitschrift für Analytische Chemie*, **22**(1), 366–383 (1883).

16. M. Koziol, Chemical composition and nutritional evaluation of quinoa (*Chenopodium quinoa* Willd.), *Journal of Food Composition and Analysis*, **5**(1), 35–68 (1992).

17. M.S. Mora-Ocación, A.C. Morillo-Coronado, E.H. Manjarres-Hernández, Extraction and quantification of saponins in Quinoa (*Chenopodium quinoa* Willd.) genotypes from Colombia, *International Journal of Food Science*, **2022**, 7287487 (2022).

18. C. Nieto, C. Vimos, C. Monteros, C. Caicedo, M. Rivera, *Obtención de dos variedades de quinua de bajo contenido de saponina, para la Sierra ecuatoriana*, Plegable 228, Estacion Experimental Santa Catalina, INIAP, Quito, Ecuador, 1992, 24 p.

19. R. Lozano, I.L. Tapia, V.J. Taco, Evaluación de las propiedades funcionales del aislado proteico de quinua (*Chenopodium quinoa* Willd) variedad INIAP-Tunkahuan con potencial uso en la nutrición humana, *Revista de la Facultad de Ciencias Médicas*, **44**(1), 48–56 (2019).

20. N. Mazón, E. Peralta, C. Monar, C. Subía, M. Rivera, *"Pata de Venado" (Tarhua chaqui). Nueva variedad de quinua, precoz y de grano dulce*, Plegable 261, Estacion Experimental Santa Catalina, INIAP, Quito, Ecuador, 2005. p. 2.

21. R. Escalera-Vásquez, C. Quiroga-Ledezma, L. Arteaga-Weill, Evaluación de la calidad nutricional y morfología del grano de variedades amargas de quinua beneficiadas en seco, mediante el novedoso empleo de un reactor de lecho fluidizado de tipo surtidor, *Investigación & Desarrollo*, **10**(1), 32–48 (2010).

22. R. Castañeda, M.J. Andrade-Cuvi, Y. Argüello, M.G. Vernaza, Efecto de la adición de quinua (*Chenopodium quinoa* wild) malteada y sin maltear en la elaboración de cerveza tipo Ale a base de cebada (*Hordeum vulgare*) malteada, *Enfoque UTE*, **9**(2), 15–26 (2018).

23. I. Tabatabaei, S. Alseekh, M. Shahid, E. Leniak, M. Wagner, H. Mahmoudi, *et al.*, The diversity of quinoa morphological traits and seed metabolic composition, *Scientific Data*, **9**(1), 323 (2022).

24. N. Maldonado-Taipe, F. Barbier, K. Schmid, C. Jung, N. Emrani, High-density mapping of quantitative trait loci controlling agronomically important traits in Quinoa (*Chenopodium quinoa* Willd.), *Frontiers in Plant Science*, **13**, 916067 (2022).

25. E. Villacrés, M. Quelal, S. Galarza, D. Iza, E. Silva, Nutritional value and bioactive compounds of leaves and grains from Quinoa (*Chenopodium quinoa* Willd.), *Plants* (Basel), **11**(2), 213 (2022).

26. A.C. Morillo-Coronado, M.A. Castro-Roberto, Y. Morillo-Coronado, Caracterización de la diversidad genética de una colección de quinua (*Chenopodium quinoa* Willd), *Biotecnología en el Sector Agropecuario y Agroindustrial*, **15**(2), **49** (2017).

27. D. Bilalis, I. Kakabouki, A. Karkanis, I. Travlos, V. Triantafyllidis, D. Hela, Seed and saponin production of organic quinoa (*Chenopodium quinoa* Willd.) for different tillage and fertilization, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **40**(1), 42–46 (2012).

28. T. Moses, K.K. Papadopoulou, A. Osbourn, Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives, *Critical Reviews in Biochemistry and Molecular Biology*, **49**(6), 439–462 (2014).

29. S.N. Thanapornpoonpong, S. Vearasilp, E. Pawelzik, S. Gorinstein, Influence of various nitrogen applications on protein and amino acid profiles of amaranth and quinoa, *Journal of Agriculture and Food Chemistry*, **56**(23), 11464–11470 (2008).

30. Å Grimberg, G.V. Saripella, R.A.M. Repo-Carrasco-Valencia, T. Bengtsson, G. Alandia, A.S. Carlsson, Transcriptional regulation of quinoa seed quality: Identification of novel candidate genetic markers for increased protein content, *Frontiers in Plant Sciences*, **13**, 816425 (2022).

31. S. Granado-Rodríguez, N. Aparicio, J. Matías, L.F. Pérez-Romero, I. Maestro, I. Gracés, *et al.*, Studying the impact of different field environmental conditions on seed quality of Quinoa: The case of three different years changing seed nutritional traits in Southern Europe, *Frontiers in Plant Sciences*, **12**, 649132 (2021).

32. O. Rosero, M. Marounek, N. Břeňová, D. Lukešova, Actividad de la fitasa y comparación en la composición química, contenido de ácido fítico en cuatro variedades de quinua (*Chenopodium quinoa* Willd.), *Acta Agronómica*, **62**(1), 13–20 (2013).

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Eficacia de la pomada de caléndula 20 % en el tratamiento de la psoriasis

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Resumen

Introducción: El tratamiento tópico en el manejo de la psoriasis es primera opción en la psoriasis leve y leve/moderadas y como terapia complementaria en la psoriasis grave o intensa. La *Calendula officinalis* L. tiene propiedades antiinflamatoria, cicatrizante y antibacteriana. **Objetivo**: Determinar la eficacia de la pomada de caléndula al 20% en el tratamiento de la psoriasis. **Metodología**: Se realizó un estudio cuasi experimental en 40 pacientes del servicio de dermatología del Hospital Juan Bruno en el periodo enero - mayo del 2019, divididos en grupo control (pomada sin caléndula) y grupo estudio (pomada de caléndula al 20 %), se clasificaron las lesiones en primera semana y se evaluó evolución al concluir quinta y décima semana, finalmente se evaluó la respuesta al tratamiento. Resultados: En la quinta semana el 55 %, grupo estudio, presentaban escamas ligero mientras que en grupo control predominó intenso (75 %) el eritema en grupo estudio con igual frecuencia (35 %) para ligero y moderado, mientras que en grupo control predominó intenso (75 %); en la décima semana en grupo estudio, las escamas entre ausente y ligero representó 80 % y 75 % eritema, mientras en grupo control las ligero y ausente 15%, tanto para escamas como eritema; el tratamiento resultó eficaz en el 80 % de los pacientes del grupo estudio a diferencia del grupo control donde la respuesta excelente fue en un 15 %. **Conclusión**: La pomada de caléndula al 20 % es eficaz para el tratamiento de la psoriasis y logra alcanzar la eficacia máxima entre la quinta y decima semana de tratamiento.

Palabras clave: Pomada de caléndula, psoriasis, mácula, eritema, escamas, eficacia

Summary

Efficacy of marigold pomade to 20% in the treatment of psoriasis

Introduction: The external use treatment in the handling of psoriasis is first option in the light and light moderated psoriasis and as complementary therapy in severe or intense psoriasis. *Calendula officinalis* L. has anti-inflammatory, healing and antibacterial properties. **Objective:** To determine the Efficacy of marigold pomade to 20 % in the treatment of psoriasis. **Methodology:** A quasi experimental study was carried out in 40 patients from the dermatology service at Juan Bruno Zayas Hospital in the period corresponding to January - May of 2019; divided in a control group (pomade without Marigold) and a study group (Marigold pomade to 20 %). Injuries in the first week were classified and evolution was assessed when concluding fifth and tenth weeks. Finally, response to treatment was also assessed. **Results:** In the fifth week the 55 % of patients from study group showed light flakes while in the control group predominated intense erythema (75 %) and in the study group with equal frequency (35 %) to light and moderated, while the control group predominated intense (75 %). In the tenth week in the study group, flakes between absentee and light represented 80 % and 75 % of erythema; while in the control group the light and missing 15 %, as for flakes as for erythema. The treatment proved to be effective in 80 % of the patients from the study group, in contrast to the control group where the excellence response was in 15 %. **Conclusion:** Marigold pomade to 20 % is effective for the treatment of psoriasis and succeeds in attaining the maximum efficiency among the fifth and tenth weeks of treatment.

Keywords: Marigold Pomade, psoriasis, sunspot, erythema, efficacy, flakes

Resumo

Eficácia da pomada de calêndula 20% no tratamento da psoríase

Introdução: O tratamento tópico no manejo da psoríase é a primeira opção na psoríase leve e leve/moderada e como terapia complementar na psoríase grave ou intensa. *Calendula officinalis* L. possui propriedades antiinflamatórias, cicatrizantes e antibacterianas. **Objetivo:** Determinar a eficácia da pomada de calêndula 20 % no tratamento da psoríase. **Metodologia:** Foi realizado um estudo quase experimental com 40 pacientes do serviço de dermatologia do Hospital Juan Bruno no período de janeiro a maio de 2019, divididos em grupo controle (pomada sem calêndula)

e grupo de estudo (pomada de calêndula 20 %). As lesões foram classificadas na primeira semana e a evolução avaliada ao final da quinta e décima semanas e, por fim, foi avaliada a resposta ao tratamento. **Resultados:** Na quinta semana, 55 % do grupo de estudo apresentou escamas leves, enquanto no grupo controle predominou eritema intenso (75 %) no grupo de estudo com igual frequência (35 %) para leve e moderado, enquanto no grupo controle predominou o grupo intenso (75 %); na décima semana no grupo estudo as escalas entre ausente e leve representaram 80 % e 75 % de eritema, enquanto no grupo controle as escalas leve e ausente representaram 15 %, tanto para escamas quanto para eritema. O tratamento foi eficaz em 80% dos pacientes do grupo de estudo, diferentemente do grupo controle onde a resposta excelente foi de 15 %. **Conclusão:** A pomada de calêndula 20% é eficaz no tratamento da psoríase e atinge eficácia máxima entre a quinta e a décima semana de tratamento.

Palavras-chave: Pomada de calêndula, psoríase, mácula, eritema, escamas, eficácia

Introducción

La psoriasis es una enfermedad inflamatoria crónica de la piel que se caracteriza por lesiones escamosas y eritematosas en la misma, la prevalencia es variable en diversas partes del mundo, oscila ordinariamente entre un 1 y 3 % de la población, se presentan en todas las latitudes, es propia de todo sexo, ocupación y se observa con menos frecuencia en la raza negra [1].

La historia de la humanidad ha sido testigo de los efectos curativos de las plantas. Por eso en el siglo XXI la rigurosidad científica nos obliga a una visión más exacta y medible de la eficacia y seguridad de las drogas en la práctica médica [2].

En la actualidad el tratamiento tópico en el manejo de psoriasis, continúa representando un pilar fundamental en la psoriasis leve y leve/moderadas y como terapia complementaria en la psoriasis grave o intensa, las cremas esteroideas o corticoesteroides, por su capacidad antiinflamatoria y antiproliferativa constituyen la primera opción [3].

Estudios químicos a la *Calendula officinalis* L (caléndula) arrojan que esta posee numerosas familias químicas entre las que sobresalen los carotenoides, los flavonoides del tipo β llamados flavonas o xantonas, los triterpenos, las saponinas, los ácidos fenólicos y taninos, las coumarinas, los polisacáridos, las sustancias pectídicas, las hemicelulosas, aceites esenciales o volátiles, etc. [2, 4-7]; lo cual le da la propiedad antinflamatoria, acción farmacológica que está valorada experimentalmente en piel y mucosa [8, 9].

Otros beneficios de la caléndula descritos, son su poder de hidratar, suavizar y reparar la piel debido a su contenido de betacarotenoides primarios como son la luteína, zeaxantinas y licopenos todos precursores de la vitamina A; ayuda en la producción de colágeno por lo que estimula que la regeneración de la piel sea más rápida y por otra parte los componentes terpénicos junto a los fitoesteroles y el ácido salicílico otorgan a la piel protección y nutrición [10-13].

Por todo lo anterior la selección de nuestra planta, en concordancia con la variedad de propiedades farmacológicas que presenta, basado en los conocimientos etnobotánicos y estudios experimentales revisados, justificando que la misma es medicinal, oficial y reconocida por la Farmacopea de los EE. UU, España y estar aprobada por el Ministerio de Salud Publica en Cuba.

En la actualidad muchos especialistas centran sus aplicaciones en su uso tópico para diferentes lesiones cutáneas como eccemas, erupciones, úlceras, quemaduras, pieles agrietadas, picaduras de insectos e inflamaciones además de lesiones inflamatorias de la mucosa oral, rectal y vaginal; pero no se describe su utilidad en inflamaciones crónicas de piel como la psoriasis, donde por la características de la enfermedad, en la que el tratamiento estaría dirigido a espaciar los brotes y retardar su aparición, la utilización de la caléndula podría constituir una excelente opción terapéutica debido a sus acciones farmacológicas y a su perfil de seguridad [6, 13, 14].

Teniendo en cuenta lo antes expuesto sobre las características de las lesiones de la psoriasis, las propiedades antiinflamatoria, cicatrizante y antibacteriana de la *Calendula officinalis* L. y el hecho de existir pocas investigaciones sobre el tema nos motivamos a la realización de este estudio con el objetivo de determinar la eficacia de la pomada de caléndula al 20 % en el tratamiento de la psoriasis.

Metodología

Se realizó un estudio cuasi experimental prospectivo en 40 pacientes diagnosticados con psoriasis, tratados en el servicio de dermatología del Hospital Clínico Quirúrgico Juan Bruno Zayas, de Santiago de Cuba, en el periodo comprendido de enero a abril del 2019.

Asignación de los tratamientos

Se conformaron dos grupos de tratamiento con 20 pacientes cada uno, el grupo I Tratamiento control y el grupo II Tratamiento estudio, asignándose al azar los tratamientos, de forma paralela partiendo de una lista única al que se asignó a los pacientes los

frascos rotulados con el código de identificación según la asignación de tratamiento establecida previamente por los investigadores, los médicos y pacientes desconocían el tratamiento.

Selección de sujetos

Criterio diagnóstico o universo de estudio:

- Pacientes con diagnóstico de psoriasis.

Criterio de inclusión:

- Paciente de 18 hasta 60 años, de cualquier sexo.
- Presencia de lesiones con no más de dos meses de aparición del nuevo brote o cuadro inicial.
- Extensión de las lesiones hasta el 10% de superficie corporal afectada

Criterios de exclusión:

- Paciente que se niegue a participar o no cumplan criterios de inclusión.
- Embarazo o lactancia.
- Pacientes bajo tratamiento con esteroides o citostáticos, tópico o sistémico, durante las cuatro últimas semanas antes del estudio.
- Enfermedades infecciosas agudas.
- Paciente con alguna condición que impida adherencia al tratamiento

Criterios de salida:

- Pacientes que abandonen el tratamiento

Tratamiento

Formaron parte del grupo I control aquellos que recibieron la pomada sin principio activo, placebo, durante 10 semanas y el grupo II los que recibieron el tratamiento con el fitofármaco pomada de caléndula al 20 %, durante 10 semanas, administrado de forma tópica 2 veces al día.

Producto control o Placebo

Se utilizó un ungüento inerte, formulación con iguales características organolépticas que el producto a evaluar e igual presentación para evitar sesgos por efecto psicológico

Producto a evaluar

El fitofármaco caléndula 20 % para uso tópico se presenta en forma de pomada dérmica que como base se utiliza la oleaginosa, vaselina, lanolina y partiendo del extracto fluido se obtuvo el extracto blando 1:4 con un 20 % del principio activo (flor de caléndula). Esta concentración se escogió partiendo de las dosis dadas por la Norma Ramal de Salud Pública (NRSP 323) [15].

Para determinar la intensidad de las lesiones eritemato-escamosa se confeccionó una escala por parte del equipo investigador y a partir de las revisiones realizadas donde se consideró a partir de los principales índices descritos por el grupo de trabajo de Psoriasis AEDV. Los más utilizados son GPs, PASI, PGA, más recientemente el IGA mod 2011 y el BSA [16]. Considerando un palmo de la mano un 1%, nuestra escala según gravedad quedo de la forma siguiente:

Signo ausente: expresión ausente

Ligero: Psoriasis estable, en placas, que afecta a menos del 3 % de la superficie cutánea.

Moderada: Psoriasis que afecta entre el 3 y 8 % de la superficie cutánea, excepto si afecta a cara, manos y pies, genitales o pliegues, y siempre que no exista afectación articular. Estado psicológico del paciente no excesivamente afectado

Intenso: Psoriasis que afecta a más del 10 % de la superficie corporal o si afecta a cara, manos y pies, genitales o pliegues.

Evaluación de la eficacia

Respuesta al tratamiento

La respuesta al tratamiento se codifico de acuerdo al tiempo que demoro la desaparición de las lesiones respecto al tiempo de evolución designado, estableciéndose las siguientes categorías:

- Excelente: cuando las lesiones desaparecen en un 90 % a un 100% al finalizar el periodo del tratamiento.
- Bueno: Las lesiones disminuyen en un 50 a un 89 % al terminar el periodo del tratamiento.
- Regular: Las lesiones disminuyen en un 30 a un 49 %% al terminar el periodo del tratamiento.
- Malo: cuando las lesiones no se modifican o desaparecieron menos del 30 %

Considerándose eficacia para las categorías excelente y bueno.

Criterio principal de evaluación de eficacia

Que los pacientes tratados con la pomada de caléndula al 20 % logren que desaparezcan o disminuyan más del 50 % las lesiones durante el tiempo de tratamiento, 10 semanas.

Recolección y procesamiento de datos

Se realizó la revisión documental de fuentes bibliográficas de bases de datos especializadas como: PubMed, EMBASE, LILACS, Web of Knowledge, entre otros, de las cuales se extrajo la información concerniente al tema de investigación. Luego se confeccionaron las plantillas para la recolección de los datos primarios en Excel, los que se procesaron y analizaron estadísticamente en un microprocesador personal, los resultados se muestran en tablas de doble entrada validadas aplicando los estadígrafos de Chicuadrado y Mantel-Haenszel con un nivel de significación del 5 % (0,05) para probar homogeneidad.

El análisis y discusión de los resultados se realizó mediante la triangulación de los datos obtenidos en las bibliografías revisadas, los obtenidos en nuestra investigación y el criterio de expertos, lo cual garantizo dar cumplimiento a el objetivo trazado y con la utilización de procedimientos lógicos del pensamiento que incluyeron análisis-síntesis e inducción-deducción arribamos a conclusiones.

Consideraciones éticas

De acuerdo a los principios éticos de la Declaración de Helsinki, la investigación fue revisada y aprobada por el Comité de Ética para la Investigación Científica de la Facultad de Medicina No 1 de la Universidad de Ciencias Médicas de Santiago de Cuba.

Los pacientes incluidos bajo su voluntariedad expresaron su intención de participar en la investigación, después de haber comprendido la información dada en cantidad y calidad suficientes, sobre los objetivos, beneficios, riesgos, molestias, alternativas, sus derechos y responsabilidades mediante del consentimiento informado.

Resultados

En las tablas 1 y 2 se muestra la clasificación de las lesiones dermatológicas, escamas y eritemas, en pacientes en ambos grupos en la primera semana del tratamiento, el 100 % de los pacientes presentaban escamas consideradas como lesiones moderadas e intensas, no ocurriendo lo mismo con el eritema donde el 95 % tenía lesiones intensas y el

5 % ligera, en el grupo control, mientras que 100 % de los pacientes del grupo estudio presentaban lesiones eritema moderadas e intensa, con una frecuencia de 15% y 85% respectivamente.

Tabla 1. Clasificación de las lesiones (escamas) en la primera semana

Escama	Control		Estudio		Total	
	Nº	%	Nº	%	Nº	%
Ausente	0	0	0	0	0	0
Ligero	0	0	0	0	0	0
Moderado	4	20	6	30	10	25,0
Intenso	16	80	14	70	30	75,0
Total	20	100	20	100	40	100

P=0,0006

Tabla 2. Clasificación de las lesiones (eritemas) en la primera semana

Eritema	Control		Estudio		Total	
	Nº	%	Nº	%	Nº	%
Ausente	0	0	0	0	0	0
Ligero	1	5	0	0	1	2,5
Moderado	0	0	3	15	3	7,5
Intenso	19	95	17	85	36	90,0
Total	20	100	20	100	40	100

P=0,0000

En las tablas 3 y 4 se muestra la evolución de las lesiones tanto escamosas como eritematosas, en la quinta semana de tratamiento y se observa diferencias estadísticas significativas en la evolución de los síntomas en los pacientes del grupo estudio respecto a los del grupo control, el 55 % de los tratados, grupo estudio, presentaban lesiones escamas ligeras mientras que en grupo control predominaron los pacientes con estas lesiones clasificadas como intensas (75 %), es interesante que el 15 % de los pacientes del grupo estudio no tenían lesiones (escamas). En cuanto a las lesiones eritema en el grupo estudio predominaron con igual frecuencia (35 %) lesiones ligero y moderado, mientras que en los pacientes del grupo control en este momento de la evaluación predominaron con un 75 % las lesiones eritema clasificadas como intensa.

Tabla 3. Evolución de las lesiones (escamas) a la quinta semana de tratamiento

Escama	Control		Estudio		Total	
	Nº	%	Nº	%	Nº	%
Ausente	0	0	3	15	3	7,5
Ligero	2	10	11	55	13	32,5
Moderado	3	15	5	25	8	20,0
Intenso	15	75	1	5	16	40,0
Total	20	100	20	100	40	100

P=0. 0000

Tabla 4. Evolución de las lesiones (eritemas) a la quinta semana de tratamiento

Eritema	Control		Estudio		Total	
	Nº	%	Nº	%	Nº	%
Ausente	0	0	4	20	4	10,0
Ligero	1	5	7	35	8	20,0
Moderado	4	20	7	35	11	27,5
Intenso	15	75	2	10	17	42,5
Total	20	100	20	100	40	100

P=0,0000

Al mostrar la evolución de las lesiones en la décima semana (tablas 5 y 6), observamos que se alcanza una evolución de las lesiones en el grupo estudio, entre ausentes y ligeras de un 80% en el caso de las escamas y un 75 % el eritema, mientras que en el grupo control evolucionaron las lesiones a ligeras y ausentes el 15 % de los paciente, tanto para las escamas como para las lesiones eritema, evolución clínica estadísticamente significativa entre ambos grupos, P = 0,00012 y = 0,00006 respectivamente.

Tabla 5. Evolución de las lesiones (escamas) en la semana 10 según tratamiento

Escama	Control		Estudio		Total	
	Nº	%	Nº	%	Nº	%
Ausente	1	5	10	50	11	27,5
Ligero	2	10	6	30	8	20
Moderado	3	15	3	15	6	15
Intenso	14	70	1	5	15	37,5
Total	20	100	20	100	40	100

P=0,00012

Tabla 6. Evolución de las lesiones (eritema) en la semana 10 según tratamiento

Eritema	Control		Estudio		Total	
	Nº	%	Nº	%	Nº	%
Ausente	1	5	11	55	12	30
Ligero	2	10	4	20	6	15
Moderado	2	10	4	20	6	15
Intenso	15	75	1	5	16	40
Total	20	100	20	100	40	100

P=0,0006

La respuesta final de los pacientes según tratamiento, se muestra en la tabla 7 donde podemos observar que en el grupo estudio el 50% de los pacientes estuvieron una respuesta excelente pues se eliminaron en su totalidad las lesiones, mientras que en el grupo control esto ocurrió en el 5% de los pacientes.

Tabla 7. Respuesta final de los pacientes según tratamiento

Respuesta al tratamiento	Control		Estudio		Total	
	Nº	%	Nº	%	Nº	%
Excelente	1	5	10	50	11	27,5
Bueno	2	10	6	30	8	20
Regular	3	15	3	15	6	15
Malo	14	70	1	5	15	37,5
Total	20	100	20	100	40	100

P=0,0001

Discusión

La fitoterapia hasta mediados del siglo XIX constituyó una importante alternativa terapéutica para el tratamiento de la psoriasis, a la luz de los conocimientos de estos días donde se exploran científicamente las plantas medicinales, no solo en países de recursos limitados sino también en países desarrollados el uso de la fitoterapia constituye más que una medicina alternativa un tratamiento coadyuvante de disimiles enfermedades. Este es el caso de la planta de caléndula con sus propiedades antinflamatorias, cicatrizantes y antibacteriana [17].

Se ha encontrado que la acción antiinflamatoria de la *Calendula officinalis L.* es de la misma magnitud que la de la indometacina, se atribuye, principalmente, a la presencia de triterpenos (especialmente del faradiol), la que parece ser mediada por la inhibición de citoquinas proinflamatorias, de la ciclooxigenasa-2 y la subsecuente síntesis de prostaglandinas que intervienen en el proceso inflamatorio; además, se ha evidenciado en estudios el poder cicatrizante de los extractos de caléndula en animales de experimentación y en humanos, pues con el extracto se ha incrementado el contenido de hidroxiprolina y de hexosamina, sustancias presentes en el tejido de granulación, lo que pudiera incrementar la síntesis del colágeno debido a la presencia de flavonoides en el extracto potenciando, de ahí la epitelización y la regeneración de la piel que esta ofrece [17, 18].

La caléndula posee la presencia de los ácidos fenólico, tanino, oleanólico y salicílico; se responsabilizan de la actividad antibacteriana algunos de estos ejemplo el ácido oleanólico, que inhibe el crecimiento bacteriano y provoca la autolisis de las bacterias Gram positivas, mientras que la acción emoliente y antimicótica se le atribuye al ácido salicílico que forma parte de los componentes de su flor [19, 20].

Estas propiedades podrían responder a las acciones farmacológicas del producto de nuestra investigación, que además es una preparación relativamente no irritante formada principalmente por componentes oleosos (lanolina y vaselina), al igual que el placebo que solo carece de la carece de la flor de caléndula, elementos que a juicio de los autores contribuye a la evolución satisfactoria de la mayoría de los pacientes en nuestro estudio, independientemente de la intervención recibida, con la presentación que contenía el principio farmacológicamente activo (*Calendula officinalis*) o no (el placebo) [20].

En nuestra investigación hubo una respuesta eficaz al tratamiento en el 20 % de los pacientes del grupo estudio quienes no presentaban lesiones eritematosas a la quinta semana de tratamiento y las mejores respuestas se presentaron en la décima semana, cuando el 80% de los pacientes tratados con el fitofármaco, logró mejoría o ausencia de los síntomas, por lo que en este estudio la eficacia máxima se evidenció entre la quinta y la décima semana de tratamiento; al compararlo con la investigación de Carracosa *et al.* [21], donde con tratamientos tópicos con corticoesteroides se logra alcanzar la eficacia máxima entre la segunda y la cuarta semana de tratamiento, se impondría entonces a partir de los criterios de selección de los medicamentos el fitofármaco en estudio en lo referente a la seguridad entre ambos fármacos.

Es importante conocer que el éxito del tratamiento exige adherencia por parte del paciente, quien debe tener conocimiento de que se trata de una enfermedad crónica cuyo objetivo terapéutico es aliviar los síntomas y espaciar los brotes.

La novedad está en que no se ha encontrado estudios similares en nuestro entorno y encontramos pocos trabajos publicados, es el caso del estudio de Hurtado-Pérez *et al.* [22] con la tintura de caléndula al 20% en afecciones dermatológicas; y la pomada en estudio podría constituir más que una alternativa terapéutica un coadyuvante del tratamiento tópico de la psoriasis. La principal limitación de esta investigación es su carácter no controlado, ni aleatorizado.

Conflicto de interés

Los autores declaran no presentar ningún conflicto de interés

Referencias

1. S. Das, Psoriasis, *Manual MSD. Versión para profesionales*. URL: https://www.msdmanuals.com/es/professional/trastornos-dermatol%C3%B3gicos/psoriasis-y-enfermedades-descamativas/psoriasis, accesado en marzo de 2023.

2. G. Dhingra, P. Dhakad, S. Tanwar, Review on phytochemical constituents and pharmacological activities of plant *Calendula officinalis* Linn, *Biological Sciences*, **2**(2), 216-228 (2022).

3. Grupo de trabajo de Psoriasis AEDV, ¿Cómo se mide la gravedad de la psoriasis? Principales índices. URL: https://aedv.es/grupos-de-trabajo/psoriasis/accesado en marzo de 2023.

4. República de Cuba, Ministerio de Salud Pública, *Formulario Nacional de Fitofármacos y Apifármacos*, Editorial de Ciencias Médicas, La Habana, Cuba, 2017. URL: http://www.bvscuba.sld.cu/libro/formulario-nacional-de-fitofarmacos-y-apifarmacos-segunda-edicion/, accesado en marzo de 2023.

5. H. Lastra-Valdés, R. Piquet-García, *Calendula officinalis*, *Revista Cubana de Farmacia*, **33**(3), 188-194 (1999).

6. O. Givol, R. Kornhaber, D. Visentin, M. Cleary, J. Haik, M. Harats, A systematic review of Calendula officinalis extract for wound healing, *Wound Repair and Regeneration*, **27**(5), 548-561 (2019).

7. K. Shahane, M. Kshirsagar, S. Tambe, D. Jain, S. Rout, M.K.M. Ferreira, R. Lima, An updated review on the multifaceted therapeutic potential of *Calendula officinalis* L, *Pharmaceuticals* (Basel), **16**(4), 611 (2023).

8. B. Tamayo-Ortíz, M.E. Romero-Pérez, D.A. Joseph-Montero, J.M. Rosales-Álvarez, Y. Rosales-García, La efectividad de la crema *Caléndula officinalis* L como tratamiento de la estomatitis aftosa recurrente, *Correo Científico Médico*, **23**, 175-186 (2019).

9. F.F. Contero-Bedoya, M. Quesada-Fernández, J.O. Barrera-Ramos, *Efectividad del tratamiento con terapia floral y una crema natural en pacientes con psoriasis*, Instituto Universitario Misael Acosta, 2020. URL: https://www.istmas.edu.ec/efectividad-del-tratamiento-con-terapia-floral-y-una-crema-natural-en-pacientes-con-psoriasis/, accesado en marzo de 2023.

10. M. Palacios de Diego, Las tinturas madre homeopáticas de *Caléndula officinalis* y Echinacea angustifolia como antiséptico oral, *Revista Médica de Homeopatía*, **6**(3), 112-126 (2013).

11. G. Ak, G. Zengin, R. Ceylan, M.F. Mahomoodally, S. Jugreet, A. Mollica, A. Stefanucci, Chemical composition and biological activities of essential oils from *Calendula officinalis* L. flowers and leaves, *Flavour and Fragrance Journal*, **36**(5), 554-563 (2021).

12. A. Cañada-Rodríguez, J. Antúnez-Coca, J.C. Escalona-Arranz, Estandarización de parámetros para el control de la calidad del extracto blando de Caléndula officinalis L., *Revista Cubana de Tecnología de la Salud*, **9**(2), 65-76 (2018).

13. D.C. Aguilera-Jiménez, O.J. Bedoya-Quevedo, X. Urquiza-Pérez, *Impacto psicosocial de la psoriasis*, Trabajo de Grado, Facultad de Ciencias de la Salud, Unidad Central del Valle del Cauca, Tulúa, Colombia, 2013. 49 p.

14. D. Silva, M.S. Ferreira, J.M. Sousa-Lobo, M.T. Cruz, I.F. Almeida, Anti-inflammatory activity of *Calendula officinalis* L. flower extract, *Cosmetics*, **8**(2), 31 (2021).

15. L.M. Leal, M.A. Andrade, L.A. Batista, V.M. Brianezi, M. Pacheco, R. De Souza, *et al.*, Angiogenic activity of *Calendula officinalis* flowers L. in rats, *Acta Cirúrgica Brasileira*, **26**(1), 19-24 (2011).

16. C.M. Ojeda, D.A. Ramos, I.C. Núñez, Cuantificación de fenoles y flavonoides totales en un extracto blando de flores de *Calendula officinalis* Linn, *Orange Journal*, **2**(3), 20-31 (2020).

17. J.A. Hernández-Díaz, J.J. Garza-García, J.M. León-Morales, A. Zamudio-Ojeda, J. Arratia-Quijada, G. Velázquez-Juárez, S. García-Morales, Antibacterial activity of biosynthesized selenium nanoparticles using extracts of *Calendula officinalis* against potentially clinical bacterial strains, *Molecules*, **26**(19), 5929 (2021).

18. R.R. Cepeda, N.Y.A. Suarez, Actividad antimicrobiana del extracto hidroalcohólico de *Calendula officinalis* L., *Revista Ion*, **34**(1), 97-110 (2021).

19. D. Sahingil, GC/MS-olfactometric characterization of the volatile compounds, determination antimicrobial and antioxidant activity of essential oil from flowers of calendula (*Calendula officinalis* L.), *Journal of Essential Oil Bearing Plants*, **22**(6), 1571-1580 (2019).

20. E. Valencia, I.I. Figueroa, E. Sosa, M.C. Bartolomé, H.E. Martínez, M.E. García, M.E. (2017). Polifenoles: propiedades antioxidantes y toxicológica, *Revista de la Facultad de Ciencias Químicas*, **16**, 15-29 (2017).

21. J.M. Carracosa, F. Vanaclocha, L. Borrego, *et al.*, Revisión actualizada del tratamiento tópico de la psoriasis, *Actas Dermo-Sifiliográficas*, **100**(3), 190-200 (2009).

22. A. Hurtado-Pérez, M. Castro-Gutiérrez, L.B. Pérez de Corcho, Efectividad de la tintura de caléndula al 20% en afecciones dermatológicas, *Acta Médica del Centro*, **8**(3), 28-36 (2014).

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Propuesta del listado de medicamentos para enfermedades huérfanas en Colombia

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Resumen

Introducción: Colombia se sitúa en una posición desfavorable y poco alentadora respecto a políticas públicas relacionadas con Medicamentos para Enfermedades Huérfanas (MEH), por tanto, la definición de un listado de MEH es un primer paso para generar un avance mejorando el contenido de los requisitos que una política pública de enfermedades huérfanas (EH) debe considerar. **Objetivo:** definir el listado de MEH en Colombia a partir de la revisión del listado de enfermedades huérfanas establecido por la Resolución 023 de 2023. **Metodología:** se realizó una revisión de la Resolución 023 de 2023, que permitió la relación de las indicaciones allí descritas con las indicaciones de las bases de datos de medicamentos del INVIMA. **Resultados:** se encontró tratamiento farmacológico para 338 (15,1%) sobre un total de 2236 EH. Respecto a los tratamientos farmacológicos, se evidencia que la mayoría están disponibles para las enfermedades endocrinas, nutricionales y metabólicas con un 37,6% sobre la oferta farmacéutica y respecto a la clasificación ATC la que se sitúa en primer lugar son los agentes antineoplásicos e inmunomoduladores. **Conclusiones:** se obtuvo el primer listado de medicamentos para enfermedades huérfanas para Colombia con su respectiva metodología de actualización, el cual será sometido a consulta pública para lograr una construcción colectiva.

