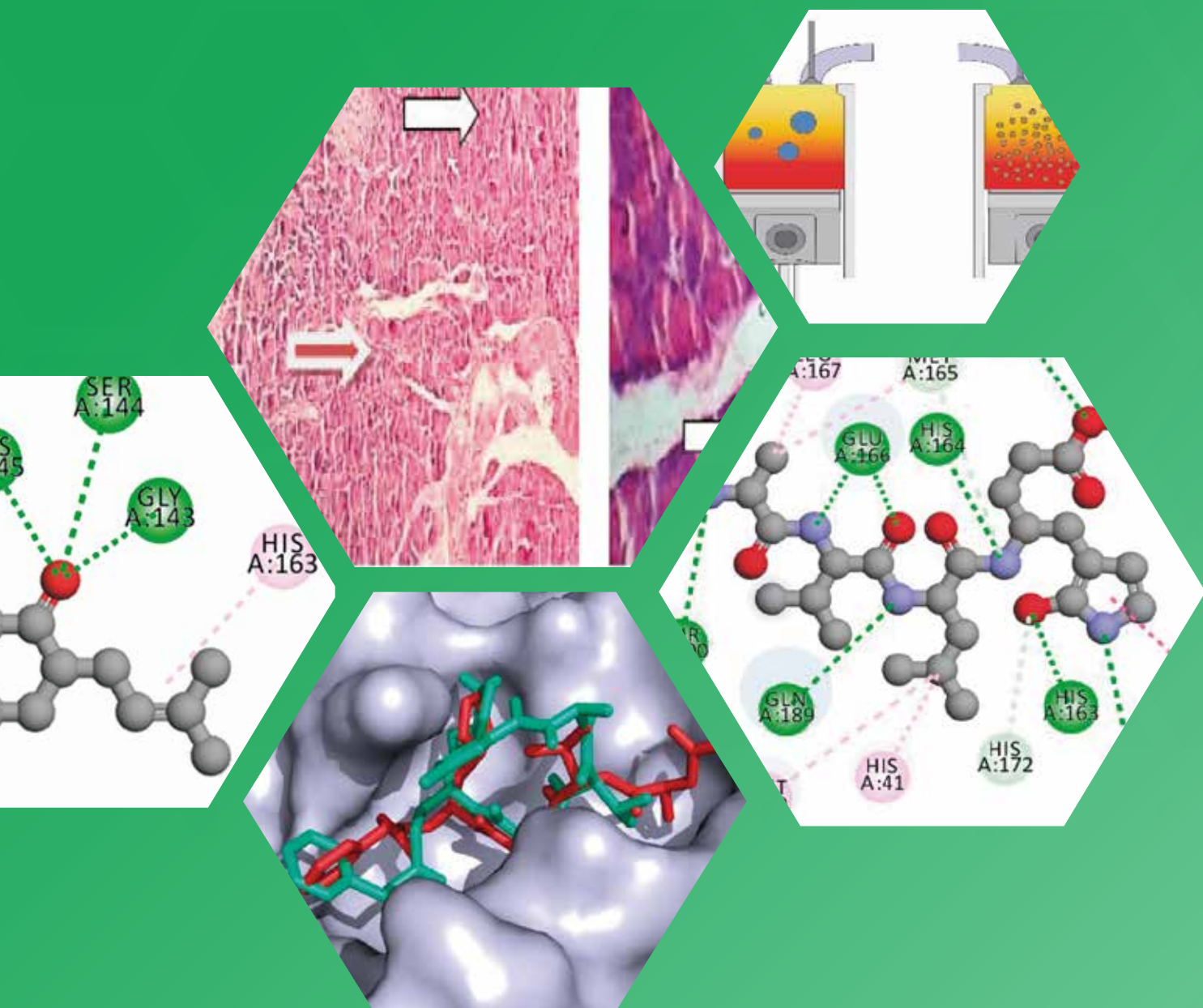




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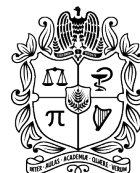
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**Design, synthesis, *in silico* studies, and antidiabetic activity of several sulfanilamide incorporated 2,3-disubstituted thiazolidin-4-ones****Abstract**

A panel of 2,3-disubstituted thiazolidin-4-ones **4a-n** was synthesised from Schiff bases **3a-n** derived from sulfanilamide, by reaction with thioglycolic acid. The compounds were characterised by means of IR, NMR, and Mass spectral data. Compounds **4a-n** were screened for DPPH scavenging assay and compounds **4e**, **4h**, **4i**, and **4n** exhibited moderate activity. Compounds **4e**, **4h**, and **4i** were tested at 200 mg/kg and **4e** at 50 mg/kg b.w. orally for antidiabetic activity in fructose induced diabetic rats. They exhibited significant antidiabetic activity compared to the control group. Pioglitazone was used as a standard drug. The tested compounds exhibited better and significant serum cholesterol lowering activity when compared with the control and standard groups. They also reduced the triglyceride level after the 21st day; however, it was insignificant when compared to the control group. Compound **4n** displayed the highest binding energy when docked with PPAR- γ followed by compounds **4e**, **4h**, and **4i** when compared to pioglitazone. The physicochemical, drug likeness and ADME properties of the title compounds were found to be satisfactory.

Keywords: Schiff bases; DPPH; antioxidant; serum glucose; total serum cholesterol; serum triglycerides; PPAR- γ ; ADME; Drug Likeness.

Diseño, síntesis, estudios *in silico* y actividad antidiabética de algunas sulfanilamidas 2,3-disustituidas tiazolidina-4-onas incorporadas**Resumen**

Se sintetizó un panel de tiazolidinas-4-onas 2,3-disustituidas **4a-n** a partir de las bases de Schiff **3a-n** derivadas de la sulfanilamida por reacción con ácido tioglicólico. Los compuestos se caracterizaron por IR, RMN y datos espectrales de masa. Los compuestos **4a-n** se analizaron para DPPH y los compuestos **4e**, **4h**, **4i** y **4n** mostraron una actividad moderada. Los compuestos **4e**, **4h** y **4i** se probaron a 200 mg/kg y **4e** a 50 mg/kg b.w. oralmente para la actividad antidiabética en ratas diabéticas, inducida por fructosa. Los compuestos mostraron una actividad antidiabética muy significativa en comparación con el grupo control. La pioglitazona se utilizó como fármaco estándar. Los compuestos ensayados mostraron una mejor y significativa actividad reductora del colesterol sérico en comparación con los grupos control y estándar. Estos compuestos también redujeron el nivel de triglicéridos después del 21^o día, aunque fue insignificante en comparación con el grupo control. El compuesto **4n** mostró la mayor afinidad de unión cuando se acopló a PPAR- γ , seguido de **4e**, **4h** y **4i** en comparación con la pioglitazona. Las propiedades fisicoquímicas, la similitud con el fármaco y las propiedades ADME de los compuestos fueron satisfactorias, lo que los convierte en útiles agentes antidiabéticos.

Palabras clave: Bases de Schiff; DPPH; antioxidante; glucosa sérica; colesterol total sérico; triglicéridos séricos; triglicéridos séricos; PPAR- γ ; ADME; similitud de fármacos.

Concepção, síntese, em estudos de *silico* e actividade antidiabética de poucas sulfanilamidas incorporado 2,3-disubstituído tiazolidina-4-ones**Resumo**

Um painel de 2,3-disubstituído tiazolidina-4-ones **4a-n** foram sintetizados a partir de bases Schiff **3a-n** derivado da sulfanilamida por reação com ácido tioglicólico. Os compostos eram caracterizado por IR, NMR e dados espectrais de massa. Os compostos **4a-n** foram rastreados para O ensaio DPPH de limpeza radical e os compostos **4e**, **4h**, **4i** e **4n** exibiram actividade moderada. Os compostos **4e**, **4h** e **4i** foram testados a 200 mg/kg e **4e** a 50 mg/kg de peso corporal por via oral para antidiabéticos. actividade em ratos diabéticos induzidos por frutose. Exibiram uma actividade antidiabética altamente significativa actividade em comparação com o controlo. A pioglitazona foi utilizada como droga padrão. Os compostos testados exibiu uma melhor e significativa actividade de redução do colesterol sérico quando comparado com de triglicéridos após o 21^o dia; no entanto, foi insignificante quando comparado com o controlo. O composto **4n** mostrou a maior afinidade de ligação quando acoplado com PPAR- γ seguido de **4e**, **4h**, **4i** quando comparado com pioglitazona. O propriedades físico-químicas, de semelhança com drogas e ADME dos compostos do título de propriedade também foram encontrados paraser satisfatórios, tornando-os agentes antidiabéticos úteis.

Palavras-chave : bases Schiff; DPPH; antioxidante; glucose sérica; colesterol total sérico; soro triglicéridos; PPAR- γ ; ADME; Probabilidade de drogas.



Introduction

Diabetes mellitus is a spectrum of metabolic disorders resulting from myriad pathogenic mechanisms, all of which lead to hyperglycemia [1]. In recent years, diabetes has become one of the leading causes of death worldwide. In 2016, the World Health Organisation reported around 1.6 million deaths globally due to diabetes. The number of diabetes cases worldwide in 2019 was 463 million and by 2045 it could increase to 629 million. In India, 77 million suffer from diabetes and could be further increased and India has the second highest number of cases in the world [2]. Oxidative stress plays an important role in the pathogenesis of typical long-term diabetic complications such as neuropathy and microangiopathy. Further, dynamic modification of intracellular redox sensors by way of reactive oxygen species in addition to other post-translational modifications such as protein phosphorylation, acetylation or ubiquitination also play a role in the development of the diabetes [3]. Thiazolidinone is one of the important and fundamental central structures for the synthesis of biologically active compounds [4]. Thiazolidin-4-one is a well-known scaffold for producing the desired biological activities: Antidiabetic [5], antioxidant [6], anticancer [7], antitubercular [8], antimicrobial [9], anticonvulsant [10], analgesic and anti-inflammatory [11] and antihyperlipidemic [12].

The abovementioned facts triggered an interest in taking up this investigation, wherein molecular architecture having a 2,3-disubstituted 4-thiazolidinone core derived from sulfanilamide was designed with an anticipation of good antidiabetic activity. Spectral characterisation, docking studies with peroxisome proliferative activated receptor- γ (PPAR- γ), and *in silico* ADME parameter studies were also undertaken.

Materials and Methods

Chemicals employed were of laboratory reagent grade and used as received. Sulphanilamide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and *p*-hydroxybenzaldehyde were purchased from Loba Chemie Pvt Limited, Mumbai, India. The other substituted aldehydes, 1,4-dioxane, thioglycolic acid, anhydrous zinc chloride, ascorbic acid, and D-fructose were procured from SD Fine Chemicals, Mumbai, India. *p*-Tolualdehyde was purchased from Sigma Aldrich, Steinheim, USA. *p*-Fluorobenzaldehyde was obtained from Spectrochem, Mumbai, India; and 2,4-dichlorobenzaldehyde, 2,6-dichlorobenzaldehyde, and *p*-bromobenzaldehyde from HiMedia Laboratories Pvt. Ltd. Mumbai, India. The glucose, cholesterol, and triglyceride kits were purchased from Erba Mannheim, Sikkim, India. Pioglitazone hydrochloride was provided by USV Private Limited, Solan, India. Thin layer chromatography using pre-coated aluminium sheets with silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany) was performed using a combination of solvents in different ratios as mobile phase. Spots were visualised by iodine vapor and/or UV light. Yields refer to crude products.

Melting points were determined on an open capillary apparatus (Veego VMP-DS Mumbai, India) and are uncorrected. The compounds were characterised by ultraviolet (UV) spectra (UV 1601 Shimadzu, Kyoto, Japan) and infrared (IR) spectra as KBr pellets (Shimadzu and Thermo Scientific Nicolet iS5, Japan). Proton nuclear magnetic resonance (¹H NMR) was recorded by means of DMSO-*d*₆ (Bruker Avance Neo 500 MHz, Germany) while ¹³C NMR was recorded at 125 MHz. Mass spectra (MS) were registered by chemical ionisation technique (Waters-SynaptG2, Massachusetts, USA).

Animals

Sprague Dawley rats of either sex weighing between 250 and 350 g and Swiss albino female mice weighing from 25 to 40 g were obtained from the central animal house of the HSK College of Pharmacy, Bagalkote. The animals were housed under standard conditions (temperature 25 ± 1°C,

relative humidity (50-55%)) for 12 h dark and light cycle. They were given standard laboratory feed (Pranava Agro Industries Ltd., Sangli, India) and water *ad libitum* and acclimatised for 7 days before experimentation. Food was withdrawn 18 h before the experiment, but free access to water was allowed. The experiments were performed on randomly formed groups during the light phase of the cycle and the animals were used for one experiment only. The test compounds and standard drug were administered *p.o.* as aqueous suspension in 0.5% Tween-80. The animals in the vehicle control group received 0.5% aqueous Tween-80. Blood samples were collected from retro orbital flexus under ether anaesthesia. The study was approved and conducted under the guidelines of institutional animal ethics committee constituted as per committee for the control and supervision of experiments on animals, Ministry of Environment and Forestry, Government of India (F.No. HSK College of Pharmacy Bagalkot/IAEC// HSKCOP/AUG2021/PG13).

Synthesis of Schiff bases 3a-n

Sulfanilamide (40 mmol) 1 and substituted aldehydes (40 mmol) 2a-n were dissolved in 20 mL ethanol and the reaction mixture was refluxed for 4-5 h. Completion of reaction was monitored by TLC (n-hexane: ethyl acetate 1:2). The excess of ethanol was distilled, and the product 3a-n was settled after several hours and was filtered and washed with cold ethanol multiple times [13].

4-(benzylideneamino)benzenesulfonamide 3a

White crystals; Yield: 38%; MP: 192-194 °C; *R*_f: 0.77; IR (KBr, cm⁻¹): 3296.35 (NH str), 3012.81 (Ar-CH str), 1624 (C = N str), 1575 (Ar-C = C str), 1334 (SO₂ str).

4-((4-methylbenzylidene)amino)benzenesulfonamide 3b

White crystals; Yield: 43.93%; MP: 198-200 °C; *R*_f: 0.78; IR (KBr, cm⁻¹): 3277 (NH str), 2993 (Ar-CH str), 1622 (C = N str), 1581 (Ar-C = C str), 1330 (SO₂ str).

4-((4-methoxybenzylidene)amino)benzenesulfonamide 3c

White crystals; Yield: 46.11%; MP: 200 °C; *R*_f: 0.69; IR (KBr, cm⁻¹): 3277 (NH str), 2954 (Ar-CH str), 1610 (C = N str), 1577 (Ar-C = C str), 1322 (SO₂ str).

4-((2-hydroxybenzylidene)amino)benzenesulfonamide 3d

Yellowish orange crystals; Yield: 45.0%; MP: 218 °C; *R*_f: 0.76; IR (KBr, cm⁻¹): 3342 (OH str), 3230 (NH str), 3012.81 (Ar-CH str), 1618 (C = N str), 1562 (Ar-C = C str), 1301 (SO₂ str).

4-((4-hydroxybenzylidene)amino)benzenesulfonamide 3e

Light yellowish powder; Yield: 69.05%; MP: 212 °C; *R*_f: 0.67; IR (KBr, cm⁻¹): 3346 (OH str), 3266 (NH str), 3011.66 (Ar-CH str), 1620 (C = N str), 1576 (Ar-C = C str), 1326 (SO₂ str).

4-((4-(dimethylamino)benzylidene)amino)benzenesulfonamide 3f

Yellowish powder; Yield: 6.82%; MP: 220 °C; *R*_f: 0.83; IR (KBr, cm⁻¹): 3277.06 (NH str), 2900 (Ar-CH str), 1604.77 (C = N str), 1571 (Ar-C = C str), 1328 (SO₂ str).

4-((3,4-dimethoxybenzylidene)amino)benzenesulfonamide 3g

White powder; Yield: 57.98%; MP: 200-202 °C; *R*_f: 0.55; IR (KBr, cm⁻¹): 3277 (NH str), 2954 (Ar-CH str), 1608 (C = N str), 1568 (Ar-C = C str), 1330 (SO₂ str), 1278 (Ar-C-O-C str).

4-((4-chlorobenzylidene)amino)benzenesulfonamide 3h

White crystals; Yield: 86.16%; MP: 206 °C; *R*_f: 0.75; IR (KBr, cm⁻¹): 3320 (NH str), 3010 (Ar-CH str), 1615 (C = N str), 1566 (Ar-C = C str), 1296 (SO₂ str), 846 (C-Cl str).

4-((2,4-dichlorobenzylidene)amino)benzenesulfonamide 3i

Light yellowish crystals; Yield: 44.93%; MP: 210 °C; R_f : 0.80; IR (KBr, cm^{-1}): 3348 (NH str), 3050 (Ar-CH str), 1618 (C = N str), 1565 (Ar-C = C str), 1298 (SO_2 str), 831 (C-Cl str).

4-((2,6-dichlorobenzylidene)amino)benzenesulfonamide 3j

White powder; Yield: 34.55%; MP: 178 °C; R_f : 0.83; IR (KBr, cm^{-1}): 3347 (NH str), 3056 (Ar-CH str), 1619 (C = N str), 1568 (Ar-C = C str), 1299 (SO_2 str), 833 (C-Cl str).

4-((4-bromobenzylidene)amino)benzenesulfonamide 3k

White crystals; Yield: 98.3%; MP: 222 °C; R_f : 0.75; IR (KBr, cm^{-1}): 3290 (NH str), 2987 (Ar-CH str), 1622 (C = N str), 1577 (Ar-C = C str), 1330 (SO_2 str), 840 (C-Br str).

4-((4-nitrobenzylidene)amino)benzenesulfonamide 3l

Yellowish orange powder; Yield: 18.83%; MP: 178-180 °C; R_f : 0.85; IR (KBr, cm^{-1}): 3263 (NH str), 3091 (Ar-CH str), 1627 (C = N str), 1591 (Ar-C = C str), 1340 (SO_2 str), 1294 (C-NO₂).

4-((4-fluorobenzylidene)amino)benzenesulfonamide 3m

Yellowish white crystals; Yield: 55.58%; MP: 156 °C; R_f : 0.76; IR (KBr, cm^{-1}): 3286 (NH str), 2996 (Ar-CH str), 1622 (C = N str), 1582 (Ar-C = C str), 1310 (SO_2 str), 1130 (C-F).

4-((3-phenylallylidene)amino)benzenesulfonamide 3n

White crystals; Yield: 30.64%; MP: 220-222 °C; R_f : 0.56; IR (KBr, cm^{-1}): 3283.53 (NH str), 2992.18 (Ar-CH str), 1614 (C = N str), 1580 (Ar-C = C str), 1335 (SO_2 str).

Synthesis of 2,3-disubstituted thiazolidin-4-ones (4a-n)

The substituted Schiff bases **3a-n** (10 mmol), thioglycolic acid (20 mmol), and anhydrous zinc chloride (0.2 g) were added to 10 mL 1,4-dioxane and the mixture was refluxed for 20 h. The progress of the reaction was monitored by TLC (n-hexane: ethyl acetate 3:2). The reaction mixture was allowed to cool and poured into crushed ice. The crude solid of 2,3-disubstituted thiazolidin-4-ones **4a-n** obtained was filtered, washed thoroughly with sodium bicarbonate followed by ice cold brine solution. The compounds were recrystallised with aqueous alcohol (30:70) [14].

4-(4-oxo-2-phenylthiazolidin-3-yl)benzenesulfonamide 4a

Orange powder, Yield: 28.74%; MP: 122 °C; R_f : 0.33; IR (KBr, cm^{-1}): 3422.20 (NH str), 1630.63 (C = O str), 1384.70 (SO_2 str), 618 (C-S str), ¹H NMR (500 MHz, δ , ppm): 4.10 (q, 2H, 5-CH₂, Thiaz) 6.39 (s, 1H, 2-CH, Thiaz) 7.03 (s, 2H, SO_2NH_2) 7.10 (m, 1H, 4" ArH) 7.21 (m, 2H, 3" & 5" ArH) 7.35 (m, 2H, 2" & 6" ArH) 7.42 (d, 2H, 2' & 6' ArH) 7.76 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ , ppm): 32.53 (1C, 5C, Thiaz) 62.64 (1C, 2C, Thiaz) 121.5 (2C, 2' & 6' ArC) 125.8 (2C, 2" and 6" ArC) 126.2 (1C, 4" ArC) 128.4 (2C, 3" and 5" ArC) 129.3 (2C, 3' & 5' ArC) 135.4 (1C, 4' ArC) 140.6 (1C, 1" ArC) 143.8 (1C, 1' ArC) 170.40 (1C, C = O); Mass (m/z): 335.01 (M+ +1), 336.01 (M++2).

4-(4-oxo-2-(p-tolyl)thiazolidin-3-yl)benzenesulfonamide 4b

Orange powder, Yield: 31%; MP: 182-184 °C; R_f : 0.37; IR (KBr, cm^{-1}): 3406.18 (NH str), 2850 (Ali CH str), 1650 (C = O str), 1384.71 (SO_2 str), 618.52 (C-S str), ¹H NMR (500 MHz, δ , ppm): 2.39 (s, 3H, CH₃) 4.4 (q, 2H, 5-CH₂, Thiaz) 5.7 (s, 1H, 2-CH, Thiaz) 6.86 (s, 2H, SO_2NH_2) 7.34 (m, 2H, 3" & 5" ArH) 7.38 (m, 2H, 2" & 6" ArH) 7.5 (m, 2H, 2' & 6' ArH) 7.8 (m, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ , ppm): 20.4 (1C, CH₃) 32.76 (1C, 5C, Thiaz) 65.68 (1C, 2C, Thiaz) 121.6 (2C, 2' & 6' ArC) 127.9 (2C, 2" and 6" ArC) 128.4 (2C, 3" and 5" ArC) 129.5 (2C, 3' & 5' ArC) 136.4 (1C, 4' ArC) 137.1 (1C, 1" ArC) 137.6 (1C, 4" ArC) 144.8 (1C, 1' ArC) 169.38 (1C, C = O); Mass (m/z): 349.07 (M+ +1).

4-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4c

Orange powder, Yield: 23.07%; MP: 220 °C; R_f : 0.77; IR (KBr, cm^{-1}): 3418.04 (NH str), 2852 (Ali CH str), 1655 (C = O str), 1384.86 (SO_2 str), 1333.29 (Ar-C-O-C str), 618.33 (C-S str), ¹H NMR (500 MHz, δ , ppm): 3.76 (s, 3H, OCH₃) 4.4 (q, 2H, 5-CH₂, Thiaz) 6.36 (s, 1H, 2-CH, Thiaz) 6.71 (m, 2H, SO_2NH_2) 6.72 (m, 2H, 3" & 5" ArH) 7.40 (d, 2H, 2' & 6' ArH) 7.72 (d, 2H, 3' & 5' ArH) 7.82 (d, 2H, 2" & 6" ArH); ¹³C NMR (125 MHz, δ , ppm): 33.76 (1C, 5C, Thiaz) 55.8 (1C, OCH₃) 70.53 (1C, 2C, Thiaz) 113.8 (2C, 3" and 5" ArC) 120.2 (2C, 2' & 6' ArC) 128.3 (2C, 3' & 5' ArC) 129.9 (2C, 2" and 6" ArC) 130.3 (1C, 1" ArC) 135.4 (1C, 4' ArC) 144.8 (1C, 1' ArC) 157.6 (1C, 4" ArC) 170.38 (1C, C = O); Mass (m/z): 365.02 (M+ +1), 366 (M+2).

4-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4d

Orange powder, Yield: 47.14%; MP: 202-204 °C; R_f : 0.7; IR (KBr, cm^{-1}): 3345.60 (OH str), 3248.17 (NH str), 2923 (Ali CH str) 1618.84 (C = O str), 1384.77 (SO_2 str), 618.57 (C-S str), ¹H NMR (500 MHz, δ , ppm): 4.4 (q, 2H, 5-CH₂, Thiaz) 5.7 (s, 1H, 2-CH, Thiaz) 6.99 (m, 2H, 3" & 5" ArH) 7.0 (m, 1H, 4" ArH) 7.4 (m, 1H, 6" ArH) 7.39 (s, 2H, SO_2NH_2) 7.56 (d, 2H, 2' & 6' ArH) 7.8-7.9 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ , ppm): 32.6 (1C, 5C, Thiaz) 65.3 (1C, 2C, Thiaz) 115.8 (1C, 5" ArC) 119.2 (1C, 1" ArC) 121.9 (1C, 3" ArC) 122.2 (2C, 2' & 6' ArC) 127.8 (1C, 2" ArC) 128.3 (1C, 4" ArC) 129.4 (2C, 3' & 5' ArC) 136.4 (1C, 4' ArC) 145.8 (1C, 1' ArC) 152.9 (1C, 6" ArC) 170.6 (1C, C = O); Mass (m/z): 351.05 (M+ +1).