Palabras clave: Medicamentos para enfermedades huérfanas, políticas públicas, enfermedades huérfanas, acceso, regulación.

Summary

Proposal of list of drugs for orphan diseases for Colombia

Colombia is in an unfavorable and discouraging position regarding public policies related to drugs for orphan diseases, therefore, the definition of a list of medications for orphan diseases as a first step is necessary to generate progress by improving the content of the requirements related with public policy on orphan diseases. **Aim:** to define the list of drugs for orphan diseases in Colombia based on the review of the list of orphan diseases established by Resolution 023 of 2023. **Methodology:** A review of Resolution 023 of 2023 was conducted which allowed the relation of the indications described there with the indications of the INVIMA drug databases. **Results:** pharmacological treatment was found for 338 (15,1%) out of a total of 2236 Orphan Diseases. Regarding pharmacological treatment, it is evident that the majority of medications are available for endocrine, metabolic and nutritional diseases, with 37.6% of the pharmaceutical market and regarding the Anatomical Therapeutic Chemical Classification (ATC) the drugs that ranks first are antineoplastic and immunomodulatory agents. Conclusions: the first list of drugs for orphan diseases for Colombia was obtained with its respective updated methodology which will be submitted for public consultation to achieve collective construction.

Keywords: Drugs for orphan diseases, public policy, orphan diseases, access, regulation.

Resumo

Proposta de lista de medicamentos para doenças órfãs na Colômbia

Introdução: A Colômbia encontra-se numa posição desfavorável e pouco inspiradora no que diz respeito às políticas públicas relacionadas com Medicamentos para Doenças Órfãs (MEH), portanto, a definição de uma lista de MEH é um primeiro passo para gerar progresso através da melhoria do conteúdo dos requisitos. Uma política pública sobre doenças órfãs (DH) deve ser considerada. **Objetivo**: definir a lista de MEH na Colômbia com base na revisão da lista de doenças órfãs estabelecida pela Resolução 023 de 2023. Metodologia: foi realizada uma revisão da Resolução 023 de 2023, que permitiu a relação das indicações ali descritas com as indicações das bases de dados de medicamentos INVIMA. Resultados: foi encontrado trata-

mento farmacológico para 338 (15,1%) de um total de 2.236 HE. Em relação aos tratamentos farmacológicos, fica evidente que a maioria está disponível para doenças endócrinas, nutricionais e metabólicas com 37,6% da oferta farmacêutica e com relação à classificação ATC, os que estão em primeiro lugar são os antineoplásicos e imunomoduladores. Conclusões: foi obtida a primeira lista de medicamentos para doenças órfãs para a Colômbia com sua respectiva metodologia de atualização, que será submetida a consulta pública para alcançar uma construção coletiva.

Palavras-chave: Medicamentos para doenças órfãs, políticas públicas, doenças órfãs, acesso, regulação.

Introduccion

Las enfermedades huérfanas (EH) son patologías extremadamente raras que tienen una baja prevalencia. Su definición varía de un país a otro ya que, por ejemplo, en la Unión Europea se define como las enfermedades que afectan a menos de 5 personas por cada 10.000, en Estados Unidos menos de 5 personas por cada 200.000, en Japón menos de 5 personas por cada 50.000, en Taiwán menos de una por cada 10.000 y por último para Colombia se define como 1 por cada 5.000 personas [1].

Por su parte, un medicamento para una enfermedad huérfana (MEH) puede definirse como un producto medicinal destinado al manejo de una EH la cual no tiene métodos de diagnóstico, prevención y tratamiento claramente definidos [2]. Al igual que para las EH, existen diferentes definiciones para MEH, sin embargo, en Colombia no hay una definición concertada.

En cuanto a acceso y cobertura, a nivel mundial es posible dividir en dos estas variables en los diferentes países: a) aquellos que no tienen políticas públicas claramente establecidas o las tienen, pero son incipientes y b) aquellos que las tienen claramente definidas brindando un panorama claro para los pacientes con este tipo de patologías. Es importante reconocer que desde la expedición de la Ley 1392 de 2010 "*Por medio de la cual se reconocen las Enfermedades Huérfanas como de especial interés y se adoptan normas tendientes a garantizar la protección social por parte del Estado colombiano a la población que padece de Enfermedades Huérfanas y sus cuidadores*", en Colombia se han generado avances sustanciales en cuanto a políticas de EH; sin embargo, en comparación con otros países es evidente que Colombia aún cuenta con oportunidades de mejora en la materia, y que las experiencias exitosas en otras latitudes, podrían guiar el desarrollo de dichas políticas públicas relacionadas con el tema.

Dentro de las políticas públicas para MEH, en la publicación realizada por Chan *et al.* [3] se analizan las dimensiones para medicamentos huérfanos en 194 países, en donde se ubica a Colombia en una posición desfavorable en la evaluación de dichas dimensiones al tener pocos avances en los temas de designación de medicamentos huérfanos, autorización de comercialización, e incentivos y pocos avances en la dimensión de requerimientos de seguridad y eficacia, regulación de precios de entrada e incentivos que fomenten la I&D.

A pesar de lo anterior, Colombia ha avanzado en los últimos 10 años en la definición de políticas públicas encaminadas a proteger los derechos de las personas que padecen EH. El primer avance específico fue la expedición de la Ley 1392 de 2010 "*Por medio de la cual se reconocen las Enfermedades Huérfanas como de especial interés y se adoptan normas tendientes a garantizar la protección social por parte del Estado colombiano a la población que padece de Enfermedades Huérfanas y sus cuidadores"* cuyo objeto es reconocer que las personas con EH merecen especial interés en salud, reconociendo la baja prevalencia en la población colombiana pero que por su elevado costo requieren un mecanismo de aseguramiento diferente al de las enfermedades generales y procesos de atención especializados [4].

Posteriormente se expide la Resolución 1438 de 2011 [5] "*Por medio de la cual se reforma el Sistema General de Seguridad Social en Salud y se dictan otras disposiciones"* la cual en su artículo 2 define las EH así:

> **Artículo 2°. Denominación de las enfermedades huérfanas***. Las enfermedades huérfanas son aquellas crónicamente debilitantes, graves, que amenazan la vida y con una prevalencia menor de 1 por cada 5.000 personas, comprenden, las enfermedades raras, las ultrahuérfanas y olvidadas. Las enfermedades olvidadas son propias de los países en desarrollo y afectan ordinariamente a la población más pobre y no cuentan con tratamientos*

Luego de la Resolución 1438 de 2011, se expide el Decreto 1954 de 2012 "*Por el cual se dictan disposiciones para implementar el sistema de información de pacientes con enfermedades huérfanas*" [6]. Seguido de este decreto se han expedido una serie de actos administrativos adicionales relacionados con los criterios técnicos de reporte que permiten tener un censo de los pacientes con EH, sin embargo, exceden el alcance de la presente revisión.

Teniendo en cuenta lo anterior, se entiende en el marco normativo del Sistema General de Salud que las personas con EH tienen especial protección por parte del Estado y no debe presentarse ningún tipo de restricción administrativa o económica.

A partir de lo anterior, se han expedido una serie de Resoluciones que definen el listado de EH, entre ellas la Resolución 5265 de 2018 "*Por la cual se actualiza el listado de enfermedades huérfanas y se dictan otras disposiciones*" [7] siendo la última la Resolución 023 de 2023 *"Por medio de la cual se actualiza el listado de enfermedades huérfanas – raras*" [8] en la cual se disponen en su Anexo Técnico 2236 EH con su respectivo nombre y CIE-10.

Es importante considerar que en Colombia no existe como tal un listado de MEH [9], sin embargo, existe el Listado de Medicamentos Vitales no disponibles -MVND- que se encuentran definidos por el Decreto 481 de 2004 como "*un medicamento indispensable e irremplazable para salvaguardar la vida o aliviar el sufrimiento de un paciente o un grupo de pacientes y que, por condiciones de baja rentabilidad en su comercialización, no se encuentra disponible en el país o las cantidades no son suficientes.* [10]" Toda la normatividad relacionada con los MVND está bajo la responsabilidad del Instituto Nacional de Vigilancia de Medicamentos y Alimentos – INVIMA, encontrando que la definición de MVND es la que más se aproxima a las reconocidas a nivel internacional "*aquellos que se utilizan para el tratamiento y diagnóstico de enfermedades de baja prevalencia o enfermedades huérfanas"* [11].

El listado de MVND podría considerarse entonces como una base de partida para la construcción del listado de MEH teniendo en cuenta su definición, sin embargo, no es posible afirmar que sea el listado oficial de MEH en Colombia. Así las cosas, se realizó una revisión del estado de los MH en Colombia mediante un estudio observacional descriptivo de tipo indicación - prescripción de corte transversal con recolección retrospectiva de información con el objetivo principal de proponer el primer listado de MEH para el territorio colombiano.

Metodología

Esta investigación fue de carácter observacional descriptivo de tipo indicación - prescripción de corte transversal con recolección retrospectiva de datos para el año 2022. Se revisó el listado de EH establecido en la Resolución 023 de 2023 y su Anexo Técnico, para realizar la búsqueda por medio de la indicación allí descrita, en las bases de datos de medicamentos de INVIMA y el listado de medicamentos con Usos No Indicados en el Registro Sanitario - UNIRS (ultimo disponible), con el objetivo de identificar los Códigos Únicos de Medicamentos – CUMS con indicaciones aprobadas que incluyan la enfermedad huérfana en revisión considerando además variables como clasificación anatómico terapéutica química (ATC) por grupo relevante definidos como el conjunto de medicamentos pertenecientes a la misma clasificación ATC a nivel 5

(principio activo) e igual forma farmacéutica si tienen otras indicaciones diferentes a EH o son exclusivas, fuente de financiación (con recursos de la UPC) y si se encuentran sujetos a control directo de precios por parte de la Comisión Reguladora de Precios de Medicamentos y Dispositivos Médicos.

Es importante considerar que el listado de MVND no tiene descripción de indicación por lo que, para la revisión del mismo, se realizó una revisión de tipo indicación-prescripción en la cual se examinaron todas las actas del INVIMA que permitieron la inclusión de estos medicamentos al listado respecto a la indicación aprobada de uso y la norma farmacológica colombiana, para hacer la respectiva comparación con el listado de EH descrito en la Resolución 023 de 2023. Por otro lado, para el caso del listado de los medicamentos importados bajo la modalidad de MVND, se descargó la respectiva base de datos de la página de datos abiertos y se revisó si la indicación allí contenida corresponde a alguna incluida en el listado previamente descrito.

Finalmente, se realizó un análisis comparativo con el Informe de evento 362 "Enfermedades huérfanas – raras. Colombia, 2022 (hasta periodo epidemiológico XII)" con el objetivo de caracterizar el tratamiento farmacológico de las EH disponible en el territorio colombiano.

Resultados y discusión

La definición que a nivel internacional se ha aceptado para Medicamento Huérfano (MH) corresponde a "*aquellos que se utilizan para el tratamiento y diagnóstico de enfermedades de baja prevalencia o enfermedades huérfanas*" [11], por lo cual es posible adoptar para la presente investigación la definición de MEH o MH y **proponer para el territorio colombiano** como "*aquellos medicamentos que se utilizan para el tratamiento y diagnóstico de enfermedades huérfanas definidas por la Resolución 023 de 2023 o aquella que la modifique o sustituya*".

La fuente oficial de información de Enfermedades Huérfanas en el país es el "*Registro Nacional de Pacientes con Enfermedades Huérfanas*" que agrupa todas las fuentes de información en el tema, incluido el Sistema de Vigilancia en Salud Publica -SIVIGILA- y es administrado por el Ministerio de Salud y Protección Social [12].

El Instituto Nacional de Salud tiene dentro de los eventos de vigilancia de interés en salud pública el evento 342 "*Enfermedades Huérfanas – Raras*" el cual considera el listado de enfermedades huérfanas según la Resolución 5265 de 2018 actualizada por la Resolución 023 de 2023 "*Por medio de la cual se actualiza el listado de enfermedades huérfanas - raras*" en su Anexo Técnico, se encuentran 2236 enfermedades reconoci-

das como enfermedades huérfanas para el territorio colombiano. En el último informe del evento Colombia, 2022 (hasta periodo epidemiológico XII) reportan 13227 casos notificados al SIVIGILA desde el 2016 hasta el periodo epidemiológico XII de 2022 en donde las enfermedades del sistema nervioso son las EH más frecuentes en el territorio colombiano seguidas de malformaciones congénitas, deformidades y anomalías cromosómicas y enfermedades del sistema osteomuscular y del tejido conectivo centrando en estas tres primeras categorías el 50,1% de la población colombiana con EH. En la revisión de los medicamentos con indicaciones para EH en Colombia, se encontró tratamiento farmacológico para 338 (15,1%) sobre un total de 2236 definidas por la Resolución 023 de 2023.

La mayoría de tratamientos farmacológicos están disponibles para las enfermedades endocrinas, nutricionales y metabólicas las cuales no son las más prevalentes en el territorio colombiano, por el contrario, se localizan en el sexto lugar en su orden según el informe del evento 342. Al revisar al interior de esta categoría, el medicamento que más tiene mayor disponibilidad de oferta comercial es la ATORVASTATINA-TABLETA O CAPSULA para Hiperlipoproteinemia no especificada, Hiperlipoproteinemia tipo 3 e Hipercolesterolemia familiar homocigota seguido de ESOMEPRAZOL-TABLETA O CAPSULA para Síndrome de Zollinger-Ellison. Con los anteriores resultados, se concluye que son las indicaciones de los medicamentos que se consideran como *'huérfanas'*, ya que un principio activo con indicaciones de alta prevalencia como por ejemplo Atorvastatina y esomeprazol para dislipoproteinemias y reflujo gastroesofágico respectivamente pueden ser considerados como medicamentos para enfermedades huérfanas ya que según la autoridad competente también tienen indicación para EH [13].

Así mismo, se encuentran con mayor disponibilidad de tratamientos farmacológicos las enfermedades del sistema nervioso las cuales se ubican en primer lugar de prevalencia. Dentro de esta categoría se halla, en primer lugar, la TOXINA BOTULINICA-POLVO PARA RECONSTITUIR A SOLUCION O SUSPENSION INYECTABLE para el tratamiento de todas las paraplejias espásticas seguido de MEMANTINA-TABLETA O CAPSULA para el tratamiento de enfermedad de Alzheimer autosómica dominante de aparición temprana.

Continuando con el análisis del último informe del evento 342 (hasta periodo epidemiológico XII), resultan las siguientes 21 enfermedades como las más prevalentes [14], para las cuales se analizó si se encontraba tratamiento farmacológico disponible o no así:

Tabla 1. Proporción de notificación de enfermedades huérfanas raras, Colombia, 2022 (hasta periodo epidemiológico XII)

No.	Enfermedad Huérfana - Rara	Casos	%	Tratamiento Farmacológico*
1	Reumatismo psoriásico	891	6,7	NO
2	Esclerosis sistémica cutánea limitada	760	5,7	NO
3	Síndrome de Guillain-Barre	494	3,7	SI
4	Esclerosis Múltiple	489	3,7	SI
5	Displasia broncopulmonar	449	3,3	SI
6	Drepanocitosis	341	2,5	NO
7	Esclerosis sistémica cutánea difusa	288	2,1	SI
8	Esclerosis lateral amiotrófica	279	2,1	SI
9	Enfermedad de Von Willebrand	255	1,9	SI
10	Fibrosis pulmonar idiopática	248	1,8	SI
11	Hipertensión arterial pulmonar idiopática	239	1,8	SI
12	Enfermedad de Devic	219	1,6	NO
13	Artritis juvenil idiopática de inicio sistémico	217	1,6	SI
14	Miastenia grave	210	1,5	SI
15	Dermatomiositis	188	1,4	NO
16	Enfermedad de Crohn	188	1,4	SI
17	Microtia	174	1,3	NO
18	Polimiositis	172	1,3	SI
19	Hepatitis crónica autoinmune	169	1,2	SI
20	Hipertensión Pulmonar Tromboembólica Crónica	161	1,2	SI
21	Déficit congénito del factor VIII	159	1,2	SI

Fuente: Tomado de Instituto Nacional de Salud. Informe de evento 342. Enfermedades huérfanas – raras. Colombia, 2022 (hasta periodo epidemiológico XII) y *adaptación propia.

Para estas 21 enfermedades se encuentra tratamiento farmacológico para 15 de ellas lo que muestra que al analizar de forma individual y detallada cada una de las enfermedades, hay tratamiento farmacológico para el 71% de las EH más prevalentes en Colombia. En cuanto al reumatismo psoriásico, en la resolución 023 de 2023 ya no se encuentra definida como una EH para Colombia.

Cumpliendo con el objetivo de definir el listado de medicamentos para enfermedades huérfanas en Colombia a partir de la revisión del listado de EH establecido por la Resolución 023 de 2023, se encontraron 331 grupos relevantes (GR), así como los mercados relevantes regulados por la Comisión Nacional de Precios de Medicamentos y Dispositivos Médicos – CNPMDM [15 - 16]. Para el análisis de los medicamentos identificados, la clasificación ATC muestra que los agentes antineoplásicos e inmunomoduladores se encuentran en primer lugar de frecuencia, siendo el de mayor importancia el IMATINIB-TABLETA O CAPSULA para el tratamiento de la Mastocitosis, Mielodisplasia con hipogammaglobulinemia y síndrome del injerto contra huésped seguido de METOTREXATO-SOLUCION O SUSPENSION INYECTABLE para artritis juvenil idiopática de inicio sistémico, enfermedad de Crohn, esclerosis sistémica cutánea difusa, enfermedad de Behçet, micosis Fungoide y osteosarcoma. En este punto, es conveniente especificar que la designación de MEH en regiones como Europa y Norte América se realiza mediante la solicitud directa a las respectivas agencias reguladoras EMA (European Medicines Agency) y la FDA (U.S. Food & Drug Administration) respectivamente por parte de los Titulares de los Registros Sanitarios a los Grupos técnicos de enfermedades raras con el objetivo de obtener incentivos como protección de patentes, beneficios tributarios, obtención de registro sanitario de manera rápida (conocido como fast-track) [3, 17], entre otros, procedimiento que no está establecido en Colombia de esta manera por lo cual la definición de un primer listado de MEH es un avance en el marco de una política pública de EH. Por otro lado, al comparar a Colombia con países de ingresos similares en la región como por ejemplo Argentina, Brasil y Ecuador la situación es parecida respecto a políticas públicas para MEH, por lo que se espera que los resultados de esta investigación sean considerados a nivel regional, siguiendo a Chile como el único país que muestra políticas relacionadas claramente definidas en la región [3, 17].

Respecto a la financiación de los MEH en el Sistema General de Seguridad Social en Salud - SGSSS, puede estar dada en primer lugar con recursos de la UPC [18] la cual es una prima que mancomuna los riesgos derivados de las necesidades en salud de las personas utilizando instrumentos para inferir y reconocer un presupuesto de manera ex ante, en segundo lugar se presenta el reconocimiento del Presupuesto Máximo que busca gestionar el riesgo en salud de manera integral financiando aquellos servicios y tecnologías en salud que no son financiadas con cargo a la UPC y en tercer lugar se cuenta con otro componente a través del cual se financia el acceso a servicios y tecnologías que aún no hacen parte del aseguramiento, los cuales son financiados con recursos dispuestos por la Administradora de Recursos del Sistema General de Seguridad Social en Salud (ADRES). Teniendo en cuenta lo anterior, independientemente del mecanismo de financiación, el Estado colombiano garantiza a la población del territorio nacional el acceso a la totalidad de servicios y tecnologías de salud autorizados en el país.

Respecto al acceso a MEH, se muestran escenarios diferentes al realizar comparaciones entre los países en donde para la mayoría, el acceso es limitado por el alto costo de los mismos y por no contar con alternativas terapéuticas, lo que permite que se cree una demanda inelástica que propicia los altos precios en un mercado con competencia limitada o monopólica para la mayoría de ellos [17]. En general, los países con PIB altos son los que presentan un mejor acceso a los MEH considerando que como se mencionó anteriormente, ya tienen implementadas políticas públicas al respecto, además que la industria farmacéutica prefiere canalizar esfuerzos en el mercado de estos países considerando mejores escenarios de negociación en comparación con países con PIB bajos, haciendo que puedan negociar precios más altos debido a la disponibilidad a pagar, que luego servirá como referenciación de precios internacionales cuando presenten los MEH en otros países [19]. En otros casos, los MEH no están financiados por los sistemas de salud, generando problemas de acceso para los pacientes [17, 20] o, en el caso de estar financiados, el paciente debe asumir un porcentaje del valor del mismo (Canadá, Suiza, EEUU); sin embargo, y afortunadamente para otros gobiernos, el acceso a los MEH es visto como un derecho adquirido, que debe ser garantizado por los sistemas de seguridad social en salud [2], como es el caso de Colombia, que independientemente de la fuente de financiación o de su precio, los MEH están garantizados para las personas que los requieran.

Así las cosas, al realizar la respectiva clasificación de los GR se tiene que la mayoría (58%) de los MEH son financiados con recursos de la UPC lo que supone una mayor eficiencia en la gestión integral de los medicamentos para este tipo de enfermedades por parte de las EPS/EOC.

En lo relacionado con la regulación directa de precios en Colombia, la Circular 13 de 2022 "*Por la cual se establece el listado de los medicamentos sujetos al régimen de control directo de precios, se fija el precio máximo de venta y el precio por unidad de regulación de Medicamentos Vitales No Disponibles y se dictan otras disposiciones*" [16] permite establecer cuáles de los 331 GR identificados tienen un precio regulado (PRI) para lo cual la mayoría con el 55% no cuentan con un control de precios directo, lo que puede generar un cobro deliberado por parte de los titulares de los Registros Sanitarios. Sin embargo, en este punto es importante destacar que a pesar que la mayoría de estos medicamentos no cuentan con PRI, en el marco de la Resolución 1139 de 2022 se fijan los valores de referencia (VR) para el cálculo del Presupuesto Máximo considerándolos como un insumo fundamental para que los agentes de la cadena de formación de valor puedan acordar las condiciones de los esquemas de gestión y prestación de los servicios de salud. Al revisar dentro de los GR no financiados con recursos de la UPC (139), 105 (75,5%) tienen PRI o VR lo que indica que la mayoría de ellos están siendo monitoreados por parte del SGSSS.

Los medicamentos son bienes de consumo, sin embargo, desde el punto de vista económico poseen algunas particularidades que los hacen específicos. En general, la información relacionada con la toma de decisiones económicas alrededor de los medicamentos es asimétrica relacionada con la alta dispersión e inelasticidad de precios y el poco conocimiento de quienes los eligen, es decir quien los prescribe no los financian y quienes los consumen y financian no los prescriben [21].

Los MEH habitualmente son tecnologías en salud con reciente llegada al país, con patentes de uso vigentes y altos costos relacionados con su adquisición. Es por esto que para garantizar la sostenibilidad financiera del aseguramiento en Colombia y ante la llegada de nuevas e innovadoras tecnologías sanitarias al país, es imprescindible la generación de políticas públicas que optimicen el uso de los recursos públicos para su eficiente adquisición. Esta tendencia se inició en países europeos y miembros de la OECD ante la presencia de nuevas tecnologías en salud que hicieron necesario el uso de mecanismos innovadores para su adquisición, debido a su impacto financiero y a la incertidumbre sobre sus resultados. Por lo anterior, se debe adoptar y desarrollar un componente regulatorio normativo que permita su implementación y operación en el contexto del Sistema General de Seguridad Social en Salud –SGSSS–.

Un ejemplo de estos mecanismos innovadores son los acuerdos que se realizan entre el productor o comercializador de una tecnología en salud y el pagador o la autoridad regulatoria. Este tipo de arreglos son conocidos como Acuerdos de Acceso Administrado (AAA) y son herramientas que permiten a los afiliados al SGSSS acceder de manera más oportuna a tecnologías recientemente incorporadas en el mercado, y a los pagadores (públicos o privados) introducir mecanismos que permitan mitigar el riesgo financiero y propender por la sostenibilidad del sistema de salud. A causa de lo anterior, se revisó si los GR tienen dentro de sus indicaciones exclusivamente EH o si tienen otras indicación aparte de EH, para los cuales se encontró que 75 GR (29%) mostraron incluir dentro de sus indicaciones aprobadas solo EH, por lo que cobran especial atención considerando que todo el uso relacionado con los mismos refleja de forma exclusiva el contexto de tratamiento farmacológico sin sesgos por otras indicaciones. Teniendo en cuenta las variables previamente analizadas, respecto a la financiación, 60 GR (80,0%) no están financiados con recursos de la UPC y 54 GR (72,0) tienen regulación directa de precios. De lo anterior es posible concluir que se presenta una gran oportunidad de realizar AAA para estos 75 GR o centrar esfuerzos para diseñar mecanismos de financiación expeditos que no representen barreras de acceso a este tipo de tecnologías.

Las publicaciones relacionadas con EH en Colombia son escasas. En una publicación realizada por Pareja (2017) [22] se describe la situación de las EH para ese año y se

concluye que a pesar de la Ley 1392 de 2010 y demás actos administrativos posteriores vigentes, aún falta la definición de modelos y rutas integrales de atención en salud para los pacientes con EH, considerando falencias tanto en el talento humano especializado que atiende este tipo de población, deficiencias en las IPS especializadas, escaso diagnóstico, seguimiento y dificultades en el acceso de los tratamientos farmacológicos y no farmacológicos para tal fin. Por otro lado, en una publicación realizada por Olivares, Vargas y López (2022) se realiza un análisis comparativo de la normatividad colombiana de medicamentos vitales no disponibles con la regulación de otros países concluyendo que para el caso de países latinoamericanos la regulación se ha centrado en los procedimientos que faciliten la importación de este tipo de medicamentos. Se observan para estos mismos países falencias en el sistema como falta de seguimiento a los resultados de los tratamientos, conocimiento por parte del paciente y responsabilidad por parte del médico prescriptor [11].

Finalmente, teniendo en cuenta lo anterior, es posible concluir que el resultado de este trabajo permite avanzar hacia la construcción de una política pública para EH considerando que: 1) se propuso una definición de MEH y 2) fue posible definir el listado de medicamentos para enfermedades huérfanas en Colombia y su metodología de actualización. Para esta última, se propone que se mantenga en constante estudio por parte del Ministerio de Salud y Protección Social mediante el proceso de revisión de las bases de datos de medicamentos relacionadas en la metodología y que sea sometido a consulta pública que permita la construcción colectiva y de este modo asemejarse a la metodología de construcción de agencias reguladoras como EMA y FDA.

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Conflicto de interés

Los autores declaran no presentar ningún conflicto de interés

Referencias

1. M. Gombocz, S. Vogler, Public spending on orphan medicines: a review of the literature, *Journal of Pharmaceutical Policy and Practice*, **13**, 66 (2020).

2. A. Côté, B. Keating, What is wrong with orphan drug policies? *International Society for Pharmacoeconomics and Outcomes Research (ISPOR)*, **15**, 1185-1191 (2012).

3. A.Y.L. Chan, V.K.Y. Chan, S. Olsson, M. Fan, M. Jit, M. Gong, *et al.*, Access and unmet needs of orphan drugs in 194 countries and 6 areas: A global policy review with content analysis, *Value in Health*, **23**(12), 1580-1591 (2020).

4. Ministerio de Salud y Protección Social, Ley 1392 de 2010 "por medio de la cual se reconocen las enfermedades huérfanas como de especial interés y se adoptan normas tendientes a garantizar la protección social por parte del estado colombiano a la población que padece de enfermedades huérfanas y sus cuidadores", Bogotá D. C., Colombia. URL https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/DE/DIJ/ley-1392-de-2010.pdf, consultado en noviembre de 2022.

5. República de Colombia, Ministerio de Salud y Protección Social, Ley 1438 de 2011 "por la cual se reforma el sistema de Seguridad Social en Salud y se dictan otras disposiciones", Bogotá D. C., Colombia. URL: https://www.minsalud.gov.co/Normatividad_Nuevo/LEY%201438%20DE%202011.pdf, consultado en enero de 2023.

6. República de Colombia, Ministerio de Salud y Protección Social, Decreto 1954 de 2012 "por el cual se dictan disposiciones para implementar el sistema de información de pacientes con enfermedades huérfanas", Bogotá D. C., Colombia. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/DE/DIJ/Decreto-1954-de-2012.PDF, consultado en noviembre de 2022.

7. República de Colombia, Ministerio de Salud y Protección Social. Resolución 5265 de 2018 "por la cual se actualiza el listado de enfermedades huérfanas y se dictan otras disposiciones", Bogotá D. C., Colombia. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/DE/DIJ/resolucion-5265-de-2018.pdf, consultado en noviembre de 2022.

8. República de Colombia, Ministerio de Salud y Protección Social. Resolución 023 de 2023 "por medio de la cual se actualiza el listado de enfermedades huérfanas – raras", Bogotá D. C., Colombia. URL: https://www.minsalud.gov.co/Normatividad_Nuevo/Resoluci%C3%B3n%20No.%20023%20de%202023.pdf, consultado en febrero de 2023.

9. L. Olivares, C. Vargas, J. López, Análisis comparativo de la regulación de los medicamentos huérfanos y los medicamentos vitales no disponibles en Colombia, en: M. Uribe-Arbeláez (editor), *Investigaciones de la Maestría en Biociencias y Derecho*, Universidad Nacional de Colombia, Bogotá, 2021, pp. 19-52.

10. República de Colombia, Ministerio de Salud y Protección Social, Decreto 481 de 2004 "por el cual se dictan normas tendientes a incentivar la oferta de medicamentos vitales no disponibles en el país", Bogotá D. C., Colombia. URL: https://www.invima.gov.co/documents/20143/453029/decreto_481_2004.pdf/003ec005-0b1f-c8fb-addb-aac2c5fba617?t=1541014936274, consultado en noviembre de 2022.

11. L.A. Olivares-Escobar, *Medicamentos Vitales no Disponibles: Análisis de la Regulación Nacional e Internacional y Caracterización de las Solicitudes de Importación, acorde al Decreto 481 de 2004, radicadas en el Instituto Nacional de Vigilancia de Medicamentos y Alimentos-INVIMA, en los años 2016 y 2017*, Tesis de Maestría, Facultad de Derecho, Ciencias Políticas y Sociales, Universidad Nacional de Colombia, Bogotá D.C., Colombia, 2019.

12. República de Colombia, Instituto Nacional de Salud, *Protocolo de Vigilancia en Salud Pública: Enfermedades Huérfanas – Raras, Código 342*, Bogotá D. C., Colombia. URL: http://www.saludcapital.gov.co/CTDLab/Publicaciones/2021/Protocolo_VSP-Enfermedades_huerfanas.pdf, consultado en noviembre de 2022.

13. J. Garjón-Parra, Medicamentos huérfanos: regulación y controversias, *Boletín de Información Farmacoterapéutica de Navarra*, **23**(1), 1-13 (2015). URL: https://www.navarra.es/NR/rdonlyres/031845D7-9D51-43BD-9F49-DDCF8F92DD7D/325783/Bit_v23n1.pdf, consultado en febrero de 2023.

14. República de Colombia, Instituto Nacional de Salud, *Informe de evento 342: Enfermedades Huérfanas-Raras Colombia, 2022* (hasta periodo epidemiológico XII), Bogotá D. C., Colombia. URL: https://www.ins.gov.co/buscador-eventos/Paginas/Info-Evento.aspx, consultado en febrero 2023.

15. República de Colombia, Ministerio de Salud y Protección Social, Resolución 1139 de 2022 "Por la cual se establecen disposiciones en relación con el presupuesto máximo para la gestión y financiación de los servicios y tecnologías en salud no financiados con recursos de la Unidad de Pago por Capitación -UPC y no excluidos de la financiación con recursos del Sistema General de Seguridad Social en Salud –SGSSS", Bogotá D. C., Colombia. URL: https://www.minsalud.gov.co/Normatividad_Nuevo/Resoluci%C3%B3n%20No.1139%20de%202022.pdf, consultado en noviembre de 2022.

16. República de Colombia, Ministerio de Salud y Protección Social, Comisión Nacional de Precios de Medicamentos y Dispositivos Médicos, Circular 13 de 2022 "Por la cual se establece el listado de los medicamentos sujetos al régimen de control directo de precios, se fija el precio máximo de venta y el precio por unidad de regulación de medicamentos vitales no disponibles y se dictan otras disposiciones", Bogotá D. C., Colombia. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/DE/DIJ/cnpmdm-circular-13-de-2022.pdf, consultado en noviembre de 2022.

17. R. Rodriguez-Monguio, T. Spargo, E. Seoane-Vazquez, Ethical imperatives of timely access to orphan drugs: is possible to reconcile economic incentives and patients' health needs? *Orphanet Journal of Rare Diseases*, **12**(1), 1 (2017).

18. República de Colombia, Dirección de Regulación de Beneficios, *Costos y Tarifas del Aseguramiento en Salud, Informe de la actualización de los servicios y tecnologías de salud financiados con recursos de la (UPC) año 2022*, Bogotá D. C., Colombia. URL: https://www.minsalud.gov.co/salud/POS/Paginas/resultados-pos.aspx, consultado en marzo de 2023.

19. I. Degtiar, A review of international coverage and pricing strategies for personalized medicine and orphan drugs, *Health Policy*, **121**, 1240-1248 (2017)

20. T. Gammie, C.Y. Lu, Z.U. Babar, Access to orphan drugs: A comprehensive review of legislations, regulations and policies in 35 countries, *PLoS One*, **10**(10), e0140002 (2015).

21. F. Tobar, Economía de los medicamentos genéricos en América Latina, *Revista Panamericana de Salud Pública*, **23**, 59-67 (2008).

22. M.L. Pareja-Arcila, Situación actual de las enfermedades huérfanas en Colombia 2017, *Revista CES Derecho*, **8**(2), 231-241 (2017).

Anexo

Tabla A1. Propuesta de listado de medicamentos para EH en Colombia y su clasificación si tiene otras indicaciones diferentes a EH

GR	Descripción	Tiene otras indicaciones diferentes a EH
1	ACETIL SALICILICO ACIDO-TABLETA O CÁPSULA	SÍ
2	ACETIL SALICILICO ACIDO-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
3	ÁCIDO ALENDRONICO-TABLETA O CÁPSULA	SÍ
4	ÁCIDO CARGLUMICO-TABLETA O CÁPSULA	NO
5	ÁCIDO COLICO-TABLETA O CÁPSULA	NO
6	ÁCIDO FÓLICO-TABLETA O CÁPSULA	SÍ
7	ÁCIDO IBANDRONICO-SOLUCION O SUSPENSIÓN INYECTABLE	SÍ
8	ÁCIDO IBANDRONICO-TABLETA O CÁPSULA	SÍ
9	ÁCIDO QUENODESOXICÓLICO-TABLETA O CÁPSULA	SÍ
10	ÁCIDO RISEDRONICO-TABLETA O CÁPSULA	SÍ
11	ÁCIDO TRANEXAMICO-SOLUCION O SUSPENSION INYECTABLE	SÍ
12	ÁCIDO TRANEXAMICO-TABLETA O CÁPSULA	SÍ
13	ÁCIDO URSODEOXICOLICO-TABLETA O CÁPSULA	SÍ
14	ÁCIDO ZOLEDRONICO-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSION INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
15	ACITRETINA-TABLETA O CÁPSULA	SÍ
16	ADALIMUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
17	AGALSIDASA ALFA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
18	AGALSIDASA BETA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
19	ALEMTUZUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO

GR	Descripción	Tiene otras indicaciones diferentes a EH
20	ALFA 1 ANTITRIPSINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
21	ALGLUCOSIDASA ALFA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
22	ALOPURINOL-TABLETA O CÁPSULA	SÍ
23	AMBRISENTAN-TABLETA O CÁPSULA	SÍ
24	ANAGRELIDA-TABLETA O CÁPSULA	SÍ
25	ANAGRELIDA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
26	ANAKINRA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
27	ANFOTERICINA B-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
28	ANFOTERICINA B-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
29	ARGININA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
30	ARGININA-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
31	ARMODAFINILO-TABLETA O CÁPSULA	SÍ
32	ASFOTASA ALFA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
33	ATALUREN-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
34	ATORVASTATINA-TABLETA O CÁPSULA	SÍ
35	ATORVASTATINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
36	AZACITIDINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
37	AZATIOPRINA-TABLETA O CÁPSULA	SÍ
38	BACLOFENO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
39	BACLOFENO-TABLETA O CÁPSULA	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
40	BETAINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	NO
41	BETAMETASONA DIPROPIONATO+BETAMETASONA FOSFATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
42	BETAMETASONA FOSFATO+BETAMETASONA ACETATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
43	BETAMETASONA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
44	BETANECOL-TABLETA O CÁPSULA	SÍ
45	BEXAROTENO-TABLETA O CÁPSULA	SÍ
46	BICARBONATO DE SODIO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
47	BOSENTAN-TABLETA O CÁPSULA	SÍ
48	BOSENTAN-TABLETA O CÁPSULA	SÍ
49	BRIVARACETAM-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
50	BRIVARACETAM-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
51	BRIVARACETAM-TABLETA O CÁPSULA	SÍ
52	BROMOCRIPTINA-TABLETA O CÁPSULA	SÍ
53	BUDESONIDA-EMULSION RECTAL	SÍ
54	BUDESONIDA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
55	BUROSUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
56	CABOZANTINIB-TABLETA O CÁPSULA	SÍ
57	CALCITRIOL-TABLETA O CÁPSULA	SÍ
58	CANAKINUMAB-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
59	CANNABIDIOL-SOLUCIÓN O SUSPENSIÓN ORAL	NO
60	CANNABIDIOL-TABLETA O CÁPSULA	NO
61	CAPECITABINA-TABLETA O CÁPSULA O TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
62	CAPLACIZUMAB-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
63	CARBONATO DE CALCIO-TABLETA O CÁPSULA	SÍ
64	CARBONATO DE CALCIO-TABLETA O CÁPSULA	SÍ
65	CASIMERSEN-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
66	CERLIPONASA ALFA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE / SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
67	CERTOLIZUMAB PEGOL-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
68	CIANOCOBALAMINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
69	CICLOFOSFAMIDA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
70	CICLOFOSFAMIDA-TABLETA O CÁPSULA	SÍ
71	CIPROFIBRATO-TABLETA O CÁPSULA	SÍ
72	CISPLATINO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
73	CISTEAMINA-TABLETA O CÁPSULA	NO
74	CLADRIBINA-TABLETA O CÁPSULA	NO
75	CLOBAZAM-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
76	CLONAZEPAM-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
77	CLONAZEPAM-TABLETA O CÁPSULA	SÍ
78	CLORAMBUCILO-TABLETA O CÁPSULA	SÍ
79	CLORMETINA-CREMAS O GELES O UNGUENTOS O POMADAS O PASTAS O JALEAS	SÍ
80	COLCHICINA-TABLETA O CÁPSULA	SÍ
81	CONESTAT ALFA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
82	CONESTAT ALFA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
83	CREATINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
84	CREATINA-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
85	DAPSONA-TABLETA O CÁPSULA	SÍ
86	DEFERASIROX-TABLETA O CÁPSULA	SÍ
87	DEFEROXAMINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
88	DEFLAZACORT-TABLETA O CÁPSULA	SÍ
89	DESMOPRESINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
90	DESMOPRESINA-SOLUCIÓN O SUSPENSIÓN PARA INHALACION	SÍ
91	DESMOPRESINA-SOLUCIÓN O SUSPENSIÓN SUBLINGUAL	SÍ
92	DESMOPRESINA-TABLETA O CÁPSULA	SÍ
93	DEXAMETASONA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
94	DEXAMETASONA-TABLETA O CÁPSULA	SÍ
95	DEXTROSA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
96	DEXTROSA-TABLETA O CÁPSULA	SÍ
97	DIAZOXIDO-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
98	DIMETILFUMARATO-TABLETA O CÁPSULA	NO
99	DONEPEZILO-TABLETA O CÁPSULA	SÍ
100	DORNASA ALFA-SOLUCIÓN O SUSPENSIÓN PARA INHALACION	NO
101	DOXORUBICINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE/ SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
102	DOXORUBICINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
103	ECALANTIDA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
104	ECULIZUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
105	EDARAVONA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
106	EDROFONIO CLORURO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
107	ELEXACAFTOR/TEZACAFTOR/IVACAFTOR-TABLETA O CÁPSULA	SÍ
108	ELIGLUSTAT-TABLETA O CÁPSULA	NO
109	ELOSULFASA ALFA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
110	EMICIZUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
111	EPOPROSTENOL-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
112	ESOMEPRAZOL-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
113	ESOMEPRAZOL-TABLETA O CÁPSULA	SÍ
114	ESOMEPRAZOL-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
115	ETANERCEPT-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
116	ETEPLIRSEN-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
117	EVEROLIMUS-TABLETA O CÁPSULA	SÍ
118	EVEROLIMUS-TABLETA O CÁPSULA	SÍ
119	EVOLOCUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
120	EZETIMIBA-TABLETA O CÁPSULA	SÍ
121	FACTOR DE COAGULACION HUMANO XI-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
122	FACTOR II DE COAGULACION+FACTOR VII DE COAGULACION+FACTOR IX DE COAGULACION+FACTOR X DE COAGULACION+PROTEINA C+PROTEINA S+HEPARINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO

GR	Descripción	Tiene otras indicaciones diferentes a EH
123	FACTOR IX DE COAGULACIÓN PROTEINA DE FUSION FC [RFIXFC]-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
124	FACTOR IX DE LA COAGULACION-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
125	FACTOR VII DE LA COAGULACION ACTIVADO-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
126	FACTOR VII DE LA COAGULACION-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
127	FACTOR VIII DE LA COAGULACION PEGILADO-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
128	FACTOR VIII DE LA COAGULACION+FACTOR DE VON WILLEBRAND-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
129	FACTOR VIII DE LA COAGULACION+FACTOR DE VON WILLEBRAND-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
130	FACTOR VIII DE LA COAGULACION+FACTOR DE VON WILLEBRAND-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
131	FACTOR VIII DE LA COAGULACION-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
132	FACTOR VIII INHIBIDOR ACTIVADO POR BYPASS-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
133	FACTOR VON WILLEBRAND-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
134	FACTOR XIII-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
135	FAMOTIDINA-TABLETA O CÁPSULA	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
136	FAMPRIDINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	NO
137	FEBUXOSTAT-TABLETA O CÁPSULA	SÍ
138	FELBAMATO-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
139	FELBAMATO-TABLETA O CÁPSULA	SÍ
140	FENILACETATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
141	FENILBUTIRATO DE GLICEROL-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
142	FENILBUTIRATO DE SODIO-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	NO
143	FENOFIBRATO DE COLINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
144	FENOFIBRATO DE COLINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
145	FENOFIBRATO-TABLETA O CÁPSULA	SÍ
146	FIBRINOGENO COAGULABLE-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
147	FIBRINOGENO HUMANO-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
148	FILGRASTIM-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
149	FINGOLIMOD-TABLETA O CÁPSULA	NO
150	FLECAINIDA-TABLETA O CÁPSULA	SÍ
151	FLUDARABINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
152	FLUDROCORTISONA-TABLETA O CÁPSULA	SÍ
153	FOLITROPINA ALFA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
154	FOLITROPINA BETA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
155	FOSFATO DE SODIO Y POTASIO EQUIVALENTES A FOSFORO-TABLETA O CÁPSULA	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
156	GALANTAMINA-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
157	GALANTAMINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
158	GALSULFASA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
159	GEMCITABINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
160	GIVOSIRAN-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
161	GLATIRAMERO ACETATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
162	GLUCAGON-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
163	GOLIMUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
164	GOLODIRSEN-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
165	GONADORELINA ACETATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
166	GONADOTROPINA CORIONICA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
167	GONADOTROPINA MENOPAUSICA HUMANA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
168	HEMINA HUMANA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE/ SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
169	HIDROCORTISONA (ACEPONATO)-CREMAS O GELES O UNGUENTOS O POMADAS O PASTAS O JALEAS	SÍ
170	HIDROCORTISONA-TABLETA O CÁPSULA	SÍ
171	HIERRO BISGLICINA QUELATO-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
172	HIERRO GLICINATO QUELATO-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
173	HIERRO POLIMALTOSADO-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
174	HORMONA LIBERADORA DE CORTICOTROPINA (CRH) -POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
175	IBRUTINIB-TABLETA O CÁPSULA	SÍ
176	ICATIBANTO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
177	IDEBENONA-TABLETA O CÁPSULA	SÍ
178	IDURSULFASA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
179	IFOSFAMIDA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
180	IMATINIB-TABLETA O CÁPSULA	SÍ
181	IMIGLUCERASA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
182	INFLIXIMAB-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
183	INHIBIDOR C1 ESTERASA -POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
184	INHIBIDOR C1 ESTERASA -POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
185	INMUNOGLOBULINA HUMANA G-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
186	INMUNOGLOBULINA HUMANA G-SOLUCIÓN O SUSPENSIÓN INYECTABLE/POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE/	SÍ
187	INMUNOGLOBULINA HUMANA NORMAL-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
188	INMUNOGLOBULINA HUMANA NORMAL-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
189	INMUNOGLOBULINA HUMANA NORMAL-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
190	INMUNOGLOBULINA HUMANA NORMAL-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
191	INOTERSEN-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
192	INTERFERON ALFA 2 BETA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
193	INTERFERON BETA-1A PEGILADO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
194	INTERFERON BETA-1A-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
195	INTERFERON BETA-1A-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
196	INTERFERON BETA-1B-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
197	INTERFERON GAMMA-1B-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
198	IVACAFTOR + LUMACAFTOR-TABLETA O CÁPSULA	SÍ
199	IVACAFTOR + TEZACAFTOR-TABLETA O CÁPSULA	SÍ
200	IVACAFTOR-TABLETA O CÁPSULA	SÍ
201	LAMOTRIGINA-TABLETA O CÁPSULA	SÍ
202	LANADELUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
203	LANREOTIDA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
204	LANSOPRAZOL-TABLETA O CÁPSULA	SÍ
205	LANSOPRAZOL-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
206	LARONIDASA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
207	LEFLUNOMIDA-TABLETA O CÁPSULA	SÍ
208	LENALIDOMIDA-TABLETA O CÁPSULA	SÍ
209	LEVOCARNITINA-SOLUCIÓN O SUSPENSIÓN ORAL	NO
210	LEVOCARNITINA-TABLETA O CÁPSULA	SÍ
211	LEVODOPA+CARBIDOPA-TABLETA O CÁPSULA	SÍ
212	LOMITAPIDA-TABLETA O CÁPSULA	NO
213	MACITENTAN-TABLETA O CÁPSULA	SÍ
214	MECASERMINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
215	MEMANTINA-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
216	MEMANTINA-TABLETA O CÁPSULA	SÍ
217	MERCAPTAMINA-SOLUCIÓN O SUSPENSIÓN OFTALMICA	NO
218	MESALAZINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
219	METILFENIDATO-TABLETA O CÁPSULA	SÍ
220	METIRAPONA-TABLETA O CÁPSULA	SÍ
221	METOTREXATO-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
222	METOTREXATO-TABLETA O CÁPSULA	SÍ
223	METRELEPTIN-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
224	MICOFENOLATO-TABLETA O CÁPSULA	SÍ
225	MIFAMURTIDA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
226	MIGALASTAT-TABLETA O CÁPSULA	NO
227	MIGLUSTAT-TABLETA O CÁPSULA	NO
228	MODAFINILO-TABLETA O CÁPSULA	SÍ
229	MULTIENZIMAS (AMILASA, LIPASA, PROTEASA)-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
230	NADOLOL-TABLETA O CÁPSULA	SÍ
231	NATALIZUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
232	NEOSTIGMINA METILSULFATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
233	NIFEDIPINA-TABLETA O CÁPSULA	SÍ
234	NINTEDANIB-TABLETA O CÁPSULA	NO
235	NITISINONA-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
236	NITISINONA-TABLETA O CÁPSULA	NO
237	NUSINERSEN-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
238	OCRELIZUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
239	OCTREOTIDE-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE O SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
240	OFATUMUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
241	OMEPRAZOL-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
242	OMEPRAZOL-TABLETA O CÁPSULA	SÍ
243	OMEPRAZOL-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
244	ORNITINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
245	OSILODROSTAT-TABLETA O CÁPSULA	NO
246	PALIVIZUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
247	PANTOPRAZOL-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
248	PASIREOTIDA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE/ SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
249	PEGVISOMANT-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO

GR	Descripción	Tiene otras indicaciones diferentes a EH
250	PENICILAMINA-TABLETA O CÁPSULA	SÍ
251	PIRFENIDONA-TABLETA O CÁPSULA	NO
252	PIRIDOSTIGMINA-TABLETA O CÁPSULA	SÍ
253	PIRIDOXINA-TABLETA O CÁPSULA	SÍ
254	PLASMA HUMANO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
255	POCAPAVIR-TABLETA O CÁPSULA	SÍ
256	PREDNISOLONA-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
257	PROPRANOLOL-TABLETA O CÁPSULA	SÍ
258	QUINIDINA-TABLETA O CÁPSULA	SÍ
259	RABEPRAZOL-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
260	RANITIDINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
261	RANITIDINA-TABLETA O CÁPSULA	SÍ
262	REGORAFENIB-TABLETA O CÁPSULA	SÍ
263	RETINOL (VIT A)-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
264	RIBOFLAVINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
265	RIBOFLAVINA-TABLETA O CÁPSULA	SÍ
266	RILUZOL-TABLETA O CÁPSULA	NO
267	RIOCIGUAT-TABLETA O CÁPSULA	SÍ
268	RITUXIMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
269	RIVASTIGMINA-PARCHE	SÍ
270	RIVASTIGMINA-TABLETA O CÁPSULA	SÍ
271	ROMIDEPSIN-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
272	ROSUVASTATINA-TABLETA O CÁPSULA	SÍ
273	ROSUVASTATINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
274	RUFINAMIDA-TABLETA O CÁPSULA	NO
275	SAPROPTERINA-TABLETA O CÁPSULA	NO

GR	Descripción	Tiene otras indicaciones diferentes a EH
276	SEBELIPASA ALFA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
277	SELEXIPAG-TABLETA O CÁPSULA	SÍ
278	SILDENAFILO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
279	SILDENAFILO-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
280	SILDENAFILO-TABLETA O CÁPSULA	SÍ
281	SIMVASTATINA-TABLETA O CÁPSULA	SÍ
282	SIPONIMOD-TABLETA O CÁPSULA	NO
283	SIROLIMUS-TABLETA O CÁPSULA	SÍ
284	SODIO BENZOATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
285	SODIO BENZOATO-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
286	SOMATROPINA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
287	SOMATROPINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
288	SUCCIMERO-TABLETA O CÁPSULA	SÍ
289	SULFASALAZINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
290	SULFATO DE MAGNESIO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
291	SULFATO DE MAGNESIO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
292	SUNITINIB-TABLETA O CÁPSULA	SÍ
293	TACROLIMUS-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
294	TACROLIMUS-TABLETA O CÁPSULA	SÍ
295	TACROLIMUS-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
296	TADALAFILO-TABLETA O CÁPSULA	SÍ
297	TAFAMIDIS-TABLETA O CÁPSULA	NO
298	TALIDOMIDA-TABLETA O CÁPSULA	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
299	TALIGLUCERASA ALFA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
300	TEDUGLUTIDA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
301	TEMOZOLOMIDA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
302	TEMOZOLOMIDA-TABLETA O CÁPSULA	SÍ
303	TERIFLUNOMIDA-TABLETA O CÁPSULA	NO
304	TESTOSTERONA-CREMAS O GELES O UNGUENTOS O POMADAS O PASTAS O JALEAS	SÍ
305	TESTOSTERONA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
306	TESTOSTERONA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
307	TESTOSTERONA-TABLETA O CÁPSULA	SÍ
308	TETRABENAZINA-TABLETA O CÁPSULA	SÍ
309	TETRACOSACTIDA ACETATO O HEXAACETATO-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
310	TIAMINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
311	TIAMINA-TABLETA O CÁPSULA	SÍ
312	TINIDAZOL-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
313	TINIDAZOL-TABLETA O CÁPSULA	SÍ
314	TIOTEPA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
315	TIZANIDINA-TABLETA O CÁPSULA	SÍ
316	TIZANIDINA-TABLETA O CÁPSULA DE LIBERACIÓN MODIFICADA	SÍ
317	TOCILIZUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
318	TOLVAPTAN-TABLETA O CÁPSULA	SÍ
319	TOPIRAMATO-TABLETA O CÁPSULA	SÍ
320	TOXINA BOTULINICA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ

GR	Descripción	Tiene otras indicaciones diferentes a EH
321	TOXINA BOTULINICA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
322	TREOSULFANO-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
323	TRIENTINA CLORHIDRATO-TABLETA O CÁPSULA	NO
324	TRIHEXIFENIDILO-TABLETA O CÁPSULA	SÍ
325	UBIDECARENONA-SOLUCIÓN O SUSPENSIÓN ORAL	SÍ
326	USTEKINUMAB-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
327	VASOPRESINA-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
328	VEDOLIZUMAB-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
329	VELAGLUCERASA ALFA-POLVO PARA RECONSTITUIR A SOLUCIÓN O SUSPENSIÓN INYECTABLE	NO
330	VOLANESORSEN-SOLUCIÓN O SUSPENSIÓN INYECTABLE	SÍ
331	WARFARINA-TABLETA O CÁPSULA	SÍ

Fuente: Elaboración propia

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Thermoanaerobacter tengcongensis esterase resists denaturation by urea and sodium dodecyl sulfate

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Summary

Introduction: The broad applications of lipolytic enzymes in various industrial processes have led to increased interest in esterases with distinctive features. Thermophiles are promising source of esterases with inherent thermal and chemical stability. *Thermoanaerobacter tengcongensis* esterase (TTE) is one of such esterases with thermostable potential, however, its resistance to protein denaturants, detergents and molecular docking studies are yet to be fully characterised. **Aim:** Therefore, this study investigated the *in vitro* and *in silico* effects of urea and sodium dodecyl sulfate on TTE activity. **Experimental:** TTE activity was determined spectrophotometrically at 405 nm. TTE was active over a pH range of 3.0 to 12.0 and its activity was optimal at alkaline range of 9.0 and 12.0. **Results:** TTE was found to be most active at 60 °C with the highest thermal stability at the same temperature. Urea at 0.1 to 4.0 mM had a concentration dependent activating effect on TTE; SDS (0.5 to 4.0 mM) had similar effect on the enzyme. Urea at 0.5, 1.0 and 2.0 mM increased maximum reaction rate (V_{max}), catalytic constant (K_{cat}) and Michaelis constant (K_m) of TTE. All concentrations of SDS (0.5 to 2.0 mM) investigated increased V_{max} and K_{cat}, while the K_m value of TTE reduced in the presence of 1.0 and 2.0 mM SDS. Structural characterization of TTE substantiates the *in vitro* thermostability claim. The

molecular docking analysis revealed that donepezil demonstrated optimal binding with TTE. **Conclusion:** the findings from this study showed that TTE strongly resists denaturation by optimal concentrations of urea and SDS.

Keywords: Thermoanaerobacter tengcongensis, esterase, thermophiles, protein denaturants, para-nitrophenyl dodecanoate

Resumen

La esterasa de *Thermoanaerobacter tengcongensis* resiste la desnaturalización por la urea y el dodecilsulfato de sodio

Introducción: Las amplias aplicaciones de las enzimas lipolíticas en diversos procesos industriales han llevado a un mayor interés en esterasas con características distintivas. Los termófilos son una fuente prometedora de esterasas con estabilidad térmica y química inherente. La esterasa de *Thermoanaerobacter tengcongensis* (TTE) es una de esas esterasas con potencial termoestable; sin embargo, su resistencia a los desnaturalizantes de proteínas, los detergentes y los estudios de acoplamiento molecular aún no se han caracterizado por completo. **Objetivo:** Por lo tanto, este estudio investigó los efectos *in vitro* e *in silico* de la urea y el dodecilsulfato de sodio sobre la actividad del TTE. **Parte experimental:** la actividad TTE se determinó espectrofotométricamente a 405 nm. El TTE estuvo activo en un rango de pH de 3,0 a 12,0 y su actividad fue óptima en un rango alcalino de 9,0 y 12,0. **Resultados:** Se encontró que el TTE era más activo a 60 °C y tenía la mayor estabilidad térmica a la misma temperatura. La urea de 0,1 a 4,0 mM tuvo un efecto activador dependiente de la concentración sobre el ETT; SDS (0,5 a 4,0 mM) tuvo un efecto similar sobre la enzima. La urea en concentraciones de 0,5, 1,0 y 2,0 mM aumentó la velocidad de reacción máxima (V_{max}), la constante catalítica (K_{cat}) y la constante de Michaelis (K_m) de TTE. Todas las concentraciones de SDS (0,5 a 2,0 mM) investigadas aumentaron V_{max} y K_{cat}, mientras que el valor de K_m de TTE se redujo en presencia de SDS 1,0 y 2,0 mM. La caracterización estructural de TTE fundamenta la afirmación de termoestabilidad *in vitro*. El análisis de acoplamiento molecular reveló que donepezilo demostró una unión óptima con TTE. **Conclusión:** los hallazgos de este estudio mostraron que el TTE resiste fuertemente la desnaturalización por concentraciones óptimas de urea y SDS.

Palabras clave: Thermoanaerobacter tengcongensis, esterasa, termófilos, desnaturalizantes de proteínas, dodecanoato de paranitrofenilo

Resumo

A esterase de *Thermoanaerobacter tengcongensis* resiste à desnaturação pela uréia e dodecil sulfato de sódio

Introdução: As amplas aplicações de enzimas lipolíticas em diversos processos industriais têm levado ao aumento do interesse em esterases com características distintivas. Os termófilos são fontes promissoras de esterases com estabilidade térmica e química inerente. A esterase de *Thermoanaerobacter tengcongensis* (TTE) é uma dessas esterases com potencial termoestável, no entanto, sua resistência a desnaturantes de proteínas, detergentes e estudos de acoplamento molecular ainda não foram totalmente caracterizadas. **Objetivo:** Portanto, este estudo investigou os efeitos *in vitro* e *in silico* da uréia e dodecilsulfato de sódio na atividade do TTE. **Parte experimental:** A atividade do TTE foi determinada espectrofotometricamente a 405 nm. O TTE foi ativo em uma faixa de pH de 3,0 a 12,0 e sua atividade foi ótima na faixa alcalina de 9,0 e 12,0. **Resultados:** Descobriu-se que o TTE é mais ativo a 60 °C, com maior estabilidade térmica à mesma temperatura. A uréia em concentrações de 0,1 a 4,0 mM teve um efeito ativador dependente da concentração no ETT; SDS (0,5 a 4,0 mM) teve efeito semelhante na enzima. A uréia a 0,5, 1,0 e 2,0 mM aumentou a taxa máxima de reação (V_{max}), a constante catalítica (K_{cat}) e a constante de Michaelis (K_m) do TTE. Todas as concentrações de SDS (0,5 a 2,0 mM) investigadas aumentaram V_{max} e K_{cat}, enquanto o valor de Km de TTE reduziu na presença de 1,0 e 2,0 mM de SDS. A caracterização estrutural do TTE fundamenta a alegação de termoestabilidade *in vitro*. A análise de acoplamento molecular revelou que o donepezil demonstrou ligação ideal ao TTE. **Conclusão:** os resultados deste estudo mostraram que o TTE resiste fortemente à desnaturação por concentrações ótimas de uréia e SDS.

Palavras-chave: Thermoanaerobacter tengcongensis, esterase, termófilos, desnaturantes de proteínas, para-nitrofenil dodecanoato

Introduction

Esterases (E.C 3.1.1.x) are lipolytic enzymes of the α/β hydrolases superfamily that catalyse the hydrolysis of a variety of substrates containing ester linkages, such as aryl esters, carboxylic esters and acylglycerols, resulting in the formation of an alcohol and carboxylic acid [1, 2]. Esterases are widely distributed in nature and have been isolated from various species, however, a significant amount of the enzymes originates from bacteria [3, 4]. The structural analyses of microbial esterases revealed that they consist

of a "cap" domain that participate in substrate binding and a catalytic domain which contains a functional serine in a conserved pentapeptide Gly-Xaa-Ser-Xaa-Gly [3, 5]. The serine of the catalytic domain together with aspartate and histidine make up the highly conserved catalytic triad (Figure 1) [4]. Esterases can catalyze three basic types of reactions: esterification, interesterification and transesterification reactions [6]. Furthermore, esterases are independent of cofactors and have been observed to be active in aqueous and non-aqueous solvents [7]. Although having preference for short to medium chain monoesters, esterases are able to hydrolyse long chain water insoluble triglycerides up to twelve carbon atoms [4, 6].

Typically, esterases display a wide pH, temperature and substrate spectra, and they also exhibit tolerance to metal ion, salt and solvent to varying extents [4]. These features in addition to their regio- and enantio-selectivity make esterases attractive as a biotransformer for the synthesis of optical prodrugs, additives and polyesters [3]. Esterases are widely used in food, pharmaceutical, detergent, pulp and paper industries, biodiesel production, fats and oils production, as well as environmental applications for the degradation of lipid wastes [4, 8, 9].

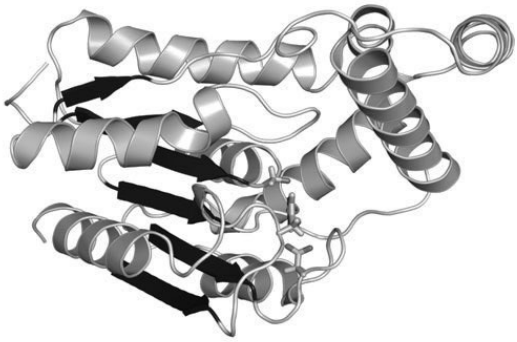

Figure 1. The overall structure of the C-terminal domain of esterase D. The central β-sheet and surrounding α-helixes are shown in black and grey, respectively. Catalytic triad residues are indicated [10].

Most industrial lipolytic hydrolase and esterification reactions occur under harsh conditions (i.e. at high temperature and presence of organic solvent) which constrain enzyme stability, thus the demand for esterases with intrinsic thermal and chemical stability has increased [3]. Extremophiles, particularly thermophiles have overtime been observed to be a promising source of esterases with high inherent stability, and over the years, some esterases with the aforementioned features have been characterised from thermophiles and hyperthermophiles such as *Archaeoglobus fulgidus*, *Pyrococcus*

furiosus, *Pyrobaculum calidifontis, Thermoanaerobacter tengcongensis* and *Thermotoga maritima* [3, 10].

Urea and Sodium dodecyl sulfate (SDS) are well established protein denaturants with denaturing effects similar to thermal denaturation above protein's melting temperature (T_m) of 75 °C [11-13]. For *Thermoanaerobacter tengcongensis* esterase (TTE) to maintain industrial relevance and competitiveness, it is necessary for it to be stable and effective in complex mixtures of compounds such as organic compounds and anionic detergent [14]. The thermostability of TTE has been established but its resistance to protein denaturants, urea and SDS, is yet to be elucidated. In this study, we investigated both *in vitro* and *in silico* the effects of protein denaturants, urea and sodium dodecyl sulfate (SDS) on *Thermoanaerobacter tengcongensis* esterase (TTE) activity.

Materials and methods

Materials

Homogenous *Thermoanaerobacter tengcongensis* esterase (TTE) was synthesized and purified at Institute of Molecular Cell and Systems Biology, University of Glasgow, Bower Building, Glasgow G12 8QQ, Scotland, UK. Para-nitrophenyl dodecanoate (pNPD) also known as 4-Nitrophenyl ester ($C_{18}H_{27}NO_4$) and sodium dodecyl sulfate (Lauryl Sulfate) sodium salt ($C_{12}H_{25}O_4SNa$) were products of Sigma-Aldrich Co, St. Louis, USA. Urea (NH_2CONH_2) is a product of NAAFCO, Scientific Supplies Ltd. New York. All other reagents used in this study were purchased from Fisher Scientific, UK, and were of analytical grade.

Methods

Overexpression and purification of Thermoanaerobacter tengcongensis esterase

The gene coding for putative esterase from *T. tengcongensis* was expressed using *E. coli* BL21 (DE3) pLysS competent cells, this produced His-tagged fusion protein. *E. coli* BL21 (DE3) pLysS culture induced with Isopropyl β-D-1-thiogalactopyranoside (IPTG) showed the over-production of His-tagged TTE. His-tagged TTE was extracted from BL21 (DE3) pLysS cells through cell lysis with the aid of a Sonicator according to the procedure earlier described by Olorunniji *et al.* [15]. His-tag purification was carried out using immobilized metal affinity chromatography (IMAC) technique. His-tagged TTE was eluted with a buffer containing imidazole since it competes with his-tag for binding to the nickel charged resin leaving the protein of interest [15].

Determination of Thermoanaerobacter tengcongensis esterase activity

Thermoanaerobacter tengcongensis esterase (TTE) activity was determined by monitoring the rate of hydrolysis of para-nitrophenyl dodecanoate (pNPD) as previously described by Gopinath *et al*. [16] with some modifications. TTE hydrolyses the colourless synthetic substrate, pNPD, to produce a yellow-coloured, para-nitrophenol (pNP). Reaction mixture containing 0.1 M Glycine-NaOH buffer (pH 9.4) and 0.001 μM TTE was pre-incubated in water bath at 37 °C for 10 minutes. The reaction was initiated by the addition of 0.08 mM pNPD and the mixture was incubated at 37 °C for 10 minutes. The reaction was then terminated by the addition of 0.5 M TCA and 0.5 M NaOH. The amount of the para-nitrophenol (pNP) released from the hydrolysis of pNPD catalysed by TTE according to Beer-Lambert's law were measured spectrophotometrically at 405 nm. All assays were carried out in triplicates.

Effect of pH on Thermoanaerobacter tengcongensis esterase activity

Reaction mixture containing 0.1 M Glycine-NaOH buffer at varying pH (3 - 12) and 0.001 μM TTE was firstly pre-incubated in a water bath at 37 °C for 10 minutes. The reaction was initiated by the addition of 0.08 mM pNPD and then incubated for 10 minutes at 37 °C. The reaction was terminated by the addition of 0.5 M TCA and 0.5 M NaOH.

Effect of temperature and thermal stability of Thermoanaerobacter tengcongensis esterase activity

To the reaction mixture containing 0.1 M Glycine-NaOH buffer (pH 9.4) and 0.001 μM TTE, 0.08 mM pNPD was added to initiate the reaction. The reaction mixture was incubated for 10 minutes at varying temperatures (20 - 100 °C) and then stopped by the addition of 0.5 M TCA and 0.5 M NaOH.

For the effect of high temperatures on stability of the enzyme, reaction mixture containing 0.1 M Glycine-NaOH buffer (pH 9.4) and 0.001 μM TTE was pre-incubated in water bath at varying temperatures (30, 35, 40, 45, 50, 60, 70, 80 °C) for 10 minutes and then placed on ice to cool. Reaction was initiated by the addition of 0.08 mM pNPD and then incubated for 10 minutes at 37 °C. Reaction was terminated by the addition of 0.5 M TCA and 0.5 M NaOH.

Effect of urea and sodium dodecyl sulfate on Thermoanaerobacter tengcongensis esterase activity

Reaction mixture containing 0.1 M Glycine-NaOH buffer (pH 9.4), varying concentrations of urea or SDS (0.1 – 4.0 mM) and 0.001 μM TTE was pre-incubated in a

water bath at 37 °C for 10 minutes. Reaction was initiated by the addition of 0.08 mM pNPD and then incubated at 37 °C for another 10 minutes. Reaction was terminated by the addition of 0.2 ml of 0.5 M TCA and 0.25 ml of 0.5 M NaOH.

Kinetics of Thermoanaerobacter tengcongensis esterase catalysed hydrolysis of para-nitrophenyl dodecanoate

Reaction mixture containing 0.1 M Glycine-NaOH buffer (pH 9.4) and 0.001 μM TTE was pre-incubated in water bath at 37 °C for 10 minutes. Reaction was initiated by the addition of 0.01 – 0.50 mM pNPD separately and incubated at 37 °C for another 10 minutes. Reaction was terminated by the addition of 0.2 ml of 0.5 M TCA and 0.25 ml of 0.5 M NaOH.

Kinetics of Thermoanaerobacter tengcongensis esterase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of urea/sodium dodecyl sulfate

Reaction mixture containing 0.1 M Glycine-NaOH buffer (pH 9.4), with 0.5, 1.0 and 2.0 mM urea or SDS and 0.001μM TTE was pre-incubated in a water bath at 37°C for 10 minutes. Reaction was initiated by the addition of 0.01 – 0.50 mM pNPD separately and incubated at 37 °C for another 10 minutes. Reaction was terminated by the addition of 0.2 ml of 0.5 M TCA and 0.25 ml of 0.5 M NaOH.