4-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4e

Brownish powder, Yield: 2.5%; MP: 230 °C; R_f : 0.28; IR (KBr, cm^{-1}): 3358.68 (OH str), 3253 (NH str), 1650 (C = O str), 1384.23 (SO_2 str), 618.34 (C-S str), ¹H NMR (500 MHz, δ , ppm): 3.98 (q, 2H, 5-CH₂, Thiaz) 6.46 (s, 1H, 2-CH, Thiaz) 6.69 (d, 2H, 3" & 5" ArH) 6.88 (s, 2H, SO_2NH_2) 7.46 (d, 2H, 2' & 6' ArH) 7.62 (d, 2H, 2" & 6" ArH) 7.73 (d, 2H, 3' & 5' ArH) 9.02 (s, 1H, OH); ¹³C NMR (125 MHz, δ , ppm): 31.5 (1C, 5C, Thiaz) 70.6 (1C, 2C, Thiaz) 114.8 (2C, 3" and 5" ArC) 121.2 (2C, 2' & 6' ArC) 129.3 (2C, 3' & 5' ArC) 131.1 (2C, 2" and 6" ArC) 131.8 (1C, 1" ArC) 136.3 (1C, 4' ArC) 143.8 (1C, 1' ArC) 156.6 (1C, 4" ArC) 168.38 (1C, C = O); Mass (m/z): 351.04 (M+ +1).

4-(2-(4-(dimethylamino)phenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4f

Dark brownish, Yield: 19.09%; MP: 158 °C; R_f : 0.57; IR (KBr, cm^{-1}): 3424.72 (NH str), 2870 (Ali CH str), 1606 (C = O str), 1384.46 (SO_2 str), 618.21 (C-S str), ¹H NMR (500 MHz, δ , ppm): 3.01 (s, 6H, N(CH₃)₂) 3.92 (q, 2H, 5-CH₂, Thiaz) 6.38 (s, 1H, 2-CH, Thiaz) 6.70 (d, 2H, 3" & 5" ArH) 6.82 (s, 2H, SO_2NH_2) 7.08 (d, 2H, 2" & 6" ArH) 7.40 (d, 2H, 2' & 6' ArH) 7.74 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ , ppm): 32.6 (1C, 5C, Thiaz) 41.6 (2C, N(CH₃)₂) 70.8 (1C, 2C, Thiaz) 112.8 (2C, 3" and 5" ArC) 122.5 (2C, 2' & 6' ArC) 126.4 (2C, 2" and 6" ArC) 127.9 (1C, 1" ArC) 128.6 (2C, 3' & 5' ArC) 136.8 (1C, 4' ArC) 143.9 (1C, 1' ArC) 148.2 (1C, 4" ArC) 170.42 (1C, C = O); Mass (m/z): 378.01 (M+ +1).

4-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4g

Dark brownish, Yield: 1.26%; MP: 90 °C; R_f : 0.46; IR (KBr, cm^{-1}): 3370.80 (NH str), 2924.94 (Ali CH str), 1674.77 (C = O str), 1384.68 (SO_2 str), 1333.29 (Ar-C-O-C str), 618.80 (C-S str), ¹H NMR (500 MHz, δ , ppm): 3.70 (s, 3H, OCH₃) 3.81 (s, 3H, OCH₃) 4.02 (q, 2H, 5-CH₂, Thiaz) 6.46 (s, 1H, 2-CH, Thiaz) 6.82 (m, 1H, 3" ArH) 6.83 (m, 2H, SO_2NH_2) 7.45 (m, 2H, 2' & 6' ArH) 7.52 (m, 1H, 2" ArH) 7.63 (m, 1H, 6" ArH) 7.74 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ , ppm): 34.46 (1C, 5C, Thiaz) 56.7 (2C, (OCH₃)₂) 72.33 (1C, 2C, Thiaz) 112.1 (1C, 3" ArC) 113.2 (1C, 6" ArC) 121.2 (2C, 2' & 6' ArC) 122.4 (1C, 2" ArC) 129.3 (2C, 3' & 5' ArC) 132.6 (1C, 1" ArC) 136.6 (1C, 4' ArC) 144.2 (1C, 1' ArC) 148.4 (1C, 4" ArC) 149.5 (1C, 5" ArC) 171.88 (1C, C = O); Mass (m/z): 395.07 (M+ +1).

4-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4h

Orange powder, Yield: 47.55%; MP: 90 °C; R_f : 0.42; IR (KBr, cm^{-1}): 3405.55 (NH str), 1630 (C = O str), 1384.76 (SO_2 str), 618.03 (C-S str), 843.25 (C-Cl str), ¹H NMR (500 MHz, δ , ppm): 3.99 (q, 2H, 5-CH₂, Thiaz) 6.2 (s, 1H, 2-CH, Thiaz) 6.8 (m, 2H, SO_2NH_2) 7.34 (m, 2H, 2" & 6" ArH)

7.36 (d, 2H, 3" & 5" ArH) 7.63 (d, 2H, 2' & 6' ArH) 7.85 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ, ppm): 31.33 (1C, 5C, Thiaz) 71.49 (1C, 2C, Thiaz) 121.22 (2C, 2' & 6' ArC) 128.28 (2C, 3" and 5" ArC) 128.43 (2C, 3' & 5' ArC) 130.9 (2C, 2" and 6" ArC) 132.1 (1C, 4" ArC) 136.3 (1C, 4' ArC) 136.2 (1C, 1" ArC) 144.3 (1C, 1' ArC) 168.26 (1C, C = O); Mass (m/z): 368.97 (M⁺+1), 370.97 (M⁺+3).

4-(2-(2,4-dichlorophenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4i

Yellow powder, Yield: 57.07%; MP: 110 °C; R_f: 0.54; IR (KBr, cm⁻¹): 3360.66 (NH str), 1680.92 (C = O str), 1384.52 (SO₂ str), 833.81 (C-Cl str), 618.10 (C-S str), ¹H NMR (500 MHz, δ, ppm): 4.0 (q, 2H, 5-CH₂, Thiaz) 5.94 (s, 1H, 2-CH, Thiaz) 6.86 (s, 2H, SO₂NH₂) 7.21 (m, 1H, 6" ArH) 7.27 (m, 1H, 5" ArH) 7.5 (m, 2H, 2' & 6' ArH) 7.74 (m, 1H, 3" ArH) 7.79 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ, ppm): 32.69 (1C, 5C, Thiaz) 67.8 (1C, 2C, Thiaz) 100.4 (1C, 1" ArC) 120.6 (2C, 2' & 6' ArC) 126.4 (1C, 5" ArC) 128.4 (2C, 3' & 5' ArC) 130.2 (1C, 3" ArC) 131.9 (2C, 6" ArC) 134.6 (1C, 4" ArC) 135.9 (1C, 2" ArC) 136.4 (1C, 4' ArC) 143.7 (1C, 1' ArC) 171.38 (1C, C = O); Mass (m/z): 402.93 (M⁺+1), 404.92 (M⁺+3).

4-(2-(2,6-dichlorophenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4j

Pale yellow powder, Yield: 44.66%; MP: 138 °C; R_f: 0.43; IR (KBr, cm⁻¹): 3314.79 (NH str), 3081.21 (Ar-CH str), 1643.96 (C = O str), 1384.73 (SO₂ str), 833.56 (C-Cl str), 618.11 (C-S str), ¹H NMR (500 MHz, δ, ppm): 4.1 (q, 2H, 5-CH₂, Thiaz) 5.8 (s, 1H, 2-CH, Thiaz) 7.38 (m, 2H, SO₂NH₂) 7.4 (m, 2H, 2' & 6' ArH) 7.52 (m, 1H, 4" ArH) 7.61 (m, 2H 3" & 5" ArH) 7.9 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ, ppm): 32.55 (1C, 5C, Thiaz) 62.43 (1C, 2C, Thiaz) 121.6 (2C, 2' & 6' ArC) 126.73 (2C, 3" and 5" ArC) 128.4 (1C, 4" ArC) 128.9 (2C, 3' & 5' ArC) 135.4 (2C, 2" and 6" ArC) 136.4 (1C, 4' ArC) 140.3 (1C, 1" ArC) 143.86 (1C, 1' ArC) 170.38 (1C, C = O); Mass (m/z): 402.93 (M⁺+1), 404.92 (M⁺+3).

4-(2-(4-bromophenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4k

Orange powder, Yield: 84.74%; MP: 212-214 °C; R_f: 0.44; IR (KBr, cm⁻¹): 3424.85 (NH str), 1640 (C = O str), 1384.77 (SO₂ str), 752.53 (C-Br str), 618.35 (C-S str), ¹H NMR (500 MHz, δ, ppm): 4.02 (q, 2H, 5-CH₂, Thiaz) 6.47 (s, 1H, 2-CH, Thiaz) 6.93 (s, 2H, SO₂NH₂) 7.21 (d, 2H, 2" & 6" ArH) 7.53 (d, 2H, 2' & 6' ArH) 7.79 (d, 2H, 3' & 5' ArH) 7.86 (d, 2H, 3" & 5" ArH); ¹³C NMR (125 MHz, δ, ppm): 32.41 (1C, 5C, Thiaz) 71.52 (1C, 2C, Thiaz) 121.6 (1C, 4" ArC) 121.4 (2C, 2' & 6' ArC) 129.3 (2C, 3' & 5' ArC) 130.9 (2C, 2" and 6" ArC) 131.8 (2C, 3" and 5" ArC) 136.4 (1C, 4' ArC) 138.3 (1C, 1" ArC) 144.6 (1C, 1' ArC) 171.37 (1C, C = O); Mass (m/z): 412.96 (M⁺+1).

4-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4l

Pale brownish powder, Yield: 98.59%; MP: 96 °C; R_f: 0.77; IR (KBr, cm⁻¹): 3380.52 (NH str), 1650 (C = O str), 1596.22 (NO₂ Asy str), 1384.77 (SO₂ str), 1346.91 (NO₂ sym str), 617.93 (C-S str), ¹H NMR (500 MHz, δ, ppm): 3.9 (q, 2H, 5-CH₂, Thiaz) 6.81 (s, 1H, 2-CH, Thiaz) 7.32 (s, 2H, SO₂NH₂) 7.59 (m, 2H, 2' & 6' ArH) 7.72 (m, 2H, 2" & 6" ArH) 7.76 (m, 2H, 3' & 5' ArH) 8.16 (m, 2H, 3" & 5" ArH); ¹³C NMR (125 MHz, δ, ppm): 32.53 (1C, 5C, Thiaz) 61.64 (1C, 2C, Thiaz) 121.38 (2C, 2' & 6' ArC) 123.96 (2C, 3" and 5" ArC) 130.53 (4C, 3', 5', 2" & 6" ArC) 139.96 (1C, 4' ArC) 141.44 (1C, 1" ArC) 147.18 (1C, 1" ArC) 147.29 (1C, 4" ArC) 170.65 (1C, C = O); Mass (m/z): 380 (M⁺+1).

4-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide 4m

Pale brownish powder, Yield: 32.65%; MP: 128 °C; R_f: 0.81; IR (KBr, cm⁻¹): 3385.74 (NH str), 1680 (C = O str), 1385 (SO₂ str), 1110.32 (C-F str), 618.28 (C-S str), ¹H NMR (500 MHz, δ, ppm): 3.97 (q, 2H, 5-CH₂, Thiaz) 6.41 (s, 1H, 2-CH, Thiaz) 6.94 (s, 2H, SO₂NH₂) 7.14 (m, 2H, 3" & 5" ArH) 7.22 (m, 2H, 2" & 6" ArH) 7.49 (d, 2H, 2' & 6' ArH) 7.8 (d, 2H, 3' & 5' ArH); ¹³C NMR (125 MHz, δ, ppm): 33.48 (1C, 5C, Thiaz) 72.9 (1C, 2C, Thiaz) 115.5 (2C, 3" and 5" ArC) 121.4 (2C, 2' & 6' ArC) 129.3 (2C, 3' & 5' ArC) 130.5 (2C, 2" and 6" ArC) 134.6 (1C, 1" ArC) 136.4 (1C, 4' ArC) 143.8 (1C, 1' ArC) 160.7 (1C, 4" ArC) 172.83 (1C, C = O); Mass (m/z): 353.01 (M⁺+1), 354.01 (M⁺+2).

4-(4-oxo-2-styrylthiazolidin-3-yl)benzenesulfonamide 4n

Pale brownish powder, Yield: 24.44%; MP: 202 °C; R_f: 0.70; IR (KBr, cm⁻¹): 3384.84 (NH str), 1670 (C = O str), 1382 (SO₂ str), 618.27 (C-S str), ¹H NMR (500 MHz, δ, ppm): 4.4 (q, 2H 5-CH₂, Thiaz) 5.7 (d, 1H 2-CH Thiaz) 6.53 (m, 1H CH) 6.88 (d, 1H CH) 6.89 (m, 2H SO₂NH₂) 7.29 (m, 2H 3" & 5" ArH) 7.30 (m, 1H 4" ArH) 7.44 (m, 2H 2' & 6' ArH) 7.72 (m, 2H 2" & 6" ArH) 7.8 (m, 2H 3' & 5' ArH); ¹³C NMR (125 MHz, δ, ppm): 34.1 (1C, 5C, Thiaz) 58.5 (1C, 2C, Thiaz) 121.3 (2C, 2' & 6' ArC) 123.2 (1C, CH) 127.8 (1C, 4" ArC) 128.6 (2C, 2" and 6" ArC) 128.8 (2C, 3" and 5" ArC) 129.6 (2C, 3' & 5' ArC) 129.8 (1C, CH) 136.5 (1C, 1" ArC) 136.6 (1C, 4' ArC) 144.7 (1C, 1' ArC) 171.63 (1C, C = O); Mass (m/z): 361.06 (M⁺+1).

Biological activity

DPPH scavenging assay

A stock solution of DPPH (0.1 mM) in ethanol (95%) was prepared. The concentrations of 40, 80, 160, 320, and 640 µg of 2,3-disubstituted thiazolidin-4-ones **4a-n** were prepared in ethanol. For the different concentration sample solutions, 2 mL DPPH stock solution was added and the volume was completed to 4 mL with ethanol. The reaction mixtures were allowed to stand for 30 min in the dark and absorbance was recorded at 517 nm against blank. Ascorbic acid was used as reference. The experiment was carried out in triplicate. The free radical scavenging activity was calculated by

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \quad \text{Eq 1}$$

The effective concentration of the sample required to scavenge the DPPH radical by 50% (IC₅₀) was obtained by linear regression analysis between concentration and absorbance [15].

Acute oral toxicity

Acute oral toxicity of 2,3-disubstituted thiazolidin-4-ones (**4a-n**) was performed as per OECD guidelines 425 [16]. The Swiss albino female mice were fasted before dosing for 18 h. The animals were administered experimental compounds at a dose of 2000 mg/kg b.w. as aqueous suspension of 0.5% Tween 80 orally and observed for 4 h after dosing and for 14 days until death.

Antidiabetic activity

The adult healthy Sprague Dawley rats of either sex was fed 30% D-fructose in water for 30 days and their serum glucose level was estimated using a glucose kit at 505 nm on the 0th, 15th and 30th days. The animals which exhibited hyperglycemia and hyperlipidemia were selected for the study. The rats were allowed to continue to receive fructose until the end of the study. The animals were divided into six groups, each consisting of six as follows: Group 1 (normal): received the water orally, Group 2 (control): received 30% fructose solution and 0.5% aqueous Tween 80 p.o., Group 3 (standard): received 30% fructose solution and pioglitazone (15 mg/kg b.w.) aqueous suspension with 0.5% Tween 80 p.o. and Groups 4, 5, 6, and 7 (Diabetic): received 30% fructose solution, compound **4e** (50 mg/kg b.w.) in 0.5% Tween 80 aqueous suspension p.o., compound **4h**, **4i**, and **4n** (200 mg/kg b.w.) in 0.5% Tween 80 aqueous suspension p.o., respectively [17]. Approximately 2 mL of blood was collected and centrifuged at 3500 RPM for 15 min to obtain the serum. The serum glucose was estimated on the 0th, 3rd, 7th, 14th and 21st days. The serum triglyceride and total cholesterol was estimated before and after treatment.

Estimation of serum glucose

The serum glucose was estimated by means of Trinder's method [18]. Glucose reagent 1 mL was added into blank, standard and test. 10 μ L of distilled water was appended to blank, standard with 10 μ L to standard solution and 10 μ L sample serum was added to test. The mixture was mixed well and incubated for 10 min at 37 °C. The absorbance was read against blank at 505 nm. The glucose level was expressed as:

$$\text{Concentration of glucose (mg/dL)} = \frac{\text{absorbance of sample}}{\text{absorbance of glucose standard}} \times \text{concentration of standard} \quad \text{Eq. 2}$$

Estimation of total serum cholesterol

The total serum cholesterol was estimated by the CHOD-PAP method [19]. Into each blank, standard and test 1000 μ L of working reagent was added. Blank was supplemented with 10 μ L distilled water, standard with 10 μ L standard solution and test was appended 10 μ L sample serum, mixed well and incubated for 10 min at 37 °C. The absorbance was read against blank at 505 nm. The cholesterol level was calculated by:

$$\text{Cholesterol} \left(\frac{\text{mg}}{\text{dL}} \right) = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{Concentration of standard} \quad \text{Eq 3}$$

Estimation of serum triglycerides

The serum triglycerides level was estimated by means of GPO-Trinder's method [20]. Working reagent 1000 μ L was added into each blank, standard, and test. Blank was supplemented with 10 μ L distilled water, standard with 10 μ L standard solution, and test was supplemented with 10 μ L sample serum, mixed well and incubated for 10 min at 37 °C. The absorbance was read at 546 nm against blank. Triglycerides level was calculated by:

$$\text{Triglycerides} \left(\frac{\text{mg}}{\text{dL}} \right) = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{Concentration of standard} \quad \text{Eq 4}$$

Histopathology

The rats were anaesthetised and exsanguinated. The pancreas from control, standard, and test groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal sections of 4 mm thickness. The sections were stained with haematoxylin and eosin dye for histopathological observation.

Molecular docking

The three-dimensional crystal structure of PPAR- γ with PDB ID: 4PRG [22] was downloaded from the RCSB protein data bank. Water molecules were removed followed by the addition of Kollman's charges and polar hydrogens using AutoDock 1.5.6 tools [23]. The structures of ligands **4a-n** were drawn by ChemDraw 16.0 and copied as SMILES. The Avogadro tool [24] was used to generate the .pdb format of ligand with energy minimisation. The .pdb file was converted into .pdbqt format for input into AutoDock 1.5.6 tools. The grid file was generated and saved as .gpf. The Lamarckian Genetic Algorithm was used to find the conformers with the

lowest binding energies and the .dpf file was generated to dock. The final docking task was performed by Autodock vina [25]. The docked file was saved as .dlg format and analysed for the binding interaction of ligand with target in different conformations. The highest binding energy conformation of ligand was selected and its interactions were visualised in Biovia discovery studio 2021 [26].

Physicochemical, drug likeness and ADME properties

The ADME properties and drug likeness of 2,3-disubstituted thiazolidin-4-ones (**4a-n**) was predicted by Swiss Prediction web tool [27]. The structures of compounds **4a-n** and pioglitazone were drawn in ChemDraw 16.0 and copied as SMILES and then submitted one by one to web tool to predict the physicochemical, ADME, and drug likeness properties.

Statistics

The data of animal experiments were expressed as mean $\pm$ standard error of mean (SEM) and analysed by means of Graphpad prism version 8.0.0 (Graphpad Software, San Diego, USA) [21]. The statistical difference between the treatments and various groups were tested by analysis of variance (ANOVA), followed by Dunnett's multiples comparison test. Data with *p*-value < 0.01 was considered significant.

Results and discussion

The hypoglycemic side effect of antimicrobial sulfonamides led to the development of the sulfonyl urea class of hypoglycemic agents such as tolbutamide and chlorpropamide. Glitazone antidiabetics such as rosiglitazone and pioglitazone show their activity due to the presence of acidic thiazolidinedione head. Enthused by this fact, the title compounds which have both the features of sulfonamide and thiazolidin-4-one (that closely resembles the thiazolidinedione present in glitazone antidiabetics) in the same molecule were designed by way of the molecular hybridisation strategy [28] with an anticipation of better hypoglycemic activity [29].

Chemistry

The design and synthesis of the title compounds 2,3-disubstituted thiazolidin-4-ones (**4a-n**) are illustrated in Figure 1. A series of Schiff bases **3a-n** was prepared by condensing substituted aromatic aldehydes with sulphanilamide in absolute alcohol with moderate to excellent yield.

The IR spectra of compounds **3a-n** showed the characteristic C = N stretching frequency between 1604.77 and 1627 cm⁻¹ among others. Further, compounds **3a-n** were reacted with thioglycolic acid in presence of anhydrous zinc chloride to yield compounds **4a-n** in moderate to excellent yield. The 2,3-disubstituted thiazolidinones **4a-n** displayed the methylene protons of the C-5 thiazolidinone ring at δ 3.9-4.4 ppm as quartet in ¹H NMR. The proton of thiazolidinone C-2 resonated at δ 5.7-6.81 ppm among other aromatic protons. The thiazolidinone C-2 appeared at δ ~ 62 ppm while C-5 resonated at δ ~ 33 ppm and C-4 showed signal at δ ~ 171 ppm amidst other carbons in ¹³C NMR. Further, the presence of C-S stretching at 617.93-618.80 cm⁻¹, SO₂ stretching at 1382-1385 cm⁻¹ and C = O stretching at 1606-1680.92 cm⁻¹ in IR spectrum confirmed their structure. Compounds **4a-n** displayed M⁺ and M⁺+1 ion peaks in their required value in mass spectrum suggesting their formation.

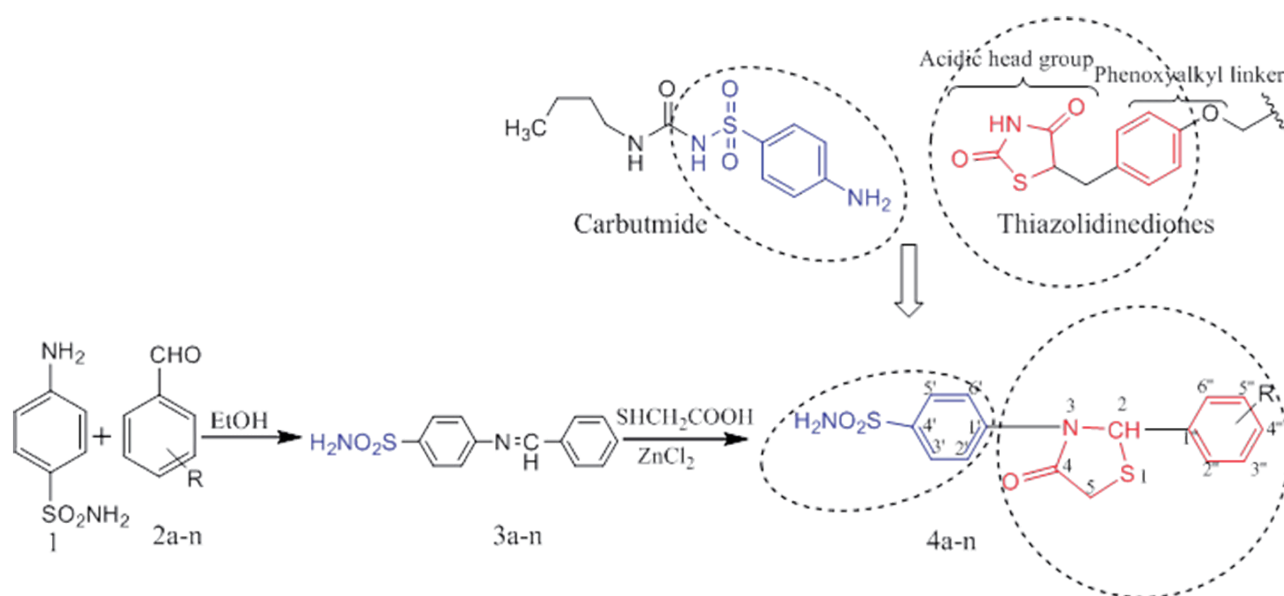


Figure 1. Design and synthesis of 2,3-disubstituted thiazolidin-4-ones.

R = a : H; b : 4-CH₃; c : 4-OCH₃; d : 2-OH; e : 4-OH; f : 4-N(CH₃)₂; g : 3,4-(OCH₃)₂; h : 4-Cl; i : 2,4-Cl₂; j : 2,6-Cl₂; k : 4-Br; l : 4-NO₂; m : 4-F.

DPPH scavenging assay

The DPPH radical scavenging activity of 2,3-disubstituted thiazolidin-4-ones 4a-n is presented on Table 1. The DPPH radical scavenging % was calculated as per equation 1. The *p*-chloro substituted compound 4h exhibited weak DPPH scavenging activity (IC₅₀ = 355.83 µg) followed by 2,4-dichloro substituted 4i (IC₅₀ = 359.101 µg), 4n with vinyl group (IC₅₀ = 372.28 µg), and *p*-hydroxy substituted 4e (IC₅₀ = 440.84 µg) when compared with standard, ascorbic acid (IC₅₀ = 9.25 µg). The remainder of the compounds did not exhibit good DPPH scavenging activity. Compounds with electron attracting substituents exhibited better DPPH scavenging than those with electron releasing substituents.

Table 1. DPPH Scavenging Activity of 2,3-disubstituted thiazolidin-4-ones (4a-n).