In silico study

In silico study was done to gain better insight on the binding affinity of TTE active site to urea, SDS and pNPD compared to donepezil. The selected ligands (urea and SDS) are known molecules with high enzyme denaturation potentials, while donepezil is a standard inhibitor. The protein and ligands were converted into dockable pdbqt format using Autodock tools. Pdbqt format of the protein, as well as those of the ligands, was dragged into their respective columns and the software was run. Blind docking of the ligands to the protein target was done and binding scores determination was carried out using PyRx-Python Prescription 0.8 (The Scripps Research Institute) [17]. The dimensions for TTE were set as grid center: x = -21.9200, y = 10.7710, z = -41.6009 and size: x = 66.7970, y = 61.4321, z = 39.3460. The binding scores of evaluated ligands and pNPD were compared to the binding score of donepezil.

Ligands and protein preparation

The three-dimensional (3D) SDF structures of urea, SDS, pNPD and donepezil with CIDs: 1176, 8778, 74778 and 3152 respectively were retrieved from PubChem database (www.pubchem.ncbi.nlm.nih.gov) [18]. The direct investigation of TTE through

in silico approach was hindered due to non-availability of its 3D crystal structure in the protein data bank. Thus the theoretical characterization of TTE was carried out. The TTE primary sequence with UniProtKD ID: Q8RC83 was retrieved from UniProt database (https://www.uniprot.org/) [19]. The obtained primary sequence was used for the modeling of the TTE tertiary structure using the swiss model webserver (https://swissmodel.expasy.org/interactive/) [20]. The quality of the foreseen TTE model for structural characterization was checked using the online PROCHECK webserver (https://saves.mbi.ucla.edu/) [21, 22] via the Ramachandran plot.

Results

Effect of pH on Thermoanaerobacter tengcongensis esterase activity

The pH dependency of *Thermoanaerobacter tengcongensis* esterase (TTE) was investigated at different pH ranging from 3.0 – 12.0 in this study. TTE was observed to be active over a pH range of 3.0 – 12.0 and the activity of the enzyme progressively increased with increase in pH from 3.0 to 9.0. Between pH 9.0 and 12.0, there was no noticeable increase in TTE activity (Figure 2). The TTE activity at pH 9.0 was observed to be 12% higher when compared with the activity at pH 3.0.

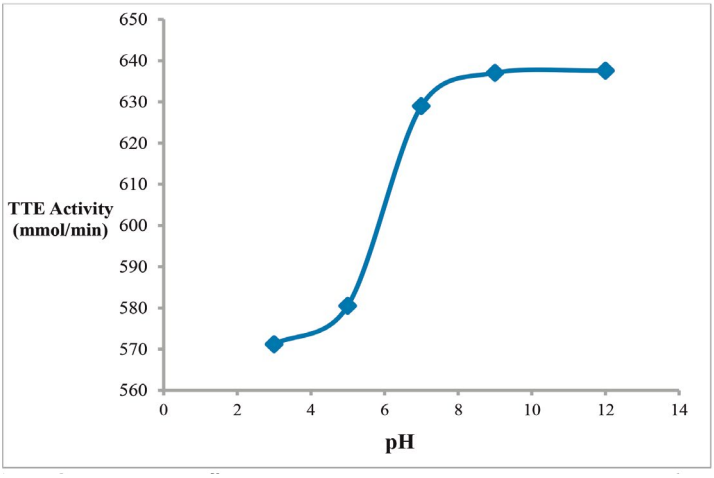

Figure 2. Effect of varying pH on *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

Effect of temperature on Thermoanaerobacter tengcongensis esterase activity

The effects of varying assay temperatures (20 – 100 °C) and varying pre-incubation temperatures (30 – 80 °C) at assay temperature of 37 °C on TTE activity were investigated in this study. TTE activity increased steadily with increase in assay temperature from 20 °C to 60 °C, at 60 °C activity spiked up by 124% when compared with activity at 20 °C (Figure 3). As assay temperature approached 100 °C, TTE activity declined by approximately 17% when compared with the peak observed at 60 °C. Increasing the pre-incubation temperature from 30 °C to 60 °C resulted in a corresponding increase in activity, which peaked at 60 °C with a 44% increase when compared with the activity recorded at 30 °C (Figure 4). At 80 °C pre-incubation temperature, TTE activity was observed to have dropped by approximately 30% when compared with the peak at 60 °C.

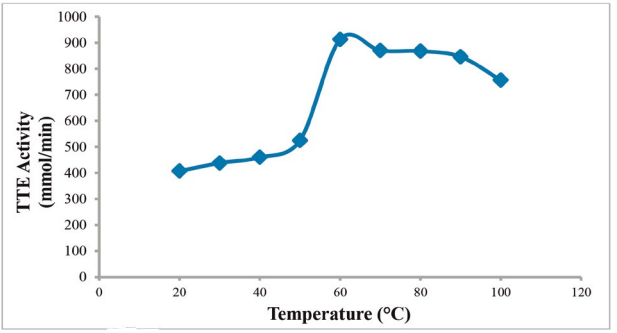

Figure 3. Effects of varying assay temperatures (20 – 100 °C) on *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

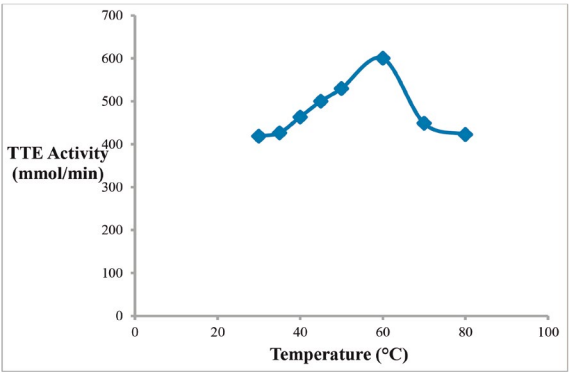

Figure 4. Effects of varying pre-incubation temperatures (30 – 80 °C) at assay temperature of 37 °C on *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

Effect of urea and sodium dodecyl sulfate on Thermoanaerobacter tengcongensis esterase activity

In this study, the modulatory effect of varying concentrations of urea and sodium dodecyl sulfate (SDS) on TTE activity was examined in the presence of 0.08 mM pNPD. In the presence of 0 to 4.0 mM urea, TTE activity increased in a concentration dependent manner (Figure 5). TTE activity peaked in the presence of 4.0 mM urea with an 82% increase compared with the activity observed in the absence of urea. Similarly, SDS also had a concentration dependent activating effect on TTE; activity peaked in the presence of 4.0 mM SDS with 119% increase when compared to activity in the absence of SDS (Figure 6).

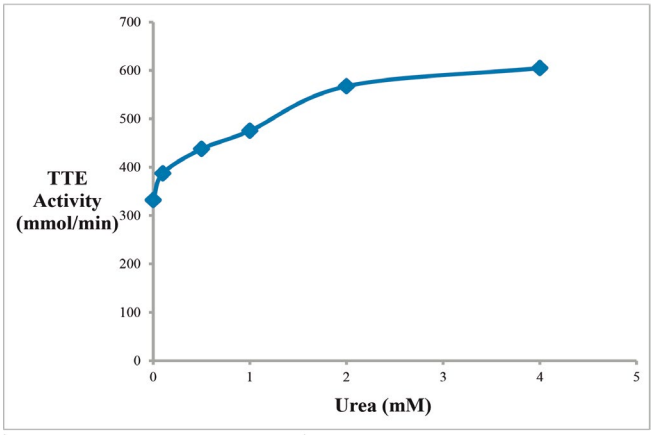

Figure 5. Effects of varying concentrations of urea on *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

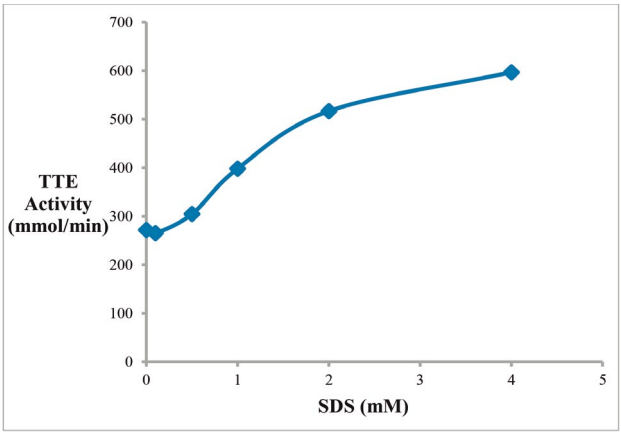

Figure 6. Effects of varying concentrations of sodium dodecyl sulfate (SDS) on *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

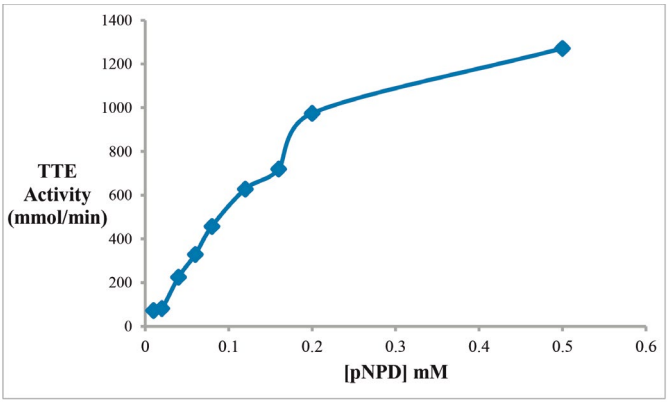

Figure 7. Michealis-Menten curve of *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

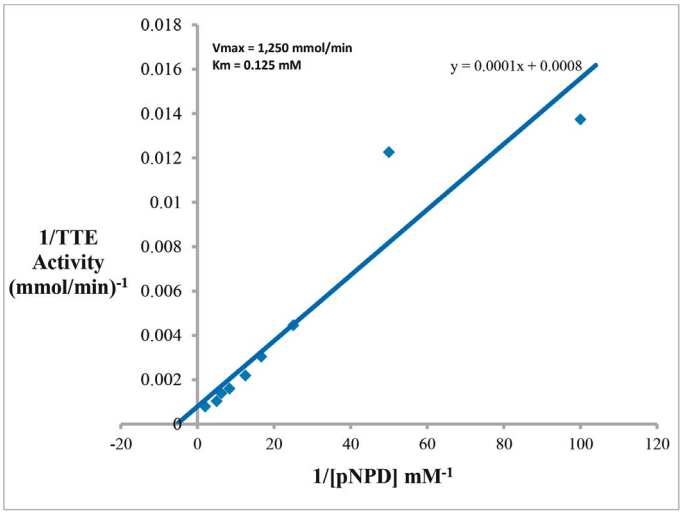

Figure 8. Lineweaver-Burk plot of *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate. Data are the means of three independent assays.

Kinetics of Thermoanaerobacter tengcongensis esterase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of urea

The substrate kinetics of TTE catalysed hydrolysis of pNPD in the presence of urea follows the classical Michealis-Menten hyperbola curve (Figure 9). From the Lineweaver-Burk plot in Figure 10, V_{max}, K_m and K_{cat} values were calculated and are shown in Table 1. The result showed that 0.5 and 1.0 mM urea concentrations, had similar effect on TTE with both showing approximately 14.3% increase in V_{max} and K_{cat} when compared with

what was observed in the absence of urea. The K_m value of TTE was also observed to increase slightly with 0.5 and 1.0 mM urea. At 2.0 mM concentration, urea had peak effect on TTE, yielding 33.3 and 33.5% increase in V_{max} and K_{cat}, respectively.

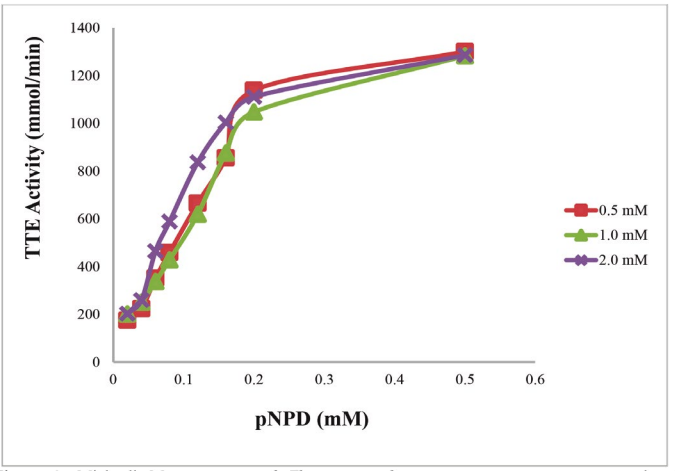

Figure 9. Michealis-Menten curve of *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of urea. Data are the means of three independent assays.

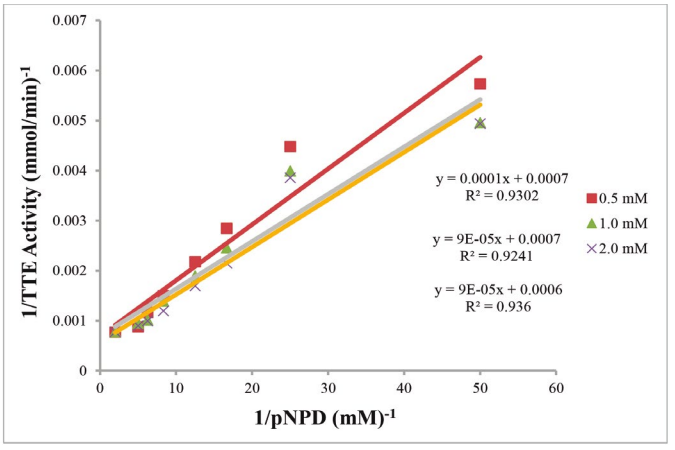

Figure 10. Lineweaver-Burk plot of *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of urea. Data are the means of three independent assays. Correlation coefficient (R^2) values > 0.90.

Kinetics of Thermoanaerobacter tengcongensis esterase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of sodium dodecyl sulfate (SDS)

The substrate kinetics of TTE catalysed hydrolysis of pNPD in the presence of sodium dodecyl sulfate (SDS) follows the classical Michealis-Menten hyperbola curve (Figure 11). V_{max}, K_m and K_{cat} values were calculated from the Lineweaver-Burk plot in Figure 12 and are shown in Table 1. SDS at 0.5, 1.0 and 2.0 mM increased the V_{max} and K_{cat} values of TTE catalyzed hydrolysis of pNPD. Also, SDS at 0.5, 1.0 and 2.0 mM increased the V_{max} of TTE, with the peak in the presence of 0.5 mM (approximately 300% increase) when compared to the V_{max} in the absence of SDS. Similarly, the K_m value of TTE was also observed to be the highest (with about 300% increase) in the presence of 0.5 mM SDS; concentrations beyond this resulted in decrease in K_m up to 20%. The K_{cat} of TTE also increased in the presence of SDS when compared to its absence, highest at 0.5 mM with 300% increase.

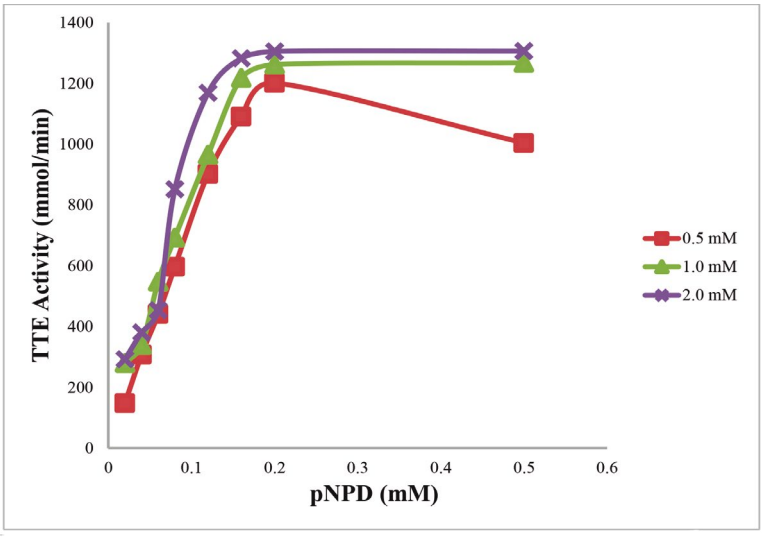

Figure 11. Michealis-Menten curve of *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of sodium dodecyl sulfate (SDS). Data are the means of three independent assays.

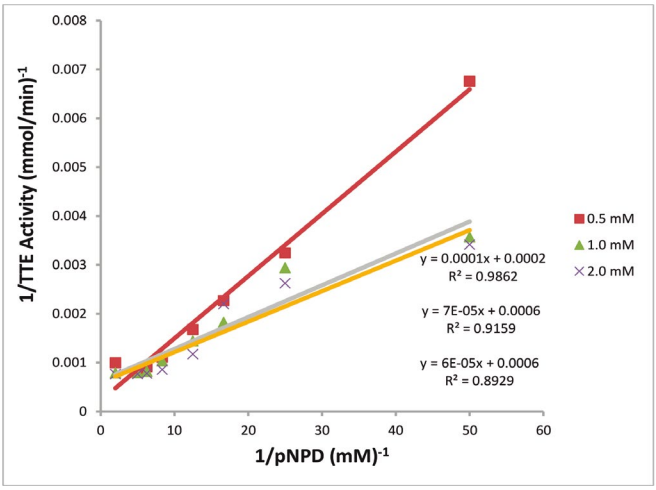

Figure 12. Lineweaver-Burk plot of *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of para-nitrophenyl dodecanoate in the presence of sodium dodecyl sulfate (SDS). Data are the means of three independent assays. Correlation coefficient (R^2) values > 0.88.

Table 1. Kinetic parameters of *Thermoanaerobacter tengcongensis* esterase catalysed hydrolysis of pNPD in the presence of urea and sodium dodecyl sulfate

Reaction	V_{max} (mmol/min)$^{-1}$	K_m (mM)	K_{cat} (sec^{-1}) × 10^4
TTE	1,250.000	0.125	3.940
TTE + 0.5 mM Urea	1,428.570	0.143	4.510
TTE + 1.0 mM Urea	1428.570	0.128	4.510
TTE + 2.0 mM Urea	1666.67	0.15	5.26
TTE + 0.5 mM SDS	5000.000	0.500	15.780
TTE + 1.0 mM SDS	1666.670	0.117	5.260
TTE + 2.0 mM SDS	1666.670	0.100	5.260

Theoretical characterization of Thermoanaerobacter tengcongensis esterase

The swiss model for TTE with GMQE and QMEAN Z-Scores 0.15 and -3.58 using *Burkholderia stabilis* cholesterol esterase (PDB ID: 7COG; resolution 3.0 Å) as template was used for the modeling (Figure 13). Also, the PROCHECK summary result for TTE without refinement via Ramachandran plot is 91.1% with 123 amino acid residues (91.1%) found in favored region (A, B, and L; Red color), 10 amino acid residues (7.4%) found in the additional allowed region (a, b, l and p; yellow color), 1 amino acid residue (0.7%) found in the generously allowed region (~a, ~b, ~l, and ~p; light green and cream colors) and 1 amino acid residue (0.7%) found in the disallowed region (white color) (Figure 14).

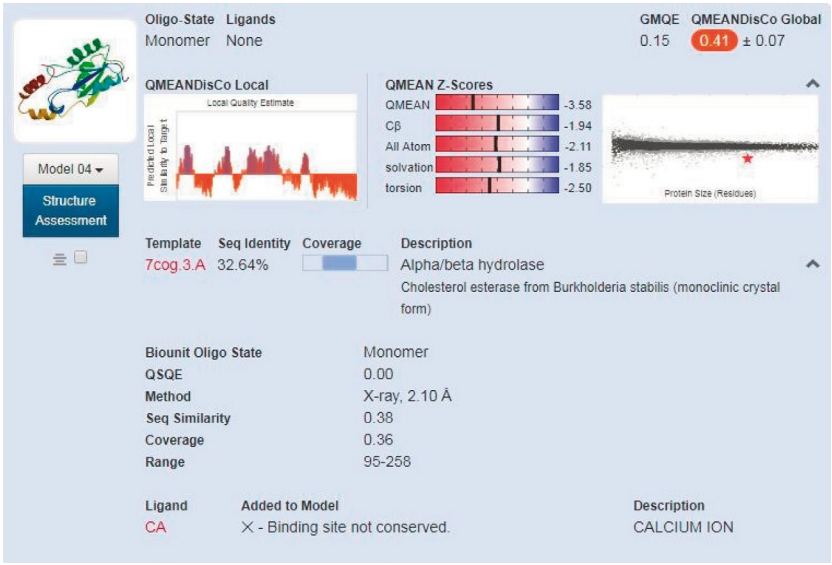

Figure 13. The Swiss model 3D crystal structure of TTE

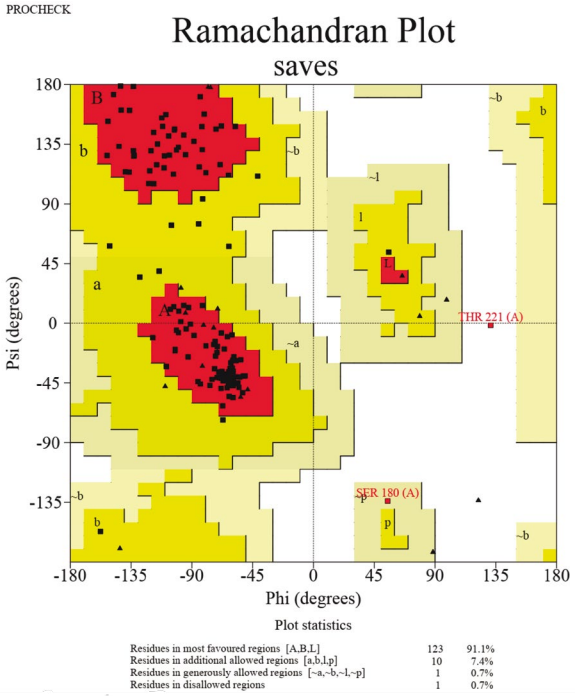

Figure 14. The PROCHECK Ramachandran plot of TTE

Molecular docking

The molecular docking study was carried on the predicted TTE model where the binding scores and interacting residues were obtained as well as the type of non-covalent interactions between the respective ligand towards the TTE model as shown in Table 2 and Figures 15a and 15b.

Table 2. Molecular docking binding scores of pNDP, urea, SDS, and donepezil towards TTE and the non-covalent interacting residues

Parameters / Ligands CIDs	Binding Scores (Kcal/mol)	Conventional Hydrogen Bond	Other non-covalent bonds
74778 1176	-5.37±0.21[d] -3.20±0.20[a]	Thr126, His179 Asp122, Gly249, Tyr252, Asn253	Leu105, His107, Phe115, Phe116
8778 3152	-4.70±0.00[c] -7.37±0.15[b]	Ser124, Thr126 Thr126	Leu105, Met129, Ala141, His179 Leu105, His107, Phe116, Asp122, Leu128, Val177

ANOVA followed by Duncan multiple range test for correlation. Different superscript indicates significant different at $P < 0.05$

Discussion

The ongoing global demand for novel biocatalyst with impeccable biochemical characteristics was expected to have reached approximately $6.2 billion as of 2020 [4]. The bid to meet the continuous high demand for sustainable and environmental-friendly biocatalyst has driven the need to explore new environments for these enzymes [23]. Microbial communities which are adapted to extreme environment such as temperature, salinity, pressure, and low levels of light have overtime been proven to be promising source of enzymes that may be uniquely suited for various industrial processes [24]. One of such extreme environment is the Tengcong hot spring in China where extreme thermophile, *Thermoanaerobacter tengcongensis*, was isolated [6].

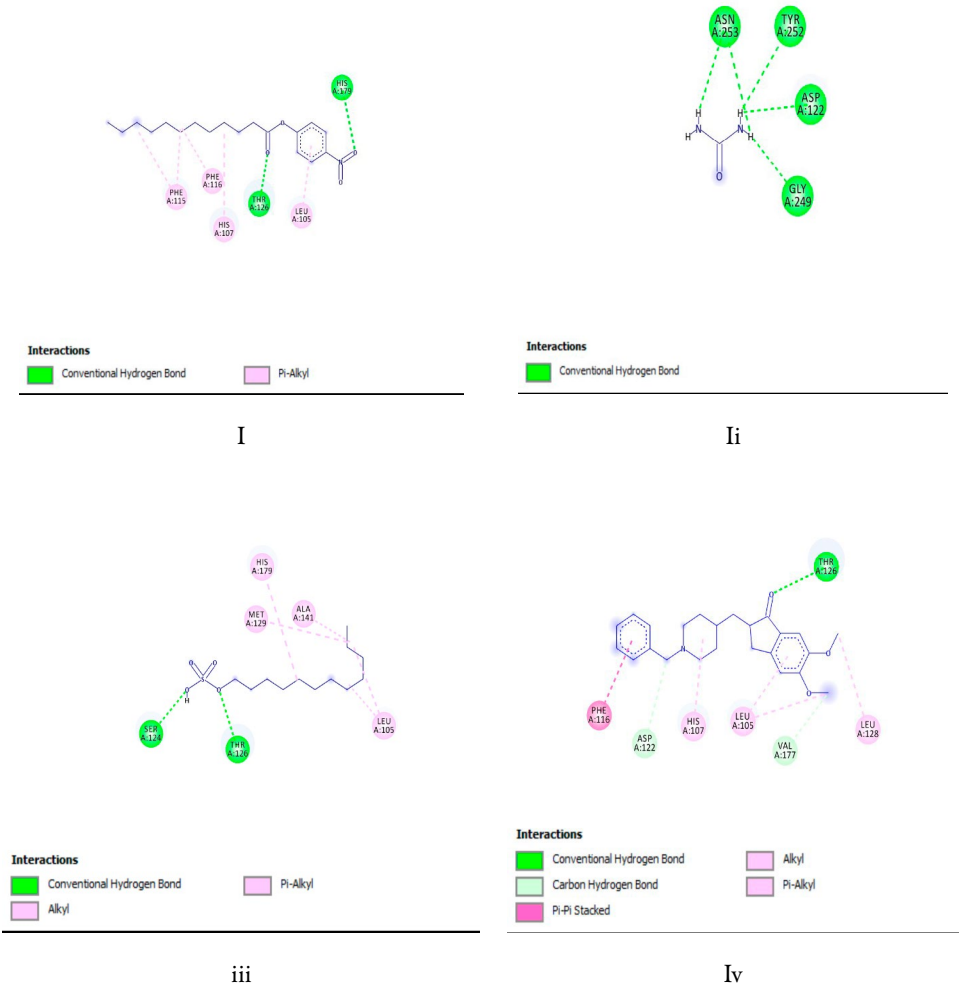

Figure 15a. 2D molecular binding structures of TTE with pNPD (i), urea (ii), SDS (iii) and Donepezil (iv)

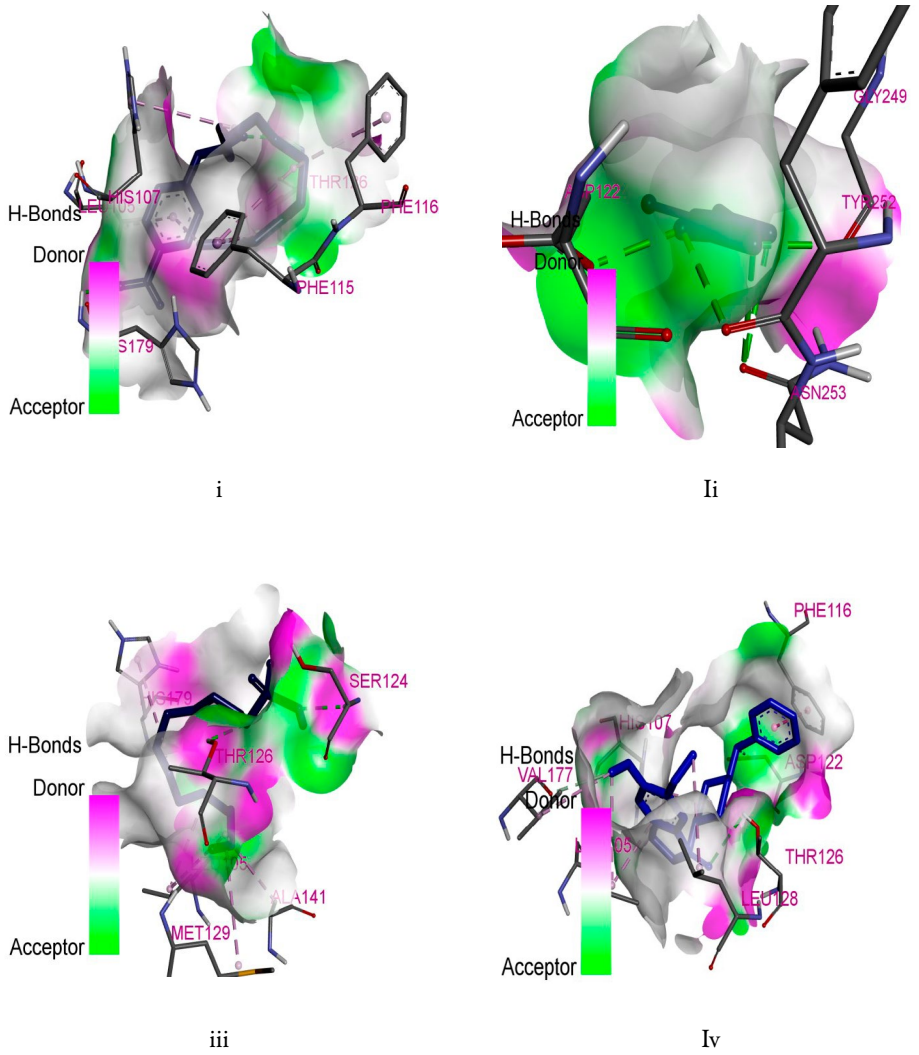

Figure 15b. 3D molecular binding structures of TTE with pNPD (i), urea (ii), SDS (iii) and Donepezil (iv)

Esterases from thermophilic sources present higher industrial potentials than those from other sources and are usually stable in a broad pH range [25]. pH stability in addition to reasonable thermostability reinforces esterases suitability for various industrial applications [6]. In this study, *Thermoanaerobacter tengcongensis* esterase (TTE) was active over a range of acidic and neutral pH and also showed excellent stability in pH range of 9.0 – 12.0. This result is in agreement with the findings of Rao *et al*. [6],

where the activity of TTE was reported to peak at pH 9.5 and stable within the pH range of 8.0 – 10.0. Similarly, Zhang *et al*. (2005) reported optimal activity for TTE at pH 9.0; however unlike the findings here, no activity was recorded at pH below 6.0. Similar to the findings herein, Borchert *et al*. [4] reported *Stelletta normani* esterase activity at all pH values investigated, however, 80 % activity was observed at alkaline pH of 9.0 and 10.0.

Esterases typically exhibit a broad temperature spectrum and several research groups have reported successful identification and development of thermostable esterases from extremophiles particularly thermophiles [4, 6]. This coupled with other factors such as tolerance to metal ion, salts and solvent, regio-, chemo-, and stero-selectivity make esterases desirable industrial biocatalyst [26]. *Thermoanaerobacter tengcongensis* esterase (TTE) displayed optimal activity at 60 °C and like other esterases from thermophiles, TTE maintained high and stable activity between 70 to 90 °C, however at 100 °C, TTE maintained 83 % of its maximal activity. The optimal temperature obtained in this study falls within the survival temperature range for *Thermoanaerobacter tengcongensis* which is between 50 to 80 °C [27]. In this study, the optimal temperature reported for TTE was 10 °C lower than what was reported by Rao *et al*. [3], where optimal activity for *T. tengcongensis* esterase was reported at 70 °C, and this might be due to the difference in other assay conditions. Similar to the findings in this study, *Geobacillus* specie esterase, a thermostable enzyme, has been reported to display optimal activity at 60 °C [25]. Thermostability assays in this study showed that TTE was most stable at 60 °C after 10 minutes pre-incubation and displayed moderate stability at higher temperatures, 70 and 80 °C, where it maintained 74 and 70 % of its maximal activity, respectively. The findings of this study are in line with earlier reports on the thermostability of *T. tengcongensis* esterase by Rao *et al*. [3] where the enzyme was reported to have retained its original activity at 60 °C for longer duration.

Besides being thermostable, the structural characteristics of esterases from thermophilic organisms enables them to have high resistance against organic solvents as well as different denaturing conditions [25]. This, in addition to other factors, reinforces the suitability of esterases for application in detergent industry, waste treatment, oil biodegradation, biodiesel production and pharmaceutical industry [26]. Small organic molecules in aqueous solutions have been observed to have significant effect on the structure, stability and function of proteins and one of such is urea [11]. Overtime, urea has been used to access protein stability and its characteristic denaturing effect has been attributed to its ability to cause unfolding of polypeptides [11]. Many manufacturing industries especially those involved in the production of fertilizers, detergent, glue and feed supplements make use of urea, as such potential biocatalysts are required to have certain degree of stability to be effective in these industries [28]. Findings of

this study showed that concentrations of urea from 0.1 to 4.0 mM progressively activated TTE activity (Figure 5). This finding showed that TTE resisted denaturation by urea up to 4.0 mM and as opposed to denaturation, urea in this test condition activated the enzyme. Urea is known to cause polypeptide unfolding and denaturation at high concentrations [11]; as such the low concentration dependent activating effect on TTE observed in this study might be as a result of mild urea-induced unfolding of TTE thereby exposing the active site to substrate. Similarly, Igunnu and co-researchers reported the activating effect of urea within the concentration range of 0.1 to 4.0 mM on *Thermomyces lanuginosus* lipase [29]. Zhu *et al*. [25] also reported the stability of *Geobacillus* sp. esterase in the presence of urea, with the enzyme maintaining 79.4 % of its activity in 2.5 M urea. High concentrations of urea (1 to 5 M) were reported to induce conformational changes at the active site of *Exiguobacterium antarcticum* esterase resulting in progressive activity decline [1].

The kinetic analysis of *Thermoanaerobacter tengcongensis* esterase in the absence of denaturant/detergent showed that the enzyme had a high turnover of product per unit time and appreciable affinity for the alternative substrate (pNPD). The K_{cat} and K_m value of TTE reported here, 0.125 mM and 3.9×10^4 s^{-1}, respectively, were observed to be higher than those reported by Rao *et al*. [6] and this might be as a result of the difference in experimental conditions. In similar manner, Levisson *et al*. [10] reported a low K_{cat} and K_m value of 1.3 s^{-1} and 0.072 mM, respectively, for *Thermotoga maritima* esterase.

Furthermore, the kinetic analysis of TTE revealed that urea at 0.5 and 1.0 mM had same impact on maximum reaction rate (V_{max}) and catalytic constant (K_{cat}) with a noticeable 14.2% increase when compared with those recorded in the absence of urea (Table 1). However, in the presence of 1.0 mM urea, TTE had higher affinity (lower K_m) for pNPD as a result of increased unfolding. Urea at 2.0 mM had the highest increasing effect on V_{max} and K_{cat}, this implies that at this concentration of urea, TTE had the highest turnover of product per seconds.

With the emergence of biotechnology, esterases have been widely used in various industrial processes; however, their use is restricted by industrial conditions that require the use of complex mixtures of compounds including anionic and nonionic detergents [14, 30]. Therefore the ability to remain stable in the presence of detergents confers an advantage and might be crucial for certain industrial applications [14]. Anionic surfactant such as sodium dodecyl sulfate (SDS) is able to destroy non-covalent bonds within enzyme molecules, thereby resulting in conformational changes that lead to loss of activity [30]. For TTE to be efficient in detergent industry as a facilitator of fat stain removal in washing powders, its ability to be stable in detergents is imperative. From this study, it was observed that TTE was stable at all tested concentrations of

SDS with 0.5 to 4.0 mM showing a concentration dependent activating effect on the enzyme. The findings in this study showed that TTE strongly resisted denaturation by SDS up to 4.0 mM and may be applicable in industries where moderate concentrations of anionic detergent are necessary for manufacturing. The activating effects at low concentrations of detergents have earlier been reported for *Thermomyces lanuginosus* lipase, where concentrations between 0.1 to 5.0 mM were observed to have led to increase in activity [29, 31]. The increased TTE activity observed in the presence of SDS in this study might be due to the increased exposure of the active site as the surfactant unfolds the protein. In a like manner, a recombinant esterase from *Geobacillus sp*. was reported to show good tolerance for SDS [25]. Zhang *et al*. [5] reported that TTE was unstable in 1% SDS which shows that TTE was unable to resist the denaturing effect of SDS at a concentration as high as 34 mM. Similarly, the presence of 1% SDS was reported to completely inhibit *Thermotoga maritima* esterase [10]; likewise, Gao *et al*. [30] reported the strong inhibitory effect of SDS on alkaline-stable esterase from *Stenotrophomonas maltophilia*.

Furthermore, the findings in this study showed that in the presence of abundant supply of pNPD, all concentrations of SDS (0.5 to 2.0 mM) increased the maximum reaction velocity and turnover rate of TTE. This finding is in line with the previous observation here in that SDS within concentration 0.5 to 4.0 mM had activating effect on TTE. It was also deduced from the kinetic analysis that 0.5 mM had the highest impact on the turnover of product. Similar to the findings here in, Igunnu *et al*. [29] had earlier reported that 2.0 mM SDS increased the V_{max} and K_{cat} of *Thermomyces lanuginosus* lipase. On the other hand, the K_m value of TTE increased in the presence of 0.5 mM SDS and decreased as concentration approached 2.0 mM. This finding shows that in the presence of 0.5 mM SDS, the affinity of TTE for pNPD was slightly reduced indicating that at this concentration, the surfactant reduced enzyme binding affinity but enhanced its catalytic power.

To substantiate the thermostability study, the structural characterization result revealed closest GMQE, QMEANDisco (between 0 and 1) and QMEAN Z-score (between -4.0 and 0) values for TTE model suggesting a good quality, reliability and the degree of nativeness of the built model to the experimental structure of similar size [32-34]. In addition, the TTE model quality check result using Ramachandran plot depicted a good quality check with 91.1% amino acid residues in the favored region and less than 1% in the disallowed region. Interestingly, the amino acid residue (THR221) in the disallowed region did not interact with any of the docked molecule towards TTE. This result suggested that the phi and psi backbone dihedral angles in the predicted structure of TTE model is reasonably accurate [35, 36]. Furthermore, the molecular docking study showing the molecular interactions between the investigated ligands

and TTE complement its homology assessment. It is evidence from the docking result that none of the investigated compounds (urea and SDS) showed better binding score than pNPD except donepezil (-7.37 ± 0.15 Kcal/mol), a known inhibitor for esterase.

Conclusion

In conclusion, findings from this study showed that TTE strongly resists denaturation by optimal concentrations of urea and SDS. In fact, moderate concentrations of the denaturants (urea and SDS) increased the activity of *Thermoanaerobacter tengcongensis* esterase. More so, TTE was stable within a broad range of temperature and pH values; these impeccable biochemical characteristics make it an ideal enzyme for use in detergent industry, waste treatment, oil biodegradation, biodiesel production, glue and feed supplements industry where harsh operating conditions might be required.

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Conflicts of interest

The authors declare no conflict of interest.

References

1. C. W. Lee, S. Kwon, S. Park, B. Y. Kim, W. Yoo, B. H. Ryu, H. W. Kim, S. C. Shin, S. Kim, H. Park, T. D. Kim, J. H. Lee, Crystal structure and functional characterization of an esterase (EaEST) from *Exiguobacterium antarcticum*, *PLoS One*, **12**(1), e0169540 (2017).

2. C. Oh, T. D. Kim, K. K. Kim, Carboxylic ester hydrolases in bacteria: Active site, structure, function and application, *Crystals*, **9**, 597 (2019).

3. L. Rao, Y. Xue, C. Zhou, J. Tao, G. Li, J. R. Lu, Y. Ma, The thermostable esterase from *Thermoanaerobacter tengcongensis* opening up a new family of bacterial lipolytic enzymes, *Biochimica et Biophysica Acta*, **1814**, 1695–1702 (2011).

4. E. Borchert, J. Selvin, S.G. Kiran, S.A. Jackson, F. O'Gara, A.D.W. Dobson, A novel cold active esterase from a deep sea sponge *Stelletta normani* metagenomic library, *Frontiers in Marine Science*, **4**, 287 (2017).

5. J. Zhang, J. Liu, J. Zhou, Y. Ren, X. Dai, H. Xiang, Thermostable esterase from *Thermoanaerobacter tengcongensis*: High-level expression, purification and characterization, *Biotechnology Letters*, **25**, 1463–1467 (2003).

6. T.E. Rao, M. Imchen, R. Kumavath, Marine enzymes: Production and applications for human health, *Advances in Food Nutrition Research*, **80**, 149–163 (2017).

7. L.F. Godinh, C.R. Reis, P.G. Tepper, G.J. Poelarends, W.J. Quax, Discovery of an *Escherichia coli* esterase with high activity and enantioselectivity towards 1,2-O-isopropylideneglycerol esters, *Application Environment Microbiology*, **77**(17), 6094–6099 (2011).

8. L. Ramnath, B. Sithole, R. Govinden, Classification of lipolytic enzymes and their biotechnological applications in the pulping industry, *Canadian Journal of Microbiology*, **63**, 179–192 (2017).

9. F. Sasso, A. Natalello, S. Castoldi, M. Lotti, C. Santambrogio, R. Grandori, *Burkholderia cepacia* lipase is a promising biocatalyst for biofuel production, *Biotechnology Journal*, **11**, 954–960 (2016).

10. M. Levisson, J.V. Oost, S.W.M. Kengen, Characterization and structural modeling of a new type of thermostable esterase from *Thermotoga maritime*, *FEBS Journal*, **274**, 2832–2842 (2007).

11. D.R. Canchi, A.E. Garcia, Cosolvent effects on protein stability, *The Annual Review of Physical Chemistry*, **64**, 273–293 (2013).

12. H. Wang, K.K. Andersen, P. Sehgal, J. Hagedorn, P. Westh, K. Borch, D.E. Otzen, pH regulation of the kinetic stability of the lipase from *Thermomyces lanuginosus*, *Biochemistry*, **52**(1), 264–276 (2013).

13. H. Monhemi, R.M. Housaindokht, A.A. Moosavi-Movahedi, M.R. Bozorgmehr, How a protein can remain stable in a solvent with high content of urea: insights from molecular dynamics simulation of *Candida antartica* lipase B in urea: Choline chloride deep eutectic solvent, *Physical Chemistry Chemical Physics*, **16**(28), 14882–14893 (2014).

14. R. Fernandez-Lafuente, Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst, *Journal of Molecular Catalalysis B: Enzymatic*, **62**, 197–212 (2010).

15. F.J. Olorunniji, A.L. McPherson, S.J. Rosser, M.C.M. Smith, S.D. Colloms, W.M. Stark, Control of serine integrase recombination directionality by fusion with the directionality factor, *Nucleic Acids Research*, **45**(14), 8635–8645 (2017).

16. S.C.B. Gopinath, Extracellular enzymatic activity profiles in fungi isolated from oil-rich environments, *Mycoscience*, **46**(2), 119–126 (2005).

17. O. Trott, A.J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry*, **31**(2), 455–461 (2010).

18. S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P.A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E.E. Bolton, PubChem 2019 update: Improved access to chemical data, *Nucleic Acids Research*, **47**(1), 1102–1109 (2019).

19. The UniProt Consortium, UniProt: The universal protein knowledgebase in 2021, *Nucleic Acids Research*, **49**(D1), D480–D489 (2021).

20. A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny F.T. Heer, T.A.P. de Beer, C. Rempfer, L. Bordoli, R. Lepore, T. Schwede, SWISS-MODEL: Homology modelling of protein structures and complexes, *Nucleic Acids Research*, **46**(W1), W296–W303 (2018).

21. R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, PROCHECK: A program to check the stereochemical quality of protein structures, *Journal of Applied Crystallography*, **26**(2), 283–291 (1993).

22. R.A. Laskowski, J.A. Rullmannn, M.W. MacArthur, R. Kaptein, J.M. Thornton, AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR, *Journal of Biomolecule NMR*, **8**(4), 477–486 (1996).

23. M.A. Salameh, J. Wiegel, Effects of detergents on activity, thermostability and aggregation of two alkalithermophilic lipases from *Thermosyntropha lipolytica*, *The Open Biochemistry Journal*, **4**, 22–28 (2010).

24. R. Singh, M. Kumar, A. Mittal, P.K. Mehta, Microbial enzymes: Industrial process in 21st century, *3 Biotech*, **6**, 174 (2016).

25. Y. Zhu, W. Zheng, H. Ni, H. Liu, A. Xiao, H. Cai, Molecular cloning and scharacterization of a new and highly thermostable esterase from *Geobacillus* sp. JM6, *Journal of Basic Microbiology*, **55**, 1219–1231 (2015).

26. A.K. Singh, M. Mukhopadhyay, Overview of fungal lipase: A review, *Applied Biochemistry and Biotechnology*, **166**, 486–520 (2012).

27. Z. Qian, J. Zhao, X. Bai, W. Tong, Z. Chen, H. Wei, Q. Wang, S. Liu, Thermastability of glucokinases in *Thermoanaerobacter tengcongensis*, *BioMed Research International*, **2013**, 646539 (2013).

28. J.H. Meessen, H. Petersen, Urea, *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim, 2010.

29. A. Igunnu, A. Aberuagba, S.O. Oyeyipo, B.T. Bakare, E.E. Onwurah, S.O. Malomo, Effects of urea and sodium dodecyl sulphate on stability profile of *Thermomyces lanuginosus* lipase, *Bioscience Research Journal*, **33**(2), 55–67 (2020).

30. X. Gao, X. Mao, P. Lu, F. Secundo, C. Xue, J. Sun, Cloning, Expression, and characterization of a novel thermostable and alkaline-stable esterase from *Stenotrophomonas maltophilia* OUC_Est10 catalytically active in organic solvents, *Catalysts*, **9**(5), 401 (2019).

31. J.E. Mogensen, P. Sehgal, D.E. Otzen, Activation, inhibition, and destabilization of *Thermomyces lanuginosus* lipase by detergents, *Biochemistry*, **44**, 1719–1730 (2005).

32. M. Alcaide, P.J. Stogios, A. Lafraya, Á. Tchigvintsev, R. Flick, R. Bargiela, Pressure adaptation is linked to thermal adaptation in salt-saturated marine habitats, *Environmental Microbiology*, **17**, 332–345 (2015).

33. J.M. Cardoso, L. Fonseca, C. Egas, I. Abrantes, Cysteine proteases secreted by the pinewood nematode, *Bursaphelenchus xylophilus*: *In silico* analysis, *Computational Biology and Chemistry*, **77**, 291–296 (2018).

34. B. Patel, V. Singh, D. Patel, *Essentials of Bioinformatics*, vol. I., Springer, Cham, Switzerland, 2019, pp. 169–199.

35. H. Yamaguchi, T. Akitaya, Y. Kidachi, K. Kamiie, H. Umetsu, Homology modeling and structural analysis of human γ-glutamylcysteine ligase catalytic subunit for antitumor drug development, *Journal of Biophysical Chemistry,* **3**(3), 238–248 (2012).

36. I. Olaoye, B. Oso, A. Aberuagba, Molecular insights into the binding mechanisms of human and mouse Glutamate-cysteine ligases, *Revista Colombiana de Ciencias Químico Farmacéuticas*, **52**(1), 311–336 (2023).

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Evaluación del uso de la radiación UV y ozono en la degradación de algunas sulfonamidas

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Resumen

Introducción: los antibióticos en el medio acuático son un problema importante debido a la aparición de resistencia bacteriana. Se desconoce el impacto ecológico a largo plazo en el medio ambiente acuático. Muchas fuentes permiten la entrada de antibióticos al medio ambiente, incluidas las plantas de tratamiento de aguas residuales (PTAR), la escorrentía agrícola, los efluentes hospitalarios y los lixiviados de vertederos. **Objetivo:** se presenta un estudio que evalúa la degradación de la sulfadiazina (SD), sulfamerazina (SMR) y sulfametazina (SMT) al ser expuesta a condiciones de estrés por radiación UV, ozonificación y UV + ozonificación. **Metodología:** todos los análisis se realizaron mediante espectrofotometría UV/Vis luego de verificar mediante análisis de HPLC que los productos de degradación no interferían de manera significativa la absorbancia de los fármacos en estudio. **Resultados:** se demostró que las tres sulfonamidas son sensibles a todos los métodos de degradación estudiados, siendo el método más eficiente la combinación de radiación UV+O_3, y el menos eficiente el tratamiento con ozono.

Palabras clave: contaminantes emergentes, fármacos, tratamiento de aguas, ozonización, radiación UV.

Summary

Evaluation of the use of UV radiation and ozone in the degradation of some sulfonamides

Introduction: antibiotics in the aquatic environment are an important problem due to the emergence of bacterial resistance. The long-term ecological impact on the aquatic environment is unknown. Many sources allow antibiotics to enter the environment, including wastewater treatment plants (WWTPs), agricultural runoff, hospital effluent, and landfill leachate. **Objective:** a study is presented that evaluates the degradation of sulfadiazine (SD), sulfamerazine (SMR) and sulfamethazine (SMT) when exposed to stress conditions due to UV radiation, ozonation and UV + ozonation. **Methodology:** all analyzes were performed by UV/Vis spectrophotometry after verifying by HPLC analysis that the degradation products did not significantly interfere with the absorbance of the drugs under study. **Results:** it was shown that the three sulfonamides are sensitive to all the degradation methods studied, the most efficient method being the combination of UV+O_3 radiation, and the least efficient being the treatment with ozone.

Keywords: emerging contaminants, drugs, water treatment, ozonation, UV radiation.

Resumo

Avaliação do uso da radiação UV e do ozônio na degradação de algumas sulfonamidas

Introdução: antibióticos no ambiente aquático são um problema importante devido ao surgimento de resistência bacteriana. O impacto ecológico a longo prazo no ambiente aquático é desconhecido. Muitas fontes permitem que antibióticos entrem no meio ambiente, incluindo estações de tratamento de águas residuais (ETEs), escoamento agrícola, efluentes hospitalares e lixiviados de aterros sanitários. **Objetivo:** é apresentado um estudo que avalia a degradação da sulfadiazina (SD), sulfamerazina (SMR) e sulfametazina (SMT) quando expostas a condições de estresse devido à radiação UV, ozonização e UV + ozonação. **Metodologia:** todas as análises foram realizadas por espectrofotometria UV/Vis após verificação por análise de HPLC que os produtos de degradação não interferiram significativamente

na absorbância dos fármacos em estudo. **Resultados:** foi demonstrado que as três sulfonamidas são sensíveis a todos os métodos de degradação estudados, sendo o método mais eficiente a combinação da radiação UV+O_3, e o menos eficiente o tratamento com Ozônio.

Palavras-chave: contaminantes emergentes, drogas, tratamento de água, ozonização, Radiação UV.

Introducción

Además de tratamiento de patologías en humanos, los antibióticos se utilizan en sectores como la acuicultura, la agricultura y la veterinaria [1, 2], lo cual ha causado un aumento de la presencia de estas sustancias en aguas superficiales [3-5].

Se esperaría que una vez ingeridos, los antibióticos sean totalmente metabolizados, sin embargo, una fracción de los mismos es excretados sin cambios [5-8]. Así, inicialmente, una vez los antibióticos lleguen al sistema de drenajes y posterior entrada a plantas de tratamiento (PTAR) pueden tener tres destinos: Biodegradación, adsorción en lodos o salida sin cambios [9-11], por lo cual tanto la principal entrada de antibióticos a los ecosistemas acuáticos es la fracción no metabolizada que pasa a través de las PTAR sin sufrir cambios, debido a que los sistemas de tratamientos no cuentan con tecnologías que eliminen completamente los antibióticos [12].

Otras fuentes de contaminación por antibióticos son los lixiviados vertederos de basura y la eliminación inadecuada de medicamentos no consumidos [13, 14], al ingresar al medio ambiente, el destino de los antibióticos está condicionado a las propiedades fisicoquímicas del medio, como pH, composición del suelo, además de las condiciones ambientales del entorno, teniendo el potencial de transformarse en metabolitos lo que dificulta predecir el comportamiento y destino final de los antibióticos [6, 13].

Los antibióticos se clasifican de manera general en agentes bactericidas y agente bacteriostáticos, los primeros destruyen las bacterias y los bacteriostáticos evitan la proliferación bacteriana impidiendo su división [15].

Dentro del grupo de los agentes bactericidas, las sulfonamidas (Figura 1) son unos de los antibióticos más utilizados sobre todo en medicina veterinaria; sin embargo, debido al aumento de la resistencia bacteriana a los antibióticos de primera línea, las sulfamidas han vuelto a tomar protagonismo en tratamientos en humanos [16, 17], lo que a su vez ha generado alertas ambientales por la presencia de estos fármacos en sistemas

acuáticos, a tal punto que organizaciones como la red NORMAN, haya clasificado a la Sulfadiazina (SD), la sulfamerazine (SMR) y la sulfametazina (SMT) como contaminantes emergentes de alta peligrosidad ambiental [18].

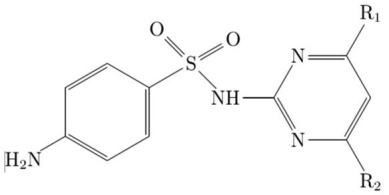

Figura 1. Formula química de las sulfonamidas (Sulfadiazina: R_1: H, R_2: H; Sulfamerazina: R_1: CH_3, R_2: H; Sulfametazina R_1: CH_3, R_2: CH_3).

En este contexto, el objetivo de la presente investigación es evaluar el uso de la radiación UV y el ozono en la degradación de la SD, SMR y SMT como alternativa de tratamiento adicional de aguas residuales, dos métodos de bajo costo y de fácil implementación en PTAR ya construidas.

Metodología

Reactivos

Sulfadiazina, sulfamerazina y sulfametazina (Sigma-Aldrich), Metanol, Etanol (Merck), Acetonitrilo (Merck). Agua destilada

Equipos

Espectrofotómetro UV-Vis (EMC-11- UV), Equipo de HPLC (Agilent 1200 Series), con automuestrador Agilent 1260 Infinity, desgasificador Agilent 1200 Series, bomba cuaternaria Agilent 1200 Series, Detector UV-Vis con arreglo de diodos e integrados Agilent 1200 Series, Columna Eclipse XDB-C18 (150 mm × 4,6 mm, 3,5-5 μm). Reactor ozono, Lampara UV ECOFILTER, bomba de recirculación, Baño de recirculación (Medingen K22/T100), Balanza analítica (RADWAG AS 220.R2, sensibilidad ±0,1 mg).

Evaluación de técnicas en la degradación de fármacos

Se preparó una solución acuosa de 25 μg/mL de cada una de las sulfonamidas evaluadas (SD, SMR y SMT) teniendo en cuenta la solubilidad de cada fármaco a 20 °C (Temperatura de laboratorio 25 °C) garantizando una sola fase [19-23]

Cada fármaco fue sometido a tres métodos de degradación 1) Radiación UV, 2) Ozonificación y 3) Radiación UV + O_3. Para el primer análisis, en un erlenmeyer de 1000 mL se tomaron 500 mL de cada una de las soluciones, los cuales fueron recirculados continuamente por un sistema comercial de lampara de luz ultravioleta, el cual consiste en un contenedor de acero inoxidable de 52 cm de largo por 7 cm de diámetro, con dos conexiones de ½ pulgada, el cual contiene en su interior un tubo de vidrio de 46 cm de largo y 2,5 cm de diámetro el cual a su vez contiene una lampara que irradia luz UV entre 200 y 295 nm de acuerdo a especificaciones comerciales. El segundo análisis, en un frasco de color ámbar de capacidad de 1000 mL, se depositaron 500 mL de cada una de las soluciones y se burbujeo ozono mediante un generador ozono, finalmente para el tercer ensayo, se acopló el generador de ozono al sistema de lámpara UV y se recirculó la solución de cada una de las sulfonamidas estudiadas [24].

La degradación de cada uno de los fármacos se evaluó contrastando la concentración de la solución de cada sulfonamida en función del tiempo de exposición a cada uno de los factores, es decir luz UV, ozono, y UV + ozono. Debido a que la exposición de las sulfonamidas a la radiación UV y/o al ozono puede generar productos de degradación que interfieran en la cuantificación del fármaco, se evaluaron dos técnicas para la determinación del cambio de concentración. La primera técnica de cuantificación fue HPLC, se determinó la concentración cada muestra de muestras sometidas a radiación + ozonificación durante 5, 15, 30 y 60 minutos a 268 nm, evaluando la altura de pico, el tiempo de retención y la pureza de pico.

La segunda técnica analítica, fue la de espectrofotometría UV-Vis, una técnica rápida, sencilla y que requiere menos recursos en comparación al HPLC, sin embargo, no es posible identificar de forma precisa la interferencia de posibles productos de degradación. En este ensayo la concentración se determinó a 268 nm sin realizar diluciones de acuerdo al método reportado por Delgado *et al.* [25].

Condiciones HPLC

Se empleó un cromatógrafo líquido modular Agilent 1200 Series, con automuestreador Agilent 1260 Infinity, desgasificador Agilent 1200 Series, bomba cuaternaria Agilent 1200 Series, detector UV/VIS con arreglo de diodos e integrador Agilent 1200 Series, así como una columna Eclipse XDB-C18 (4,6 mm × 150 mm, 3,5-5 μm) cuya temperatura de operación fue de 25 °C. El volumen de inyección fue de 10 μL; la fase móvil utilizada fue sistema de gradiente lineal de dos solventes con agua/acetonitrilo, ambos con ácido trifluoroacético (TFA) al 0,05% cuya composición de partida fue 90/10 a cero minutos, luego se cambió a 85/15 a los diez minutos. La velocidad de flujo fue de 2,5 mL/min, y la longitud de onda de cuantificación fue de 268 nm.

Resultados y discusión

La degradación de cada una de las sulfonamidas se evaluó analizando los cambios de concentración en función del tiempo de exposición de cada fármaco a las diferentes condiciones de estrés. En principio, para evaluar el uso de espectrofotometría UV en el análisis de degradación se realizó un estudio de degradación mediante radiación UV + ozonificación, la cuantificación de cada fármaco se realizó median HPLC con el fin de identificar posibles interferencias de los productos de degradación en la cuantificación. Así, se tomo una solución de 25 μg/mL de cada sulfonamida y se sometió a estrés por radiación UV + Ozonificación.

En la tabla 1 se muestran los resultados, a partir de los cuales se pude demostrar que los tres fármacos son sensibles a radiación UV + Ozonificación, puesto que a medida que aumenta tiempo de exposición el área de pico se reduce manteniendo el tiempo de retención, indicando degradación del fármaco, en los primeros 5 minutos de exposición a radiación UV y ozono, la SD, se degrada en un 12%, la SMR 21% y la SMT 17%, a los 15 minutos de exposición la SD aumenta su degradación a 28%, la SMR 37% y la SMT 33% siendo nuevamente la SMR el fármaco que presenta mayor porcentaje de degradación. A los 30 minutos, la SD alcanza el 57% de degradación, la SMR 63% y la SMT 53%, pasados 60 minutos, la SD se degrada 71%, la SMR 76% y la SMT 73%, demostrado la eficiencia del tratamiento UV + Ozonificación en la degradación de los tres fármacos; otro análisis importante es la pureza de pico, la cual en el caso de la SD pasa de 99,9% a 98,7% variando tan solo 1.2%, en cuanto al SMR, factor de pureza de pico varia 1,1% y en la SMT 1,6%, porcentajes que permiten concluir que los productos de degradación no interfieren de forma significativa en la cuantificación del fármaco luego de someterlo a estrés por radiación UV + Ozonificación, por lo que el estudio de degradación por radiación UV, O3, o ambas técnicas combinadas, puede realizarse mediante espectrofotometría UV-Vis. Si bien mediante espectrofotometría UV-Vis o HPLC no es posible identificar los productos de degradación, García-Galán *et al.* indican que muchos de estos productos de degradación son el resultado de la ruptura de los anillos aromáticos por lo no tienen grupos cromóforos que permitan su cuantificación mediante técnicas espectrofotométricas [5], lo cual es respaldado por Li *et al.* [26].

Tabla 1. Áreas, tiempo de retención (TR) y factor de similitud o de pureza de pico del estándar de cada sulfonamida y de las muestras expuestas a condiciones de estrés (radiación UV + Ozonificación; solución 25 μg/mL).

Fármaco	Tiempo de exposición /minutos	Área /mAU	Tiempo de retención	Pureza de pico
Sulfadiazina	0	745	3,52	999,21±0,85
	5	652	3,41	995,25±0,58
	15	532	3,65	992,54±0,25
	30	321	3,55	989,14±0,21
	60	213	3,42	987,15±0,12
Sulfamerazina	0	801	4,32	999,78±0,75
	5	625	4,21	997,45±0,32
	15	502	4,35	993,47±0,95
	30	298	4,42	990,64±0,75
	60	189	4,25	988,85±0,37
Sulfametazina	0	754	5,12	999,56±0,28
	5	625	5,21	996,18±0,24
	15	498	5,24	991,75±0,69
	30	351	5,28	985,72±0,74
	60	201	5,32	983,78±0,45

Evaluación de métodos de degradación

Se analizaron tres métodos para degradar las sulfonamidas, el primero consistió en irradiar la muestra mediante una lampara de UV, recirculando la muestra mediante una bomba a una rapidez de flujo 4 L/min, por entre un reactor UV; el segundo método, consistió en burbujear O_3 a la muestra y el tercer método consistió en una técnica combinada de radiación UV + O_3.

En todos los casos de tomaron 500 mL de solución de 25 μg/mL de cada una de las sulfonamidas estudiadas. Cada muestra se sometió a estrés durante 80 minutos, una vez se inició el proceso de degradación se tomaron muestras cada 5 minutos durante los primeros 30 minutos y cada 10 minutos durante los restantes 50 minutos.

En la Figura 2, se muestra el porcentaje de degradación en función del tiempo, el tratamiento con ozono, mostró ser el método menos eficiente, sin embargo, la SMR y SMT alcanzan porcentajes de degradación cercanos al 50%, y en cuanto a la SD solo se logra degradar aproximadamente un 30%.

Al irradiar las muestras con luz ultravioleta, se alcanzan porcentajes de degradación entre 62-65%, mayores a las alcanzadas mediante burbujeo de ozono en donde el porcentaje de degradación no supera el 50% para la SMR y SMT y tan solo llega a un 30% en la SD, sin embargo, al someter las muestras a la técnica combinada UV + O_3 el porcentaje de degradación de las tres sulfonamidas alcanzan valores entre el 80 y el 83%. Al analizar el porcentaje de degradación de las tres sulfonamidas en función de su estructura molecular solo se notan diferencias en el tratamiento por O_3, posiblemente debido a que la longitud de onda de máxima absorbancia de las tres sulfonamidas es similar (263-264 nm), por lo que en los métodos de radiación UV y UV + O_3 los resultados sean mas homogéneos en relación al tratamiento por O_3.

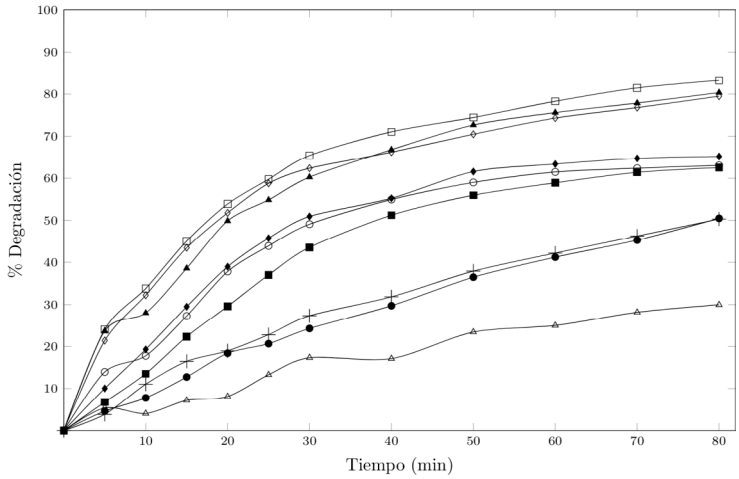

Figura 2. Porcentaje de degradación de la SD (Δ: Ozono, ○: Radiación UV, ◊: Radiación UV + Ozono.) SMR (●: Ozono, ♦: Radiación UV, □: Radiación UV + Ozono.) SMT (+: Ozono, ▪: Radiación UV, ▲: Radiación UV + Ozono).

Al determinar la cinética de degradación (Tabla 2, Figura 3) se puede establecer que el mejor orden que describe la degradación de las sulfonamidas es la cinética de orden 2. Indicando dos principios importantes, el primero es que la velocidad de degradación depende la concentración inicial, y el segundo, la vida media de los agentes terapéuticos aumenta a medida que la concentración disminuye, es decir, para eliminar trazas es necesario mayor tiempo de tratamiento [27].

Esto demuestra la dificultad relacionada con la eliminación total de estos fármacos, más aún cuando se ha demostrado que concentraciones del orden de 2,9 ng/mL presentan efectos biológicos graves [1, 28-30].

Al verificar los datos de r^2 para la cinética de las sulfonamidas expuesta a radiación UV, se observa un posible seudo orden 2, a diferencia de los tratamientos con ozono y radiación UV + O_3 en donde se obtienen tendencias lineales con r^2 aproximados a 1,0 conservándose claramente una cinética de orden 2.

Tabla 2. Cinética de degradación de la SD, SMR y SMT expuestas a diferentes condiciones de estrés.

Tratamiento	Sulfonamida	Ecuación	R^2
Ozono	SD	1/C(µg/mL)= 0,00022 t(min) + 0,04027	0,9794
	SMR	1/C(µg/mL)= 0,00050 t(min) + 0,03860	0,9933
	SMT	1/C(µg/mL)= 0,00050 t(min) + 0,03962	0,9982
Radiación UV	SD	1/C(µg/mL)= 0,00093 t(min) + 0,04447	0,9495
	SMR	1/C(µg/mL)= 0,00103 t(min) + 0,04384	0,9560
	SMT	1/C(µg/mL)= 0,00092 t(min) + 0,04004	0,9796
Radiación UV + O_3	SD	1/C(µg/mL)= 0,00189 t(min) + 0,04323	0,9953
	SMR	1/C(µg/mL)= 0,00249 t(min) + 0,03765	0,9981
	SMT	1/C(µg/mL)= 0,00207 t(min) + 0,03819	0,9976

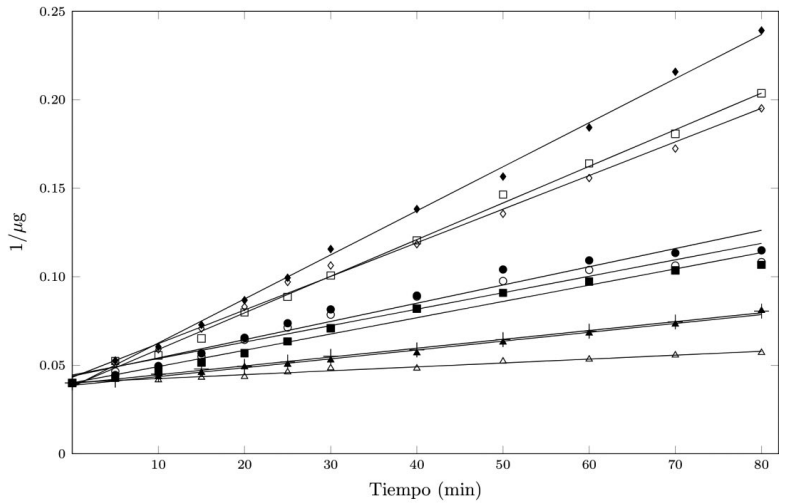

Figura 3. Cinética de degradación de la SD (Δ: Ozono, ○: Radiación UV, ◊: Radiación UV + Ozono.) SMR (▲: Ozono, ●: Radiación UV, □: Radiación UV + Ozono.) SMT (+: Ozono, ▪: Radiación UV, ♦: Radiación UV + Ozono).

Conclusiones

Las tres sulfonamidas son sensibles a la degradación por radiación y ozono, en todos los casos, se muestra un mínimo del 50% de degradación en un lapso de tiempo de 80 minutos. El método de tratamiento menos eficiente es la ozonificación sin embargo al combinar este método con radiación UV se obtienen mejores resultados.

Si bien los tres métodos permiten degradar las sulfonamidas (SD, SMR y SMT), el que el proceso siga un orden cinético de segundo orden sugiere que la eliminación del 100% de residuos de estos fármacos es un proceso complejo, puesto que la vida media de los fármacos aumentaría a medida que su concentración disminuye como consecuencia de la degradación.