Compound	IC ₅₀ , µg
4a	9920
4b	1349.70
4c	2280
4d	1016.77
4e	440.84
4f	1857.36
4g	952.28
4h	355.83
4i	359.101
4j	1975.95
4k	6556
4l	786.85
4m	2737.27
4n	372.28
Asc acid	9.25

Acute oral toxicity

Compounds **4e**, **4h**, **4i**, and **4n** were found to be safe at the dose of 2000 mg/kg b.w. Thus, they were tested at 200 mg/kg b.w. and compound **4e** at 50 mg/kg b.w. orally for *in vivo* antidiabetic activity.

Antidiabetic activity

It is a well-known fact that, diabetes mellitus is associated with oxidative stress [30] and compounds having the antioxidant property will be of great value in the treatment of diabetes. Therefore, the thiazolidin-4-ones that have exhibited good antioxidant activity in the DPPH assay namely, **4e**, **4h**, **4i** and **4n** were selected for antidiabetic activity. The antidiabetic activity was carried out by estimating the serum glucose level as per equation 2 in rats with fructose induced diabetes. The estimation of the serum glucose level was carried out by Trinder's method in which, glucose oxidase reacts with glucose, water, and oxygen to form gluconic acid and hydrogen peroxide. The enzyme peroxidase catalyses the oxidative coupling of 4-aminoantipyrine with phenol in the presence of hydrogen peroxide to yield a purple coloured quinoneimine complex with absorbance proportional to the concentration of glucose [31].

The administration of fructose resulted in the induction of diabetes in rats when compared to the normal group. The tested compounds exhibited highly significant antidiabetic activity when compared with the control group. Compound **4e** with *p*-hydroxy substitution reduced serum glucose by 67.93% followed by compound **4h** (*p*-Cl) by 66.03%, compound **4i** (2,4-Cl₂) by 65.29%, and compound **4n** by 62.68% on the 21st day of the treatment. All the compounds exhibited higher glucose lowering activity than the standard drug pioglitazone. Contrary to DPPH scavenging activity, *p*-OH substitution seems to favour the activity more than *p*-Cl and 2,4-Cl₂. The reduction in the serum glucose level by way of compounds was found to be significant when compared with pioglitazone on the 3rd day, compound **4e** exhibited a significant reduction in the serum glucose level on the 14th day. The results are tabulated on Table 2.

Table 2. Antidiabetic Activity of Compounds **4e**, **4h**, **4i** and **4n**.

Groups	Serum glucose, mg/dl				
	0 th Day	3 rd Day	7 th Day	14 th Day	21 st Day
Normal	85.74±7.09	89.26±5.49	85.28±4.05	90.08±3.60	90.56±4.38
Control	186.5±19.37	202.6±11.11a	193.5±8.39a	191.7±7.66a	199.0±13.95a
Compound 4e (% reduction of serum glucose)	129.3±7.06 (30.67)	83.71±4.64*** ### (58.68)	70.47±0.98*** # (63.58)	67.37±0.48*** ## (64.85)	63.80±0.90*** (67.93)
Compound 4h (% reduction of serum glucose)	110.8±3.85 (40.58)	79.21±2.34*** ### (60.90)	77.52±2.23*** (59.93)	72.52±1.26*** (62.17)	67.59±1.18*** (66.03)
Compound 4i (% reduction of serum glucose)	128.7±10.69 (30.99)	110.2±5.15*** ## (45.60)	82.18±0.98*** (57.52)	76.20±1.70*** (60.25)	69.06±1.79*** (65.29)
Compound 4n (% reduction of serum glucose)	135.3±10.93 (27.45)	112.1±5.50*** ## (44.66)	93.88±3.43*** (51.48)	83.99±2.12*** (56.15)	74.26±0.93*** (62.68)
Pioglitazone (% reduction of serum glucose)	151.0±20.97 (19.03)	144.8±9.25*** (28.52)	89.66±3.96*** (53.66)	86.40±4.18*** (54.92)	85.29±3.47*** (57.14)

All the values are expressed as mean ± SEM, n = 6, ^a*p* < 0.001 as compared to the normal group, ****p* < 0.001 as compared to the control group and ###*p* < 0.001, ##*p* < 0.01 and #*p* < 0.05 as compared to the standard group [ANOVA followed by multiple comparison Dunnett's test].

Total serum cholesterol

Cholesterol esterase catalyses the conversion of cholesterol ester to cholesterol in the presence of water. The released cholesterol is then converted into cholest-4-en-3-one and hydrogen peroxide in the presence of oxygen by cholesterol oxidase. The released hydrogen peroxide reacts with 4-aminoantipyrine and phenol to yield a purple complex of quinoneimine that was measured spectrophotometrically [19]. The serum cholesterol was calculated as per equation 3. The tested compounds exhibited better and significant serum cholesterol lowering activity than pioglitazone, when compared with the control group. Compound **4e** possessed the highest activity followed by **4h**, **4i**, and **4n**. The activity pattern displayed by the compounds is the same as that of antidiabetic activity. The reduction in total cholesterol by tested compounds was found to be insignificant when compared with pioglitazone; only compound **4e** has shown significant activity on the 21st day. The results are tabulated on Table 3.

Table 3. Effect of compounds **4e**, **4h**, **4i** and **4n** on serum total cholesterol and triglycerides.

Group	Total cholesterol level (mg/dl)		Triglycerides level (mg/dl)	
	Before treatment (0 th day)	After treatment (21 st day)	Before treatment (0 th day)	After treatment (21 st day)
Normal	35.96±7.99	22.05±2.05	51.01±9.50	95.75±13.40
Control	65.18±3.58	98.20±4.50a	91.15±9.55	119.5±12.78b
Compound 4e	86.25±8.03	41.20±5.41*** ##	175.7±22.67	91.05±16.07
Compound 4h	69.78±5.01	51.86±0.94***	163.6±19.71	77.47±4.61
Compound 4i	71.82±10.42	52.79±3.76***	156.6±29.25	104.0±15.16#
Compound 4n	71.43±7.26	52.54±6.54***	139.3±19.97	86.03±5.63
Pioglitazone	97.65±20.84	64.10±4.94***	153.0±11.68	52.32±4.51**

All the values are expressed as mean ± SEM, n = 6, ^a*p* < 0.001 as compared to the normal group, ****p* < 0.001 as compared to the control group and ##*p* < 0.01 as compared to the standard group. [One way ANOVA followed by multiple comparison Dunnett's test].

Serum triglycerides

The determination of serum triglycerides was done by means of the GPO-Trinder method. Lipase hydrolyses serum triglycerides to glycerol and free fatty acids. In the presence of ATP and glycerol kinase, the glycerol

is converted to glycerol-3-phosphate. The glycerol-3-phosphate is then oxidised by glycerol phosphate oxidase to dihydroxy acetone phosphate and hydrogen peroxide. The condensation of hydrogen peroxide with TOOS reagent (sodium 3-(ethyl(m-tolyl)amino)-2-hydroxypropane-1-sulfonate) and 4-aminoantipyrine yielded a coloured complex of quinoneimine which absorbs at 546 nm [20]. The serum triglycerides level was calculated as per equation 4. The tested compounds reduced the triglyceride level after the 21st day. However, it was found to be insignificant, whereas pioglitazone significantly reduced triglycerides when compared to the control group. Compound **4h** exhibited the highest serum triglyceride reduction followed by **4n**. Compound **4e** displayed moderate activity. Compound **4i** with 2,4-Cl₂ substitution has significantly reduced serum triglycerides after the 21st day when compared with standard pioglitazone. The results are tabulated on Table 3.

Body weight

The tested compounds were found to decrease the elevated body weight when tested at different time intervals. However, it was insignificant when compared with the control and standard groups. The results are tabulated on Table 4.

Table 4. Effects of compounds **4e**, **4h**, **4i** and **4n** on the body weight of animals.

Groups	Body Weight (gm)				
	0 th Day	3 rd Day	7 th Day	14 th Day	21 st Day
Normal	288.2 ± 9.35	295.8 ± 10.00	282.7 ± 11.74	283.3 ± 12.38	287.7 ± 13.10
Control	317.5 ± 9.90	325.8 ± 9.404	316.3 ± 10.19	322.3 ± 11.86	321.3 ± 10.62
Compound 4e	308.3 ± 14.45	291.0 ± 6.70	285.3 ± 6.03	285.3 ± 7.72	282.7 ± 10.57
Compound 4h	308.7 ± 18.82	299.3 ± 18.99	297.0 ± 19.28	292.7 ± 22.16	291.3 ± 21.57
Compound 4i	294.8 ± 24.19	283.0 ± 21.61	282.7 ± 24.97	285.2 ± 29.32	281.0 ± 273.0
Compound 4n	280.0 ± 19.0	278.7 ± 21.24	276.7 ± 20.88	275.0 ± 21.98	273.0 ± 23.18
Pioglitazone	316.8 ± 7.59	320.2 ± 10.22	295.5 ± 12.53	311.3 ± 10.79	326.0 ± 12.37

All the values are expressed as mean ± SEM, n = 6, as compared to the control and standard group. [One way ANOVA followed by multiple comparison Dunnett's test].

Histopathology

The histopathological investigation of the pancreas of rats belonging to the normal group showed a normal architecture of islets of Langerhans with pale, rounded and ovoid β cells in the centre embedded in the exocrine portion of the pancreas. Whereas, shrinkage of islets of Langerhans with degeneration and necrosis of cells with their nucleus appearing densely basophilic with predominant karyolysis was observed in rats of the control group. The pancreas of diabetic rats treated with pioglitazone showed a normal architecture of islets of Langerhans with pale, large, round to ovoid shape containing cells embedded in the exocrine portion of the pancreas. In the case of a pancreas treated with compounds **4h** and **4i** normal sized islets of Langerhans but some degeneration of β cells in the centre were noticed, suggesting a protective role of compounds **4h** and **4i** on the islets of Langerhans. The histopathological changes are presented in Figure 2.

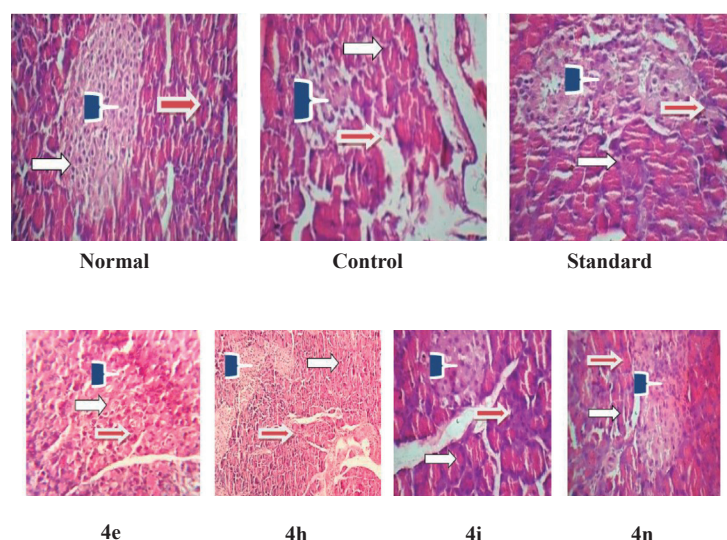


Figure 2. Effect of 2,3-disubstituted thiazolidin-4-ones on pancreatic cell.

Normal: Pancreatic islet of rat showed intact cluster of β -cells and architecture of islets of Langerhans.

Control: Showed destruction of pancreatic islets of β -cells, islet shape and number of islets are reduced.

Standard: Showed apparently reduced degranulation architecture, moderately restored pancreatic cells, islet shape and increased number of islets.

Treatment: 4e, 4h, 4i and 4n treated showing regeneration of β -cells, restoration of architecture, shape and increase in the number of islets.

→ Architecture of β cells of Islets

■ Islets shape

→ Number of β cells of islets

Molecular docking

The compounds that were tested for antidiabetic activity were docked with PPAR- γ (PDB ID: 4PRG) using autodock tools 1.5.6 to explore their interaction. The PPAR- γ plays an important role in the regulation of glucose and homeostasis and was used as a molecular target for the 2,4-thiazolidinedione class (glitazones) of antidiabetic agents [32]. The binding energies and amino acid residues with which compounds **4e**, **4h**, **4i**, **4n** and pioglitazone interacted are presented on Table 5 and in Figure 3. The compounds exhibited more binding energy with PPAR- γ than with pioglitazone.

Table 5. Interactions of compounds **4e**, **4h**, **4i** and **4n** with PPAR- γ .

Compound	Binding energy <i>Kcal/mol</i>	Interacting amino acid residues
4e	-7.6	GLU(B:343), ARG(B:288), LEU(B:340), LEU(B:228), LEU(B:330)
4h	-7.3	GLU(B:460), LEU(B:453), VAL(B:450)
4i	-8.3	ASP(B:475), ILE(B:279), GLU(B:460)
4n	-10.3	ILE(A:267), ARG(A:280), PHE(A:264), LYS(A:265), PHE(A:287)
Pioglitazone	-7.12	GLU(B:343), LYS(C:263), LEU(C:255), MET(C:256), ILE(C:281), ARG(C:280), PHE(C:264), PHE(C:287)

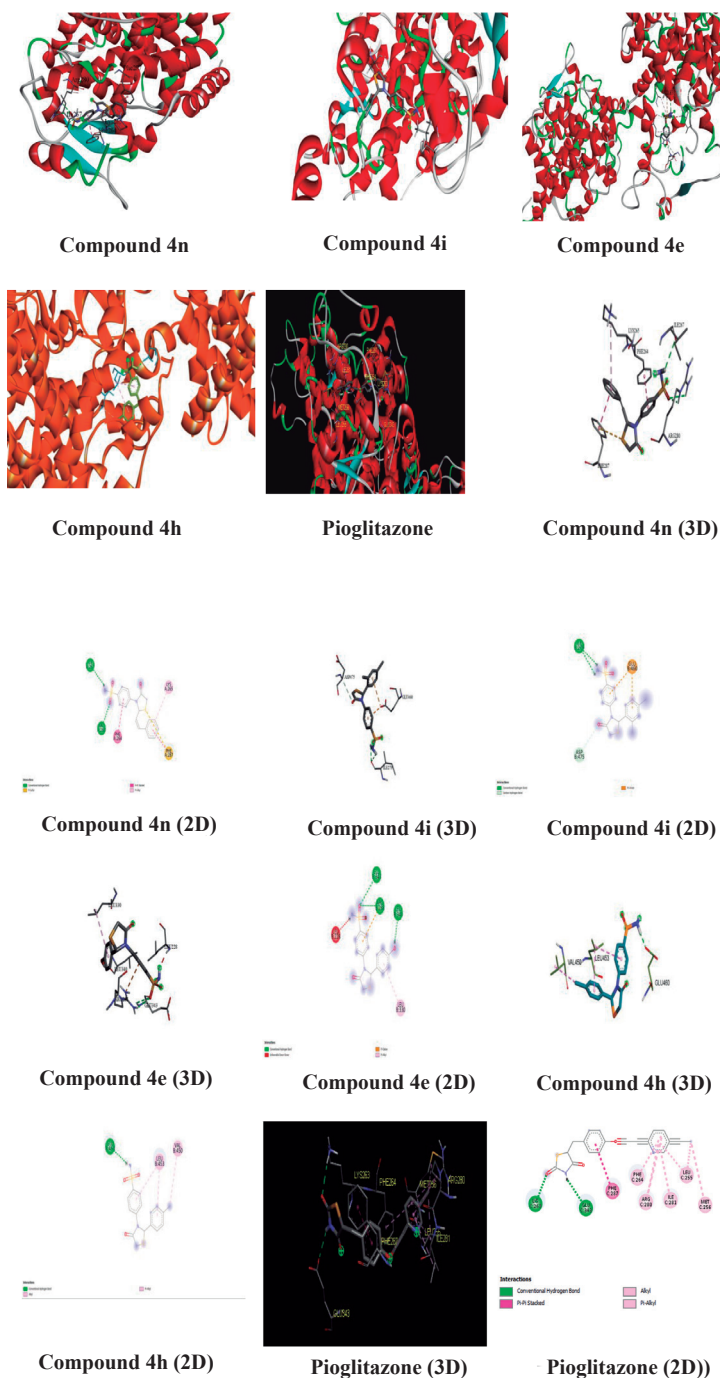


Figure 3. Docking of Compounds and Standard drug with PPAR- γ .

Table 6. *In silico* parameters of 2,3-disubstituted thiazolidin-4-ones (4a-n).

Compounds	Physicochemical properties							Lipophilicity						Drug likeness					Pharmacokinetics	
	MW	Fsp3	RB	HBA	HBD	MR	TPSA	iLOGP	X LOGP3	W LOGP	M LOGP	SILICOS-IT	Consensus Log P	Lipinski	Ghose	Veber	Egan	Muegge	Log KP (cm/s)	F
4a	334.41	0.13	3	4	1	89.55	114.15	1.45	1.93	2.49	1.51	1.12	1.70	0	0	0	0	0	-6.97	0.55
4b	348.44	0.19	3	4	1	94.52	114.15	1.62	2.29	2.80	1.76	1.62	2.02	0	0	0	0	0	-6.80	0.55
4c	364.44	0.19	4	5	1	96.04	123.38	2.13	1.90	2.50	1.21	1.16	1.78	0	0	0	0	0	-7.17	0.55
4d	350.41	0.13	3	5	2	91.57	134.38	1.51	1.57	2.19	0.96	0.64	1.37	0	0	0	1	0	-7.32	0.55
4e	350.41	0.13	3	5	2	91.5	134.38	1.49	1.57	2.19	0.96	0.64	1.37	0	0	0	1	0	-7.32	0.55
4f	377.48	0.24	4	4	1	103.76	117.39	1.76	2.05	2.55	1.45	0.77	1.72	0	0	0	0	0	-7.15	0.55
4g	394.47	0.24	5	6	1	102.53	132.61	2.26	1.87	2.51	0.92	1.21	1.75	0	0	0	1	0	-7.38	0.55
4h	368.86	0.13	3	4	1	94.56	114.15	2.13	2.55	3.14	2.02	1.75	2.32	0	0	0	0	0	-6.74	0.55
4i	403.30	0.13	3	4	1	99.57	114.15	2.41	3.18	3.80	2.53	2.40	2.86	0	0	0	0	0	-6.50	0.55
4j	403.30	0.13	3	4	1	99.57	114.15	1.77	3.18	3.80	2.53	2.40	2.74	0	0	0	0	0	-6.50	0.55
4k	413.31	0.13	3	4	1	97.25	114.15	1.84	2.62	3.25	2.15	1.79	2.33	0	0	0	0	0	-6.96	0.55
4l	379.41	0.13	4	6	1	98.37	159.97	1.54	1.75	2.40	0.59	-1.04	1.04	0	0	1	1	1	-7.37	0.55
4m	352.40	0.13	3	5	1	89.51	114.15	1.94	2.03	3.05	1.90	1.54	2.09	0	0	0	0	0	-7.01	0.55
4n	360.45	0.12	4	4	1	99.48	114.15	1.86	2.57	3.04	1.66	1.68	2.16	0	0	0	0	0	-6.67	0.55
Pioglitazone	356.44	0.32	7	4	1	99.48	93.59	2.61	3.75	2.78	2.01	4.28	3.09	0	0	0	0	0	-5.81	0.55

MW: Molecular Weight, Fsp3: Fraction of sp³ Carbon atoms, RB: Rotatable Bonds, HBA: Hydrogen Bond Acceptor, HBD: Hydrogen Bond Donor, MR: Molar Refractivity, TPSA: Topological Polar Surface Area, LogP: Indicator of Lipophilicity, Log KP: Skin Permeation, F: Bioavailability Score

It is interesting that, the tested compounds exhibited higher binding energy with PPAR- γ . The compound **4n** demonstrated the highest binding energy with -10.3 *kcal/mol* among the tested compounds and formed two hydrogen bonds with amino acid residues ILE (A:267) and ARG (A:280), and also formed other bonds by interacting with other amino acid residues PHE (A:264), LYS (A:265), and PHE (A:287). Likewise, compound **4i** showed good binding energy of -8.3 *kcal/mol* and formed two hydrogen bonds with ASP (B:475) and ILE (B:279), and also formed another bond with GLU (B:460). Compound **4e** displayed good binding energy of -7.6 *kcal/mol* and formed three hydrogen bonds with GLU (B:343), ARG (B:288), and LEU (B:340), and formed other bonds with LEU (B:228) and LEU (B:330). Compound **4h** also showed binding energy of -7.3 *kcal/mol* and formed a hydrogen bond with GLU (B:460), and other bonds with LEU (B:453) and VAL (B:450). Compound **4n** interacted with the A chain while, others with the B chain of PPAR- γ . All the results are compared with pioglitazone which had binding energy of -7.12 *kcal/mol* and formed two hydrogen bonds with GLU (B:343) and LYS (C:263), and other bonds with PHE (C:287), LEU (C:255), MET (C:256), ILE (C:281), PHE (C:264), and ARG (C:280) with the B and C chains. Thus, compound **4n** exhibited excellent binding energy and interaction with PPAR- γ followed by **4i**, **4e** and **4h** when compared with pioglitazone. It is interesting to note that, the compounds which showed good antidiabetic activity interacted with a lower binding energy with PPAR- γ .

Physicochemical, ADME, and drug likeness properties

For a molecule to be effective as a potent drug, it must reach its target in the body in sufficient concentration and stay there in a bioactive form long enough for the expected biologic events to occur. Drug development involves assessments of pharmacokinetic properties such as absorption, distribution, metabolism, and excretion (ADME); in this context computer models constitute valid alternatives to experiments. The Swiss ADME web

tool provides easy efficient inputs and free access to a pool of past yet robust predictive models for the physicochemical properties, pharmacokinetics, and drug likeness of compounds. The ADME and drug likeness of 2,3-disubstituted thiazolidin-4-ones (**4a-n**) were determined by the Swiss ADME web tool and the results are tabulated on Table 6.

The filters used to evaluate the drug likeness of the title compounds were i) Lipinski filter: MW $\leq$ 500, MLogP $\leq$ 4.15, HBA $\leq$ 10, HBD $\leq$ 5 [33] ii) Ghose filter: 160 $\leq$ MW $\leq$ 480, -0.4 $\leq$ WlogP $\leq$ 5.6, 40 $\leq$ MR $\leq$ 130, 20 $\leq$ atoms $\leq$ 70 [34] iii) Veber (GSK) filter: RB $\leq$ 10, TPSA $\leq$ 140 [35] iv) Egan (Pharmacia) filters: WLogP $\leq$ 5.88, TPSA $\leq$ 131.6 [36] v) Muegge (Bayer) filter : 200 $\leq$ MW $\leq$ 600, -2 $\leq$ XLogP $\leq$ 5, TPSA $\leq$ 157; HBD $\leq$ 5, RB $\leq$ 15, number of rings $\leq$ 7; number of carbons $>$ 4, number of heteroatoms $\leq$ 1 [37]. The compounds did not violate the Lipinski and Ghose rule but, some violated the Veber, Egan, and Muegge rules. To summarise, 2,3-disubstituted thiazolidin-4-ones met the criteria of drug likeness and exhibited satisfactory ADME parameters.

Conclusions

A series of 2,3-disubstituted thiazolidinones **4a-n** were synthesised from Schiff base intermediates **3a-n** that have been derived from sulfanilamide. The final products were characterised by physicochemical parameters and spectral data.

Compounds **4e**, **4h**, **4i**, and **4n** exhibited weak DPPH radical scavenging activity. The acute toxicity study revealed the safety of compounds **4e**, **4h**, **4i** and **4n** at the dose of 2000 mg/kg b.w. p.o. Compounds **4h**, **4i** and **4n** were tested at 200 mg/kg b.w. and compound **4e** at 50 mg/kg b.w. orally for *in vivo* antidiabetic activity. Compounds **4e** with p-hydroxy substitution, **4h** with p-chloro substitution, **4i** with 2,4-dichloro substitution, and **4n** with vinyl substitution exhibited highly significant antidiabetic activity when compared with the control group. The tested compounds exhibited better and significant serum cholesterol lowering activity with reference to the control group. Compound **4e** possessed the highest cholesterol lowering activity followed by **4h**, **4i**, and **4n**. The tested compounds also

reduced the serum triglyceride level after the 21st day, however this was found to be insignificant in comparison to the control group. Compound **4h** exhibited the highest serum triglyceride reducing activity followed by **4n** and **4e**. The tested compounds reduced elevated body weight but it was found to be insignificant when compared with the control group. The histopathology demonstrated the protective role of compounds **4h** and **4i** on the β cells of islets of Langerhans. The molecular docking showed compound **4n** binds with high energy more than pioglitazone with PPAR- γ . The physicochemical, ADME, and drug likeness properties of most of the synthesised compounds were found to be within permitted limits. Thus, the molecular architecture developed and synthesised in this study would be of great interest with good antidiabetic potential warranting further work.

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**Síntesis y actividad antifúngica *in vitro* frente a *Fusarium oxysporum* de amidas N-alquilsustituídas derivadas de 2-aminoácidos****Resumen**

Una serie de amidas N-alquilsustituídas **1-16** fueron sintetizadas a partir de malonato de dietilo y ésteres de alquilo derivados de los aminoácidos *L*-triptófano, *L*-alanina, *L*-fenilalanina y *L*-tirosina. Los métodos de síntesis empleados involucraron calentamiento por irradiación de microondas empleando tanto un ácido de Lewis (AlCl_3) o 4-dimetilaminopiridina (DMAP) como catalizador y auxiliar nucleofílico, respectivamente. Los resultados sugieren que el uso de irradiación de microondas y de DMAP conlleva mejores rendimientos en un tiempo de reacción más corto. Para ilustrar las diferencias observadas, se presentan las propuestas mecanísticas de cada método de reacción para la formación de amidas N-alquilsustituídas. Finalmente, las amidas sintetizadas se evaluaron en condiciones *in vitro* frente a *Fusarium oxysporum*; mostraron actividad antifúngica a diferentes niveles ($0,40 \text{ mM} < \text{IC}_{50} < 29,1 \text{ mM}$), lo cual indicó que las variaciones de la actividad observada de este grupo de compuestos pueden deberse al efecto de la amida acíclica como bioisótero no clásico de algunas fitoalexinas heterocíclicas.