Conflicto de interés

Los autores declaran no presentar ningún conflicto de interés

Referencias

1. J. Harrower, M. McNaughtan, C. Hunter, R. Hough, Z. Zhang, K. Helwig, Chemical fate and partitioning behavior of antibiotics in the aquatic environment—A review, *Environmental Toxicology and Chemistry*, **40**(12), 3275–3298 (2021). https://doi.org/https://doi.org/10.1002/etc.5191

2. K. Kümmerer, Antibiotics in the aquatic environment – A review – Part I, *Chemosphere*, **75**(4), 417–434 (2009). https://doi.org/10.1016/j.chemosphere.2008.11.086

3. D.I. Caviedes-Rubio, D.R. Delgado, Regulación ambiental sobre los productos farmacéuticos residuales en ambientes acuáticos, *Entornos*, **28**(1), 76–80 (2015). https://doi.org/10.25054/01247905.1225

4. D.I. Caviedes-Rubio, D.R. Delgado, A. Olaya-Amaya, Normatividad ambiental dirigida a regular la presencia de los productos farmacéuticos residuales en ambientes acuáticos, *Revista Jurídica Piélagus*, **16**(1), 121–130 (2017). https://doi.org/10.25054/16576799.1445

5. M.J. García-Galán, M. Silvia Díaz-Cruz, D. Barceló, Identification and determination of metabolites and degradation products of sulfonamide antibiotics, *TrAC Trends in Analytical Chemistry*, **27**(11), 1008–1022 (2008). https://doi.org/10.1016/J.TRAC.2008.10.001

6. I. Gozlan, A. Rotstein, D. Avisar, Amoxicillin-degradation products formed under controlled environmental conditions: Identification and determination in the aquatic environment, *Chemosphere*, **91**(7), 985–992 (2013). https://doi.org/10.1016/j.chemosphere.2013.01.095

7. C.P. Ortiz, R.E. Cardenas-Torres, M. Herrera, D.R. Delgado, Numerical analysis of sulfamerazine solubility in acetonitrile + 1-propanol cosolvent mixtures at different temperatures, *Sustainability*, **15**(8), 6596 (2023). https://doi.org/10.3390/su15086596

8. C.P. Ortiz, R.E. Cardenas-Torres, M. Herrera, D.R. Delgado, Thermodynamic analysis of the solubility of propylparaben in acetonitrile–water cosolvent mixtures, *Sustainability*, **15**(6), 4795 (2023). https://doi.org/10.3390/su15064795

9. Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, *Science of The Total Environment*, **473–474**, 619–641 (2014). https://doi.org/10.1016/j.scitotenv.2013.12.065

10. C.E. Chen, H. Zhang, G.G. Ying, L.J. Zhou, K.C. Jones, Passive sampling: A cost-effective method for understanding antibiotic fate, behaviour and impact, *Environment International*, **85**, 284–291 (2015). https://doi.org/10.1016/j.envint.2015.10.001

11. M.B. Ahmed, J.L. Zhou, H.H. Ngo, W. Guo, Adsorptive removal of antibiotics from water and wastewater: Progress and challenges, *Science of The Total Environment*, **532**, 112–126 (2015). https://doi.org/10.1016/j.scitotenv.2015.05.130

12. I. Michael, L. Rizzo, C.S. McArdell, C.M. Manaia, C. Merlin, T. Schwartz, C. Dagot, D. Fatta-Kassinos, Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review, *Water Research*, **47**(3), 957–995 (2013). https://doi.org/10.1016/j.watres.2012.11.027

13. B.A. Otoo, I.A. Amoabeng, G. Darko, L.S. Borquaye, Antibiotic and analgesic residues in the environment – Occurrence and ecological risk study from the Sunyani municipality, Ghana, *Toxicology Reports*, **9**, 1491–1500 (2022). https://doi.org/10.1016/j.toxrep.2022.07.003

14. H. Luo, Y. Zeng, Y. Cheng, D. He, X. Pan, Recent advances in municipal landfill leachate: A review focusing on its characteristics, treatment, and toxicity assessment, *Science of The Total Environment*, **703**, 135468 (2020). https://doi.org/10.1016/j.scitotenv.2019.135468

15. N. Milić, M. Milanović, N.G. Letić, M.T. Sekulić, J. Radonić, I. Mihajlović, M.V. Miloradov, Occurrence of antibiotics as emerging contaminant substances in aquatic environment, *International Journal of Environmental Health Research*, **23**(4), 296–310 (2013). https://doi.org/10.1080/09603123.2012.733934

16. E.N. Krylov, M.S. Gruzdev, U.V. Chervonova, L.V. Virzum, Synthesis, reactivity, acidic and pharmaceutical properties of sulphonamides, en: D. Sarkar (editor), *Sulfonamides: An Overview*, Nova Science Publishers, Odisha, 2020.

17. D.R. Delgado, M.Á. Peña, F. Martínez, Preferential solvation of some sulfonamides in propylene glycol + water solvent mixtures according to the IKBI and QLQC methods, *Journal of Solution Chemistry*, **43**(2), 360–374 (2014). https://doi.org/10.1007/s10953-014-0130-2

18. NORMAN network, NORMAN Substance Database, NORMAN Substance Database, 2023. URL: https://www.norman-network.com/nds/susdat/ accesado el 16 de abril de 2023.

19. D.R. Delgado, *Estudio termodinámico de la solubilidad de algunas sulfonamidas en mezclas cosolventes,* Tesis de Doctorado, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá D.C., 2014. URL: https://repositorio.unal.edu.co/handle/unal/53095, accesado el 25 de agosto de 2020.

20. D.R. Delgado, F. Martínez, Solution thermodynamics of sulfadiazine in some ethanol + water mixtures, *Journal of Molecular Liquids*, **187**, 99–105 (2013). https://doi.org/10.1016/j.molliq.2013.06.011

21. D.R. Delgado, F. Martínez, Solubility and solution thermodynamics of sulfamerazine and sulfamethazine in some ethanol + water mixtures, *Fluid Phase Equilibria*, **360**, 88–96 (2013). https://doi.org/10.1016/j.fluid.2013.09.018

22. D.R. Delgado, F. Martínez, Solution thermodynamics and preferential solvation of sulfamerazine in methanol + water mixtures, *Journal of Solution Chemistry*, **44**(2), 360–377 (2015). https://doi.org/10.1007/s10953-015-0317-1

23. D.R. Delgado, O.A. Almanza, F. Martínez, M.A. Peña, A. Jouyban, W.E. Acree, Solution thermodynamics and preferential solvation of sulfamethazine in (methanol + water) mixtures, *Journal of Chemical Thermodynamics*, **97**, 264–276 (2016). https://doi.org/10.1016/J.JCT.2016.02.002

24. Y.K. Álvarez-Gómez, D.R. Delgado, Y.K. Álvarez-Gómez, D.R. Delgado, Evaluación del uso de la radiación UV y ozono en la degradación de metilparabeno, *Revista de Ciencias Ambientales*, **57**(2), 1–17 (2023). https://doi.org/10.15359/RCA.57-2.7

25. D.R. Delgado, G. Rodríguez, J. Martínez, J. Rojas, F. Martínez, Validación de una metodología analítica empleando espectrofotometría ultravioleta para el estudio de la solubilidad de algunas sulfonamidas en mezclas cosolventes alcohol + agua, *Revista Colombiana de Química*, **42**(3), 31–40 (2013). URL: https://revistas.unal.edu.co/index.php/rcolquim/article/view/53429, accesado el 10 de marzo de 2020.

26. J. Li, L. Zhao, M. Feng, C.H. Huang, P. Sun, Abiotic transformation and ecotoxicity change of sulfonamide antibiotics in environmental and water treatment processes: A critical review, *Water Research*, **202**, 117463 (2021). https://doi.org/10.1016/j.watres.2021.117463

27. L. Wu, Z. Wu, Y. Wang, L. Han, H. Mao, Z. Gu, The effect of chemical composition on the degradation kinetics of high molecular weight poly(trimethylene carbonate-co-L-lactide), *Polymer Degradation and Stability*, **206**, 110183 (2022). https://doi.org/10.1016/j.polymdegradstab.2022.110183

28. V. Vishnu-Priyan, N. Kumar, H.K. Rajendran, J. Ray, S. Narayanasamy, Sequestration and toxicological assessment of emerging contaminants with polypyrrole modified carboxymethyl cellulose (CMC/PPY): Case of ibuprofen pharmaceutical drug, *International Journal of Biological Macromolecules*, **221**, 547–557 (2022). https://doi.org/10.1016/j.ijbiomac.2022.09.046

29. J.C. Carrizo, S. Vo Duy, G. Munoz, G. Marconi, M.V. Amé, S. Sauvé, Suspect screening of pharmaceuticals, illicit drugs, pesticides, and other emerging contaminants in Argentinean *Piaractus mesopotamicus*, a fish species used for local consumption and export, *Chemosphere*, **309**(Part 1), 136769 (2022). https://doi.org/10.1016/j.chemosphere.2022.136769

30. R. Wang, J. Luo, C. Li, J. Chen, N. Zhu, Antiviral drugs in wastewater are on the rise as emerging contaminants: A comprehensive review of spatiotemporal characteristics, removal technologies and environmental risks, *Journal of Hazardous Material*, **457**, 131694 (2023). https://doi.org/10.1016/j.jhazmat.2023.131694

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Acute effect of pregabalin on depressive- and anxiety-like behaviors in adult rats in a pharmacologic model of depression

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Summary

Introduction: Due to lack of effective and fast-acting therapy, depression can become a life-threatening condition if not treated adequately. In fibromyalgia animal models, pregabalin has shown antidepressant properties. Compared to fluoxetine, the unusual fast-acting antidepressant properties of pregabalin offers the possibility to explore this GABA analogue in the context of depression. **Objective:** we evaluated the acute effect of pregabalin in a reserpine-induced animal models of depression. **Method:** rats were organized into four groups regarding their planned drug regimen: control (C), reserpine (R), reserpine + fluoxetine (RF) and reserpine + pregabalin (RP). The C group received only saline throughout the study. Depressive and anxiety-like behavior were tested using the force swimming test (FST) and the open field test (OFT), respectively. **Results:** Our data shows that both RP and RF groups have a significant longer mobility time (seconds) than the C group during the FST (RP: 44.76 ± 8.37 and RF: 65.86 ± 34.10 vs C: 21.80 ± 11.10, $p<0.05$ for both). No significant differences were observed in immobility time and climbing across all groups ($p>0.05$). On the other hand, RF and RP groups showed a reduced time spent in the center of the arena compared to control ($p<0.05$) in

OFT, suggesting increased anxiety-like behavior. **Conclusion:** Our results show that pregabalin has an acute effect on depressive and can also acutely exert an unexpected anxiety-like behavior as well. Our work reveals an unexpected outcome in the search for fast-acting antidepressants.

Keywords: Reserpine; pregabalin; depression; anxiety; animal model.

Resumen

Efecto agudo de la pregabalina sobre los comportamientos tipo depresivo y ansioso en ratas adultas en un modelo farmacológico de depresión

Introducción: Debido a la falta de un tratamiento eficaz, el trastorno depresivo puede ser riesgoso para la vida si no es tratado adecuadamente. En modelos animales de fibromialgia, la pregabalina ha demostrado propiedades antidepresivas. En comparación con la fluoxetina, la inesperada acción antidepresiva aguda de la pregabalina ofrece la posibilidad de explorar este análogo del GABA en el contexto de la depresión. **Objetivo:** evaluar el efecto agudo de la pregabalina en un modelo animal de depresión inducida por reserpina. **Método:** las ratas fueron agrupadas en cuatro grupos según su régimen terapéutico: control (C), reserpina (R), reserpina + fluoxetina (RF) y reserpina + pregabalina (RP). El grupo C recibió sólo solución salina durante el estudio. Los comportamientos tipo depresivo y ansioso fueron evaluados mediante la prueba de nado forzada (PNF) y la prueba de campo abierto (PCA), respectivamente. **Resultados:** Nuestros datos mostraron que tanto el grupo RP como el RF tienen un tiempo de movilidad significativamente mayor que el grupo C durante la PNF (RP: 44,76s ± 8,37 y RF: 65,86s ± 34,10 vs C: 21,80s ± 11,10, $p<0,05$ para ambos). No se observaron diferencias significativas en el tiempo de inmovilidad y escalada entre los grupos ($p>0,05$). Por otro lado, en la PCA, los grupos RF y RP mostraron una reducción del tiempo en el centro de la arena en comparación con el control ($p<0,05$), lo que sugiere un mayor comportamiento tipo ansioso. **Conclusión:** Nuestros resultados sugieren que la pregabalina tiene un efecto agudo sobre los comportamientos tipo depresivo y ansioso.

Palabras clave: Reserpina; pregabalina; depresión; ansiedad; modelo animal.

Resumo

Efeito agudo da pregabalina nos comportamentos depressivos e ansiosos em ratos adultos em um modelo farmacológico de depressão

Introdução: Devido à falta de terapia eficaz e de ação rápida, a depressão pode tornar-se uma condição potencialmente fatal se não for tratada adequadamente. Em modelos animais de fibromialgia, a pregabalina demonstrou propriedades antidepressivas. Em comparação com a fluoxetina, as propriedades antidepressivas incomuns de ação rápida da pregabalina oferecem a possibilidade de explorar este análogo do GABA no contexto da depressão. **Objetivo:** avaliamos o efeito agudo da pregabalina em modelos animais de depressão induzidos por reserpina. **Método:** os ratos foram organizados em quatro grupos quanto ao regime medicamentoso planejado: controle (C), reserpina (R), reserpina + fluoxetina (RF) e reserpina + pregabalina (RP). O grupo C recebeu apenas solução salina durante todo o estudo. Os comportamentos depressivos e ansiosos foram testados por meio do teste de natação forçada (FST) e do teste de campo aberto (OFT), respectivamente. **Resultados:** Nossos dados mostram que ambos os grupos RP e RF apresentam um tempo de mobilidade (segundos) significativamente maior do que o grupo C durante o FST (RP: 44,76 ± 8,37 e FR: 65,86 ± 34,10 vs C: 21,80 ± 11,10, $p<0,05$ para ambos). Não foram observadas diferenças significativas no tempo de imobilidade e escalada em todos os grupos ($p>0,05$). Por outro lado, os grupos RF e RP apresentaram redução do tempo gasto no centro da arena em comparação ao controle ($p<0,05$) no OFT, sugerindo aumento do comportamento semelhante à ansiedade. **Conclusão:** Nossos resultados mostram que a pregabalina tem um efeito agudo na depressão e também pode exercer de forma aguda um comportamento inesperado semelhante à ansiedade. Nosso trabalho revela um resultado inesperado na busca por antidepressivos de ação rápida.

Palavras-chave: Reserpina; pregabalina; depressão; ansiedade; modelo animal.

Introduction

Depression is a complex mood disorder shaped by an imbalance in the interaction between monoamine networks in the brain. Consequently, individuals struggling with depression tend to display behaviors of helplessness, negativity, and despair that ultimately impact their physical health as well. This disorder is characterized by changes

in appetite, abnormal social behavior, insomnia, fatigue, lack of energy, headaches, and inability to enjoy life [1]. Depression prevalence affects up to of 350 million people around the world; however, this condition has not yet been treated successfully due to the lack of effective therapeutic modalities and the social stigma that this entails [2].

One of the main reasons why depression remains poorly responsive to antidepressant treatment is its heterogeneous aetiology [3]. Studies have shown that close to 50% of depression cases are mediated by genes [4, 5]. This has provided a potential explanation for recurrent patients where vulnerability to depressive events constantly triggers the resurgence of the pathology. On the other hand, there are also clear sex differences [6]. Evidence has shown that women are 2-3 times more likely to develop Major Depressive Disorder (MDD) compared to men [7]. Even worse, it is the female sex who presents a greater severity of symptoms, greater functional impairment, more atypical depressive symptoms, and higher rates of comorbid anxiety [8, 9]. Recent investigations have evidenced the existence of denoted sexual dimorphism at the transcriptional level in MDD and highlight the importance of studying sex-specific treatments [6, 10, 11]. This clear evidence at the transcriptional level has not only shed new light on the understanding of the body's response to depression but has also helped explain the resistance to treatment present in many patients.

Through the last years depression symptoms have been largely associated with monoamine neurotransmitters. Among the main symptoms, sadness, appetite, aggression, suicidal ideation, guilt, and feelings of worthlessness are mainly associated with serotonin while motivation and sociability are dopamine-related. Moreover, it is important to note that many other symptoms are not caused by a single neurotransmitter, but rather the result of the interaction between them. This is the case of mood, sleep disturbances, psychomotor retardation, anhedonia, anxiety, fatigue and concentration deficiency that arise as a result of the imbalance between serotonin, dopamine and norepinephrine [12]. This central knowledge has dictated the path mainly used for the development of pharmacological treatments against depression in the last 30 decades.

Unfortunately, the current antidepressant treatments present drawbacks. People must wait at least 4 weeks before the treatment to produce significant results, meanwhile, they must also deal with the occurrence of side effects. Constipation, decreased vision, sexual dysfunction, high blood pressure, cognitive impairment, loss of libido, headache, agitation, and anxiety are just some of the most common reported after the use of monoamine modulators [13].

Interestingly, a recent study suggest that antiepileptic drugs, such as pregabalin, could ameliorate depressive symptoms [14]. Pregabalin is advised as first-line pharmacological agent for neuropathic pain [15], being intensely used for fibromyalgia syndrome

(FMS). Despite pregabalin prescriptions have increased over the last few years [16] yet our knowledge is still scarce about its potential application on mood disorders such as depression and anxiety. Initial investigations showed that while pregabalin significantly reduces the symptoms and side effects of many drug treatments, it does not have a significant effect in relieving depressive states [17-19]. Nevertheless, recent strong meta-analysis evidence has suggested a crucial role of pregabalin in the treatment of anxiety disorders [20], demonstrating its key influence in many of the most related symptoms and characteristics that also configure depression spectrum. Here, we evaluated the acute effects of pregabalin in a well stablished depression animal model induced by reserpine in rats.

Method

Animals

A total of twenty-four male adult Wistar rats of 3 – 4 months of age were purchased from "Instituto Nacional de Salud (INS)", Lima, Peru. Rats weighed in average between 300 – 400 g. The animals were housed in conventional plastic-steel cages in a 12 h light/dark cycle at room temperature of 22 ± 1 °C. Water and food were given *ad libitum* during all the procedures.

Experimental design

Upon arrival at our colony space, adult rats were randomly assigned to 4 groups (6 animals each) and acclimated for 5 days before experiments were performed. Groups were named regarding with planned drug regimen (Table 1): control (C) group, Reserpine (R) group, Reserpine + Pregabalin (RP), and Reserpine + Fluoxetine (RF).

Table 1. Drug regimen of the study

Groups	Number of Animals	Reserpinization (s)	Drug administration (o)
Control	6	Saline solution	Saline solution
Reserpine	6	1 mg/kg	Saline solution
Reserpine + Pregabalin	6	1 mg/kg	Pregabalin 30 mg/kg
Reserpine + Fluoxetine	6	1 mg/kg	Fluoxetine 10 mg/kg

Abbreviations: s: subcutaneous route; o: oral route. Saline solution was administrated subcutaneously in all cases.

On day 6, reserpine (Sigma-Aldrich, Buchs, Switzerland) was diluted in saline solution and administered subcutaneously (1 mg/kg) three consecutive days [14]. All animals received reserpine, except those in the C group. Two days after reserpinization, saline and treatments were administered orally (oral gavage technique). No fasting was required to proceed with oral gavage. RP mice was treated with pregabalin (Lyrica™; Pfizer, Madrid, Spain) with a single oral dose of 30 mg/kg [14], while the RF mice received fluoxetine (Prozac™, Eli Lilly Interamerica, Indianapolis, USA) in a single oral dose of 10 mg/kg [21, 22]. Behavioral assessment including forced swimming and open field testing were performed 2 hours after treatment administration. Finally, the animals were euthanized with two anesthetics: 100 mg/kg of ketamine and 10 mg/kg of xylazine injected intraperitoneally. Biological waste was discarded according to national policy following the 'Norma Tecnica de Salud' from the Peruvian Ministry of Health (NTSN°144-MINSA/2018/DIGESA). The experimental design is resumed in Figure 1.

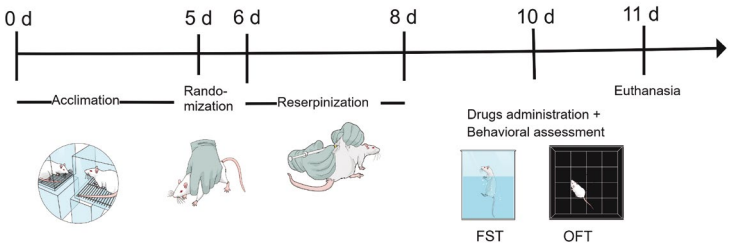

Figure 1. The experimental design for the study. Abbreviation: rand: randomization; FST: forced swimming test; OFT: Open field test.

Behavioural assessments

Forced swimming test (FST): 24 hours after the last reserpinization, all animals were individually placed for 5 minutes in a 23 litters glass cylinder of 34.1 cm in diameter and 38.1 cm in height, previously filled with water acclimated to 25 °C. This session prior to the test allows us to eliminate acute stress and the reaction to novelty in the animals, in addition to allowing them to know the challenging conditions of the task before their actual evaluation. One day after, the test was repeated rand immobility, climbing and swimming behaviors were evaluated during the first 3 minutes using a video camera located above the cylinder [23].

Open field test (OFT): animals were individually placed in the center of an arena (94 cm × 42 cm) and allowed to explore freely for 10 minutes. Total distanced travelled and velocity average were measured to study locomotor activity. On the other hand, the number of entrances to the center and time spent in the center were considered

to evaluate anxiety-like behavior [24]. The recordings were analysed using EthoVision video tracking software (Noldus, Leesburg, USA).

Statistical plan

All data were expressed as average ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by Tukey *post-hoc* tests, were used with GraphPad Prism version 6 (GraphPad Software Inc., San Diego, CA, USA) to evaluate differences between groups. $p<0.05$ was considered statistically significant.

Ethical Considerations

All procedures were approved by "Comité Institucional de Ética para el Uso de Animales (CIEA)" de la Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru (Protocol number 205561).

Results and discussion

Forced swimming test

We found significant differences between all groups in swimming time ($p=0.021$). *In* Tukey *post-hoc* test, RP and RF groups showed a longer swimming time compared to C group ($p=0.047$ and $p=0.037$, respectively). On the other hand, immobility and climbing time did not differ across all groups ($p>0.05$) (Figure 2).

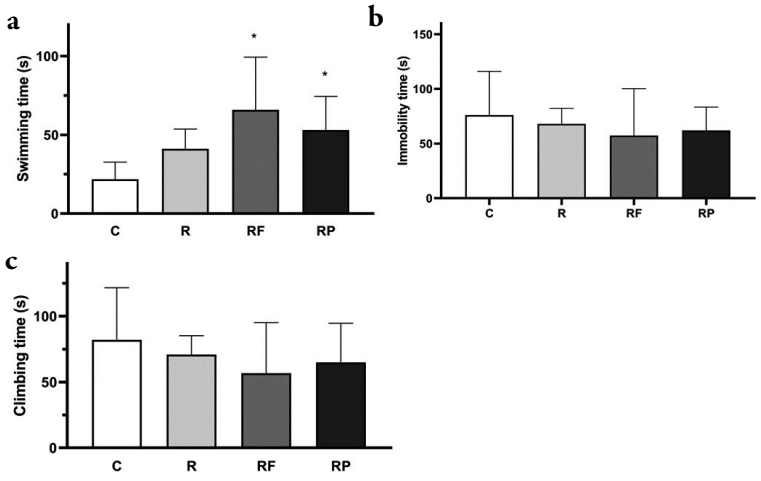

Figure 2. Effect of pregabalin and fluoxetine in reserpine pharmacological model. Forced swimming test: a) swimming time, b) immobility time, and c) climbing time. Bars represents mean ± SD. Legend: *$p<0.05$ in comparison to C group. ANOVA followed by Tukey *post-hoc* test.

Open Field Test

Regarding locomotor activity, we found differences in total distance travelled and average velocity during the test between all groups (p=0.003 for both). In Tukey *post-hoc* analysis, animals from RF and RP groups showed a lower traveled distance than C group (p=0.004 and p=0.022, respectively) and R group (p=0.001 and p=0.005, respectively). Similarly, RF and RP groups also displayed a reduction in average velocity in comparison that C group (p=0.004 and p=0.021, respectively) and R group (p=0.001 and p=0.006, respectively) (Figure 3).

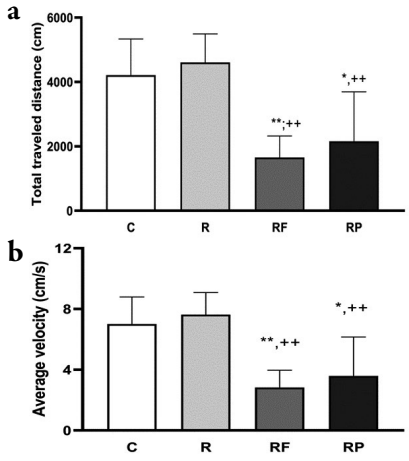

Figure 3. Effect of pregabalin and fluoxetine in reserpine pharmacological model. Open Field test (locomotor activity): a) total traveled distance, and b) average velocity. Bars represents mean ± SD. Bars represents mean ± SD. Legend: $^{**}p<0.001$ and $^{*}p<0.05$ in comparison to C group. $^{++}p<0.001$ in comparison to R group. ANOVA followed by Tukey *post-hoc* test.

In relation to anxiety-like behavior, all groups exhibited differences in the number of entrances to the center (p<0.0001) and the time spent in the center of the arena (p=0.002). In multiple comparison analysis, the RF and RP groups showed a reduction in the number of entrances to the center compared to control (p=0.0006 and p=0.0003, respectively) and the reserpine group (p=0.006 and p=0.0028, respectively). On the other hand, in another parameter, the RF and RP groups only displayed a decrease of time spent in the center with respect to the C group (p=0.006 and p=0.005, respectively, but not to the R group (p>0.05 for both) (Figure 4).

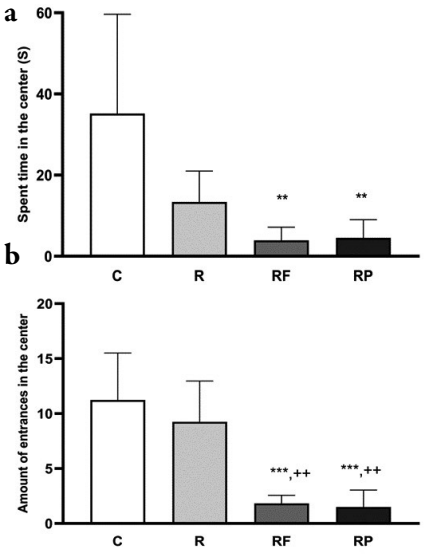

Figure 4. Effect of pregabalin and fluoxetine in reserpine pharmacological model. Open Field test (Anxiety-like behavior): a) spent time in the center, and b) amount of entrances in the center. Bars represents mean ± SD. Legend: ***p<0.0001 and **p<0.001 in comparison to C group. ++p<0.001 in comparison to R group. ANOVA followed by Tukey *post-hoc* test.

Major depression steadily increases in prevalence worldwide with more than 75% of the population from low- and middle-income countries without adequate treatment. A major barrier to treat patients with depression includes extant ineffective antidepressant drugs [25]. In this study, we set to investigate the acute effect of pregabalin in a rat model of reserpine-induced depression using the FST and OFT.

Molecularly, reserpine-induced depression may stem from the irreversible inhibition of the vesicular monoamine transporter 2 receptor leading to leakage and degradation of biogenic amines stored in pre-synaptic vesicles [26]. The degradation of these amines includes neurotransmitters such as serotonin, dopamine and norepinephrine and causes depletion of transmitters at pre-synaptic terminals.

Serotonin has been implicated in depression for decades due to the pharmacological properties of antidepressants such as fluoxetine [27]. The FST was developed to test depressive-like states and the well-characterized effects of fluoxetine on this behavioral assay provides a proxy to serotonin involvement.

In the FST, we only observed longer swimming time in the RP and RF group compared to C group. Notably, pregabalin mimicked fluoxetine antidepressant effect as swimming time in both groups were similar. Even though the R group did not show the expected behavior in this test, we attribute it to inter-strain variability, animal age, and reserpine dose.

We followed González-Soler's protocol [14] to acutely induce depressive-like behavior using a 3-consecutive day regime of 1 mg/kg reserpine subcutaneously on male Sprague-Dawley rats. On her study, it is possible to assume that rats showed depressive-like behavior because duloxetine improved swimming time and reduced immobility time; however, the authors did not show a vehicle group without reserpine to set a baseline level for the forced swimming test. Even though it has been shown that acute administration of reserpine can induce depressive-like behavior using appropriate control groups [28], we speculate that 1 mg/kg of reserpine for 3 consecutive days may not exert a profound impact on learned hopelessness on the FST compared to no reserpine. The acute administration of reserpine in adult Wistar rats may differently affect depressive-like and anxiety-like behaviors with certain degree of variability. This variability can depend on the susceptibility of rats to reserpine due to maturation of brain circuitry. For instance, Ruiz et al. has recently explored the effects of reserpine-induced depression and fluoxetine on adolescent alcohol intake [29]. They showed that 1 mg/kg of reserpine for 4 days drove depressive-like behaviors on adolescent post-weaned 1 month-old male and female Wistar rats.

The effect of reserpine may be affected by dose. Ahmed and col. used a high-dose of reserpine to induce depressive-like behaviors acutely in adult male Wistar rats [30]. In the study, rats receiving reserpine intraperitoneally 6 mg/kg showed a significant increase in immobility time in the forced swimming test compared to control, 1 day after the injection. Another study that aimed to use a reserpine-induced model of depression also showed acute depressive-like behaviors only 1-hour post-injection with reserpine doses from 4 to 8 mg/kg [28].

Ahmed *et al.* [30] showed that fluoxetine administered for 3 days after reserpinization restored immobility time suggesting that this drug can acutely display antidepressant effects. Despite the paradoxical effect of reserpine in the FST, the intervention with fluoxetine and pregabalin acted on learned helplessness and behavioral despair. To extensively dissect the effects of reserpine and pregabalin, we recommend coupling behavioral analysis to molecular correlates.

Given the dopamine depleting effects of reserpine and the known co-morbidity of depression and anxiety, we extended our analysis to the open field test. Reserpine

at 1 mg/kg dose in our study did not reduce overall traveled distance or speed suggesting that dopamine signaling still acts to perform motor activity primarily via striatum [31]. In addition, no significant effects were observed in the time spent at the center between the R and C groups strengthening the notion that lack of complete dopamine depletion may explain no notable effects on anxiety-like behaviors. Interestingly, acute treatment with pregabalin in our model exerted an unexpected anxiogenic effect.

In the study of Ruiz *et al.* [29], they showed that 4 consecutive doses of reserpine at 1 mg/kg reduced travelled distance and reduced the time spent in the center of the open field chamber. The levels of dopamine in the insular cortex were also reduced pointing out that lack of dopamine signaling could explain locomotor deficits and anxiogenic behavior [29]. In the work of Ahmed *et al.* [30], the lack of overall motor activity could be explained by ablation of dopaminergic signaling due to high-dose reserpine (6 mg/kg). The reason for the lack of effect on anxiety-like behaviors in the reserpine group may lie on remaining dopaminergic signaling.

High doses of fluoxetine administered acutely may also have a sedative effect reducing locomotor activity shown in the elevated plus-maze test [32]. This also explains the reduced activity in our study. We need to note that pregabalin shows a similar effect as acute fluoxetine treatment increases anxiety-like behavior. Even though both drugs act on different targets, acute pharmacological effect may converge in the nucleus for motor and anxiety control.

Our limitations in this work were related to the lack of molecular, histological, and electrophysiological studies, which would offer more information about potential targets or molecule that could explain these outcomes. Another limitation was that we cannot observed differences in depressive or anxiety-like behavior between R and C group. This fact could be explained because reserpine acts on multiple pathways and not only those related to depression [33].

Our results suggests that acute pregabalin administration can ameliorate depressive-like behavior in a pharmacological model in rats. This could be explained by an acute action on 5-HT, DA, NA or GABA system. However, further studies are needed in order to confirm these results and elucidate monoaminergic pathways that could participate in this process.

Conflicts of interest

The authors declare no conflict of interest.

References

1. K. Smith, Mental health: A world of depression, *Nature*, **515**, 180–181 (2014).

2. C. Otte, S.M. Gold, B.W. Penninx, C.M. Pariante, A. Etkin, M. Fava, D.C. Mohr, A.F. Schatzberg, Major depressive disorder, *Nat. Rev. Dis. Prim.*, **2**, 16065 (2016).

3. R.H. Belmaker, G. Agam, Major depressive disorder, *N. Engl. J. Med.*, **358**, 55–68 (2008).

4. B.D. Hare, R.S. Duman, Prefrontal cortex circuits in depression and anxiety: Contribution of discrete neuronal populations and target regions, *Mol. Psychiatry*, **25**, 2742–2758 (2020).

5. J. Flint, K.S. Kendler, The genetics of major depression, *Neuron*, **81**, 484–503 (2014).

6. B. Labonté, O. Engmann, I. Purushothaman, C. Menard, J. Wang, C. Tan, J.R. Scarpa, *et al.*, Sex-specific transcriptional signatures in human depression, *Nat. Med.*, **23**, 1102–1111 (2017).

7. R.C. Kessler, W.T. Chiu, O. Demler, K.R. Merikangas, E.E. Walters, Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication, *Arch. Gen. Psychiatry*, **62**, 617–627 (2005).

8. S.G. Kornstein, A.F. Schatzberg, M.E. Thase, K.A. Yonkers, J.P. McCullough, G.I. Keitner, *et al.*, Gender differences in chronic major and double depression, *J. Affect. Disord.*, **60**, 1–11 (2000).

9. R.C. Kessler, K.A. McGonagle, M. Swartz, D.G. Blazer, C.B. Nelson, Sex and depression in the National Comorbidity Survey I: Lifetime prevalence, chronicity and recurrence, *J. Affect. Disord.*, **29**, 85–96 (1993).

10. M. Touchant, B. Labonté, Sex-specific brain transcriptional signatures in human MDD and their correlates in mouse models of depression, *Front. Behav. Neurosci.*, **16**, 845491 (2022).

11. M. Maitra, H. Mitsuhashi, R. Rahimian, A. Chawla, J. Yang, L.M. Fiori, *et al.*, Cell type specific transcriptomic differences in depression show similar patterns between males and females but implicate distinct cell types and genes, *Nat. Commun.*, **14**, 2912 (2023).

12. E. Beurel, M. Toups, C.B. Nemeroff, The bidirectional relationship of depression and inflammation: Double trouble, *Neuron*, **107**, 234–256 (2020).

13. S. Marwaha, E. Palmer, T. Suppes, E. Cons, A.H. Young, R. Upthegrove, Novel and emerging treatments for major depression, *Lancet*, **401**, 141–153 (2023).

14. E.M. González-Soler, A. Blasco-Serra, G.M. Alfosea-Cuadrado, M. Igual-López, J. Orduña-Valls, C. Tornero-Tornero, *et al.*, Chronic pregabalin treatment ameliorates pain, but not depressive-like behaviors, in a reserpine-induced myalgia model in rats, *Pain Physician*, **23**, E581–E590 (2020).