Palabras clave: amidas N-alquilsustituídas; derivados de 2-aminoácidos; *Fusarium oxysporum*.

Synthesis and *in vitro* antifungal activity against *Fusarium oxysporum* of N-alkyl-substituted amides derived from 2-amino acids**Abstract**

N-alkyl substituted amides **1-16** were synthesized from diethyl malonate and alkyl esters derived from the amino acids *L*-tryptophan, *L*-alanine, *L*-phenylalanine, and *L*-tyrosine. In addition, a microwave-assisted protocol was employed using a Lewis acid (AlCl_3) or dimethylaminopyridine (DMAP) as a catalyst and nucleophilic auxiliary, respectively, affording the desired compounds. The results suggest that DMAP-catalyzed reactions under microwave irradiation yield higher during short reaction times. Each reaction method's mechanistic proposals for forming N-alkyl-substituted amides are presented to illustrate the observed differences. The synthesized amides were evaluated under *in vitro* conditions against *Fusarium oxysporum*. The compounds exhibited antifungal activity at different levels ($0.40 \text{ mM} < \text{IC}_{50} < 29.1 \text{ mM}$). These results indicated that the observed activity variations of this compound group might be due to the effect of acyclic amide as a non-classical bioisostere of some heterocyclic phytoalexins.

Keywords: N-alkyl-substituted amides; 2-amino acid derivatives; *Fusarium oxysporum*.

Síntese e atividade antifúngica *in vitro* contra *Fusarium oxysporum* de amidas N-alquil-substituídas derivadas de 2-aminoácidos**Resumo**

Uma série de amidas N-alquil substituídas foram sintetizadas a partir de malonato de dietila e ésteres alquílicos derivados dos aminoácidos *L*-triptofano, *L*-alanina, *L*-fenilalanina e *L*-tirosina. Os métodos de síntese empregados foram realizados usando aquecimento por irradiação de micro-ondas empregando um ácido de Lewis (AlCl_3) ou dimetilaminopiridina (DMAP) como catalisador. Os resultados sugerem que a irradiação de micro-ondas usando DMAP leva a melhores rendimentos em um tempo de reação mais curto. Para ilustrar as diferenças observadas, são apresentadas as propostas mecanísticas de cada método de reação para a formação de amidas N-alquilsustituídas. Finalmente, as amidas sintetizadas foram avaliadas *in vitro* contra *Fusarium oxysporum*, mostrando atividade antifúngica em diferentes níveis ($0.40 \text{ mM} < \text{IC}_{50} < 29.1 \text{ mM}$), o que indica que as variações observadas na atividade desse grupo de compostos podem ser devidas ao efeito de amida acíclica como um bioisótero não clássico de algumas fitoalexinas heterocíclicas.

Palavras-chave: amidas N-alquilsustituídas; Derivados de 2 aminoácidos; *Fusarium oxysporum*.



Introducción

Las fitoalexinas son metabolitos secundarios (actualmente conocidos como especializados) producidos por las plantas para protegerse contra patógenos, metales pesados y radiación ultravioleta; se constituyen como compuestos cruciales de los mecanismos de defensa de las plantas [1]. Entre las fitoalexinas liberadas por las crucíferas (Brassicaceae), la brassinina tiene un papel fundamental, no solo como respuesta defensiva, sino también en la vía biosintética de aquellas derivadas de *L*-triptófano, dado que es un precursor importante de varias fitoalexinas, por ejemplo, ciclobrassinina, brasillexina, espirobrassinina, entre otras [2]. Este tipo de compuestos ha generado gran importancia por su actividad biológica frente a patógenos como *Alternaria brassicicola*, *Botrytis cinerea*, *Leptosphaeria maculans*, *Rhizoctonia solani*, entre otros [3], [4]. Recientemente, el grupo Integrado de Investigaciones en Química Bioorgánica (InQuiBio), de la Universidad Militar Nueva Granada, ha orientado sus investigaciones hacia la síntesis de análogos de fitoalexinas indólicas, utilizando 2-aminoácidos convencionales como precursores sintéticos, los cuales puedan comportarse como bioisómeros de fitoalexinas heterocíclicas para ser usados como agentes antifúngicos contra *Fusarium oxysporum* [5]-[8]. Estos antecedentes han inspirado la búsqueda de nuevas moléculas bioactivas que tengan núcleos estructurales interesantes, de los cuales ya se cuenta con un conocimiento previo sobre su potencial actividad biológica.

Dando continuidad a nuestro propósito, hemos estudiado varios grupos funcionales; buscando identificar su relevancia en la actividad biológica de las fitoalexinas. Particularmente, hemos iniciado la exploración de grupos funcionales nitrogenados, como las bases de Schiff y en especial las amidas *N*-alquilsustituidas, las cuales han sido identificadas como farmacóforos [9]-[14]. Dada su relevancia, la síntesis de amidas se ha convertido en un tema de investigación relevante en la síntesis química, dirigido al desarrollo de moléculas biológicamente activas con potenciales actividades anticancerígenas, antibacteriales, analgésicas, antiinflamatorias y antifúngicas [15]-[21]. Las metodologías tradicionales para la síntesis de amidas involucran el uso de aminas primarias y secundarias, y derivados de ácidos carboxílicos activados [22], por ejemplo, cloruros de acilo o anhídridos. Sin embargo, el uso de agentes de acoplamiento C-N, a partir de la activación del grupo ácido carboxílico, como la formación de ésteres activados con carbodiimidas, con EDC y DCC, la formación de cloruro de ácido con cloruro de tionilo y cloruro de oxalilo como reactivos preferidos, y CDI, cloruro de pivaloilo (PivCl), cloroformiato de isobutilo (IBCF) y anhídrido de ácido *n*-propanofosfónico (T3P) para la preparación de anhídridos mixtos [23], resulta tóxico, costoso y genera gran cantidad de residuos nocivos para el medioambiente. Algunas de estas metodologías suelen presentar dificultades debido a los tiempos largos de reacción y bajos rendimientos, y dan lugar a grandes cantidades de subproductos que conllevan procedimientos de purificación difíciles [24]. Por tal motivo, en los últimos años se han planteado nuevas estrategias que involucran el uso de catalizadores ácidos de Lewis o empleando catálisis básica mediante aminas [25]-[27]. Por otro lado, la utilización de la irradiación por microondas (MW) ha permitido mejoras significativas en los porcentajes de rendimiento y cortos tiempos de reacción [28], [29], específicamente para la síntesis de *N*-acilendiaminas [30], [31], amidas monosustituidas [32], amidas *N*-heterocíclicas [33], entre otras.

Basándonos en los antecedentes descritos anteriormente y la estructura molecular de algunas fitoalexinas indólicas biológicamente activas, en este artículo se discute la síntesis asistida por irradiación de microondas, de amidas *N*-alquilsustituidas derivadas de 2-aminoácidos (*i. e.*, *L*-triptófano, *L*-alanina, *L*-fenilalanina y *L*-tirosina); se evalúa el uso de tricloruro de aluminio (que actúa como catalizador) y el de 4-dimetilaminopiridina (con un rol como auxiliar nucleofílico). Se discuten las propuestas mecanísticas de cada método de reacción para la formación de amidas *N*-alquilsustituidas. Finalmente, los compuestos obtenidos fueron evaluados frente a *Fusarium oxysporum* en un medio suplementado; se determinó el valor del IC₅₀ de cada compuesto, demostrando su actividad antifúngica a diferentes niveles, lo cual se interpretó en razón del efecto de la amida acíclica como bioisómero de fitoalexinas heterocíclicas.

Materiales y métodos

Información general

Todos los reactivos y productos químicos se adquirieron comercialmente (Merck KGaA y/o Sigma-Aldrich) y fueron empleados sin purificación adicional. Todos los compuestos obtenidos fueron purificados mediante cromatografía flash empleando sílica gel (Mesh 230-400) y como fase móvil éter de petróleo/acetato de etilo en proporción variable. La pureza de los compuestos se comprobó mediante cromatografía en capa fina realizada con láminas de aluminio Merck Silica Gel 60 F254. Las manchas se detectaron por su absorción bajo luz UV (254 nm) y relevado empleando yodo molecular, ninhidrina y sulfato cérico amónico. Los experimentos de RMN unidimensionales y bidimensionales se registraron en un espectrómetro Bruker Avance III HD Ascend de 400 MHz utilizando CDCl₃ como disolvente con tetrametilsilano (TMS) como patrón interno a temperatura ambiente. Los desplazamientos químicos se indican en δ (ppm) con respecto al TMS y las constantes de acoplamiento *J* se indican en hercios (Hz). Los experimentos de cromatografía líquida acoplada a espectrometría de masas (LC/MS) para corroborar la pureza de las muestras se realizaron en un espectrómetro LCMS 2020 (Shimadzu, Columbia, MD), que comprende un sistema de cromatografía líquida de alto rendimiento (HPLC) Prominence acoplado a un analizador de cuadrupolo simple con ionización por electrospray (ESI). Se utilizó una columna Synergi (150 × 4,6 mm, 4,0 μ m) para el análisis a 0,6 mL/min utilizando mezclas de acetonitrilo (A) y ácido fórmico al 1% v/v (B) en elución en gradiente. La ESI-MS se operó simultáneamente en los modos de iones positivos y negativos (barrido 100-2000 *m/z*), una temperatura de la línea de desolvatación a 250 °C, nitrógeno como gas nebulizador a 1,5 L/min, un gas de secado a 8 L/min, y un voltaje del detector a 1,4 kV, determinando el espectro de masas de cuatro compuestos seleccionados aleatoriamente. Se registraron datos de masa exacta a los compuestos **12**, **14**, **15** y **16**, mediante mediciones por MS de alta resolución (HRMS) en un espectrómetro de masas micrOTOF-Q II (Bruker, Billerica, MA, EE. UU.). La ESI también se operó en los modos de iones positivos y negativos (barrido 100-2000 *m/z*), una temperatura de la línea de desolvatación a 250 °C, nitrógeno como gas nebulizador a 1,5 L/min, un gas de secado a 8 L/min, una energía cuadrupolar a 7,0 eV y una energía de colisión a 14 eV. La rotación óptica específica para cada compuesto se determinó con un polarímetro Jasco P-2000 (Jasco Co., Ltd., Mary's Court, PA, EE. UU.) en una celda de cuarzo (1,0 cm).

Síntesis de amidas N-alquilsustituidas

La síntesis de las amidas **1-16** se realizó empleando dos métodos bajo irradiación de microondas (MW): en el método A se empleó un ácido de Lewis como el tricloruro de aluminio (AlCl₃) y en el método B se utilizó un auxiliar nucleofílico como la 4-dimetilaminopiridina (DAMP).

Método A. Síntesis de amidas con ácido de Lewis (AlCl₃)

Las amidas se prepararon basándose en la metodología previamente reportada en la literatura con algunas modificaciones [34]. Los 2-aminoésteres se prepararon bajo irradiación por microondas (MW): se mezclaron los 2-aminoácidos (1 mmol) con TMSCl (4 mmol) y el alcohol respectivo (1 mL). La mezcla se calentó a 120 °C durante 20 min bajo irradiación de MW en atmósfera cerrada. Posteriormente, se dispuso la mezcla en un balón de fondo redondo y se concentró a presión reducida. El 2-aminoéster de alquilo, en forma de clorhidrato crudo, se dispuso en un tubo reactor del equipo de MW, se adicionó Et₃N (4 mmol) y malonato de dietilo (DEM: 1 mmol), tolueno

(1,5 mL), AlCl_3 (0,2 mmol) y ácido acético (0,5 mmol). La mezcla de reacción se llevó a irradiación por MW a 150 °C por 20 min. Finalizado este tiempo, y al no detectar el reactivo de partida por CCD, el crudo de reacción se acidificó con HCl diluido hasta obtener un pH débilmente ácido (entre 5 y 6). A continuación, se realizó extracción con acetato de etilo (5×10 mL), se secó con sulfato de sodio anhidro y, posteriormente, se concentró bajo presión reducida. El crudo de reacción se purificó por cromatografía en columna flash, eluyendo con mezclas de éter de petróleo/acetato de etilo de polaridad variable, para dar los productos **1-16**. Los rendimientos de cada compuesto se muestran en la Figura 2.

Método B. Síntesis de amidas con DAMP

Los 2-aminoésteres se prepararon bajo irradiación por MW: se mezclaron los 2-aminoácidos (1 mmol) con TMSCl (4 mmol) y el alcohol respectivo (1 mL). La mezcla se calentó a 120 °C durante 20 min bajo irradiación de MW en atmósfera cerrada. La mezcla de reacción se concentró a presión reducida y se redisolvió en THF (1 mL). Por último, esta disolución se calentó a 140 °C durante 20 min bajo MW tras la adición de DEM (1 mmol) y DMAP (0,5 mmol). La mezcla de reacción se llevó a sequedad por destilación al vacío y el crudo se purificó por cromatografía en columna flash; se eluyó con mezclas éter de petróleo-acetato de etilo de polaridad variable, para obtener los productos **1-16**. Los rendimientos de los compuestos se exponen a continuación.

N^a -(3-etoxi-3-oxopropanoil)-L-triptofanato de metilo (**1**, $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 8,12 (s, 1H); 7,54 (d; $J = 7,9$ Hz; 1H); 7,35 (d; $J = 8,1$ Hz; 1H); 7,26 (s; 1H); 7,21–7,16 (m; 1H); 7,13–7,08 (m; 1H); 7,06 (d; $J = 2,4$ Hz; 1H); 4,93 (dt; $J = 7,5$; 5,6 Hz; 1H); 4,12 (q; $J = 7,1$ Hz; 2H); 3,69 (s; 3H); 3,34 (t; $J = 4,9$ Hz; 2H); 3,28 (d; $J = 3,6$ Hz; 2H); 1,61 (s; 3H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 172,2; 169,0; 164,9; 136,2; 127,7; 123,0; 122,3; 119,8; 118,7; 111,4; 110,1; 77,5; 77,2; 76,8; 61,7; 53,2; 52,6; 41,5; 29,8; 27,7; 14,1; $[\alpha]^{25}_{\text{D}} = +5,89^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-triptofanato de etilo (**2**, $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_5$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 8,27 (s; 1H); 7,55 (d; $J = 7,9$ Hz; 1H); 7,50 (d; $J = 7,5$ Hz; 1H); 7,33 (d; $J = 8,1$ Hz; 1H); 7,19–7,14 (m; 1H); 7,09 (t; $J = 7,6$ Hz; 1H); 7,04 (d; $J = 2,4$ Hz; 1H); 4,92 (dt; $J = 7,6$; 5,6 Hz; 1H); 4,14–4,07 (m; 4H); 3,34 (t; $J = 5,7$ Hz; 2H); 3,26 (d; $J = 2,8$ Hz; 2H); 1,21 (td; $J = 7,1$; 4,4 Hz; 6H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,8; 168,9; 165,0; 136,2; 127,8; 123,0; 122,2; 119,6; 118,7; 111,3; 110,1; 61,7; 53,3; 41,5; 27,7; 14,2; $[\alpha]^{25}_{\text{D}} = +9,55^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-triptofanato de isopropilo (**3**, $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_5$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 8,79 (d; $J = 7,9$ Hz; 1H); 7,58–7,53 (m; 2H); 7,31 (d; $J = 8,0$ Hz; 1H); 7,19–7,08 (m; 1H); 7,00 (s; 1H); 4,92 (dq; $J = 27,6$; 6,3 Hz; 2H); 4,07 (q; $J = 7,1$ Hz; 1H); 3,31 (qd; $J = 14,8$; 5,8 Hz; 2H); 1,21–1,15 (m; 8H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 172,3; 169,9; 167,0; 69,1; 48,5; 42,5; 21,7; 17,8; $[\alpha]^{25}_{\text{D}} = +2,46^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-triptofanato de n-butilo (**4**, $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_5$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 8,69 (t; $J = 3,5$ Hz; 1H); 7,53 (d; $J = 7,9$ Hz; 2H); 7,47 (d; $J = 7,5$ Hz; 0H); 7,31 (d; $J = 8,1$ Hz; 1H); 7,15 (t; $J = 7,5$ Hz; 1H); 7,08 (t; $J = 7,4$ Hz; 1H); 7,00 (d; $J = 2,3$ Hz; 1H); 4,93 (q; $J = 5,9$ Hz; 1H); 4,14–4,00 (m; 4H); 3,32 (t; $J = 5,8$ Hz; 1H); 3,24 (d; $J = 2,2$ Hz; 2H); 1,58–1,48 (m; 3H); 1,37–1,23 (m; 3H); 1,19 (t; $J = 7,1$ Hz; 1H); 0,89 (q; $J = 7,3$ Hz; 4H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,8; 168,8; 165,1; 136,2; 127,6; 123,1; 121,9; 119,4; 118,4; 111,4; 109,5; 65,4; 61,6; 53,3; 41,4; 30,4; 27,6; 19,0; 13,7; $[\alpha]^{25}_{\text{D}} = +5,13^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-fenilalaninato de metilo (**5**, $\text{C}_{15}\text{H}_{19}\text{NO}_5$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,48 (d; $J = 7,6$ Hz; 1H); 7,33–7,10 (m; 5H); 4,87 (dt; $J = 7,5$; 6,0 Hz; 1H); 4,18 (q; $J = 7,1$ Hz;

2H); 3,72 (s; 3H); 3,29 (d; $J = 2,7$ Hz; 2H); 3,22–3,04 (m; 2H); 1,26 (t; $J = 7,2$ Hz; 4H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,8; 168,9; 164,8; 135,9; 129,4; 128,7; 127,3; 61,8; 53,6; 52,5; 41,4; 37,9; 14,1; $[\alpha]^{25}_{\text{D}} = +6,50^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-fenilalaninato de etilo (**6**, $\text{C}_{16}\text{H}_{21}\text{NO}_5$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,46 (d; $J = 7,6$ Hz; 1H); 7,36–7,09 (m; 4H); 4,85 (dt; $J = 7,6$; 6,1 Hz; 1H); 4,24–4,07 (m; 4H); 3,30 (d; $J = 2,2$ Hz; 1H); 3,14 (qd; $J = 13,9$; 6,1 Hz; 1H); 1,29–1,23 (m; 6H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,3; 168,9; 164,8; 136,0; 129,5; 128,6; 127,2; 61,8; 61,7; 60,5; 53,6; 41,5; 38,0; 21,2; 14,1; $[\alpha]^{25}_{\text{D}} = +1,50^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-fenilalaninato de isopropilo (**7**, $\text{C}_{17}\text{H}_{23}\text{NO}_5$)

Aceite amarillo pálido; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,60 (dd; $J = 11,5$; 7,7 Hz; 1H); 7,25 (p; $J = 6,9$ Hz; 3H); 7,14 (t; $J = 6,0$ Hz; 2H); 6,40 (d; $J = 8,1$ Hz; 1H); 5,03–4,93 (m; 1H); 4,79 (td; $J = 21,4$; 6,7 Hz; 1H); 4,09 (q; $J = 7,1$ Hz; 2H); 3,07 (q; 4H); 1,96 (s; 3H); 1,27–1,11 (m; 9H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,3; 169,8; 166,7; 136,0; 129,3; 128,4; 126,9; 69,2; 60,3; 53,7; 53,3; 37,8; 22,9; 21,7; 21,6; 14,1; $[\alpha]^{25}_{\text{D}} = -1,73^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-fenilalaninato de n-butilo (**8**, $\text{C}_{18}\text{H}_{25}\text{NO}_5$)

Aceite amarillo pálido; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,48 (d; $J = 8,2$ Hz; 2H); 7,31–7,09 (m; 5H); 6,19 (t; $J = 7,7$ Hz; 1H); 4,91–4,77 (m; 5H); 4,09 (dtd; $J = 12,5$; 7,2; 6,5; 3,6 Hz; 7H); 3,13 (d; $J = 7,5$ Hz; 2H); 1,97 (s; 3H); 1,61–1,50 (m; 7H); 1,36–1,22 (m; 9H); 1,22–1,07 (m; 1H); 0,91 (q; $J = 7,0$ Hz; 3H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,9; 171,4; 167,0; 166,7; 136,0; 129,3; 129,3; 128,5; 127,1; 77,5; 77,2; 76,8; 65,4; 53,7; 53,2; 37,9; 30,5; 23,1; 19,1; 13,7; $[\alpha]^{25}_{\text{D}} = +8,86^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-tirosinato de metilo (**9**, $\text{C}_{15}\text{H}_{19}\text{NO}_6$)

Aceite amarillo pálido; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,58 (d; $J = 7,8$ Hz; 1H); 7,26 (s; 1H); 6,97 (d; $J = 8,4$ Hz; 2H); 6,72 (d; $J = 8,1$ Hz; 2H); 4,84 (td; $J = 6,9$; 5,5 Hz; 1H); 4,17 (q; $J = 7,2$ Hz; 2H); 3,73 (s; 3H); 3,29 (d; $J = 3,4$ Hz; 2H); 3,13–2,95 (m; 2H); 1,25 (t; $J = 7,2$ Hz; 3H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 172,0; 169,0; 165,3; 155,5; 130,4; 127,2; 115,7; 61,9; 53,8; 52,6; 41,1; 37,2; 14,1; $[\alpha]^{25}_{\text{D}} = +6,89^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-tirosinato de etilo (**10**, $\text{C}_{16}\text{H}_{21}\text{NO}_6$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,51 (d; $J = 7,8$ Hz; 1H); 7,04–6,96 (m; 2H); 6,76–6,68 (m; 2H); 5,57 (s; 1H); 4,82 (dt; $J = 7,8$; 6,1 Hz; 1H); 4,18 (qd; $J = 7,1$; 1,4 Hz; 5H); 3,29 (d; $J = 2,4$ Hz; 2H); 3,13–2,97 (m; 2H); 1,28–1,24 (m; 6H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,8; 169,3; 165,3; 155,4; 130,9; 128,1; 115,9; 62,2; 54,1; 41,6; 37,6; 14,6; $[\alpha]^{25}_{\text{D}} = +11,25^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-tirosinato de isopropilo (**11**, $\text{C}_{17}\text{H}_{23}\text{NO}_6$)

Aceite amarillo; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,60 (d; $J = 7,7$ Hz; 1H); 6,95 (d; $J = 8,2$ Hz; 2H); 6,70 (d; $J = 8,7$ Hz; 2H); 4,98 (p; $J = 6,4$ Hz; 7H); 4,74 (q; $J = 6,5$ Hz; 1H); 4,12 (q; $J = 7,1$ Hz; 2H); 3,27 (d; $J = 2,5$ Hz; 2H); 3,08–2,93 (m; 2H); 1,20 (t; $J = 6,4$ Hz; 9H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,0; 168,8; 165,4; 155,7; 130,4; 126,8; 115,5; 77,5; 77,2; 76,8; 69,6; 61,8; 53,9; 41,2; 37,1; 21,8; 14,0; $[\alpha]^{25}_{\text{D}} = +4,39^\circ \pm 0,01$ (c 0,6; MeOH).

N^a -(3-etoxi-3-oxopropanoil)-L-tirosinato de n-butilo (**12**, $\text{C}_{18}\text{H}_{25}\text{NO}_6$)

Aceite amarillo pálido; RMN ^1H (400 MHz; CDCl_3) δ_{H} 7,83 (s; 1H); 7,63 (d; $J = 7,6$ Hz; 1H); 6,91 (d; $J = 8,3$ Hz; 2H); 6,69 (d; $J = 8,1$ Hz; 2H); 4,76 (q; $J = 6,5$ Hz; 1H); 4,14–4,01 (m; 5H); 3,25 (d; $J = 2,5$ Hz; 2H); 3,07–2,91 (m; 2H); 1,60–1,47 (m; 4H); 1,33–1,24 (m; 3H); 1,18 (t; $J = 7,2$ Hz; 2H); 0,86 (t; $J = 7,4$ Hz; 3H); RMN ^{13}C (100 MHz; CDCl_3) δ_{C} 171,5; 168,7; 165,6; 155,7; 130,2; 126,6; 115,5; 77,5; 77,2; 76,8; 65,5; 61,7; 53,8; 41,1; 36,9; 30,3; 18,9; 13,9; 13,6; $[\alpha]^{25}_{\text{D}} = +6,70^\circ \pm 0,01$ (c 0,6; MeOH). ESI-HRMS m/z $[\text{M}+\text{Na}]^+$, 374,1574 (calcd. 374.1574).

N^α-(3-etoxi-3-oxopropanoil)-L-alaninato de metilo (13, C₉H₁₅NO₅)

Aceite amarillo pálido; RMN ¹H (400 MHz; CDCl₃) δ_H 7,64 (d; 1H); 4,58 (q; *J* = 7,2 Hz; 1H); 4,19 (q; *J* = 7,2 Hz; 2H); 3,73 (s; 3H); 3,32 (s; 2H); 1,41 (d; *J* = 7,2 Hz; 3H); 1,27 (t; *J* = 7,2 Hz; 3H); RMN ¹³C (100 MHz; CDCl₃) δ_C 173,2; 169,3; 164,8; 61,8; 52,6; 48,3; 41,1; 18,3; 14,1; [α]_D²⁵ = -17,97° ± 0,01 (*c* 0,6; MeOH).

N^α-(3-etoxi-3-oxopropanoil)-L-alaninato de etilo (14; C₁₀H₁₇NO₅)

Aceite amarillo pálido; RMN ¹H (400 MHz; CDCl₃) δ_H 7,64 (d; *J* = 7,6 Hz; 1H); 4,57 (q; *J* = 7,2 Hz; 1H); 4,20 (qd; *J* = 7,2; 3,2 Hz; 3H); 3,32 (s; 2H); 1,43 (d; *J* = 7,2 Hz; 3H); 1,28 (td; *J* = 7,1; 4,7 Hz; 6H); RMN ¹³C (100 MHz; CDCl₃) δ_C 172,8; 169,3; 164,7; 61,8; 61,7; 48,4; 41,2; 18,4; 14,2; 14,2; [α]_D²⁵ = -21,15° ± 0,01 (*c* 0,6; MeOH). ESI-HRMS *m/z* [M+Na]⁺; 254,1007 (calcd, 254,0999).

N^α-(3-etoxi-3-oxopropanoil)-L-alaninato de isopropilo (15, C₁₁H₁₉NO₅)

Aceite amarillo pálido; RMN ¹H (400 MHz; CDCl₃) δ_H 7,71 (dd; *J* = 16,4; 7,2 Hz; 1H); 4,96 (q; *J* = 6,2 Hz; 1H); 4,51–4,37 (m; 1H); 4,05 (q; *J* = 7,1 Hz; 2H); 3,23 (d; *J* = 3,0 Hz; 1H); 1,32 (dd; *J* = 9,7; 7,2 Hz; 4H); 1,18 (dd; *J* = 8,5; 5,4 Hz; 6H); RMN ¹³C (100 MHz; CDCl₃) δ_C 172,3; 169,9; 167,0; 77,5; 77,2; 76,8; 69,1; 48,5; 42,5; 21,7; 17,8; [α]_D²⁵ = -20,98° ± 0,01 (*c* 0,6; MeOH). ESI-HRMS *m/z* [M+Na]⁺; 268,1161 (calcd, 268,1155).

N^α-(3-etoxi-3-oxopropanoil)-L-alaninato de n-butilo (16, C₁₂H₂₁NO₅)

Aceite amarillo pálido; RMN ¹H (400 MHz; CDCl₃) δ_H 7,78 (d; *J* = 7,2 Hz; 1H); 6,51 (d; *J* = 7,5 Hz; 1H); 4,48 (dt; *J* = 14,6; 7,3 Hz; 2H); 4,06 (td; *J* = 6,6; 3,7 Hz; 4H); 3,23 (s; 1H); 1,60–1,51 (m; 4H); 1,36–1,26 (m; 8H); 0,86 (t; *J* = 7,4; 2,0 Hz; 3H); RMN ¹³C (100 MHz; CDCl₃) δ_C 173,4; 172,9; 169,9; 167,1; 65,3; 48,3; 48,1; 42,4; 30,5; 23,0; 19,0; 17,7; 13,6; [α]_D²⁵ = -19,90° ± 0,01 (*c* 0,6; MeOH). ESI-HRMS *m/z* [M+Na]⁺; 282,1322 (calcd, 282,1312).

Actividad antifúngica a partir de la inhibición del crecimiento micelial de *Fusarium oxysporum*

La evaluación de la actividad antifúngica de los compuestos **1-16** se realizó por medio de la medición del crecimiento radial del micelio del fitopatógeno *Fusarium oxysporum* siguiendo una metodología previamente reportada [35]. Brevemente, en cada pozo de una placa de vidrio de 12 pozos se adicionó agar de papa-dextrosa (PDA) suplementado separadamente con los compuestos **1-16** y controles a evaluar, utilizando concentraciones en un rango de 0,1–100 mM, en un arreglo por triplicado para cada compuesto y concentración. Una vez adicionado el agar suplementado en cada pozo, se inoculó con un fragmento cilíndrico de 3 mm de diámetro y 1 mm de altura, proveniente de un cultivo activo de *F. oxysporum*. El hongo se dejó crecer en cámara húmeda hasta que el micelio del control absoluto (*i. e.*, sin tratamiento) llegó hasta el borde del pozo. Luego de la incubación, se registraron las áreas de crecimiento micelial mediante el software ImageJ y se calcularon los porcentajes de inhibición mediante la siguiente fórmula: % de inhibición = (CA-T)*(100/CA), donde CA representa el diámetro del crecimiento micelial para el control absoluto y T representa el diámetro del crecimiento micelial del tratamiento respectivo. Los datos de inhibición (%) se emplearon para construir las correspondientes curvas dosis-respuesta con el fin de calcular la concentración inhibitoria media (IC₅₀), expresada en mM para cada compuesto, usando una regresión no lineal en el programa GraphPad Prism versión 9.0 (GraphPad Software, San Diego, CA, EE. UU.) para Windows.

Resultados y discusión

Para la síntesis de amidas N-sustituidas se planteó un modelo de estudio buscando aplicar la metodología optimizada para la síntesis de enaminas

previamente descrita en la literatura [8]. En esta etapa inicial, los 2-aminoésteres fueron obtenidos mediante la reacción entre el L-triptófano y el respectivo alcohol (MeOH, EtOH) en cloruro de trimetilsililo (TMSCl), para posteriormente adicionar trietilamina (Et₃N) y malonato de dietilo (DEM). La mezcla de reacción se calentó bajo reflujo hasta que se evidenciara consumo mayoritario de los reactivos (tiempo de reacción entre 20 y 30 h). El perfil cromatográfico por CCD evidenció la presencia de múltiples bandas difíciles de separar y una banda en el origen correspondiente al aminoácido de partida. Además, el DEM fue recuperado con acetato de etilo a partir del crudo de reacción. Estos resultados permitieron concluir que el DEM es poco reactivo bajo estas condiciones y que, posiblemente, los 2-aminoésteres de alquilo derivados del L-triptófano se hidrolizaron al permanecer en una atmósfera húmeda y en tiempos prolongados de reacción, lo que permitió deducir que la metodología evaluada no era sintéticamente adecuada. Por tal motivo, fue necesario explorar otros métodos de síntesis alternativos orientados a la activación del DEM.

El uso de ácidos de Lewis se emplea para activar el grupo carbonilo de los ésteres por polarización, ya que es uno de los métodos más confiables para inducir la formación de amidas a partir de ésteres [36]. Por otro lado, la activación de esta clase de compuestos puede realizarse con un nucleófilo tipo amina; por ejemplo, DMAP. Este compuesto ha sido utilizado ampliamente para muchas reacciones orgánicas, como la reacción de Baylis-Hillman, la síntesis de β-lactamas monocíclicas de N-sulfonilo [37] y de β-cetoamidas a partir de β-cetoésteres [38]. Teniendo en cuenta estos antecedentes, después de obtener los 2-aminoésteres de alquilo bajo irradiación por MW, se evaluó el uso tanto de AlCl₃ como de DMAP, para lo cual se plantearon dos experimentos: en el primero, se preparó una mezcla con DEM, tolueno, AlCl₃ y ácido acético (método A), la cual se dispuso en un tubo de reacción y se irradió bajo MW a diferentes temperaturas (80, 100, 120 y 150 °C) y a diferentes tiempos de reacción. Los resultados del seguimiento de las reacciones por CCD permitieron establecer que el DEM reaccionó completamente a 150 °C durante 20 min. Después de este tiempo, la reacción se acidificó con HCl diluido, hasta pH entre 5-6, para liberar el AlCl₃ y, posteriormente, se realizó extracción con acetato de etilo (5× 10 mL). El crudo de reacción se concentró a sequedad y fue purificado por cromatografía en columna, separando el producto mayoritario; en el segundo experimento (método B), se empleó DMAP como auxiliar nucleofílico. Así, después de obtener los 2-aminoésteres de alquilo, se adicionó Et₃N para neutralizar el exceso de HCl y liberar el grupo amino y mantenerlo como base libre, y garantizando medio básico. Posteriormente, se adicionó DEM y DMAP y se irradió bajo MW a distintas temperaturas (100, 120 y 150 °C) y a diferentes tiempos de reacción. Los resultados del seguimiento de las reacciones por CCD indicaron que el DEM reaccionó completamente a 140 °C y 20 min, y se formó el mismo producto que en el método A.

A continuación, se describe la caracterización por espectroscopía del compuesto **1**, el cual fue aislado y purificado tanto para el método A como para el método B. En el espectro de RMN ¹H del compuesto **1** se observaron señales que corresponden a hidrógenos aromáticos del grupo indol en la región de desplazamiento químico entre 7,00 y 7,50 ppm. A δ_H 4,93 ppm aparece una señal como multiplete que corresponde al hidrógeno del carbono quiral; la señal a campo alto como singlete a δ_H 3,63 ppm fue asignada al grupo metoxilo del éster. El fragmento etoxilo del malonato de dietilo puede verse como señales a δ_H 4,12 ppm (grupo CH₂O) y la señal triplete a δ_H 1,22 ppm del grupo CH₃. La señal tipo doblete a δ_H 7,50 ppm fue asignada al protón del grupo NH de la amida. La señal a δ_H 3,28 ppm, que aparece como doblete, corresponde al CH₂ adyacente a los grupos C=O (Figura 1a). En el espectro de RMN ¹³C del compuesto **1** se pueden apreciar 18 señales, las cuales se asignan a continuación. Las señales a δ_C 172,1; 168,9 y 164,2 ppm corresponden a los grupos carbonilo, mientras que las señales a δ_C 136,2; 127,6; 122,9; 122,3; 119,7; 118,7; 111,3 y 110,1 ppm fueron asignadas a los carbonos aromáticos del grupo indol. Así mismo, se observaron dos señales para el grupo amida, *i. e.*, el átomo de carbono directamente unido al grupo NH que resuena a δ_C 163,0 ppm y el grupo CH₂ adyacente a los dos C=O que resuena a δ_C 41,4 ppm (Figura 1b).

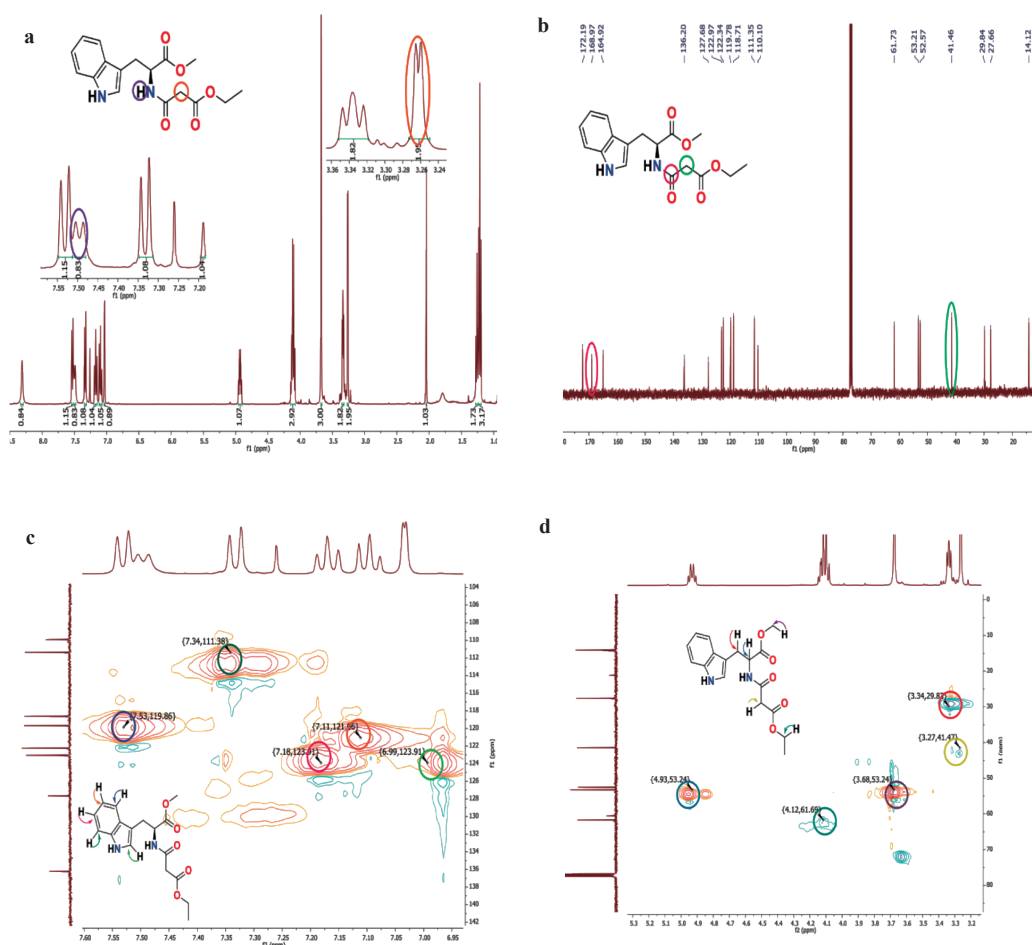


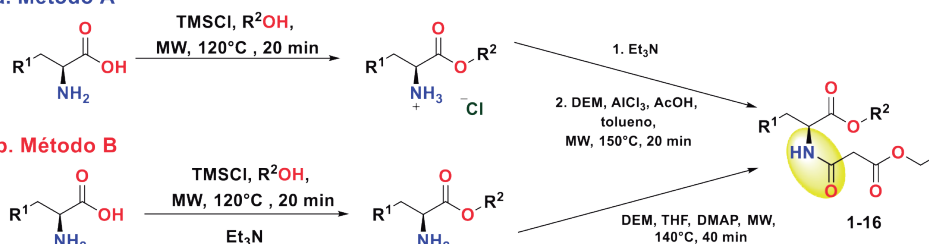
Figura 1. Experimentos RMN uni y bidimensionales del compuesto 1, a) RMN ^1H , b) RMN ^{13}C , c) HSQC, d) HMBC.

El experimento HSQC evidenció que la señal a δ_c 7,50 ppm no muestra ninguna correlación, lo que indica que esta señal corresponde al NH de la amida. La señal a δ_c 3,27 ppm mostró una correlación directa con la señal a δ_c 41,4 ppm, lo cual confirmó la asignación realizada al grupo CH_2 adyacente a los grupos $\text{C}=\text{O}$ (Figura 1c). Una vez establecidas las condiciones óptimas de reacción, estas fueron empleadas en las reacciones con los restantes precursores. Los rendimientos obtenidos para el método A se presentan en color azul y para el método B en color rojo (Figura 2). Los compuestos **2-16** fueron análogamente elucidados. Sus datos espectroscópicos se reportan en la sección Materiales y métodos. Los espectros de masas de alta resolución (HRMS) de los compuestos **12**, **14**, **15** y **16**, los cuales fueron seleccionados aleatoriamente del grupo total de compuestos para esta determinación, mostraron señales para los iones $[\text{M}+\text{Na}]^+$ con errores menores que 5 ppm, lo cual confirmó la estructura propuesta para los compuestos **1-16**.

Los rendimientos de los compuestos **1-16** variaron con respecto a la metodología empleada, a pesar de que se empleó la irradiación por MW. En el método A se observa que los rendimientos son menores del 15%, mientras que para el método B se evidencia una mejora significativa (20-60%). Los bajos rendimientos obtenidos por el método A pueden explicarse debido a la posible formación de un quelato entre el DEM y el centro metálico, lo que forma un complejo estable [39]. Es sabido que un centro metálico que se comporte como ácido de Lewis, como el AlCl_3 , tiene una fuerte tendencia a los alcóxidos metálicos para formar oligómeros con “puente alcóxido” para alcanzar números de coordinación más altos. El grado de oligomerización está inversamente correlacionado con el tamaño del grupo alcóxido. Esta tendencia a formar oligómeros se mantiene

después de la modificación con otros ligandos orgánicos como compuestos dicarbonílicos como acetilacetona, acetoacetato de etilo incluso, DEM. Lichtenberger y colaboradores [40] han reportado que, aunque DEM y otros ésteres de alquilo, derivados del ácido malónico, tienen una menor reactividad hacia los alcóxidos de aluminio a temperatura ambiente en comparación con los 2-cetoésteres, a temperaturas más altas reaccionan formando complejos estables. Dichos compuestos pueden formarse al reaccionar $\text{Al}(\text{OR})_3$ (adicionado como reactivo o formado *in situ*) con DEM en una relación estequiométrica 1:3. Dado que en nuestras condiciones de reacción estudiadas se cuenta con presencia de grupos RO^- y de un exceso de DEM, es de esperar la formación de dichos complejos, lo cual reduce significativamente el porcentaje de rendimiento de los compuestos **1-16**. Por lo tanto, a partir de los resultados por este método se deduce que la eficiencia de la reacción no está dependiendo de la naturaleza estructural del aminoácido, dado que los rendimientos son similares al comparar cada uno de dichos precursores. El mecanismo de reacción se presenta en la Figura 3. La activación catalizada por el AlCl_3 del grupo carboxilato del malonato de dietilo (DEM) inicia la reacción tras la formación del quelato, lo cual hace que se incremente la electrofilicidad del grupo $\text{C}=\text{O}$ coordinado con el aluminio y, de esta manera, permite el ataque nucleofílico del grupo NH_2 del 2-aminoéster de alquilo. Una vez ocurre este ataque, se da la formación de un intermediario catiónico, el cual cuenta con un hidrógeno ácido sobre el grupo NH_2^+ que posteriormente reacciona con Et_3N , lo que da lugar a la formación del intermediario tetraédrico. Sin embargo, este intermediario no es estable y cuenta con un buen grupo saliente como el alcóxido, el cual se libera y conlleva la formación de la amida.

a. Método A



Rendimientos (%) de Productos 1-16 por el Método A y el Método B

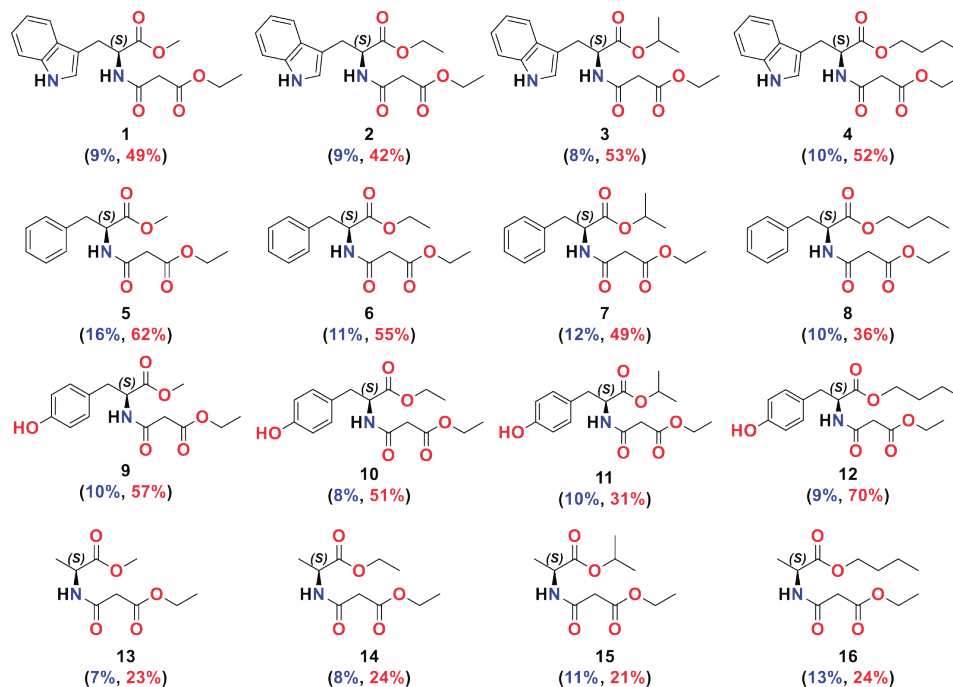
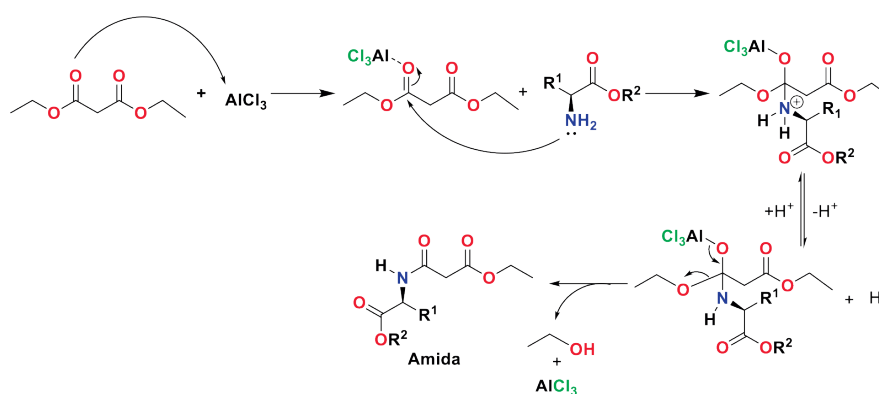

 Figura 2. Síntesis de amidas *N*-alquilsustituidas 1-16 por el método A y el método B.


Figura 3. Mecanismo de reacción propuesto para la síntesis de amidas (método A).

Para el método B, el hecho de emplear un auxiliar nucleofílico, como la DMAP, fue esencial para mejorar los rendimientos ya que, en ausencia de esta, la reacción no procedió; esto demostró una participación nucleofílica de DMAP en el avance de la reacción. Este reactivo ha sido empleado en diversas reacciones, de las cuales sobresalen las de esterificación de Steglich y la de Yamaguchi [41]-[43]. En la primera, se lleva a cabo la conversión de sustratos estéricamente impedidos usando DCC (diciclohexilcarbodiimida) y el ácido carboxílico puede formar un intermedio de *O*-acilisourea, que ofrece una reactividad similar al anhídrido de ácido carboxílico correspondiente. El alcohol puede agregarse al ácido carboxílico activado para formar la diciclohexilurea (DHU) estable y el éster. Sin embargo, los nucleófilos fuertes, por ejemplo las aminas, reaccionan fácilmente con la *O*-acilisourea, lo cual se explica por el comportamiento nucleofílico del DMAP, más fuerte que el alcohol, que reacciona con la *O*-acilisourea dando lugar a una amida reactiva. Este intermediario tipo amida no puede formar productos secundarios intramoleculares, pero reacciona rápidamente con los nucleófilos del medio, en este caso particular, con los alcoholes. Así, DMAP actúa como un reactivo de transferencia de acilo al contar con un grupo acilo polarizado. Como el DMAP es un buen grupo saliente, permite una reacción rápida con el nucleófilo del medio. En las reacciones estudiadas en el presente estudio, DEM y DAMP reaccionan dando lugar a la formación de un intermediario similar, el cual es más reactivo que el grupo éster del DEM, permitiendo el posterior ataque nucleofílico de la amina del éster de alquilo derivado del aminoácido y la salida del DMAP para dar la formación de la amida *N*-alquilsustituida. Para esta metodología se observó que, empleando el aminoácido *L*-alanina como precursor, los porcentajes de rendimiento fueron los más bajos (< 25%), lo cual puede ser explicado a partir de reacciones colaterales, tipo condensación de Claisen cruzada, que pueden ocurrir por desprotonación del hidrógeno sobre el carbono quiral, por medio de una base orgánica. Esto no se evidenció en los restantes aminoácidos; se entiende que dicho átomo de hidrógeno se encuentra menos expuesto al ataque de la base cuando la cadena lateral del aminoácido es un grupo más voluminoso que el grupo metilo. El mecanismo de reacción propuesto para el método B se presenta en la Figura 4. El DMAP hace un ataque nucleofílico sobre uno de los carboxilatos del DEM, formando un intermediario tetraédrico zwitteriónico que libera un grupo alcóxido para generar un catión 4-(dimetilamino)-1-(3-etoxi-3-oxopropanoil)piridinio estabilizado por resonancia. Posteriormente, se lleva a cabo el ataque nucleofílico del 2-aminoéster de alquilo sobre el grupo C=O tipo amida, formando el intermediario hemiacetaminal. Finalmente, dadas las condiciones básicas del medio, se da la salida del fragmento DMAP y, por ende, la formación de la amida.

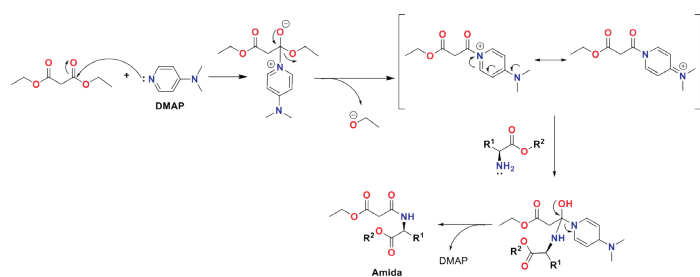


Figura 4. Mecanismo de reacción propuesto para la síntesis de amidas *N*-alquilsustituidas (método B).