15. Y. Wang, H. Yang, C. Shen, J. Luo, Morphine and pregabalin in the treatment of neuropathic pain, *Exp. Ther. Med.*, **13**, 1393–1397 (2017).

16. I.J. Onakpoya, E.T. Thomas, J.J. Lee, B. Goldacre, C.J. Heneghan, Benefits and harms of pregabalin in the management of neuropathic pain: A rapid review and meta-analysis of randomised clinical trials, *BMJ Open*, **9**, e023600 (2019).

17. W. Häuser, F. Petzke, N. Üçeyler, C. Sommer, Comparative efficacy and acceptability of amitriptyline, duloxetine and milnacipran in fibromyalgia syndrome: A systematic review with meta-analysis, *Rheumatology* (Oxford), **50**, 532–543 (2011).

18. S. Quilici, J. Chancellor, M. Löthgren, D. Simon, G. Said, T.K. Le, A. Garcia-Cebrian, B. Monz, Meta-analysis of duloxetine vs pregabalin and gabapentin in the treatment of diabetic peripheral neuropathic pain, *BMC Neurol.*, **9**, 6 (2009).

19. W. Häuser, F. Petzke, C. Sommer, Comparative efficacy and harms of duloxetine, milnacipran, and pregabalin in fibromyalgia syndrome, *J. Pain*, **11**, 505–521 (2010).

20. M.B. Generoso, A.P. Trevizol, S. Kasper, H.J. Cho, Q. Cordeiro, P. Shiozawa, Pregabalin for generalized anxiety disorder: An updated systematic review and meta-analysis, *Int. Clin. Psychopharmacol.*, **32**, 49–55 (2017).

21. S. Jayakumar, G. Raghunath, S. Ilango, J. Vijayakumar, R. Vijayaraghavan, Effect of fluoxetine on the hippocampus of wistar albino rats in cold restraint stress model, *J. Clin. Diagnostic Res.*, **11**, AF01–AF06 (2017).

22. S.C. Dulawa, K.A. Holick, B. Gundersen, R. Hen, Effects of chronic fluoxetine in animal models of anxiety and depression, *Neuropsychopharmacology*, **29**, 1321–1330 (2004).

23. D. Kuzay, E. Dileköz, Ç. Özer, Effects of thymoquinone in a rat model of reserpine-induced depression, *Braz. J. Pharm. Sci.*, **58**, e19847 (2022).

24. K.R. Lezak, G. Missig, W.A. Carlezon, Behavioral methods to study anxiety in rodents, *Dialogues Clin. Neurosci.*, **19**, 181-191 (2017).

25. F.C. Gabriel, D.O. de Melo, R. Fráguas, N.C. Leite-Santos, R.A. Mantovani da Silva, E. Ribeiro, Pharmacological treatment of depression: A systematic review comparing clinical practice guideline recommendations, *PLoS One*, **15**, e0231700 (2020).

26. T. Hedgecock, A. Phillips, B. Ludrick, T. Golden, N. Wu, Molecular mechanisms and applications of a reserpine-induced rodent model, *SSR Inst. Int. J. Life Sci*., **5**, 2160–2167 (2019).

27. J. Moncrieff, R.E. Cooper, T. Stockmann, S. Amendola, M.P. Hengartner, M.A. Horowitz, The serotonin theory of depression: A systematic umbrella review of the evidence, *Mol. Psychiatry*, **28**, 3243–3256 (2023).

28. Q. Huang, H. Jiang, X. Hao, T.R. Minor, Brain IL-1 beta was involved in reserpine-induced behavioral depression in rats, *Acta Pharmacol. Sin.*, **25**, 293–296 (2004).

29. P. Ruiz, A. Calliari, R.M. Pautassi, Reserpine-induced depression is associated in female, but not in male, adolescent rats with heightened, fluoxetine-sensitive, ethanol consumption, *Behav. Brain Res.*, **348**, 160–170 (2018).

30. R.F. Ahmed, R.F. Abdel-Rahman, O.A.H.A. Farid, S.A. El-Marasy, A.F. Hessin, Combined hepatoprotective and antidepressant effects of resveratrol in an acute model of depression, *Bull. Fac. Pharm.*, *Cairo Univ.*, **52**, 191–197 (2014).

31. Y. Li, Q. Yin, B. Wang, T. Shen, W. Luo, T. Liu, Preclinical reserpine models recapitulating motor and non-motor features of Parkinson's disease: Roles of epigenetic upregulation of alpha-synuclein and autophagy impairment, *Front. Pharmacol.*, **13**, 944376 (2022)

32. D. Drapier, D. Bentué-Ferrer, B. Laviolle, B. Millet, H. Allain, M. Bourin, J.M. Reymann, Effects of acute fluoxetine, paroxetine and desipramine on rats tested on the elevated plus-maze, *Behav. Brain Res.*, **176**, 202–209 (2007).

33. M. Cheung, M. Parmar, Reserpine, StatPearls [Internet]. URL: https://www.ncbi.nlm.nih.gov/books/NBK557767/, accessed September **1**, 2023.

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Acoplamiento molecular *in silico* de compuestos de *Moringa oleifera* Lam. con el sitio catalítico y de unión a FAD-NADPH de la tripanotiona reductasa de *Leishmania infantum*

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Resumen

Introducción: la búsqueda computacional de nuevos compuestos para futuras terapias farmacológicas contra la leishmaniasis se ha enfocado en la tripanotiona reductasa. Esta enzima es esencial para la supervivencia de *Leishmania infantum*, su ausencia en el huésped y su alta farmacobilidad la convierten en un buen objetivo para el desarrollo de nuevas drogas. El uso de plantas como fitoterapéuticos ha sido ampliamente investigado debido a que han demostrado importantes actividades leishmanicidas. **Objetivo:** analizar las interacciones de los compuestos de *Moringa oleifera* con el sitio catalítico y de unión a FAD-NADPH de la tripanotiona reductasa de *L. infantum* empleando el acoplamiento molecular. **Método:** fueron evaluados treinta compuestos descritos en la especie de *M. oleifera,* estas se sometieron a ensayos computacionales de acoplamiento molecular semiflexible con el sitio catalítico de la enzima y el dominio de unión FAD-NADPH. **Resultados:** se evidencia que los compuestos que demostraron valores favorables de energía de unión con el sitio catalítico de la enzima fueron la apigenina, la quercetina, la miricetina y la isorhamnetina. Mientras que los compuestos que presentaron uniones favorables con el dominio FAD-NADPH fueron la rutina y el ácido

5,6,7,8-tetrahidrofólico. **Conclusión:** estos hallazgos sugieren que los compuestos de *M. oleifera* mencionados presentan afinidades de unión por el sitio catalítico y el dominio de unión FAD-NADPH de la tripanotiona reductasa de *L. infantum*, pudiendo ser utilizados para pruebas *in vitro*.

Palabras clave: moringa, fitoconstituyentes, acoplamiento molecular, leishmania, tripanotiona reductasa

Summary

In silico molecular docking of *Moringa oleifera Lam*. compounds with the catalytic and FAD-NADPH-binding site of trypanothione reductase from *Leishmania infantum*

Introduction: Computational search of new compounds for future drug therapies against leishmaniasis has focused on trypanothione reductase. This enzyme is essential for the survival of *Leishmania infantum*, the absence in the host and its high druggability make it a good target for the development of new drugs. The use of plants for phytotherapeutics purpose has been widely investigated due to its important leishmanicidal activities. **Aim**: The aim of this study was to analyze the interactions of *Moringa oleifera* compounds to the catalytic and FAD-NADPH binding site of *L. infantum* trypanothione reductase using molecular docking. Method: Thirty compounds described in *M. oleifera* species were evaluated and subjected to computational semi-flexible molecular docking assays with the catalytic site and the FAD-NADPH binding domain of the enzyme. **Results**: The results show that the compounds that demonstrated favorable binding energy values with the catalytic site of the enzyme were apigenin, quercetin, myricetin and isorhamnetin. While the compounds that exhibited favorable binding with the FAD-NADPH domain were rutin and 5,6,7,8-tetrahydrofolic acid. **Conclusion**: These findings suggest that mentioned M. oleifera compounds present binding affinities for the catalytic site and the FAD-NADPH binding domain of *L. infantum* trypanothione reductase and could be used for *in vitro* assays.

Keywords: moringa, phytoconstituents, molecular docking, leishmania, trypanothione reductase.

Resumo

Docking molecular *in silico* de compostos de *Moringa oleifera* Lam. com o sítio catalítico e de ligação FAD-NADPH da tripanotiona redutase de *Leishmania infantum*

Introdução: a busca computacional por novos compostos para futuras terapias farmacológicas contra a leishmaniose tem se concentrado na tripanotiona redutase. Esta enzima é essencial para a sobrevivência de *Leishmania infantum*, sua ausência no hospedeiro e sua alta farmacobilidade a tornam um bom alvo para o desenvolvimento de novos fármacos. O uso de plantas como fitoterápicos tem sido amplamente investigado por apresentarem importantes atividades leishmanicidas. **Objetivo:** o objetivo deste estudo foi analisar as interações dos compostos da *Moringa oleifera* com o sítio catalítico e de ligação FAD-NADPH da tripanotiona redutase de L. infantum usando docking molecular. **Método:** foram avaliados trinta compostos descritos na espécie *M. oleifera*, os quais foram submetidos a testes computacionais de docking molecular semi-flexível com o sítio catalítico da enzima e o domínio de ligação FAD-NADPH. **Resultados:** fica evidente que os compostos que demonstraram valores de energia de ligação favoráveis com o sítio catalítico da enzima foram apigenina, quercetina, miricetina e isorhamnetina. Enquanto os compostos que apresentaram ligações favoráveis com o domínio FAD-NADPH foram a rutina e o ácido 5,6,7,8-tetraidrofólico. **Conclusão:** esses achados sugerem que os compostos de *M. oleifera* mencionados possuem afinidades de ligação para o sítio catalítico e o domínio de ligação FAD-NADPH da tripanotiona redutase de *L. infantum*, e podem ser usados para testes *in vitro*.

Palavras-chave: moringa, fitoconstituintes, acoplamento molecular, leishmania, tripanotiona redutase

Introducción

Paraguay forma parte de uno de los 13 países a nivel mundial en dónde esta enfermedad se considera endémica. Según un reporte de control de Leishmaniasis Visceral, ocasionado por el parásito *Leishmania infantum*, en humanos y caninos en Paraguay, entre el 2016 al 2018 por el Programa Nacional de Control de Zoonosis y Centro Antirrábico Nacional (PNCZyCAN), la ciudad de Asunción reportó el 90% de los casos, siguiendo con cifras menores pero relevantes los departamentos; de Central, Paraguarí y Cordillera [1].

La comunidad científica ha dirigido su mirada en la enzima tripanotiona reductasa (TR) en la búsqueda por nuevos compuestos y nuevas posibles terapias farmacológicas para combatir a la leishmaniasis, esta oxidorreductasa es característica de la familia Trypanosomatidae y su relevancia recae sus funciones en la supervivencia de estos parásitos, ya que protege al parásito contra el daño de los oxidantes, xenobióticos y metales pesados tóxicos, además de estar ausente en el huésped y de que presenta una alta farmacobilidad [2, 3]. Esta enzima es una flavoproteína, un homodímero simétrico doble donde cada subunidad consta de una cadena polipeptídica que se dispone en tres dominios; el dominio FAD (residuos 1–160 y residuos 289-360), el dominio de interfaz (residuos 361-488) y el dominio NADPH (residuos 161-288) [4, 5].

El tratamiento farmacológico depende de factores como la presencia de patologías de base, el estado inmunitario del paciente y principalmente la susceptibilidad del protozoario a los medicamentos [6]. Los fármacos empleados generalmente en la terapia son antimoniales pentavalentes y anfotericina B, en sus formas convencional y liposomal, sin embargo, los tratamientos disponibles no erradican la infección y por lo regular las contraindicaciones son graves, dañando órganos como el corazón, hígado y riñón, así como pérdida de la eficacia de los medicamentos, su compleja administración se suma a las tantas contrapartidas en el uso de estos fármacos [7].

La ineficacia, los efectos secundarios y el alto costo, revelan la necesidad urgente de encontrar y desarrollar potenciales compuestos con posibles aplicaciones terapéuticas con una menor toxicidad y mayor eficacia. En esto el uso de las plantas como fuentes de fitoterapéuticos ha sido ampliamente estudiado por la comunidad científica, así como por las industrias farmacéuticas debido a los compuestos presentes en diferentes órganos (raíces, tallo, hojas, flores, frutos) como los alcaloides, los flavonoides, las quinonas, las, chalconas, los terpenos, entre otros, que han demostrado actividades antiparasitarias *in vitro* [8, 9]. En este punto, se han reportado que la *Moringa oleifera* podrían tener potencial para el tratamiento de la leishmaniasis [10, 11].

Debido a los registros generados acerca de las actividades beneficiosas de la *M. olefera* contra la leishmaniasis y a los posibles principios activos presentes en esta especie, se ha propuesto como objetivo analizar las afinidades de interacción de los compuestos de *M. oleifera* con el sitio catalítico y de unión a FAD-NADPH de la tripanotiona reductasa de *L. infantum* empleando el método *in silico* del acoplamiento molecular.

Metodología

Cribado virtual y acoplamiento molecular de fitoconstituyentes de *M. oleifera* con sitios activos de tripanotiona reductasa de *L. infantum*

Se emplearon las estructuras moleculares de treinta compuestos descritos en *M. oleifera,* los cuales fueron obtenidos de la base de datos PubChem [12-15]. La estructura molecular de la enzima tripanotiona reductasa de *L. infantum* (PDB: 2X50, 4ADW) fue obtenida de la base de datos Protein Data Bank [16].

La preparación y minimización de energía de las estructuras de los compuestos de *M. oleifera* y de la tripanotiona reductasa fueron llevadas a cabo con el algoritmo de campo de fuerza universal (UFF) empleando cuatro pasos por actualización y gradientes conjugados con un ciclo consistente en 50.000 pasos y un criterio de convergencia de 0,001 $kcal{\cdot}mol^{-1}{\cdot}Å^{-1}$, también se añadieron cargas parciales y átomos de hidrógeno polares a pH 7,4, para ello se utilizó el programa Avogadro [17].

El cribado virtual se llevó a cabo empleando los programas AutoDock Vina V.01 [18] y PyRx [19], evaluando la afinidad de unión de los fitoconstituyentes de *M. oleifera* con los sitios activos de la tripanotiona reductasa (sitio catalítico y dominio de unión FAD-NADPH) para ello se utilizó una caja de interacción de 96×95×70 $Å^3$, y como controles se emplearon las moléculas de tripanotiona y de NADPH [20].

Aquellos compuestos que demostraron valores de energía libre de unión menor o igual a los obtenidos con los controles fueron seleccionados para llevar a cabo ensayos exhaustivos (exhaustividad = 16) de acoplamiento molecular, los cuales se llevaron a cabo en diez repeticiones de cada simulación, para ello se utilizó el programa AutoDock Vina V.01 [18] y cajas de interacción de dimensiones 15,1×15,2×15,2 $Å^3$ y 31,7×26,6×41,2 $Å^3$ para el sitio catalítico y el dominio de unión FAD-NADPH, respectivamente. Los cálculos de energía libre de unión (ΔG) se llevaron a cabo teniendo en cuenta variables de contribuciones energéticas en términos estéricos gaussianos, aportes energéticos de repulsiones, hidrofóbicos, puentes de hidrógenos y el número de enlaces rotables del complejo [18].

El análisis y visualización de los resultados se realizaron empleando los programas Chimera 1.15 [21], Chimera X [22] y PoseView [23, 24]. La validación de la simulación se llevó a cabo con el re-acoplamiento de los ligandos co-cristalizados (tripanotiona y NADPH) mediante la medición del RMSD de los ligandos.

Predicciones de biodisponibilidad, absorción, distribución, metabolismo, excreción y toxicidad (ADMET)

Las predicciones de las propiedades farmacocinéticas y tóxicas (ADMET) de los compuestos de *M. oleifera*, así como la biodisponibilidad mediante la regla modificada de Lipinski (peso molecular<500 g·mol^{-1}, número de aceptores de puentes de hidrógeno<10, número donadores de puentes de hidrógeno<5), coeficiente de reparto agua:octanol de Moriguchi<4,15 [25], estos análisis predictivos fueron llevados a cabo mediante el uso de la herramienta SwissADME [26-28] y ProTox-II [29].

Análisis de datos

Los datos de energía libre de Gibbs de interacción que fueron obtenidos en las simulaciones de acoplamiento molecular fueron analizados mediante el test de Kruskal-Wallis [30] y el test *post-hoc* de Dunn [31], todos los análisis se realizaron con 5% de nivel de significancia. Las pruebas y gráficos estadísticos se llevaron a cabo con el programa Past v. 4.12 [32].

Resultados y discusión

Los resultados obtenidos en el cribado virtual llevados a cabo entre las estructuras de los fitoconstituyentes de *M. oleifera* y los sitios activos de la tripanotiona reductasa se pueden observar en la Tabla 1.

Estos compuestos fueron seleccionados y sometidos a simulaciones exhaustivas para identificar a las moléculas que presentan valores de energía de interacción significativamente favorables en comparación a los controles empleados (Figura 2). Los controles empleados en este estudio fueron la tripanotiona y la molécula de NADP (Nicotinamida Adenina Dinucleótido Fosfato), los mismos son sustratos naturales de la enzima [20]. La tripanotiona se localiza en el sitio catalítico y es una análoga de la molécula de glutatión, la cual protege al parásito del estrés oxidativo generado por el hospedero mediante reacciones redox mediadas por la tripanotiona reductasa y con la participación de la NADP/NADPH, la cual se encuentra en el dominio de unión a NADP-FAD [20].

Las simulaciones de acoplamiento molecular que fueron llevados a cabo entre los fitoconstituyentes seleccionados en unión con el sitio catalítico reveló que los compuestos que demostraron valores significativamente favorables (P<0,001) de energía de unión con el sitio catalítico de la enzima fueron la apigenina, la quercetina, la miricetina, la luteolina, el kaempferol y la isorhamnetina, en comparación a lo obtenido con la tripanotiona (Figura 1A).

El complejo formado entre la apigenina y la tripanotiona reductasa en el sitio catalítico reveló una energía de unión promedio de -8,8±1,87×10^{-15} kcal·mol^{-1}. Las interacciones presentes entre el compuesto y los aminoácidos, entre estos se identificaron la formación de puentes de hidrógeno y los residuos de Thr335, Ala365, Lys60 y Ser178, también se registró interacciones de Van der Waals con los residuos Cys57 y Gly56. El complejo quercetina-tripanotiona reductasa demostró una energía libre de interacción de -8,77 ± 0,095 kcal·mol^{-1}. En el mismo se identificaron puentes de hidrógeno con los residuos Met333, His461 y Ala365, e interacciones de Van der Waals con el residuo Leu334 (Figura 2.A,B).

Tabla 1. Resultados obtenidos en el cribado virtual de los fitoconstituyentes de *M. oleifera* y la tripanotiona reductasa de *L. infantum.*

Compuestos	PubChem CID	ΔG de union (kcal·mol^{-1})	
		Sitio catalítico	Dominio NADPH-FAD
Tripanotiona*	-	-6,6	-
NADPH*	-	-	-10,6
Ácido 5,6,7,8-tetrahidrofólico	135444742	-6,3	-10,8
Ácido 5-metil-5,6,7,8-tetrahidrófólico	135415868	-5,8	-10,3
Ácido 10-formilfólico	135405023	-7,7	-10,6
Ácido a-linolénico	5280934	-5,6	-6,8
Apigenina	5280443	-8,8	-8,8
Bencilamina	7504	-4,7	-5,0
Ftalato de bis(2-etilhexilo)	8343	-7,0	-7,4
Ácido cafeico	689043	-6,1	-7,1
Ácido clorogénico	1794427	-8,2	-9,0
Ácido cumarínico	10752	-7,2	-7,4
Daidzeína	5281708	-8,3	-8,3
D-alose	439507	-5,4	-6,1
Epicatequina	72276	-8,2	-8,2
Eugenol	3314	-5,2	-6,3
Ácido ferúlico	445858	-5,9	-7,1
Ácido gálico	370	-5,7	-6,6
Genisteína	5280961	-8,4	-8,4
Hexadecanal	984	-5,1	-5,0

(Continúa)

Compuestos	PubChem CID	ΔG de union (kcal·mol^{-1})	
		Sitio catalítico	Dominio NADPH-FAD
Isorhamnetina	5281654	-8,6	-9,2
Kaempferol	5280863	-8,6	-9,0
l-galactosa, 6-desoxi	3034656	-5,0	-5,5
Ácido linoleico	5280450	-6,0	-5,7
Luteolina	5280445	-8,9	-9,0
Miricetina	5281672	-8,9	-8,6
Ácido palmítico	985	-5,5	-6,1
Quercetina	5280343	-8,5	-9,3
Rutina	5280805	-3,7	-10,9
Ácido sinápico	637775	-6,3	-7,3
Ácido siríngico	10742	-5,9	-7,0
Vanilina	1183	-5,6	-6,0

*Control

En el complejo formado entre la miricetina y la tripanotiona reductasa evidenció valores promedio de energía de unión de -8,84 ± 0,13 kcal·mol^{-1}, también se registró la formación de puentes de hidrógeno con los residuos His461, Lys60, Ser14, Ser 162 y Asp327, e interacciones de Van der Waals con los residuos Thr335 y Cys57. Así también se el complejo isorhamnetina-tripanotiona reductasa reveló un promedio de energía de unión de -8,6±1,87×10^{-15} kcal·mol^{-1}, donde se registraron puentes de hidrógeno con los residuos Ser162, Ser14 y Lys60, e interacciones de Van der Waals con el residuo Cys57 (Figura 2.C,D).

Así también, entre los fitoconstituyentes que demostraron afinidades de interacción con el dominio de unión FAD-NADPH en el cribado virtual, fueron solo el ácido 5,6,7,8-tetrahidrofólico y la rutina los compuestos que presentaron valores de energía significativamente favorables (P<0,05) en comparación a lo detectado con el control (NADPH) (Figura 1.B).

En el complejo formado por el ácido 5,6,7,8-tetrahidrofólico y el dominio de unión FAD-NADPH de la tripanotiona reductasa se registró valores de energía de interacción de -10.65 ± 0.23 kcal·mol^{-1}, en el mismo se registraron puentes de hidrógeno con los residuos Ser14, Thr51, Cys52, Gly16, Gly15, Asp35 y Gly127, interacciones hidrofóbicas con el residuo Phe126 y uniones de Van der Waals con el residuo Thr160. El complejo rutina-tripanotiona reductasa reveló valores promedios de energía de unión

de -10,78 ± 0,063 kcal·mol^{-1}, en el mismo se identificaron puentes de hidrógeno con los residuos Ser178, Val55, Lys60 y Ala365, uniones hidrofóbicas con el residuo Tyr198 e interacciones de Van der Waals con los residuos Leu334, Tyr198 y Ile199 (Figura 3.A,B).

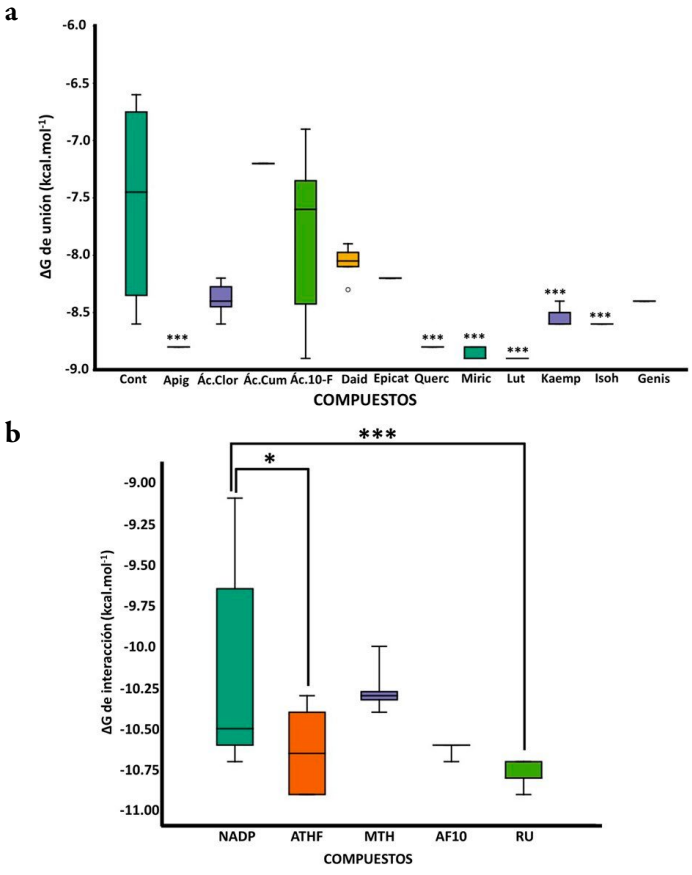

Figura 1. Análisis estadísticos de las pruebas de acoplamiento molecular entre fitoconstituyentes seleccionados de *M. oleifera* y la tripanotiona reductasa. **A.** Fitoconstituyentes en interacción con el sitio catalítico de la tripanotiona reductasa. **B.** Fitoconstituyentes en interacción con el sitio de unión FAD-NADPH de la tripanotiona reductasa. Cont: Tripanotiona, Apig: apigenina. Ác. Clor: ácido clorogenico Ác. Cum: ácido cumarinico Ác.10-F: ácido 10-formilico, Daid: daidzeina, Epicat: epicatechina, Querc: quercetina, Miric: miricetina, Lut: luteolina, Kaemp: kaempferol, Isoh: isohramnetina, Genis: genisteina, NADP: nicotinamida adenina dinucleótido fosfato, ATHF: ácido 5,6,7,8-tetrahidrofólico, MTH: ácido 5-metil-5,6,7,8-tetrahidrofólico, AF10: Ácido 10-formilfólico, Ru: rutina. * P<0,05, *** P<0,001.

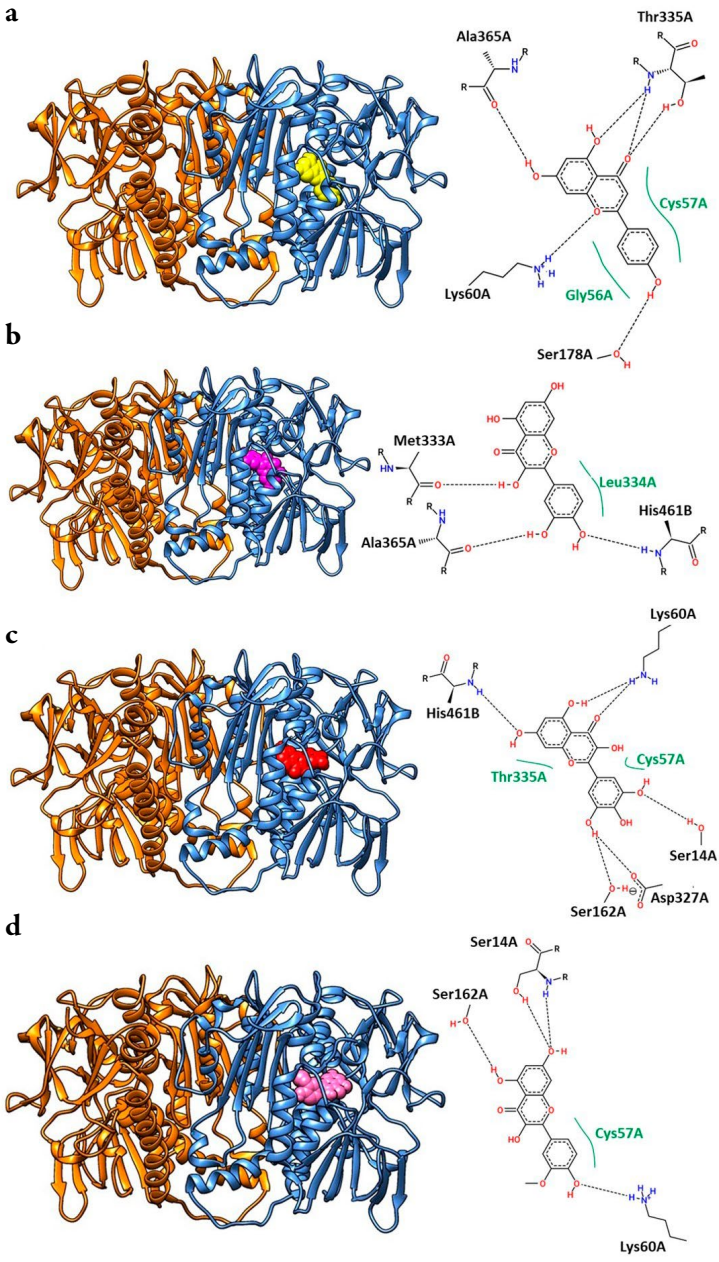

Figura 2. Representaciones tridimensionales y bidimensionales de los compuestos con el sitio catalítico de la tripanotiona reductasa. **A.** Apigenina. **B.** Quercetina. **C.** Miricetina. **D.** Isorhamnetina. Línea punteada negra: puente de hidrógeno, línea punteada verde: interacciones hidrofóbicas, línea continua verde: interacciones de Van der Waals.

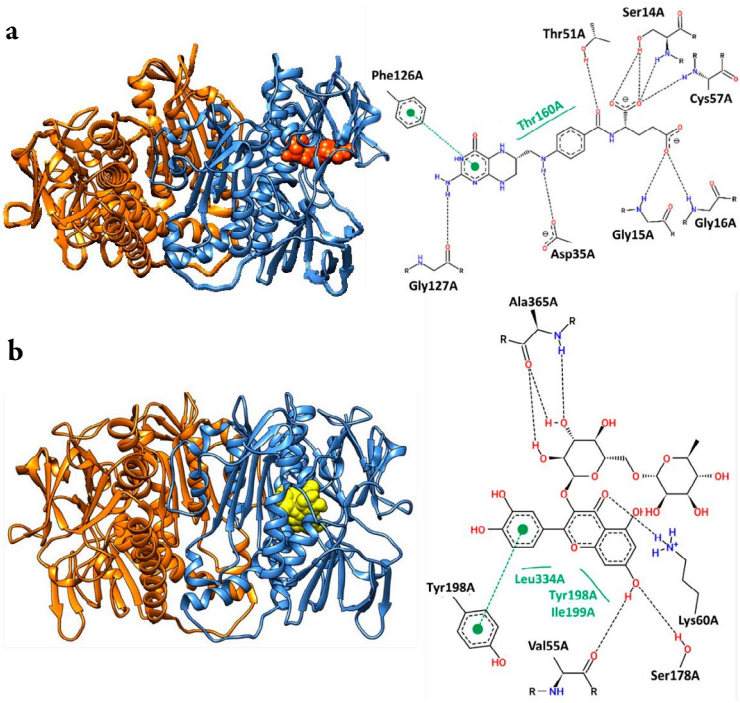

Figura 3. Representaciones tridimensionales y bidimensionales de los compuestos en unión al dominio FAD-NADPH de la tripanotiona reductasa. **A.** Ácido 5,6,7,8-tetrahidrofólico. **B.** Rutina. Línea punteada negra: puente de hidrógeno, línea punteada verde: interacciones hidrofóbicas, línea continua verde: interacciones de Van der Waals.

Las validaciones de re-docking del sistema empleado evidenciaron valores de RMSD de 0,82 Å en interacción entre la enzima y la tripanotiona.

Las predicciones de biodisponibilidad de los compuestos seleccionados, según la regla modificada de los cinco de Lipinski, revelaron que el ácido 5,6,7,8-tetrahidrofólico y la rutina infringen con dicha regla, en ambas moléculas se observa un número mayor de grupos donadores de puentes de hidrógeno recomendados por la regla (<5), sin embargo, la rutina también presenta otras infracciones como un peso molecular mayor a lo indicado en la regla (<500 g·mol^{-1}), y un número mayor de grupos aceptores de puentes de hidrogeno (<10), esta abundancia en grupos donadores y aceptores de puentes de hidrogeno se debe principalmente a la abundancia de grupos hidroxilos (-OH), aminos (-NH, -NH_2), y de átomos de oxígeno (O) en la estructura molecular del compuesto [33]. La miricetina presenta una infracción en la cantidad de grupos

donadores de puentes de hidrógeno, el cual también resultó ser mayor a lo establecido en la regla empleada (<5), debido a la presencia de un grupo hidroxilo (-OH) demás en la molécula [34].

En cuanto a la quercetina, la apigenina y la isorhamnetina no demostraron infracciones a la regla resultando favorable para su biodisponibilidad en el organismo (Tabla 2).

A su vez, la evaluación de las propiedades farmacocinéticas (ADME) de los compuestos evaluados demostró que tanto el ácido 5,6,7,8-tetrahidrofólico, la miricetina y la rutina presentaron una baja absorción gastrointestinal, lo cual representa una barrera para la eficiencia del consumo oral de estas moléculas con fines farmacológicos, de entre los cuales solo la miricetina demostró capacidades de ser inhibidoras de las isoformas del citocromo P450 CYP1A2 y CYP3A4, lo cual nos indica que este compuesto podría interactuar con ambas proteínas, en cuanto a sus actividades tóxicas la miricetina demostró probabilidades de presentar actividades mutagénicas y carcinogénicas, lo cual fue reportado por Hobbs *et al.* en el año 2015 [35] quienes reportaron actividades mutagénicas de la miricetina empleando el test de AMES (Tabla 3).

Por lo demás, las moléculas de quercetina, la apigenina y la isorhamnetina presenta alta absorción gastrointestinal, sin embargo, presentan probabilidades de ser inhibidores de las isoformas CYP1A2, CYP2D6 y CYP3A4 del citocromo P450, siendo solo la molécula de quercetina la que demuestra probabilidades de ser mutagénica y carcinogénica, lo cual fue anteriormente descrito por Vrijsen *et al.* en el año 1990 [36] quienes reportaron actividades mutagénicas *in vitro* de la molécula de quercetina (Tabla 3).

A su vez, las predicciones de actividades inhibitorias de isoformas de citocromo P450 (CYP) nos da un indicio de que los compuestos capaces de inhibir alguna de ellas podrían interferir con las funciones enzimáticas de estas proteínas alterando el metabolismo de otros fármacos/compuestos, dando paso a la acumulación de xenobióticos en el organismo pudiendo generarse efectos adversos [37].

El análisis de los complejos formados entre los compuestos evaluados de *M. oleifera* y la estructura de la tripanotiona reductasa de *L. infantum*, reveló afinidades de interacción tanto por el sitio catalítico y por el dominio de unión FAD-NAPH.