La evaluación de la actividad antifúngica *in vitro* sobre *F. oxysporum* se realizó empleando el ensayo de medio suplementado a microescala [35]. Los resultados se expresaron como la concentración inhibitoria media (IC₅₀ en mM) para cada compuesto, utilizando una regresión no lineal, cuyos valores se presentan en la Tabla 1. Los valores de IC₅₀ medidos para las amidas *N*-alquilsustituidas **1-16** oscilaron entre 0,40 y 29,1 mM.

Tabla 1. Valores de IC₅₀ (mM) para las amidas *N*-alquilsustituidas **1-16** sobre *F. oxysporum*.

Compuesto	IC ₅₀ (IC) ^a
1	3,43 (2,58–4,52)
2	0,40 (0,30–0,50)
3	3,02 (2,28–3,98)
4	2,28 (1,67–3,06)
5	8,80 (5,82–13,99)
6	16,9 (12,3–25,3)
7	2,55 (2,15–3,02)
8	16,9 (11,6–21,6)
9	19,1 (14,8–25,9)
10	0,44 (0,34–0,55)
11	3,76 (2,48–5,71)
12	5,46 (3,61–8,52)
13	8,91 (6,05–13,6)
14	17,2 (12,7–24,8)
15	29,1 (21,1–43,8)
16	25,4 (16,6–46,1)
p ^b	0,02 (0,01–0,03)

^a Los valores de IC₅₀ están expresados en mM, con su intervalo de confianza (CI) al 95% entre paréntesis; ^b p = prochloraz como control positivo.

Los resultados de la actividad antifúngica *in vitro* de las amidas *N*-alquilsustituidas mostraron que **2** y **10** presentaron menores valores de IC₅₀ (0,40 y 0,44 mM, respectivamente). Estos resultados permitieron establecer que, en algunos casos, el tamaño del fragmento aromático del aminoácido podría mejorar la actividad frente a *F. oxysporum*. Sin embargo, la actividad antifúngica se vio influenciada por la cadena lateral del aminoácido precursor, dado que los compuestos más activos presentan tanto grupo 1*H*-indol como 4-hidroxifenilo. Esto sugiere que la presencia de un sistema aromático y un grupo, tanto donador como aceptor de puente de hidrógeno (*i. e.*, NH u OH), favorece la actividad antifúngica, debido a la interacción plausible con algunos objetivos bioquímicos relevantes en el metabolismo de *Fusarium oxysporum*, como se ha reportado previamente [8]. Estos resultados también pueden explicarse a favor de la presencia del grupo amida acíclica como un bioisómero no clásico de ciertos heterocíclicos (*e. g.*, triazol, tiazol, imidazol) y ditiocarbamatos, comúnmente presentes en fitoalexinas de crucíferas [44], el cual puede modular la actividad biológica como sustitutos debido al favorecimiento en el tamaño molecular, forma, distribución electrónica, polarizabilidad, lipofiliidad, polaridad, entre otros [45]. En este sentido, diversas investigaciones han demostrado que compuestos que contienen grupo amida han sido evaluados contra fitopatógenos fúngicos [46]. Wu y colaboradores [47] sintetizaron diferentes compuestos derivados de amida de pirazol que tienen un fragmento de hidrazona. Estos compuestos se evaluaron frente a *F. oxysporum*; sus porcentajes de inhibición fueron de alrededor del 40% [47]-[49]. Recientemente, nuevos derivados de amidas de pirazol fueron sintetizados por Zhang y colaboradores, los cuales fueron evaluados a escala *in vitro* sobre *F. oxysporum* a una concentración de 20 mg/mL. Los resultados obtenidos mostraron que este tipo de compuestos presentaron una moderada actividad antifúngica con porcentajes de inhibición entre 20 y 60% [50]. Pese a que resulta necesario involucrar un espacio químico más amplio para establecer un modelo de correlación estructura-actividad que permita establecer con mayor fidelidad el efecto del grupo amida, los resultados discutidos en este manuscrito evidencian un comportamiento

promisorio de este grupo funcional en derivados de 2-aminoácidos hacia un rol como toxóforo contra *F. oxysporum* en razón de su bioisosterismo no clásico en fitoalexinas.

Conclusiones

En el presente estudio se obtuvieron nuevas amidas N-sustituidas **1-16** empleando dos metodologías de síntesis. El uso de DMAP generó mejores porcentajes de rendimiento respecto al uso de AlCl_3 como ácido de Lewis. Los resultados de la actividad antifúngica frente a *F. oxysporum* de las amidas N-sustituidas **1-16** permitieron establecer que la variabilidad estructural influencia la actividad, principalmente debido a la presencia de grupos 1*H*-indol y 4-hidroxifenilo en los ésteres de etilo de los 2-aminoácidos respectivos (*i. e.*, compuestos **2** y **10**). Lo anterior demostró el potencial de esta clase de derivados de 2-aminoácidos convencionales como bioactivos antifúngicos contra *F. oxysporum* debido al bioisosterismo no clásico de la amida acíclica con fragmentos heterocíclicos de fitoalexinas.

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A computational predicting of possible inhibitors of the main SARS-CoV-2 protease found in Algerian herbal medicines

Abstract

COVID-19 is a zoonotic viral disease caused by the SARS-CoV-2 virus. Its abrupt outbreak has caused a tremendous challenge to public health systems due to the rapid spread of the virus. In this sense, a great deal of work has been focused on finding substances from herbal plants to be used against this virus. In order to investigate the molecular interactions between natural metabolites from Algerian herbal plants and the SARS-CoV-2 protease Mpro, computational docking and molecular dynamics were used, also the drug likeness degree and *in silico* ADMET prediction were carried out in this study. warfarin and catalponol preferentially binds to a pocket of the SARS-Cov-2 Mpro active site that is made up of residues His 41 to Glu 166 and Leu 27 to His 163 with a relatively low binding energy of -7.1 and -6.6 kcal/mol respectively. Dynamic molecular assay further established that only warfarin managed to stay in the active site. The results suggest that warfarin may be an interesting candidate for development as a medical treatment of COVID-19 and more research is proposed, without disregarding its toxicity which deserves to be well studied.

Keywords: SARS-CoV-2; warfarin; ADMET; molecular docking; molecular dynamics.

Una predicción computacional de los posibles inhibidores de la principal proteasa del SARS-CoV-2 que se encuentra en las hierbas medicinales argelinas

Resumen

El COVID-19 es una enfermedad zoonótica causada por el virus SARS-CoV-2. Su abrupto brote en años recientes ha supuesto un tremendo desafío para los sistemas de salud pública, como resultado de la rápida propagación del virus. En tal sentido, muchos trabajos se han centrado en encontrar sustancias de origen vegetal, para ser utilizadas contra este virus. Se realizaron estudios de acoplamiento computacional y dinámica molecular para investigar las interacciones moleculares entre los metabolitos secundarios de las plantas herbales argelinas con la Proteasa Mpro del SARS-CoV-2, también se realizaron estudios de semejanza con drogas mediante ADMET computacional. La warfarina y el catalponol se unen preferentemente al sitio activo SARS-Cov-2 Mpro que se compone de residuos His 41 a Glu 166 y Leu 27 a His 163 con una energía de enlace relativamente baja, -7,1 y -6,6 kcal/mol respectivamente. Los ensayos de dinámica molecular establecieron además que sólo la warfarina logró permanecer en el sitio activo. Estos resultados sugieren que la warfarina puede ser un candidato interesante para el desarrollo como tratamiento médico de COVID-19 e instan a realizar más investigaciones, sin dejar de lado estudios de toxicidad respectivos.

Palabras clave: SARS-CoV-2; warfarina; ADMET; docking molecular; dinámica molecular.

Um prevendo computacional de possível inibidores da principal protease SARS-CoV-2 encontrada em fitoterápicos argelinos

Resumo

A COVID-19 é uma doença zoonótica causada pelo vírus SARS-CoV-2, cujo surto abrupto nos últimos anos representou um tremendo desafio para os sistemas de saúde pública devido à rápida disseminação do vírus. Nesse sentido, muitos trabalhos têm se concentrado em encontrar substâncias de origem vegetal, para serem utilizadas contra esse vírus. Estudos de ancoragem computacional e dinâmica molecular foram conduzidos para investigar as interações moleculares entre metabólitos secundários de ervas argelinas com o SARS-CoV-2 Protease Mpro, estudos de similaridade de drogas também foram conduzidos usando ADMET *in silico*. A warfarina e o catalponol ligam-se preferencialmente ao sítio ativo SARS-Cov-2 Mpro que é composto pelos resíduos His 41 a Glu 166 e Leu 27 a His 163 com uma energia de ligação relativamente baixa, -7,1 e -6,6 kcal/mol, respectivamente. Ensaio de dinâmica molecular estabeleceram ainda que apenas a warfarina conseguiu permanecer no sítio ativo. Esses resultados sugerem que a warfarina pode ser um candidato interessante para desenvolvimento como tratamento médico para COVID-19 e exigem mais pesquisas, incluindo os respectivos estudos de toxicidade.

Palavras-chave: SARS-CoV-2; warfarina; ADMET; docking molecular; dinâmica molecular.



Introduction

On November 17, 2019, coronavirus infection (SARS-CoV-2) was recognised for the first time in Wuhan, China. After one month, 27 cases were reported on December 15 and 60 on December 20, and since then this disease known as COVID-19 has spread worldwide [1, 2]. In this sense, the main variants of coronavirus in humans are the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1) which, through severe infections, affect the respiratory system and may finally lead to a pandemic that causes a significant quantity of deaths [3-5]. In March 2020, the World Health Organisation (WHO) declared COVID-19 to be a pandemic and declared a public health emergency [5]. The main source of transmission of COVID 19 has so far been patients with pneumonia [6]. In addition, to the best of our knowledge, coronavirus infection does not yet have a specific treatment [3, 7]. Notwithstanding, some treatments have included anti-inflammatories, bronchodilators, anticoagulants, antiparasitic, antimalarial, and antiviral drugs [1, 8-12]. Nonetheless, the WHO stopped using the two antimalarial medications, chloroquine, and hydroxychloroquine, as a COVID-19 treatment due to their secondary pharmacological and toxicological effects [1, 8, 10-12]. Indeed, there are many, direct and indirect, ways in which these drugs could inhibit SARS-CoV-2, but one of them is cogitating that the drug is able to dock with a specific active site in the major protease (Mpro) of SARS-CoV-2, which has been identified as essential in the life cycle of this virus, including its replication [13].

It is commonly acknowledged that the process of discovering and developing novel drugs is time-consuming, dangerous, and expensive [14]. A quick and efficient way to find drugs that may be successful against SARS-CoV-2 is through virtual screening (VS), a computational modeling technique [15]. One of the most popular approaches in drug development is the use of big and chemically diverse compound libraries for computational and biological screening, which has encouraged the use of VS as a quick and affordable method for assessing a variety of compound collections [16]. Applications of machine learning in drug discovery and development are well highlighted [17] and analysis of the computational methods used for COVID-19 drug finding is critiqued [18]. Drug interactions with their receptors can be studied using molecular docking and molecular dynamics (MD) simulations [19]. Molecular docking enables accurate exploration of interactions between molecules and also prediction of how these molecules will fit together as well as analyses of conformational changes of particular molecules over time. MD simulations can be utilised independently [20] since, for drug repurposing, different computational studies have been carried out that target both the structural and non-structural proteins of SARS-CoV-2 [4, 21-23]. An overview of molecular docking's principles and features are highlighted [24] and the main three types of software for molecular docking are listed and described [25].

Therefore, there is an urgent need to search for active and safe antiviral drugs with broad spectrum activity that can inhibit the major protease (Mpro) of SARS-CoV-2. Among the drugs proposed as COVID-19 antiviral agents, it is possible to consider the molecules present in medicinal plants as a potentially effective source of treatment [21] when considering that nature constitutes a potential reservoir of molecules with various purposes. They are being researched for potential application as a therapy alternative for a number of disorders [26]. Considering this in these works, the proposal is to study the interaction between natural compounds against the SARS-CoV-2 protease Mpro [27].

The primary goal of this research was to evaluate the effectiveness of various phytochemicals against COVID-19 using bioinformatics tools. In total, 129 natural compounds were docked into the SARS-CoV-2 Mpro active site (Code PDB: 6LU7) to find potential inhibitors of this virus. In tandem, docking scores were obtained to separate possible active molecules from inactive ones. A prediction of Lipinski's rule of five and ADMET parameters was then performed, and finally, a molecular dynamics study was conducted on the two best molecules derived from the docking studies to determine their interaction in the presence of water molecules and ions over a period of time, to evaluate their residence time in the active site.

Materials and methods

Phytochemicals

A total of 129 phytocompounds from 34 Algerian aromatic and medicinal plants were selected. The plants were chosen on the basis of their traditional medicinal use and the amount and type of active principles present in them. The first plants selected were those used to treat respiratory infections and illnesses.

Software programmes

In this study, ChemSpider and Drugbank were used as the databases to find all molecular structures [28-29]. The RCSB Protein Data Bank's crystal structure for the receptor was used (PDB code 6LU7) [30]. All the preparation was done using Autodock tools 1.5.6 [31]. Autodock vina was used to carry out the docking study [32, 33]. The pkCSM [34] and Swissadmet [35] online servers were carried out to calculate the Lipinski's rule of five and ADMET properties [36]. Simulation of molecular dynamics MD was carried out using Gromacs 2019 package [37] and CHARMM 2017 force field. The results were analysed applying the Discovery studio 2016 package [38].

Molecular docking

Molecular docking, which is performed prior to conducting any experiments, can show that any biochemical process is feasible [39]. Generally, there is four-step procedure to perform the molecular docking, namely, target selection, Ligand selection, preparations, and docking. In this study, molecular docking was performed to predict poses in which the different phytocompounds may be in the Mpro active site. This allows analysing possible intermolecular interactions between the target and the ligand, where various orientations and conformations of the protein interacting with its ligand are generated [20].

The extended PDB format (PDBQT) was employed for ligand and enzyme preparations. Further receptor preparation includes the elimination of cofactors, water molecules, and other ligands; and the addition of polar hydrogens. To obtain the binding site, the crystallographic structure of SARS-CoV-2 Mpro (PDB code 6LU7) was imported into the programme's "work space" in Discovery Studio 2016 [40]. Based on the co-crystallised inhibitor, the active site's centre was then identified and its coordinated extracted ($x = -10.782$, $y = 15.787$, and $z = 71.277$) [22]. For the x , y , and z points, the grid size was set to $20 \times 20 \times 20$, and a grid spacing of 1 Å was established to cover the full binding pocket.

Lipinski's rule of five and ADMET prediction

The rule of five (ROF) is a general guideline for assessing drug similarity or determining whether a chemical compound with a particular pharmacological or biological activity possesses characteristics that would make it likely to be an orally active drug in people [41]. Molecular weight (≤ 500), $\log P$ (< 5), the number of rotatable bonds (< 10), the number of hydrogen bonds (HB) acceptor (≤ 10), and the number of HB donors (< 5) are among the parameters computed (values of a given property which will not be exceeded) for the Lipinski's rule of five; so, for more than one violation of the ROF, molecules will be viewed as being unimportant for future development, according the authors themselves.

In the study, ADMET was used to predict the absorption, distribution, metabolism, excretion, and toxicity parameters of the examined compounds.

Molecular dynamic simulations

Macromolecular structure-to-function relationships can now be effectively understood using a mature method called molecular dynamics simulations. Current simulation times are close to biological times and the wealth of data collected on the dynamic properties of macromolecules allows structural bioinformatics to change its standard paradigm from studying single structures to analysing conformational ensembles [42].

For this study, simulations of molecular dynamics (MD) were carried out only on the two best molecules derived from the docking studies. The main objective of MD simulations is to evaluate the stability of the ligand-enzyme complex found in the docking study. The results obtained from the docking calculation were used as input for the MD simulation. The ligand-receptor complex was prepared by adding water molecules to solvate the system and sodium and chlorine atoms to neutralise it. Prior to the simulation, the system was relaxed for 100 ps at 300 K and 1 bar. Then, simulation was run for 20 ns.

Results and discussion

Since the emergence of the Covid-19 pandemic, many phytochemicals were evaluated to find candidates which might be able to combat the primary proteins of SARS-CoV-2 [43-52]. Several of them have already been shown to display effective activity against SARS-CoV-1 Mpro [44]; others have been evaluated from Algerian herbal medicines [43], from Indian traditional medicine [45, 48-51], from traditional Vietnamese therapy [52], or from Thailand's National List of Essential Medicines [46] as well as compounds selected from the literature with a wide range of biological activities and which were chosen based on their highest activity according to Dr. Duke's phytochemical and ethnobotanical databases [47].

Molecular docking

Among the five therapeutic protein targets of SARS-CoV-2: 3CLpro (main protease), PLpro (papain-like protease), SGp-RBD (spike glycoprotein-receptor binding domain), RdRp (RNA dependent RNA polymerase), and ACE2 (angiotensin-converting enzyme 2) the poses and potential types of interactions between the 129 compounds and SARS-CoV-2 Mpro were carried out using molecular docking. The results are reported on Table 1. Among the 129 molecules docked, warfarin (compound No. 107) was previously studied by us [43].

Warfarin has the highest docking score out of all the compounds (-7.1 kcal/mol). Here it is interesting to mention that warfarin is an anticoagulant and is used to prevent thrombosis and embolism. Its anticoagulant effects are based on blocking the vitamin K-dependent (VKD) stage in the full synthesis of several blood clotting components necessary for healthy blood coagulation [53]. In consequence, interactions between warfarin and Chinese herbal medicine were reviewed and described [54]. Additionally, some features of warfarin's anti-tumor effect are thought to be a result of its antithrombotic activity. It's also crucial to note that warfarin's inflammatory and immunomodulatory actions point to a far wider range of possible consequences [55]. Additionally, warfarin toxicity and metabolism are highlighted [56].

Other than warfarin, six additional molecules were found to have interesting docking scores which include catalponol, benzyl salicylate, Prasterone, davana ether, chamazulene, and rosifoliol (Table 2). Therefore, these seven molecules underwent further study for being the best possible candidates to inhibit SARS-CoV-2 Mpro.

Some of these compounds were found to possess biological activities, such as chamazulene, which is considered a natural anti-inflammatory [57] associated to its antioxidant properties, and contributes to the *in vivo* antiphlogistic activity by inhibiting the leukotriene synthesis, and it has antitumor activity as well [58].

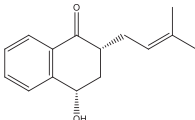
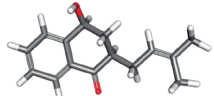
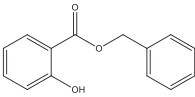
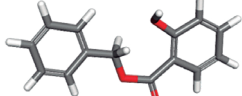
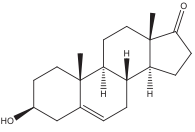
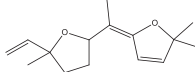
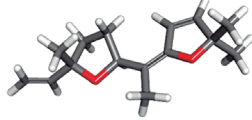
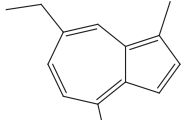
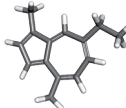
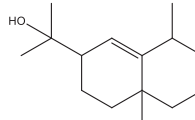
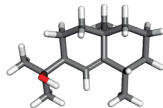
Table 1. Best conformation affinity in the SARS-CoV-2 Mpro binding pocket.

N°	Name of molecule	Score (kcal/mol)	N°	Name of Molecule	Score (kcal/mol)	N°	Name of molecule	Score (kcal/mol)
1	β-pinene	-4.6	44	Epi-cadinol	-5.9	87	7-epi-α-Eudesmol	-5.6
2	Sabinene	-4.4	45	n-Tetradecanol	-4.3	88	Spathulenol	-5.8
3	Limonene	-4.5	46	Hexadecanoic acid	-4.5	89	Rosifoliol	-6.0
4	p-Cymene	-4.6	47	Ethyl linoleate	-4.6	90	Ledene	-5.8
5	β-Myrcene	-4.8	48	Phytol	-4.8	91	n-Hexadecanoic acid	-4.4
6	(Z,E)-farnesol	-5.3	49	Neophytadiene	-4.7	92	Geranyl isovalerate	-5.3
7	Cedrol	-5.0	50	Terpenyl acetate	-5.2	93	Ledol	-5.3
8	Verbenone	-5.1	51	Tetradecanoic acid	-4.4	94	Perillaldehyde	-4.7
9	Trans-Calamenene	-5.9	52	6,10,14-trimethyl-2-pentadecanone	-4.8	95	α-Terpinyl acetate	-5.2
10	Trans, dihydrocarvone	-4.7	53	β-caryophyllene	-5.8	96	β-Selinene	-5.7
11	Geranial	-4.5	54	α-humulene	-5.7	97	Germacrene	-5.6
12	1,8-Cineol	-4.3	55	Aromadendrene	-5.3	98	β-Cadinene	-5.8
13	Acetophenone	-4.5	56	γ-Eudesmol	-5.7	99	Isoniazid	-4.8
14	α-Murolene	-5.4	57	Myristicin	-5.3	100	Methacrylonitrile	-3.2
15	β-Eudesmol	-5.7	58	Elemicin	-4.9	101	2-Propenenitrile-2-methyl	-3.2
16	Cis-peperitol acetate	-5.0	59	Linalool	-4.8	102	Diethyltoluamide	-5.0
17	γ-terpinene	-4.6	60	Benzyl salicylate	-6.6	103	Benzoic acid	-4.4
18	Germacrene D	-5.5	61	Ethyl hexadecanoate	-4.4	104	Benzene	-3.4
19	(E)-β-ocimene	-4.5	62	Decanoic acid	-4.3	105	Prasterone	-6.6
20	(Z)-β-ocimene	-4.1	63	Ethyl dodecanoate	-4.6	106	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl	-4.1
21	Carvacrol	-4.9	64	Ethyl hexadec-9-enoate	-4.9	107	Warfarin	-7.1

(Continued)

N°	Name of molecule	Score (kcal/mol)	N°	Name of Molecule	Score (kcal/mol)	N°	Name of molecule	Score (kcal/mol)
22	Davanone	-5.8	65	Dodecanoic acid	-4.6	108	Iodoquinol	-5.5
23	Davana ether	-6.1	66	Ethyl tetradecanoate	-4.3	109	Phenol,4-(2-aminopropyl)-	-4.6
24	Camphore	-4.5	67	α -Eudesmol	-5.6	110	Phenol,2-methoxy-4-(1-propenyl)-, (E)-	-5.1
25	Eucalyptol	-4.2	68	β -bourbonene	-5.3	111	Geranyl acetate	-5.2
26	Chamazulene	-6.0	69	α - terpinolene	-4.6	112	Methyl eugenol	-4.8
27	β -thujone	-4.5	70	δ -cadinene	-5.8	113	β -Linalool	-4.9
28	Catalponol	-6.6	71	1-Octen-3-ol	-3.8	114	4-Terpineol	-4.3
29	Piperitone	-4.7	72	α -copaene	-5.1	115	Elemol	-5.1
30	Borneol	-4.3	73	β -elemene	-4.9	116	β -sesquiphellandrene	-5.9
31	1-epi-cubenol	-5.8	74	1,8-cineole	-4.3	117	Bicyclogermacrene	-5.0
32	Valencene	-5.3	75	Globulol	-5.6	118	Curcumene	-5.6
33	Humulene oxide II	-5.3	76	α -phellandrene	-4.7	119	Pulegone	-5.2
34	Caryophyllene oxide	-5.5	77	β -Bisabolene	-5.6	120	Iso-pulegyl acetate	-4.9
35	Terpinen-4-ol	-4.3	78	4,8,12,16-tetramethylheptadecan-4-olid	-5.5	121	Carvone	-4.8
36	Bornyl acetate	-5.2	79	γ -Cadinene	-5.7	122	Thymol methyl ether	-4.8
37	α -Terpineol	-4.8	80	Germacrene D-4-ol	-5.7	123	Linalyl acetate	-4.8
38	Thymol	-4.7	81	β -Citronellol	-4.4	124	Allo-ocimene	-4.4
39	Cymene	-4.6	82	Trans-pinocarveol	-4.5	125	Neryl acetate	-5.1
40	Camphene	-4.0	83	Cis-verbenol	-4.4	126	α -amorphene	-5.3
41	Eugenol	-4.9	84	Manoyl oxide	-5.9	127	E-caryophyllene	-5.2
42	Isomethyl- α -ionol	-5.2	85	α -Cadinol	-5.9	128	Carvotanacetone	-4.7
43	Dihydrocarveol acetate	-5.2	86	Pinocarvone	-4.9	129	α -Pinene	-4.8

Table 2. Structures that bind to SARS-CoV-2 Mpro with the greatest affinity.

N°	Name of molecules	Plant of origin	Score (kcal/mol)	Structure 2D	Structure 3D
28	Catalponol	<i>Artemisia arborescens</i> L.	-6.6		
60	Benzyl salicylate	<i>Thapsia garganica</i> L.	-6.6		
105	Prasterone	<i>Glycyrrhiza glabra</i>	-6.6		
23	Davana ether	<i>Artemisia herba alba</i> Asso.	-6.1		
26	Chamazulene	<i>Artemisia arborescens</i> L.	-6.0		
89	Rosifoliol	<i>Pistacia atlantica</i> Desf.	-6.0		

According to Lobo [59], Prasterone has been approved by the US Food and Drug Administration (FDA) for symptoms of dyspareunia in postmenopausal women; and Heo [60] proposes intravaginal Prasterone as a treatment for vulvovaginal atrophy in postmenopausal women. Huang et al. [61] confirmed the pharmacological functions of catalponol which improved the biosynthesis of dopamine through the activation of tyrosine hydroxylase and the protection of PC12 cells cytotoxicity induced by L-DOPA.

To verify the reliability of the procedure and the software used in this work to produce reliable results, the co-crystallised ligand (N3) was re-docked into the active site and a docking score of -6.9 kcal/mol was found. Successively, the N3 structure was compared to the docked one (Figure 1a) and 16 interactions were found (Figure 1b [62]). The interactions between warfarin and the Mpro were established previously [43]. For the other interactions between compounds and SARS-CoV-2 main protease, they are shown in Figures 2 (a-f).

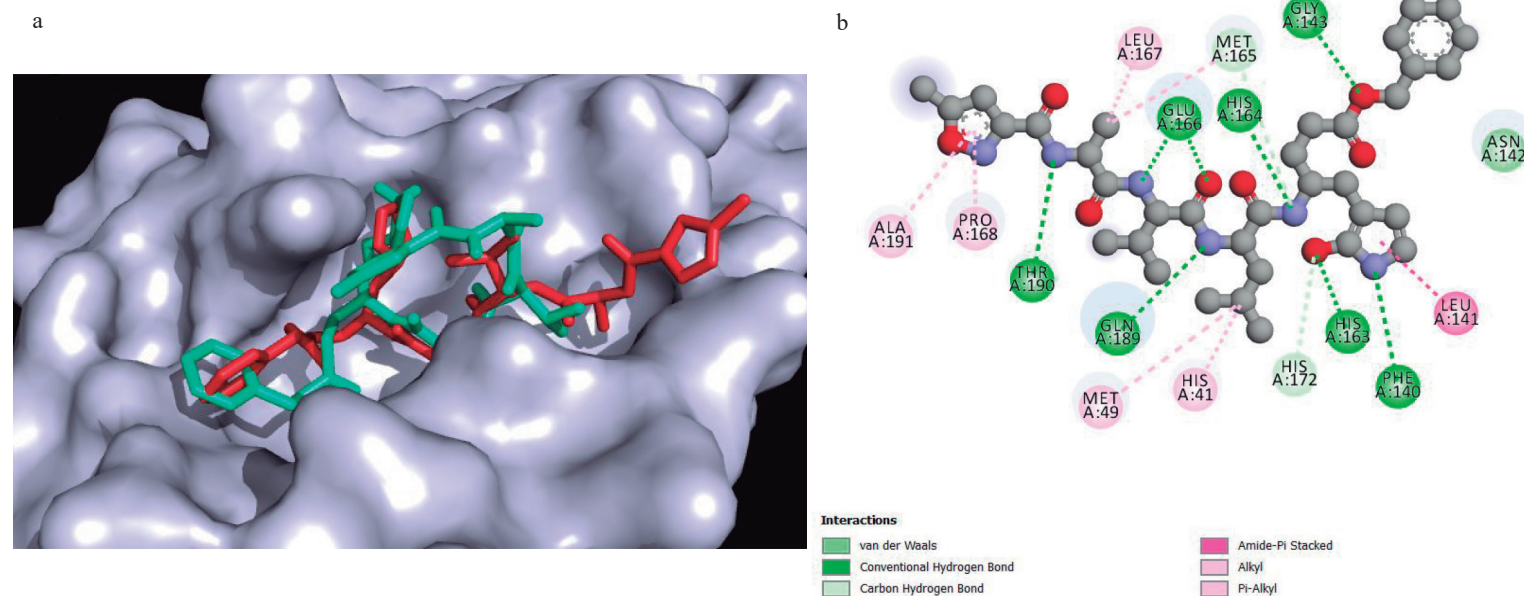
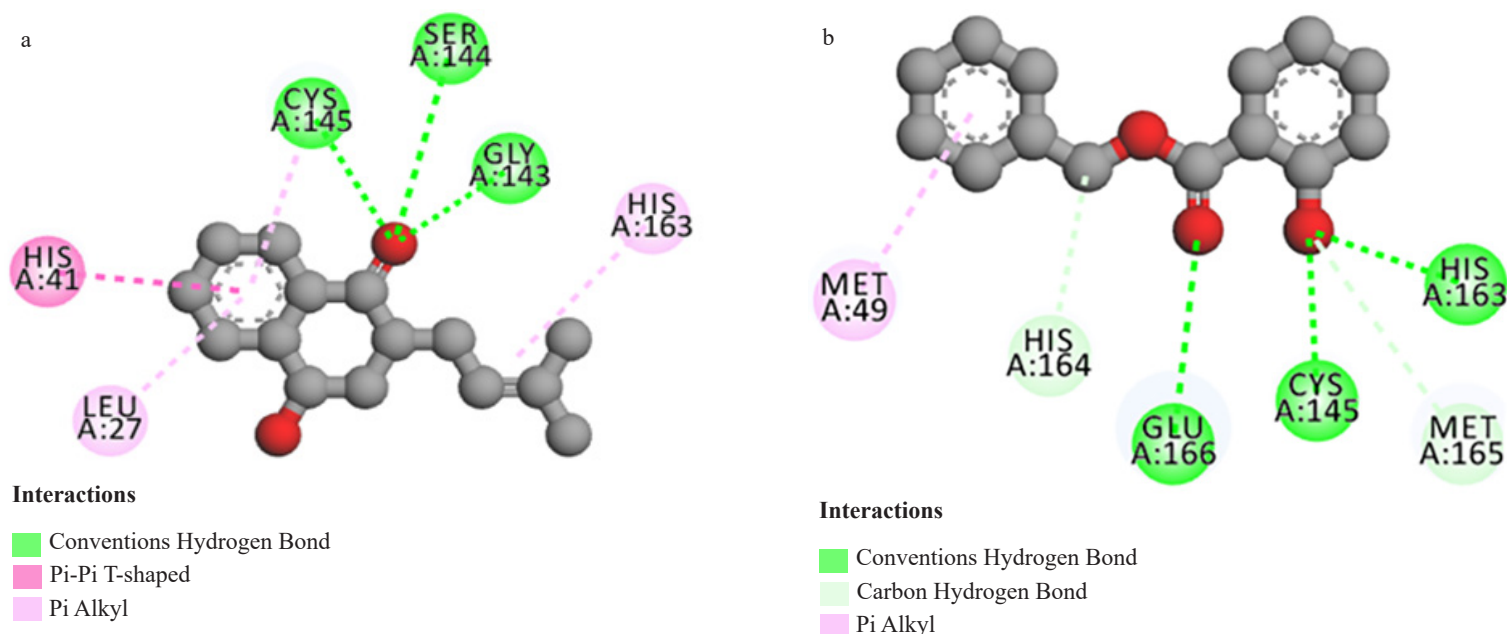


Figure 1. (a) N3 co-crystallised ligand (red) and N3 docked ligand (cyan); (b) Interactions between inhibitor N3 and SARS-CoV-2 Mpro⁵³.



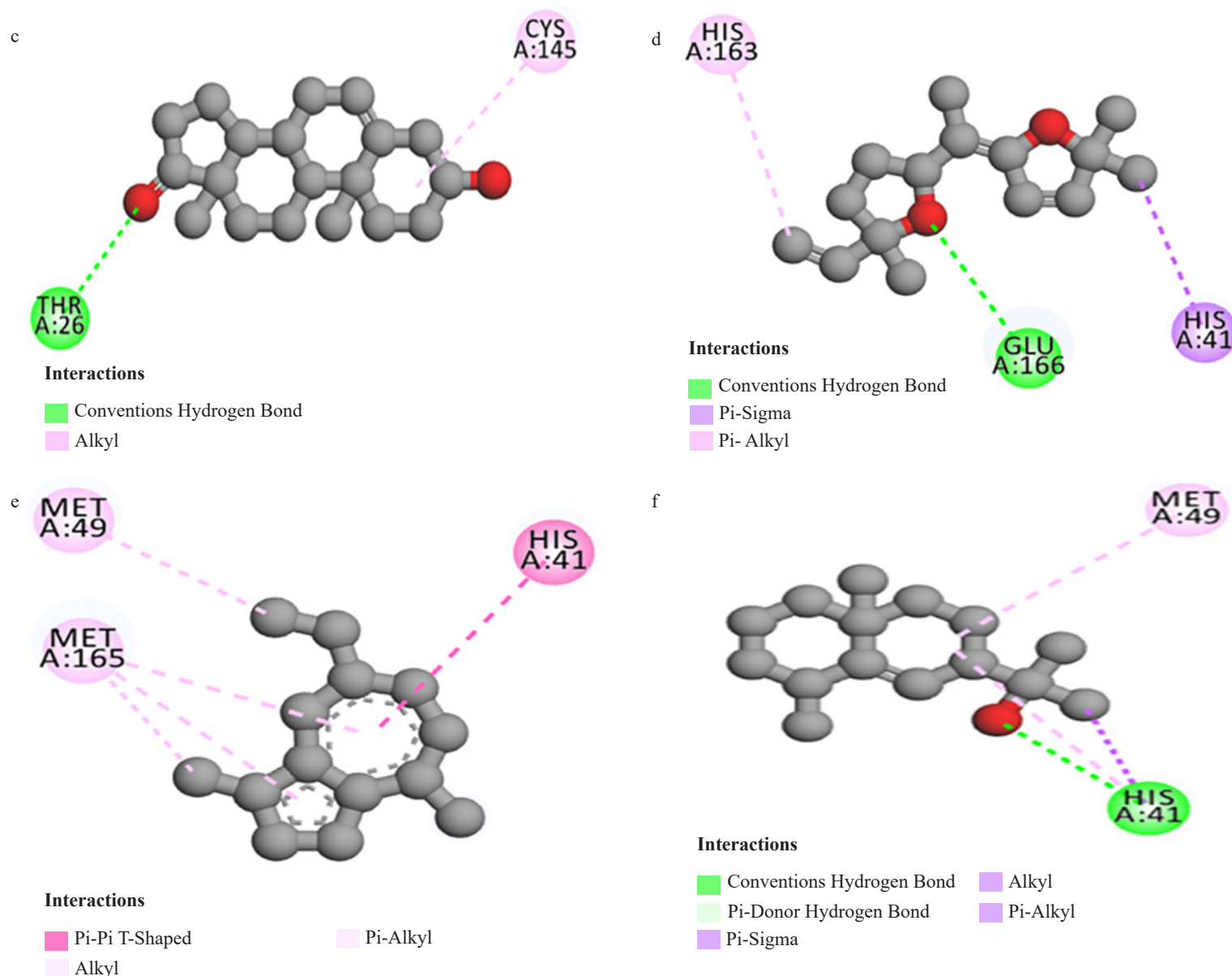


Figure 2. Interactions between Catalponol (a), Benzyl salicylate (b), Prasterone (c), Davana (d) Chamazulene (e), Rosifoliol (f) and SARS-CoV-2 main protease

The results show that both structures overlap in most of the molecule, demonstrating that the procedure employed is able to reproduce the experimental results. The difference between the two structures is noted at one end of the molecule, where the docking result shows that the chain is oriented upward due to the flexibility of the peptide losing the interaction with the receptor, while the co-crystallised structure is interacting with the whole pocket. Analysing the N3-Mpro complex, 16 interactions were found, which include a Van der Waals interaction with Asn142, HB interactions with Glu 166, His 164, Gly 143, Thr 190, Gln 189, His 163, and Phe 140 residues, amide- π Stacked interactions with Leu 141 residue, Carbon HB with Met 165 and His 172 residues, and Alkyl and/or π -Alkyl interactions with Leu 167, Ala 191, Met 167, Pro 168, Met 49 and His 41 residues [62].

This result is relevant to analyse whether the studied molecules may interact with the active site and whether those interactions may be strong enough to compete with the natural substrate inhibiting the enzyme. Based on the docking score, only warfarin shows a higher value compared to the N3 inhibitor. The docking results of the best seven molecules based on the docking score show that Warfarin interacts with five residues [43],

catalponol with five residues, benzyl salicylate with six, prasterone with two, davana ether with three, chamazulene with three, and rosifoliol with two residues. It is important to notice that the N3 inhibitor forms more than twice the interaction of any of these drug candidates which may infer a weak interaction in case any interaction at all is produced. Delving deeper into the analysis of the interactions between warfarin and the Mpro, the interactions include HB with Glu 166, Ser 144 and Cys 145 residues, π - π T-shaped with His 41 residue and π -alkyl with Met 164 residue [43]. Also, the presence of HB and π - π T-shaped interactions in the complex formed by warfarin and Mpro increase the affinity of the complex and may be related with its pharmacological activity. This is in accord with the reports in the literature where the HB role is identified with the pharmacological effect of a ligand [21,62].

The values obtained for Mpro docking with warfarin, catalponol, benzyl salicylate, prasterone, davana ether, chamazulene and rosifoliol may be related to the number and type of bonds observed in the ligand-target formed, in fact the presence of the HB suggests an important potential pharmacological effect for the Mpro inhibition of SARS-CoV-2. Thus, the inhibition of this protein will stop viral replication and may become useful

in the clinical management of COVID-19. These results are supported by many other studies where *Glycyrrhiza glabra* is used in traditional medicine to treat cough and as a laxative as mentioned by Chouitah [63]. Warfarin and catalponol, found in this study to be two of the most interesting molecules, are part of the active ingredients present in *Glycyrrhiza glabra*. Therefore, the extract of this plant could have very interesting effects on the inhibition of SARS-CoV-2 Mpro. According to Soleiman-Meigooni, liquorice (*Glycyrrhiza glabra* L.) syrup can be used as an adjuvant treatment for mild to moderate Covid-19 in addition to the recommended course of care [64]. It was found that, glyasperin A, glycyrrhizic acid, and compounds having oxane ring and chromenone ring substituted with hydroxyl 3-methylbut-2-enyl group could be considered as the best molecules and leads for the development of a new drug based on liquorice against COVID-19 [65,66].

Lipinski's Rule of five and ADMET Prediction

Using the web servers pkCSM and Swissadmet, the Lipinski's rule of five, and the ADMET parameters of the chemicals examined in this paper were computed. According to "the rule of 5", an orally active drug may have no more than one violation of the following criteria (Table 3): molecular weight less than 500; not more than 5 H-bond donors, 10 H-bond acceptors and 10 rotatable bonds; an octanol-water partition coefficient log P not greater than 5.

Table 3. Lipinski's rule of potential inhibitors.

Parameters	Log P	HB Acceptor	HB Donor	Rotatable bonds	MW g/ mol	Lipinski violations
Rule	< 5	≤ 10	< 5	< 10	≤ 500	≤ 1
Compounds						
Catalponol	2.36	2	1	2	230.30	0
Benzyl salicylate	2.80	3	1	4	228.24	0
Prasterone	3.59	2	1	0	288.42	0
Davana ether	2.54	2	0	2	234.33	0
Chamazulene	5.01	0	0	1	184.28	1
Rosifoliol	3.67	1	1	1	222.37	0

The results obtained for all the studied compounds in this work respect Lipinski's rule of five, including warfarin [43]. The N3 inhibitor has three Lipinski violations [30], which is to be expected, since it is a peptide-type inhibitor, characterised by a high MW, and many more than 10 rotational bonds. The parameters predicted (Table 4) were water solubility (WS) and human intestinal absorption (IA), volume of distribution (Vd), blood-brain barrier permeability (Bbp), cytochrome P450 (CYP450)

metabolism, total clearance (TCI), hERG I and II inhibition (hERGi) and syntetic accessibility (SA).

All the molecules exhibit a low value for the blood-brain partition coefficient. This may indicate that, there will not be any risk of central nervous system (CNS)-related toxicity because these molecules have poor potential to pass the blood-brain barrier. The WS, IA, and metabolism pharmacokinetic parameters present drug-like values with more than 92% of intestinal absorption, WS between 6.3×10^{-4} and 4.0×10^{-6} mol/l, and a hepatic metabolism for catalponol, benzyl salicylate, and Prasterone. Also, the toxicity predicted for hERG I and hERG II inhibitors indicates that any compound exhibits hERG I, while only prasterone has hERG II inhibition. SA values show the feasibility of experimentally synthesising all the studied compounds.

Molecular dynamics simulation

Although the seven studied compounds were found to have good docking scores against Mpro, following Lipinski's rule of five and exhibiting appropriate ADMET properties; only the two best compounds (warfarin and catalponol) were taken to further MD simulations to determine the possibility of keeping the ligand-receptor complex in a solvated system for a period of time. Results will increase the feasibility of these compounds for being possible COVID-19 treatments through Mpro inhibition. In Figure 3, the results for the simulation of Warfarin against Mpro is shown.

For this complex, the potential energy (Figure 3a) reaches a plateau before 500 ps showing that after this time, the system is at its lowest potential energy. The RMSD plot (Figure 3b) shows the ligand-enzyme complex maintains values lower than 0.5 nm while the ligand presents values lower than 1 nm during most part of the simulation (first 15 ns). During the last 5 ns the RMSD values in the ligands increase to around 1.1 nm. Looking at the HB formation and the number of pairs within 0.35 nm (Figures 3c and 3d) it may be observed that the maximum number of HBs and pairs is four and the minimum number is zero. In most parts of the simulation, at least one HB and one pair are formed. Finally, the Coulomb and Lennard-Jones interaction energies were evaluated (Figure 3e). While the Coulomb energies stayed constant throughout the simulation with values around -5 kcal/mol, the Lennard-Jones interaction energies increased as the simulation proceeded starting at -25 kcal/mol and ending at around -17 kcal/mol. Comparing the structure at the beginning of the simulation and at the end, it was observed that warfarin moves out of the active site cavity and stayed in the most superficial part of the pocket (Figure 4). This movement will make warfarin lose most of its potency although there is still some chance based on the interaction formed to be strong enough to compete with natural substrates.

Table 4. Computer simulation of ADMET forecast and accessibility values.

	Absorption		Distribution		Metabolism							Excretion		SA
	WS	IA	Vd	Bbp	2D6	3A4	1A2	2C19	2C9	2D6	3A4	TCI	hERGi	
	log mol/l	%	log l/kg	log Bb	yes/no							log ml/ min/kg	(I-II)	
Compounds														
Catalponol	-3.719	93.130	0.409	0.404	no	yes	yes	yes	no	no	no	0.168	No-No	3.11
Benzyl salicylate	-3.216	93.023	-0.007	0.362	no	no	yes	yes	no	no	no	0.605	No-No	1.64
Prasterone	-4.504	94.629	0.519	0.134	no	yes	no	no	no	no	no	0.936	No-Yes	4.66
Davana ether	-3.486	96.243	0.319	0.599	no	no	no	no	no	no	no	1.231	No-No	4.28
Chamazulene	-5.438	94.503	0.639	0.792	no	no	no	no	no	no	no	0.257	No-No	2.22
Rosifoliol	-4.439	92.802	0.486	0.601	no	no	no	no	no	no	no	1.029	No-No	4.14

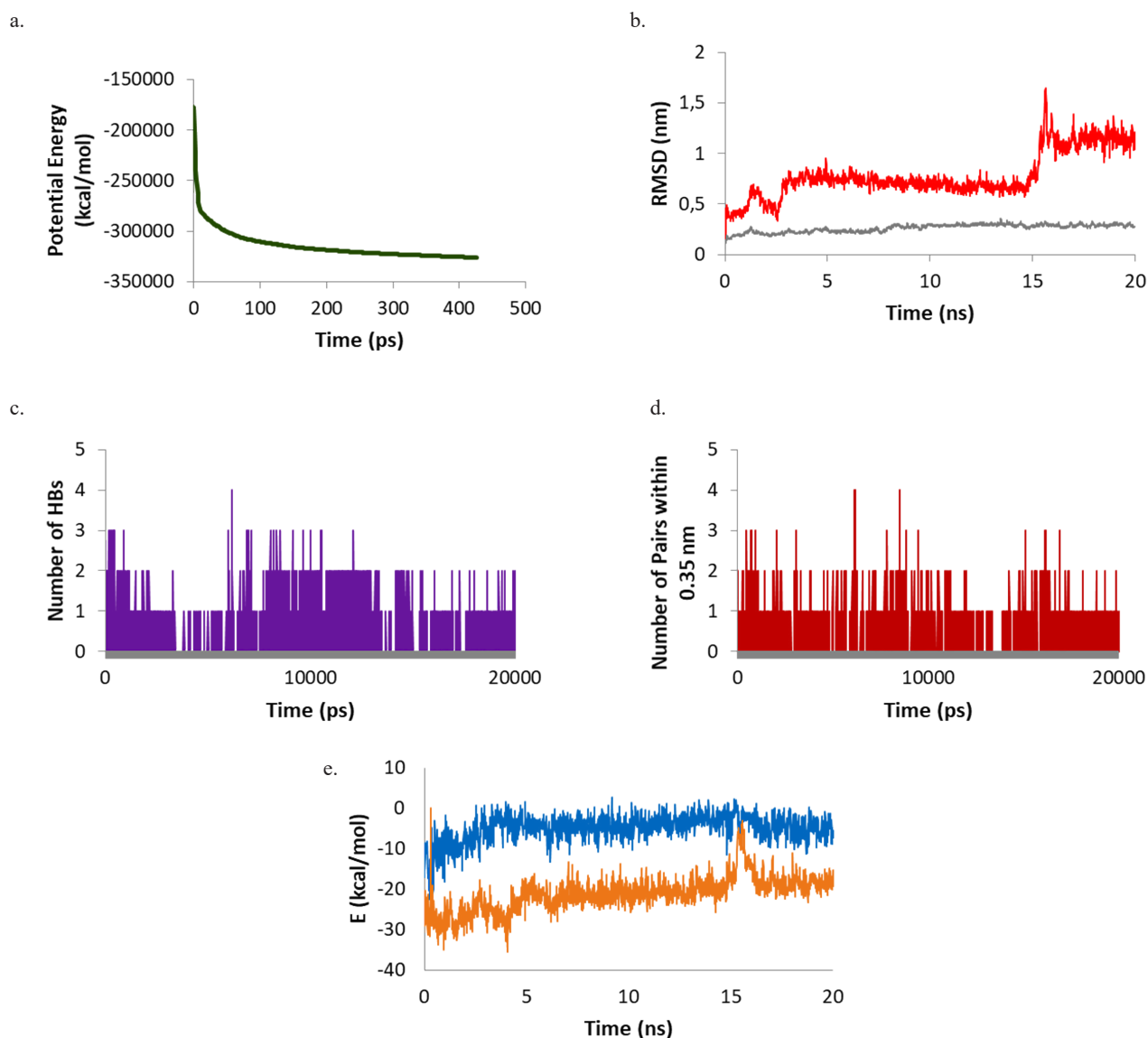


Figure 3. (a) Potential energy; (b) RMSD of Warfarin (red) and the Warfarin-Mpro complex (gray); (c) number of HBs; (d) number of pairs within 0.35 nm; (e) Coulomb (blue) and Lennard-Jones (orange) interaction energy during the simulation.

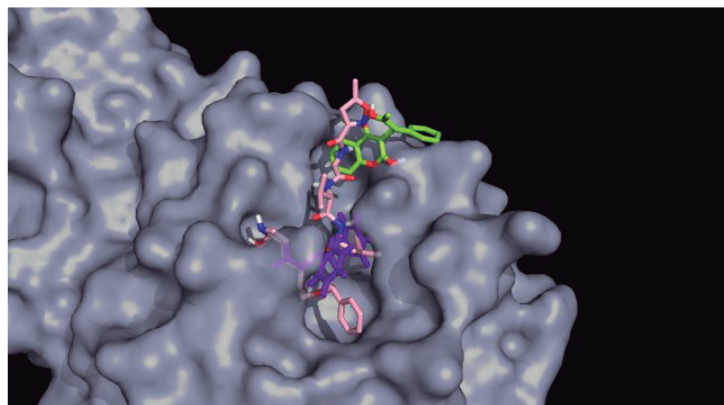


Figure 4. Docking (Purple) and MD (Green) results of the interaction between warfarin and Mpro. N3 inhibitor is shown in pink.

Analysing the simulation between catalponol and Mpro, the potential energy plateau is reached at 1181 ps. The three-dimensional structure of the complex appeared after 20 ns, the ligand could not manage to stay in the active site and leaves the system. This can be reflected in the plots found in Figure 5. The RMSD plot shows catalponol RMSD values are less than 0.5 nm in the first 5 ns of simulation. In the next 5 ns, values rise up to 2 nm. After that, in the final 10 ns, values experience a great increase reaching values of 8 nm. Looking at the HB and the number of pairs within 0.35 nm, catalponol makes interactions only during the first 10 ns of simulation. After that, all interactions are lost. In the first 10 ns, catalponol makes between 1 and 4 HBs and between 2 and 7 pairs within 0.35 nm. The same can be observed in the Coulomb and Lennard-Jones interaction energy where negative values are found up to 12 ns. After that all interaction is lost and both energies are zero.