En los complejos se registraron interacciones moleculares importantes, uno de ellos fueron los puentes de hidrógeno, con un aporte energético en la formación de los complejos de entre 0,25 kcal·mol^{-1} a 40 kcal·mol^{-1}, estas son interacciones electrostáticas entre un átomo donante de hidrógeno y un átomo aceptor de hidrógeno en una molécula vecina. Estas interacciones son importantes para la estabilización de la conformación de la proteína y para la formación de complejos proteicos, impactando en gran

medida sobre la acción farmacológica de ligandos [38-40]. Esta interacción se da principalmente entre grupos hidroxilos (-OH), aminos (-NH, -NH_2) y átomos de oxígeno (O) presentes principalmente en los fitoconstituyentes evaluados, así como también presentes en los residuos activos involucrados en la formación en la formación de los puentes de hidrógeno [33, 34, 41-43].

Otra interacción importante registrada fueron las interacciones hidrofóbicas en las moléculas de ácido 5,6,7,8-tetrahidrofólico y rutina, los cuales contienen grupos aromáticos hidrofóbicos que permiten interactuar con los residuos de igual propiedad fisicoquímica, como la fenilalanina (Phe) y la tirosina (Tyr), los cuales presentan también en sus cadenas laterales grupos aromáticos, estas interacciones son débiles con aportes de entre 1,5 a 2 kcal·mol^{-1}, sin embargo, son importantes para la unión específica de la proteína y el ligando y para la estabilización de la proteína [44].

A su vez, las fuerzas de Van der Waals son interacciones débiles entre moléculas que surgen debido a las fluctuaciones en las cargas eléctricas de estas. Estas interacciones son importantes para la estabilización de la estructura de la proteína y para la formación de complejos proteicos [45].

Tabla 2. Evaluación de la regla de los cinco modificada de Lipinski de los compuestos evaluados.

Compuesto	Peso Molecular	Donadores de PH	Aceptores de PH	MlogP	Número de infracciones
Kaempferol	270,24	3	5	0,52	0
l-galactosa, 6-desoxi	164,16	4	5	-2,1	0
Ftalato de bis(2-etilhexilo)	390,56	0	4	5,24	1
Hexadecanal	240,42	0	1	4,31	1
Ácido 5-metil-5,6,7,8-tetrahidrofólico	459,46	7	7	-0,48	1
Ácido 5,6,7,8-tetrahidrofólico	445,43	8	7	-1,12	1
Ácido 10-formilfólico	469,41	5	10	-0,39	0
Quercetina	302,24	5	7	-0,56	0
Apigenina	270,24	3	5	0,52	0
Isorhamnetina	316,26	4	7	-0,31	0
Ácido a-linolénico	278,43	1	2	4,38	1
Ácido linoleico	280,45	1	2	4,47	1
Ácido palmítico	256,42	1	2	4,19	1
Ácido clorogénico	354,31	6	9	-1,05	1

(Continúa)

Compuesto	Peso Molecular	Donadores de PH	Aceptores de PH	MlogP	Número de infracciones
Miricetina	318,24	6	8	-1,08	1
Bencilamina	107,15	1	1	1,54	0
Rutina	610,52	10	16	-3,89	3
Ácido gálico	170,12	4	5	-0,16	0
Ácido cumarínico	190,15	1	4	1,22	0
Ácido cafeico	180,16	3	4	0,7	0
Ácido siríngico	198,17	2	5	0,49	0
Luteolina	286,24	4	6	-0,03	0
Genisteína	270,24	3	5	0,52	0
Daidzeína	254,24	2	4	1,08	0
D-Alose	180,16	5	6	-2,75	0
Epicatequina	290,27	5	6	0,24	0
Ácido ferúlico	194,18	2	4	1	0
Ácido sinápico	224,21	2	5	0,73	0
Vanilina	152,15	1	3	0,51	0
Eugenol	164,2	1	2	2,01	0

PH: puente de hidrogeno; MlogP: coeficiente de reparto octanol-agua de Moriguchi.

En el sitio catalítico unos de los residuos activos identificados fueron la Cys57 y la His461, estos residuos cumplen un papel fundamental en la transferencia de electrones desde NADPH hasta la tripanotiona [46]. La Cys57 forma parte del puente disulfuro Cys52-Cys57 que se reduce durante la catálisis, mientras que His461 actúa como un ácido/base en el proceso de transferencia de protones que acompaña la transferencia de electrones [47].

Con respecto al dominio de unión FAD-NADPH, los residuos activos importantes fueron Gly11, Gly13, Ser14, Gly15, Va134, Asp35, Val36, Ala46, Ala47, Gly50, Thr51, Cys52, Lys60, Phe126, Gly127, Ala159, Thr168, Gly161, Gly196, Gly197, Tyr198, Ile199, Glu282, Tyr221, Arg222, Arg228, Asn254, Ala284, Ile285, Gly286, Arg287, Arg290, Leu294, Gly326, Asp327, Met333, Leu334, Thr335, Pro336, A1-365, Phe367.

Fonseca *et al.* (2011) [48] estudiaron la actividad leishmanicida de la quercetina y describió su actividad frente a promastigotes de *Leishmania amazonensis*, ya que este bioactivo tiene una amplia gama de efectos biológicos informados que incluyen actividades antioxidantes, antihipertensivas, antiinflamatorias, antimicrobianas y antiprotozoarias.

Así también Mercado-Camargo *et al.* (2020) [49], mencionaron que los flavonoides poseen actividad leishmanicida, entre ellos, a una concentración de 45 μM, la quercetina mostró una reducción del 70% en la carga intracelular.

Por otro lado, Das *et al.* (2013) [50] demostraron la actividad antileishmania de la quercetina en nanopartículas de oro conjugadas con el compuesto, los cuales fueron expuestas a macrófagos infectado con *L. donovani*, obteniendo una IC_{50} de 15±3 μM. La concentración inhibitoria para el aglicón de quercetina en amastigote de *L. donovani* fue de (IC_{50} 30±6 μM) [50]. Sousa *et al.* (2017) [51] evaluaron la eficacia oral mejorada de la quercetina en la leishmaniasis cutánea después de la encapsulación en nanocápsulas de núcleo lipídico a ratones infectados con *Leishmania amazonensis*, el tratamiento redujo el tamaño de las lesiones y la carga parasitaria en un 38% y un 71%, respectivamente.

En una actualización de las actividades leishmanicidas de hierbas medicinales y fitoconstituyentes *in vitro* e *in vivo* realizado por Hassan *et al.* (2022) [52] mencionaron que la apigenina podría ser un compuesto prometedor para el desarrollo de nuevos fármacos contra la leishmaniasis.

Así también, Chandrasekar *et al.* (2018) [53] plantearon la acción de este flavonoide para luchar contra la *L. infantum* y *L. amazonensis*. Otros reportes señalan también que la isorhamnetina demuestra potencial antiprotozoario contra *L. donovani* (IC_{50} 1,20 μM) [54].

De igual manera, Tasdemir *et al.* (2006) [55] validaron a la quercetina como agente leishmanicida contra amastigotes *L. donovani* en forma *in vitro* con un IC_{50} de 1,0 $\mu g \cdot mL^{-1}$ y descubrió también que la miricetina puede tener la misma actividad antiprotozoaria con un valor de (IC_{50} de 1,3 $\mu g \cdot mL^{-1}$), esto también fue mencionado por Neto *et al.* (2022) [56] y Vidal (2019) [57]. Ensayos llevados a cabo por Vila-Nova *et al.* (2012) [58] con flavonoides (quercetina y rutina) presentaron resultados en contra *L. infantum* comparables al fármaco de control positivo que fue la anfotericina B, frente a las formas amastigotes con EC_{50} para quercetina y rutina de 10,6 $\mu g.mL^{-1}$ y 43,3 $\mu g \cdot mL^{-1}$, respectivamente.

Tabla 3. Predicciones de las propiedades farmacocinéticas y toxicas de los compuestos evaluados (ADME-Tox).

Compuesto	Absorción GI	Inhibición					Toxicidad			
		CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	MU	CA	CI	HE
Kaempferol	Alto	Si	No	No	Si	Si	I	I	I	I
l-galactosa, 6-desoxi	Bajo	No	No	No	No	No	I	I	I	I
Ftalato de bis(2-etilhexilo)	Alto	No	No	Si	No	Si	I	A	I	I
Hexadecanal	Alto	Si	No	No	No	No	I	I	I	I
Ácido 5-metil-5,6,7,8-tetrahidrofólico	Bajo	No	No	No	No	No	I	I	I	I
Ácido 5,6,7,8-tetrahidrofólico	Bajo	No	No	No	No	No	I	I	I	I
Ácido 10-formilfólico	Bajo	No	No	No	No	No	I	I	I	I
Quercetina	Alto	Si	No	No	Si	Si	A	A	I	I
Apigenina	Alto	Si	No	No	Si	Si	I	I	I	I
Isorhamnetina	Alto	Si	No	No	Si	Si	I	I	I	I
Ácido a-linolénico	Alto	Si	No	Si	No	No	I	I	I	I
Ácido linoleico	Alto	Si	No	Si	No	No	I	I	I	I
Ácido palmítico	Alto	Si	No	Si	No	No	I	I	I	I
Ácido clorogénico	Bajo	No	No	No	No	No	I	I	I	I
Miricetina	Bajo	Si	No	No	No	Si	A	A	I	I

(Continúa)

Compuesto	Absorción GI	Inhibición					Toxicidad			
		CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	MU	CA	CI	HE
Bencilamina	Alto	Si	No	No	No	No	I	A	I	I
Rutina	Bajo	No	No	No	No	No	I	I	I	I
Ácido gálico	Alto	No	No	No	No	Si	I	A	I	I
Ácido cumarínico	Alto	Si	No	No	No	No	I	A	I	I
Ácido cafeico	Alto	No	No	No	No	No	I	A	I	I
Ácido siríngico	Alto	No	No	No	No	No	I	I	I	I
Luteolina	Alto	Si	No	No	Si	Si	A	A	I	I
Genisteína	Alto	Si	No	No	Si	Si	I	I	I	I
Daidzeína	Alto	Si	No	No	Si	Si	I	I	I	I
D-Alose	Bajo	No	No	No	No	No	I	I	I	I
Epicatequina	Alto	No	No	No	No	No	I	I	I	I
Ácido ferúlico	Alto	No	No	No	No	No	I	I	I	I
Ácido sinápico	Alto	No	No	No	No	No	I	I	I	I
Vanilina	Alto	No	No	No	No	No	I	I	I	I
Eugenol	Alto	Si	No	No	No	No	I	I	I	I

GI: Gastrointestinal; A: Activo; I: Inactivo.

Conclusiones

Los hallazgos *in silico* encontrados en este estudio evidenciaron que los compuestos descritos en la *M. oleifera* que presentaron potenciales afinidades de interacción con la tripanotiona reductasa de *L. infantum* y con valores de energía libre de unión significativamente favorables fueron la apigenina, la quercetina, la miricetina y la isorhamnetina en el sitio catalítico de la enzima, y las moléculas de rutina y de ácido 5,6,7,8-tetrahidrofólico en el dominio FAD-NADPH. Los resultados de biodisponibilidad y ADME-Tox demuestran infracciones, sin embargo, pueden ser considerados candidatos para llevar a cabo pruebas *in vitro* sobre las actividades enzimáticas de la tripanotiona reductasa de *L. infantum*, así como continuar llevando a cabo estudios *in silico* para el diseño de nuevos derivados de estos compuestos con la finalidad de optimizar a los compuestos mediante herramientas QSAR (relación cuantitativa estructura-actividad).

Contribuciones de los autores

SF: Diseño del estudio, marco metodológico, pruebas computacionales, análisis de resultados, escritura de artículo. EG: Diseño del estudio, marco metodológico, análisis de resultados, escritura de artículo.

Conflicto de intereses

Los autores manifiestan no poseer conflictos de intereses.

Referencias

1. República de Paraguay, Ministerio de Salud Pública y Bienestar Social, *Reporte de Leishmaniasis Visceral en humanos y caninos, Paraguay, período 2016 al 2018*, DGVS, Asunción, paraguay, 2018. URL: https://dgvs.mspbs.gov.py/files/boletines_anuales/15_2018_BoletinAnual.pdf

2. L. Turcano, E. Torrente, A. Missineo, M. Andreini, M. Gramiccia, T. Di Muccio, I. Genovese, A. Fiorillo, S. Harper, A. Bresciani, G. Colotti, A. IlariI, Identification and binding mode of a novel Leishmania Trypanothione reductase inhibitor from high throughput screening, *PLoS Neglected Tropical Diseases*, **12**(11), e0006969 (2018). https://doi.org/10.1371/journal.pntd.0006969

3. P.R.T. Romão, J. Tovar, S.G. Fonseca, R. H. Moraes, A.K. Cruz, J.S. Hothersal, A.A. Noronha-Dutra, S.H. Ferreira, F.Q. Cunha, Glutathione and the redox control system trypanothione/trypanothione reductase are involved in the protection of Leishmania spp. against nitrosothiol-induced cytotoxicity, *Brazilian Journal of Medical and Biological Research*, **39**(3), 355–363 (2006). https://doi.org/10.1590/s0100-879x2006000300006

4. B.K. Singh, N. Sarkar, M.V. Jagannadham, V.K. Dubey, Modeled structure of trypanothione reductase of Leishmania infantum, *BMB Reports*, **41**(6), 444–447 (2008). https://doi.org/10.5483/bmbrep.2008.41.6.444

5. F. Saccoliti, G. Angiulli, G. Pupo, L. Pescatori, V.N. Madia, A. Messore, G. Colotti, A. Fiorillo, L. Scipione, M. Gramiccia, T. Di Muccio, R. Di Santo, R. Costi, A. Ilari, Inhibition of *Leishmania infantum* trypanothione reductase by diaryl sulfide derivatives, *Journal of Enzyme Inhibition and Medicinal Chemistry,* **32**(1), 304-310 (2017). https://doi.org/10.1080/14756366.2016.1250755

6. J.A. Gómez-Intriago, J. Cañarte-Alcivar, Leishmaniasis: un tema siempre de actualidad, *Revista Científica Arbitrada en Investigaciones de la Salud "GESTAR"*, **5**(10), 2–21 (2022). URL: https://journalgestar.org/index.php/gestar/article/view/75/134

7. A. Kaur, P.K. Kaur, S. Singh, I. P. Singh, Antileishmanial compounds from *Moringa oleifera* Lam., *Zeitschrift für Naturforschung C*, **69**(3-4), 110–116 (2014). https://doi.org/10.5560/znc.2013-0159

8. M.K. Singh, J. Paul, T. De, T. Chakraborti, Bioactivity guided fractionation of *Moringa oleifera* Kam. flower targeting *Leishmania donovani*, *Indian Journal of Experimental Biology*, **53**, 747–752 (2015). URL: https://nopr.niscpr.res.in/bitstream/123456789/33101/1/IJEB%2053%2811%29%20747-752.pdf

9. F.A. Espinosa-Méndez, *Leishmanicidal activity of ishpingo and moringa plants*, Tesis de Maestría en Microbiología, Universidad San Francisco de Quito (USFQ), Quito, Ecuador, 2019. URL: https://repositorio.usfq.edu.ec/bitstream/23000/9185/1/140970.pdf

10. I.A. Raubilu, U. Isah, M.A. Ahmad, Antimicrobial activity of *Moringa oleifera*: A short review, *Bayero Journal of Pure and Applied Sciences*, **12**(1), 128–132 (2019). https://doi.org/10.4314/bajopas.v12i1.21S

11. K.A. Hammi, R. Essid, O. Tabbene, S. Elkahoui, H. Majdoub, R. Ksouri, Anti-leishmanial activity of *Moringa oleifera* leaf extracts and potential synergy with amphotericin B, *South African Journal of Botany*, **129**, 67–73 (2019). https://doi.org/10.1016/j.sajb.2019.01.008

12. A.R. Nur-Zahirah, H. Khairana, K. Endang, Moringa genus: A review of phytochemistry and pharmacology, *Frontiers in Pharmacology*, **9**, 108 (2018). https://doi.org/10.3389/fphar.2018.00108

13. R.K. Saini, I. Sivanesan, Y.S. Keum, Phytochemicals of *Moringa oleifera*: A review of their nutritional, therapeutic and industrial significance, *3 Biotech*, **6**(2), 203 (2016). https://doi.org/10.1007/s13205-016-0526-3

14. S.J. Stohs, M.J. Hartman, Review of the safety and efficacy of *Moringa oleifera*, *Phytotherapy Research*, **29**(6), 796–804 (2015). https://doi.org/10.1002/ptr.5325

15. S. Kim, J. Chen, T. Chen, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P.A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E.E. Bolton, PubChem in 2021: New data content and improved web interfaces, *Nucleic Acids Res.*, **49**(D1), D1388–D1395 (2021). https://doi.org/10.1093/nar/gkaa971

16. P. Baiocco, G. Poce, S. Alfonso, M. Cocozza, G.C. Porretta, G. Colotti, M. Biava, F. Moraca, B. Botta, V. Yardley, A. Fiorillo, A. Lantella, F. Malatesta, A. Ilari, Inhibition of *Leishmania infantum* trypanothione reductase by azole-based compounds: A comparative analysis with its physiological substrate by x-ray crystallography, *ChemMedChem*, **8**(7), 1175–1183 (2013). https://doi.org/10.1002/cmdc.201300176

17. M.D. Hanwell, D.E. Curtis, D.C. Lonie, T. Vandermeersch, E. Zurek, G.R. Hutchison, Avogadro: An advanced semantic chemical editor, visualization, and analysis platform, *Journal of Cheminformatics*, **4**(1), 17 (2012). https://doi.org/10.1186/1758-2946-4-17

18. O. Trott, A.J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *Journal of Computational Chemistry*, **31**(2), 455-461 (2010). https://doi.org/10.1002/jcc.21334

19. S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, *Chemical Biology: Methods and Protocols*, **1263**, 243–250 (2015). https://doi.org/10.1007/978-1-4939-2269-7_19

20. E. Gayozo, L. Rojas, Análisis estructural de interacciones *in silico* de fitoconstituyentes de *Solanum americanum* Mill., *Solanum guaraniticum* A. St.-Hil. y *Solanum lycopersicum* L. con la proteína tripanotiona reductasa de *Leishmania infantum*, *Steviana*, **12**(2), 31–54 (2022). https://doi.org/10.56152/Steviana-FacenV12N2A2_2020

21. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera—A visualization system for exploratory research and analysis, *Journal of Computational Chemistry*, **25**(13), 1605–1612 (2004). https://doi.org/10.1002/jcc.20084

22. E.F. Pettersen, T.D. Goddard, C.C. Huang, E.C. Meng, G.S. Couch, T.I. Croll, T.E. Ferrin, UCSF ChimeraX: Structure visualization for researchers, educators, and developers, *Protein Science*, **30**(1), 70–82 (2021). https://doi.org/10.1002/pro.3943

23. P.C. Fricker, M. Gastreich, M. Rarey, Automated generation of structural molecular formulas under constraints, *Journal of Chemical Information and Computer Sciences*, **44**(3), 1065–1078 (2004). https://doi.org/10.1021/ci049958u

24. K. Stierand, P. Maaß, M. Rarey, Molecular complexes at a glance: Automated generation of two-dimensional complex diagrams, *Bioinformatics*, **22**(14), 1710–1716 (2006). https://doi.org/10.1093/bioinformatics/btl150

25. C.A. Lipinski, F. Lombardo, B.W Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development, *Advanced Drug Delivery Reviews*, **46**(1-3), 3–26 (2001). https://doi.org/10.1016/S0169-409X(00)00129-0

26. A. Daina, O. Michielin, V. Zoete, iLOGP: A simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach, *Journal of Chemical Information and Modeling*, **54**(12), 3284-3301 (2014). https://doi.org/10.1021/ci500467k

27. A. Daina, V. Zoete, A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules, *ChemMedChem*, **11**(11), 1117-1121 (2016). https://doi.org/10.1002/cmdc.201600182

28. N. Daina, A. Sivakumar, J.W. Polak, Electric vehicle charging choices: Modelling and implications for smart charging services, *Transportation Research Part C: Emerging Technologies*, **81**, 36–56 (2017). https://doi.org/10.1016/j.trc.2017.05.006

29. P. Banerjee, A.O. Eckert, A.K. Schrey, R. Preissner, ProTox-II: A webserver for the prediction of toxicity of chemicals, *Nucleic Acids Research*, **46**(W1), W257–W263 (2018). https://doi.org/10.1093/nar/gky318

30. W.H. Kruskal, W.A Wallis, Use of ranks in one-criterion variance analysis, *Journal of the American Statistical Association*, **47**(260), 583–621 (1952). https://doi.org/10.1080/01621459.1952.10483441

31. O.J. Dunn, Multiple comparisons using rank sums, *Technometrics*, **6**(3), 241–252 (1964). https://doi.org/10.1080/00401706.1964.10490181

32. Ø. Hammer, D.A. Harper, P.D. Ryan, PAST: Paleontological statistics software package for education and data analysis, *Palaeontologia electronica*, **4**(1), 9 (2001).

33. P. Matteini, A. Goti, G. Agati, Theoretical conformational analysis of rutin, *Monatshefte für Chemie-Chemical Monthly*, **141**, 793–800 (2010). https://doi.org/10.1007/s00706-010-0330-4

34. X. Song, L. Tan, M. Wang, C. Ren, C. Guo, B. Yang, Y. Ren, Z. Cao, Y. Li, J. Pei, Myricetin: A review of the most recent research, *Biomedicine & Pharmacotherapy*, **134**, 111017 (2021). https://doi.org/10.1016/j.biopha.2020.111017

35. C.A. Hobbs, C. Swartz, R. Maronpot, J. Davis, L. Recio, M. Koyanagi, S.M. Hayashi, Genotoxicity evaluation of the flavonoid, myricitrin, and its aglycone, myricetin, *Food and Chemical Toxicology*, **83**, 283–292 (2015). https://doi.org/10.1016/j.fct.2015.06.016

36. R. Vrijsen, Y. Michotte, A. Boeyé, Metabolic activation of quercetin mutagenicity. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **232**(2), 243–248 (1990). https://doi.org/10.1016/0027-5107(90)90130-V

37. J.H. Lin, A. Y. Lu, Role of pharmacokinetics and metabolism in drug discovery and development, *Pharmacological Reviews*, **49**(4), 403–449 (1997).

38. P. Zhou, J. Huang, F. Tian, Specific noncovalent interactions at protein-ligand interface: Implications for rational drug design, *Current Medicinal Chemistry*, **19**(2), 226–238 (2012). https://doi.org/10.2174/092986712803414150

39. C.N. Pace, H. Fu, K.L. Fryar, J. Landua, S.R. Trevino, D. Schell, R.L. Thurlkill, S. Imura, J.M. Scholtz, K. Gajiwala, J. Sevcik, L. Urbanikova, J.K. Myers, K. Takano, E.J. Hebert, B.A. Shirley, G.R. Grimsley, Contribution of hydrogen bonds to protein stability, *Protein Science: A Publication of the Protein Society*, **23**(5), 652–661 (2014). https://doi.org/10.1002/pro.2449

40. A. Khaldan, S. Bouamrane, R. El-mernissi, H. Maghat, A. Sbai, M. Bouachrine, T. Lakhlifi, Molecular docking, ADMET prediction, and quantum computational on 2-methoxy benzoyl hydrazone compounds as potential antileishmanial inhibitors, *Biointerface Research in Applied Chemistry*, **13**(4), 302 (2022). https://doi.org/10.33263/BRIAC134.302

41. Y.Z. Zheng, Y. Zhou, Q. Liang, D.F. Chen, R. Guo, R.C. Lai, Hydrogen-bonding interactions between Apigenin and ethanol/water: a theoretical study, *Scientific Reports*, **6**(1), 34647 (2016). https://doi.org/10.1038/srep34647

42. M. Rossi, L.F. Rickles, W.A. Halpin, The crystal and molecular structure of quercetin: A biologically active and naturally occurring flavonoid, *Bioorganic Chemistry*, **14**(1), 55–69 (1986). https://doi.org/10.1016/0045-2068(86)90018-0

43. G. Gong, Y.Y. Guan, Z.L. Zhang, K. Rahman, S.J. Wang, S. Zhou, X. Luan, H. Zhang, Isorhamnetin: A review of pharmacological effects, *Biomedicine & Pharmacotherapy*, **128**, 110301 (2020). https://doi.org/10.1016/j.biopha.2020.110301

44. R. Patil, S. Das, A. Stanley, L. Yadav, A. Sudhakar, A.K. Varma, Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing, *PLoS One*, **5**(8), e12029 (2010). https://doi.org/10.1371/journal.pone.0012029

45. C. M. Roth, B.L. Neal, A.M. Lenhoff, Van der Waals interactions involving proteins, *Biophysical Journal*, **70**(2), 977–987 (1996). https://doi.org/10.1016/S0006-3495(96)79641-8

46. L. Turcano, E. Torrente, A. Missineo, M. Andreini, M, Gramiccia, T. Di Muccio, I. Genovese, A. Fiorillo, S. Harper, A. Bresciani, G. Colotti, A. IlariI, Identification and binding mode of a novel *Leishmania* trypanothione reductase inhibitor from high throughput screening, *PLoS Neglected Tropical Diseases*, **12**(11), e0006969 (2018). https://doi.org/10.1371/journal.pntd.0006969

47. T. Battista, G. Colotti, A. Ilari, A. Fiorillo, Targeting trypanothione reductase, a key enzyme in the redox trypanosomatid metabolism, to develop new drugs against leishmaniasis and trypanosomiases, *Molecules* (Basel), **25**(8), 1924 (2020). https://doi.org/10.3390/molecules25081924

48. F. Fonseca-Silva, J.D.F Inacio, M.M. Canto-Cavalheiro, E.E. Almeida-Amaral, Reactive oxygen species production and mitochondrial dysfunction contribute to quercetin induced death in *Leishmania amazonensis*, *PLoS One*, **6**(2), e14666 (2011). https://doi.org/10.1371/journal.pone.0014666

49. J. Mercado-Camargo, L. Cervantes-Ceballos, R. Vivas-Reyes, A. Pedretti, M.L. Serrano-García, H. Gómez-Estrada, Homology modeling of leishmanolysin (gp63) from *Leishmania panamensis* and molecular docking of flavonoids, *ACS Omega*, **5**(24), 14741–14749 (2020). https://doi.org/10.1021/acsomega.0c01584

50. S. Das, P. Roy, S. Mondal, T. Bera, A. Mukherjee, One pot synthesis of gold nanoparticles and application in chemotherapy of wild and resistant type visceral leishmaniasis, *Colloids and Surfaces B: Biointerfaces*, **107**, 27–34 (2013). https://doi.org/10.1016/j.colsurfb.2013.01.061

51. A. Sousa-Batista, F. Poletto, C. Philipon, S. Guterres, A. Pohlmann, B. Rossi-Bergmann, Lipid-core nanocapsules increase the oral efficacy of quercetin in cutaneous leishmaniasis, *Parasitology*, **144**(13), 1769–1774 (2017). https://doi.org/10.1017/S003118201700097X

52. A. A. Hassan, H.E. Khalid, A.H. Abdalla, M.M. Mukhtar, W.J. Osman, T. Efferth, Antileishmanial activities of medicinal herbs and phytochemicals *in vitro* and *in vivo*: An update for the years 2015 to 2021, *Molecules*, **27**(21), 7579 (2022). https://doi.org/10.3390/molecules27217579

53. R. Chandrasekar, D. Subasish, B. Sivagami, P. Jayasree, M. Niranjan Babu, Therapeutic efficacy of flavonoids and terpenoids an ongoing herbal therapy in the treatment of leishmaniasis, *Natural Products: An Indian Journal*, **14**(2), 124 (2018). URL: https://www.tsijournals.com/articles/therapeutic-efficacy-of-flavonoids-and-terpenoids-an-ongoing-herbal-therapy-in-the-treatment-of-leishmaniasis-13903.html

54. J.L. Baldim, B.G.V. de Alcântara, O.d.S. Domingos, M.G. Soares, I.S. Caldas, R.D. Novaes, T.B. Oliveira, J.H.G. Lago, D.A. Chagas-Paula, The correlation between chemical structures and antioxidant, prooxidant, and antitrypanosomatid properties of flavonoids, *Oxidative Medicine and Cellular Longevity*, **2017**, ID3789856 (2017). https://doi.org/10.1155/2017/3789856

55. D. Tasdemir, M. Kaiser, R. Brun, V. Yardley, T.J. Schmidt, F. Tosun, P. Rüedi, Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *In vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies, *Antimicrobial Agents and Chemotherapy*, **50**(4), 1352–1364 (2006). https://doi.org/10.1128/AAC.50.4.1352-1364.2006

56. P.T.P.P. Neto, T.R. Santos, C.J.M. Tellis, Desenvolvimento de novos derivados de plantas medicinais para doenças negligenciadas: uma análise bibliométrica, *Revista Fitos*, **16**(Supl. 2), 267–292 (2022). URL: https://www.arca.fiocruz.br/handle/icict/51650

57. M.G. Vidal-Sampaio, *Avaliação do perfil fitoquímico e antiparasitário dos óleos essenciais extraídos de espécies eugenia provenientes da Caatinga*, Tesis de Doctorado, Programa de Pós-Graduação em Ciências Biológicas, Universidade Federal de Pernambuco, Brasil, 2016. URL: https://repositorio.ufpe.br/handle/123456789/36898

58. N.S. Vila-Nova, S.M. Morais, M.J. Falcão, C.M. Bevilaqua, F. Rondon, M.E. Wilson, H.F. Andrade, Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds of *Dimorphandra gardneriana* and *Platymiscium floribundum*, native plants from Caatinga biome, *Pesquisa Veterinária Brasileira*, **32**(11), 1164–1168 (2012). https://doi.org/10.1590/S0100-736X2012001100015

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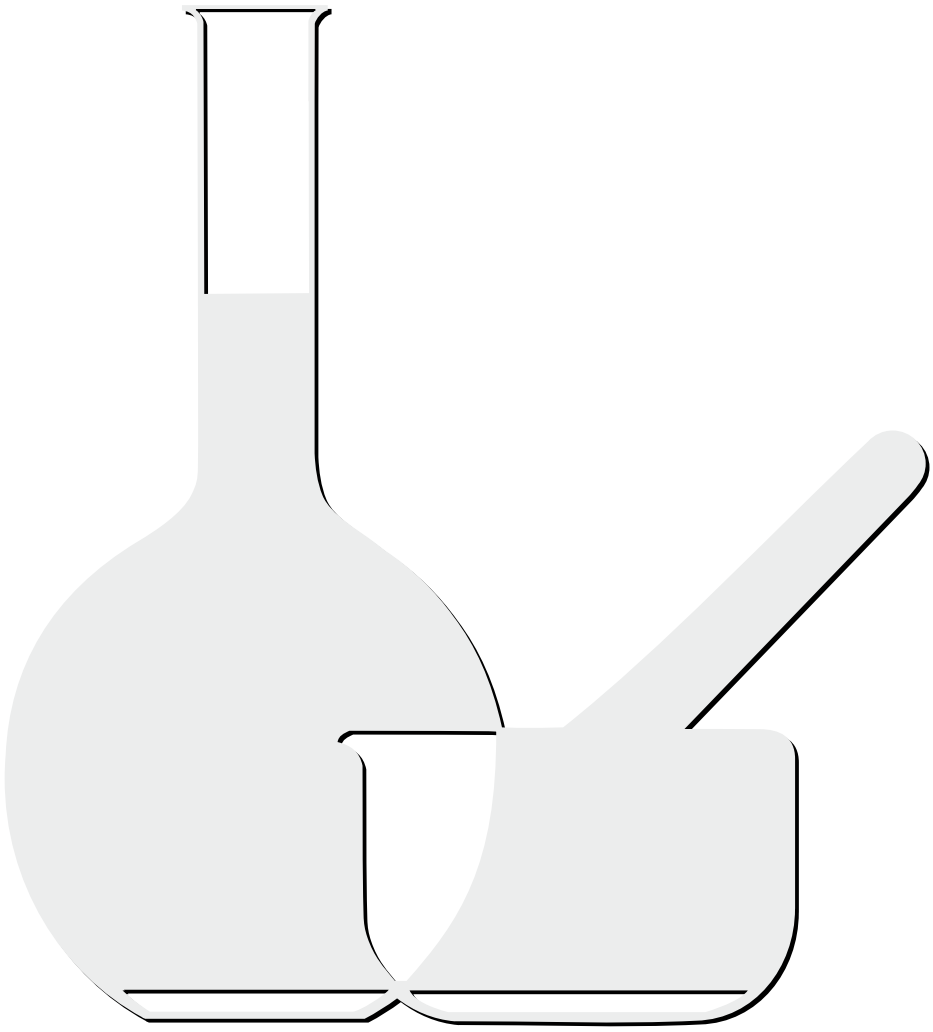

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Las abreviaturas de pesos y medidas serán las indicadas por la Farmacopea de los Estados Unidos en su edición oficial o unidades SI. Los datos espectroscópicos se deben presentar de la siguiente manera:

UV λ max (solvente ε) nm (log ε). Ej.: UV λ max (MeOH) 275 (log ε 2,94).

IR ν max (medio) cm^{-1}. Ej.: IR ν max (KBr) 1740, 1720 cm^{-1}.

EM *m/z* (% intensidad relativa). Ej.: em *m/z* (%): 340 (M^+, 100), 295 (10), 134 (26) ...

RMN ^1H (solvente, frecuencia de registro) δ ppm (integración, multiplicidad, J en Hz, asignación). Ej.: RMN ^1H ($CDCl_3$, 400 MHz) 3,84 (1H, *d*, J = 10,3 Hz, H-30).

RMN ^{13}C (solvente, frecuencia de registro) δ ppm (multiplicidad, asignación). Ej.: RMN ^{13}C ($CDCl_3$, 600 MHz) 16,60 (t, C-12).

Las abreviaturas usadas para describir la multiplicidad de las señales en RMN son: *s* = singlete, *d* = doblete, *t* = triplete, *m* = multiplete, *dd* = doble de dobletes, *ddd* = doble de doble de dobletes.

Las abreviaturas para los solventes y reactivos más comúnmente usados son: EtOH= etanol, MeOH = metanol, $CHCl_3$ = cloroformo, C_6H_6 = benceno, AcOEt = acetato de etilo, EP = éter de petróleo, Me_2CO = acetona, DMSO = dimetilsulfóxido, AcOH = ácido acético.

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5. F. Salcedo, *Contribución al estudio de las Cinchonas colombianas*, Tesis de Grado, Universidad del Valle, Cali, 1983, pp. 14-16.

Referencias de Internet: Iniciales del nombre y apellido completo del autor, título del documento, dirección URL y fecha de revisión. Ej.:

6. Lipidat, Lipid thermotropic phase transition database, Ohio State University, URL: http://www.lipidat.chemistry.ohio-state.edu, consultado en septiembre de 2001.

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