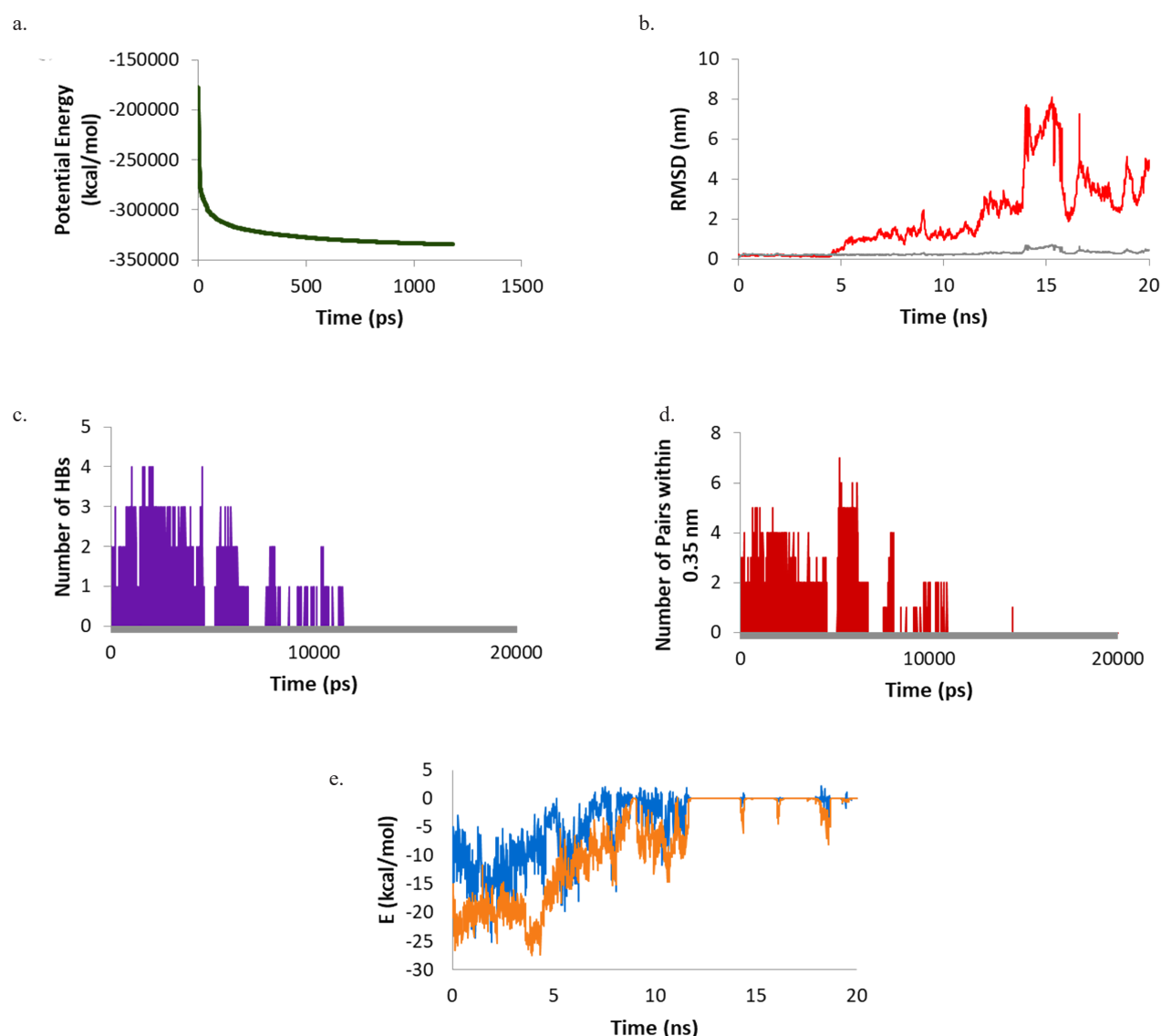


Figure 5. (a) Potential energy; (b) RMSD of catalponol (red) and the catalponol-Mpro complex (gray); (c) number of HBs; (d) number of pairs within 0.35 nm; (e) Coulomb (blue) and Lennard-Jones (orange) interaction energy during the simulation.

Conclusions

Herbal medicine, natural and safe, requires consideration by scientists. Essential oils from aromatic and medicinal plants are used by people for the treatment of infectious diseases throughout the world. Bioactive compounds contained in essential oils may be studied for drug development. Because traditional drug research and development takes a lot of time and money, computational study at multiple scales becomes a crucial instrument in drug discovery. In this sense, by using *in silico* methods, from the 129 natural compounds of Algerian essential oils, molecular docking indicated that warfarin (in this study and according our previous finding [43]), catalponol, benzyl salicylate, prasterone, davana ether, chamazulene and rosifoliol are the molecules that showed the best docking score against Mpro. Furthermore, each of them adheres to Lipinski's five requirements and provides suitable premeditated ADMET characteristics. Among these compounds, warfarin displayed the best results and presented a better docking score than Mpro inhibitor N3. A simulation time of 20 ns shows that warfarin manages to stay in the active site although it moves from the active site's cavity to a more superficial section of the active site. Furthermore, catalponol did not

stay in the active site after the simulation. Results suggest that warfarin may be an interesting candidate for performing further studies to try to find a new and effective treatment against COVID-19. However, before reaching a firm conclusion, there are still a number of measures to be taken in order to experimentally verify and validate the current result.

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Análisis de la mezcla de alcoholes en motor diésel

Resumen

Debido a que el futuro del petróleo es incierto, en la búsqueda de los combustibles alternativos se ha encontrado que el alcohol es un fuerte candidato como combustible; los alcoholes inferiores más representativos son el metanol y el etanol, los cuales han demostrado en general baja miscibilidad, por lo que pueden generar una separación de fases al ser mezclados después de un cierto tiempo. La única manera de emplearlos en un motor diésel es mezclándolos con biodiésel o con surfactantes. Por el contrario, los alcoholes superiores tienen características más adecuadas para ser mezclados o empleados en el motor diésel. Por su parte, los éteres son una buena opción cuando se trate de combustión piloto en que se considere emplear combustibles gaseosos como el biogás o el syngas en motor diésel dual. En esta investigación se exploran las alternativas que existen para mejorar las características del alcohol, por ejemplo, mezclarlo con biodiésel, biogás, agua o nanopartículas, y también se analizan las emisiones que se producen con las mezclas generadas. El objetivo es indagar las diferencias entre los alcoholes inferiores y los alcoholes superiores de cadena larga, así como las mezclas que se pueden generar para mejorar el rendimiento del motor. Los resultados afirman que los alcoholes de cadena larga tienen mejores propiedades físico-químicas que los alcoholes de cadena corta; el butanol es el único combustible que puede ser transportado y almacenado en las mismas redes de tuberías actuales de los productos petroleros existentes. También se cuenta con la opción de usar el alcohol como combustible piloto en un motor diésel dual; esto puede servir para aplicar una enorme variedad de combustibles tanto líquidos como gaseosos, lo cual hace que las opciones en su aplicación se incrementen. Como conclusión, se recomienda explorar nuevas mezclas analizando sus sinergias con varios combustibles alternativos. La posibilidad de realizar el diesterol-BED genera resultados muy alentadores, por lo que se recomienda seguir haciendo análisis sobre esa línea para encontrar la mezcla óptima.

Palabras clave: alcohol; motor diésel; energía renovable; biocombustible.

Analysis of alcohol mixture in diesel engine

Abstract

As the future of oil is uncertain and in the search for alternative fuels, it has been found that alcohol is a strong candidate as a fuel. The most representative lower alcohols are methanol and ethanol, which have generally demonstrated low miscibility so that they can generate phase separation when mixed after a certain period of time. The only way to use them in a diesel engine is to mix them with biodiesel or surfactants. By contrast, higher alcohols have more suitable characteristics to be mixed or used in the diesel engine. In the case of ethers they are a good option when it comes to pilot combustion considering using gaseous fuels such as biogas or syngas in dual diesel engine. This research explores the alternatives that exist to improve the characteristics of alcohol as it can be mixed with biodiesel, biogas, water or nanoparticles and also analyzes the emissions that are generated with the mixtures. The aim of this research is to explore the differences between lower alcohols and long chain upper alcohols, as well as the mixtures that can be generated to improve engine performance. The results of this research claim that long-chain alcohols have better physico-chemical properties than short-chain alcohols, butanol being the only fuel that can be transported and stored in the same current pipeline networks of existing oil products. There is also the option to use alcohol as a pilot fuel in a dual diesel engine. The latter can be used to apply a huge variety of both liquid and gaseous fuels, this makes the options in its application increase. As a conclusion of this work, it is recommended to explore new mixtures analyzing their synergies with various alternative fuels. The possibility of performing diesterol-BED generates very encouraging results, therefore it is recommended to continue to perform analyses on that line to find the optimal mixture.

Keywords: Alcohol; diesel engine; renewable energy; biofuel.

Análise da mistura de álcoois em motores diesel

Resumo

Na busca por combustíveis alternativos porque o futuro do petróleo é incerto, verificou-se que o álcool é um forte candidato como combustível, onde os álcoois inferiores mais representativos são o metanol e o etanol, que, em geral, demonstraram baixa miscibilidade, de modo que podem gerar separação de fase quando misturadas após um determinado período de tempo, a única forma de as utilizar num motor diesel é misturá-las com biodiesel ou tensoativos, pelo contrário, os álcoois superiores têm características mais adequadas para serem misturados ou utilizados no motor diesel, no caso dos éteres, são uma boa opção quando se trata de combustão piloto, considerando a utilização de combustíveis gasosos, como biogás ou gás de síntese em motores a diesel duplos, esta pesquisa explora as várias alternativas que existem para melhorar as características do álcool, pois pode ser misturado com biodiesel, biogás, água ou nanopartículas e também analisa as emissões que são geradas com as misturas geradas. O objetivo desta pesquisa é explorar as diferenças entre álcoois inferiores e álcoois superiores de cadeia longa, bem como as misturas que podem ser geradas para melhorar o desempenho do motor. Os resultados desta pesquisa afirmam que os álcoois de cadeia longa têm melhores propriedades físico-químicas do que os álcoois de cadeia curta, sendo o butanol o único combustível que pode ser transportado e armazenado nas mesmas redes de gasodutos atuais que os produtos petrolíferos existentes, há também a opção de usar álcool como combustível piloto em um motor diesel duplo, Este último pode ser usado para aplicar uma enorme variedade de combustíveis líquidos e gasosos, o que aumenta as opções em sua aplicação. Como conclusão deste trabalho recomenda-se explorar novas misturas analisando suas sinergias com vários combustíveis alternativos, a possibilidade de realizar diesterol-BED gera resultados muito encorajadores, portanto, recomenda-se continuar a realizar análises nessa linha para encontrar a mistura ideal.

Palavras-chave: Álcool; diesel; energia renovável; biocombustíveis.

Introducción

En el futuro los productos del petróleo serán escasos y costosos, y si se tiene en cuenta la contaminación que estos generan, representan una amenaza para el medio ambiente. Para superar esto, se buscan combustibles alternativos [1]. La biomasa proporciona aproximadamente el 13% del suministro mundial de energía primaria y más del 75% de la energía renovable mundial. De hecho, se estima que la bioenergía podría contribuir con entre 25 y 33% del suministro mundial de energía para el 2050 [2]. Los biocombustibles no son de ninguna manera una tecnología emergente: a finales del siglo XIX ya se usaban lámparas de etanol [3]; posteriormente, los vehículos que solo tenían como combustible etanol eran muy populares en la década de 1980, pero se volvieron económicamente imprácticos cuando los precios del petróleo cayeron y los precios del azúcar aumentaron a finales de esa década [4]. Entre los alcoholes más estudiados se encuentran etanol, butanol, metanol, aceite de fusel y los ésteres MTBE (éter metil tert-butílico) y DME (dimetil éter) aplicados como combustible en los motores [5]. Las mezclas alcohol-diésel pueden ser una solución prometedora como combustibles más limpios, debido a que pueden mejorar la combustión y generar temperaturas más bajas en el motor [6]. Otros autores recomiendan la adición de H_2 (hidrógeno) a la mezcla de alcoholes [7]; también se han definido algunas mezclas como la del diésel-biodiésel-etanol con un nuevo término: diesterol [8].

En este estudio se presenta un análisis sobre las alternativas que existen para mejorar las características del alcohol, y con esto hacerlo más atractivo como biocombustible, por lo que se analizan las propiedades y el rendimiento de los alcoholes superiores y los alcoholes inferiores de cadena corta, y también se analiza la posibilidad de realizar mezclas con diferentes sustancias tratando de encontrar la mezcla óptima.

Propiedades físico-químicas de alcoholes y éteres

Aunque existen numerosas tecnologías de conversión, las propiedades de los combustibles varían significativamente. Incluso cuando se utiliza la misma materia prima [9], la variación de la composición química, tal como la longitud de la cadena de carbono y el grado de saturación/insaturación, cambia las propiedades físico-químicas del combustible [10]. El alcohol tiene una estabilidad a la oxidación superior y fluidez a baja temperatura en comparación con el biodiésel. Por lo tanto, los alcoholes tienen mejores características de manejo de combustible para el uso del motor en comparación con el biodiésel [11]. Desafortunadamente, también los alcoholes tienen sus desventajas físico-químicas; estas se presentan en la Tabla 1.

Tabla 1. Desventajas en las propiedades físico-químicas del alcohol en motores.

Tipo de causa	Provoca	Tipo de alcohol	Referencias
Combustión oxigenada	Bajo punto de ebullición y altas presiones de vapor	Bioalcoholes	[12]
Alto contenido de agua y humedad	Por la pérdida de calor puede disminuir su eficiencia	Aceite de fusel y etanol	[13]
Contenido de cenizas en los combustibles	Afecta el rendimiento del motor y el entorno ambiental	Biocombustible	[14]
Tener menor carbono y más oxígeno	Bajo valor de calentamiento	Alcoholes de cadena corta	[12]
Por ser disolventes	Corrosión en algunas partes del motor	Metanol, aceite de fusel	[12]

Alcoholes inferiores de cadena corta

Los alcoholes inferiores más representativos y usados son el metanol y el etanol [15]. Los alcoholes de cadena corta en el motor diésel tienen propiedades pobres, tales como el índice de cetano inferior, valor calorífico y viscosidad cinemática [16]; también han demostrado baja miscibilidad y realizan separaciones de fases después de cierto tiempo [17]. El etanol es de difícil autoignición y no genera liberación de calor a baja temperatura [18]; además tiene poca o nula liberación de calor a temperatura intermedia, y con la presencia de agua el etanol es más difícil de iniciar [19]. El etanol puede reducir la temperatura del gas en el cilindro y proporciona una zona de enfriamiento más grande, lo que lleva a una mayor emisión de CO (monóxido de carbono) [20]. Debido a la capacidad de generar combustión completa (Figura 1), el bioetanol tiene el potencial de aumentar la eficiencia de combustión [21] y con esto reducir la emisión de HC (hidrocarburos no quemados) [20]. El etanol tiene un alto calor de vaporización; por lo tanto, reduce la temperatura máxima dentro del cilindro y esto hace reducir los NOx (óxidos de nitrógeno), lo que aumenta la potencia del motor [7]. El metanol tiene un alto número de octanaje, alta velocidad laminar de la llama, amplio límite de inflamabilidad y alto calor latente de vaporización [22]. El metanol es tóxico, líquido, sin sabor, incoloro y generalmente se conoce como alcohol de madera. Su estructura química simple puede conducir a menores emisiones y una mejor combustión [13].

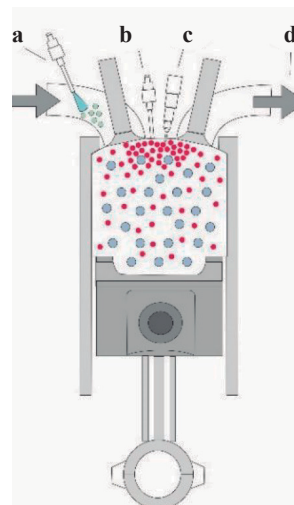


Figura 1. Inyección del alcohol en motor CI. a) Inyección de alcohol, b) inyección directa diésel, c) sensor de presión, d) escape. (Elaboración propia).

Alcoholes superiores de cadena larga

Recientemente, los investigadores han demostrado mayor interés en los alcoholes de cadena larga, ya que tienen mejores propiedades físico-químicas que los alcoholes de cadena corta [17]. A medida que aumenta el número de carbonos en la estructura química de los alcoholes, las propiedades básicas del combustible también mejoran; por ejemplo, la capacidad de disolverse, la baja polaridad y la naturaleza menos higroscópica hacen que la mezcla sea muy fácil con diésel y biodiésel; se evita así separación de fases [23] y se logra un menor retardo de encendido [24]. Además, tienen mayor valor calorífico, mayor volatilidad, menos problemas de ignición, solubilidad, mayor viscosidad, no presentan problemas de separación de fases y cuentan con mejores propiedades de flujo en frío. Además de una mejor estabilidad de mezcla y alta densidad, tiene también mejor lubricidad y menor riesgo de corrosión [25]. Por estas razones se sugieren los alcoholes altos en carbono para los motores diésel [26]. El uso del butanol tiene ventajas, ya que es el único combustible a base de alcohol que puede ser transportado fácilmente

por las redes de tuberías de productos petroleros existentes, en lugar de transportarse únicamente en camiones con cisternas y vagones de ferrocarril [27]. En la Tabla 2 se muestran las ventajas del butanol.

Tabla 2. Ventajas del butanol sobre el etanol y el metanol.

Ventaja del butanol	Beneficio	Referencia
Mejor miscibilidad con el combustible diésel	Mezcla con diésel	[28]-[32], [20]
Menor calor de evaporización	Menor pérdida	[28], [31], [20]
Mayor valor calorífico	Mejor combustión	[20], [21], [31]
No tiene separación de fases	Mejor mezcla	[32]
Menor consumo de combustible	Mejor combustión	[11]
Mejor estabilidad de mezcla	El sistema de inyección mejora y protege al motor	[11], [29], [30]
Genera menor temperatura de autoignición	Es más fácil de aplicar al motor	[11], [28]
Menor corrosión	Más durabilidad en las partes del motor	[29], [30], [33]
Mejor arranque del motor	Mejora arranque en climas fríos	[28]
No es higroscópico	No absorbe el agua	[32]
Tiene el doble de átomos de carbono que el etanol	Incrementa el valor calorífico	[33]
Tiene mayor densidad que el etanol	Reduce el consumo de combustible y mejora el rendimiento	[33]
Se pueden usar mezclas más magras	Reduce emisiones	[33]
Mejora la mezcla	Ofrece mejor rendimiento en el motor	[21]
Menos tóxico y mejor solubilidad	Mejor uso en mezcla con diésel	[28]
Mayor viscosidad y lubricidad	Protege el motor y evita el desgaste	[28]

El inconveniente de usar el butanol es que puede generar un incremento del BSFC (el consumo de combustible específico del freno), pero al mismo tiempo puede lograr disminuir las emisiones de CO, NO_x y humo, pero con una reducción de BTE (eficiencia térmica del freno) y con un aumento de las emisiones de HC [34]. El butanol muestra una capacidad de reducción de hollín más evidente y ofrece una mayor eficiencia del combustible comparándolo con el biodiésel [35], [36]. Las mezclas de butanol/diésel no requieren cosolventes o agentes mezcladores, debido a su naturaleza menos hidrófila [37]. Por otro lado, el geraniol es un alcohol de orden superior que se obtiene a partir de la reacción con solución ácida y forma turpineol el cual ayuda a mejorar el rendimiento del motor [38]. Con esto se puede llegar a concluir que el alcohol superior sería un posible sustituto del biodiésel en un motor diésel [39]. Otros alcoholes superiores como el 1-pentanol y el n-octanol se presentan en las Tablas 3 y 4 respectivamente.

Tabla 3. Ventajas del pentanol sobre el metanol y el etanol.

Ventaja	Beneficio	Referencia
Mejores propiedades de combustión	Mejora la combustión	[17], [35], [40]
Tiene baja polaridad y es hidrófilo	No muestra separación de fases	[17], [40], [41], [42]
Es miscible con diésel y aceite	Mejora la mezcla	[17], [25], [35], [40], [41], [42]
Mayor número de cetano	Mejor rendimiento	[25], [40], [41], [35], [42]
Mayor velocidad de la llama y viscosidad	Mayor valor calorífico	[25], [40], [41], [42]
Menor calor de evaporización y presión	Mejora la eficiencia	[25], [40], [35]
Menor riesgo de corrosión	Durabilidad	[25], [40], [41], [42]

Tabla 4. Beneficios del octanol sobre los alcoholes de cadena corta y el biodiésel.

Cualidad	Logro	Referencia
Altamente miscible al diésel	Mejor mezcla	[43]
Emisiones de humo ultrabajas	Reducción de humo	[44]
Mejores propiedades de flujo en frío	Punto de fluidez de 9 °C	[45]
Menos corrosivo que el resto de alcoholes	Naturaleza hidrofóbica	[46]
Tiene baja presión de vapor	Puede ser seguro en el traslado y almacenamiento	[46]
Alta tensión superficial y baja presión	Mejor pulverización	[44]
Cadena más larga y menos ramificada	Genera mayor densidad energética	[47]
Mayor número de cetanos, valor calorífico y la temperatura de autoignición	Propiedades similares al diésel	[39], [46]

Características de los éteres como biocombustibles

El DEE (éter dietílico) tiene el potencial para hacer el suplemento de combustible más adecuado para los motores diésel, debido a su número de cetano y valor de calefacción, el cual es mayor en comparación con el etanol y el butanol y además es miscible con el combustible diésel y biodiésel [48]. El DEE en modo de combustible dual puede usarse como combustible piloto, y de esta forma se podrían usar como combustible primario algunos combustibles alternativos como el gas natural comprimido, gas licuado de petróleo y biogás [49], [50]. Aunque tiene menor valor calorífico en comparación con el diésel y el biodiésel, su menor viscosidad y densidad mejoran la calidad de atomización del combustible, lo que lleva a una mejor eficiencia de combustión [51]. El DEE es de naturaleza renovable y se puede producir fácilmente mediante el etanol a un precio aceptable [50]. El DME tiene muchas propiedades y se cree que es uno de los mejores combustibles alternativos en motores CI en el futuro, debido a su alto índice de cetano que es 55, en comparación con el diésel que es 40-53, tiene bajo ruido de combustión, alto nivel de eficiencia térmica, genera combustión sin hollín y con bajos niveles de NO_x [52]. También tiene mayor contenido de oxígeno que los combustibles convencionales (aproximadamente 35%) y no tiene enlace directo carbono-carbono (C-C), por lo tanto, tiene reducciones de contaminantes tales como PM (la materia particulada) y HC [53]. Aun así, sufre de baja viscosidad y carece de buenas características de lubricación, por lo que el uso de aditivos de lubricidad como Lubrizol (539 N) o biodiésel puede mejorar la viscosidad y lubricidad del DME [53]. Por su menor valor de calentamiento (alrededor de 28,4 MJ/kg) requiere casi el doble de cantidad de combustible inyectado por ciclo para llegar a tener la misma potencia del motor [54]. Además, el bajo punto de ebullición del DME y su rápida evaporación requieren almacenarlo bajo presión constante para mantenerlo en fase líquida y así evitar su evaporación [55]. En la Figura 2 se hace un resumen de los alcoholes más representativos para los motores de combustión interna.

Mezclas en general con alcohol en motor diésel

En la mezcla de dos líquidos pueden tener diferentes propiedades químicas, por lo que pueden existir problemas de inestabilidad y separación durante el funcionamiento del motor; esta es la razón de agregar aditivos (cosolventes) y de esta manera superar el problema de separación de fases [56]. Los aditivos oxigenados pueden ayudar a encender el combustible más fácilmente y reducir la contaminación del escape del motor [57]. El hidroxitolueno butilado y el n-butanol son aditivos antioxidantes que con

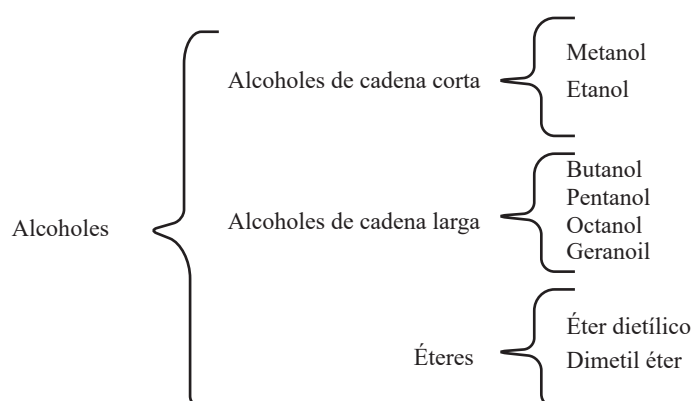


Figura 2. Cuadro sinóptico de los alcoholes aplicados en los motores de combustión interna.

Tabla 5. Desempeño del motor aplicando mezclas de aceites con alcoholes.

Emisión que reduce	Tipos de mezclas	Desempeño del motor	Referencia
NOx	1-octanol con 5% de aceite de palma y 95% de diésel y adición de 2EHN	Combustión mejorada y mayor BTE	[60]
NOx, CO y humo	1-octanol mezclado con diésel	Mejora el BTE pero con mayores emisiones de HC	[61]
CO y HC	Biodiésel con n-butanol y pentanol	Mejora el BTE y el BSFC, y se concluye que es mejor e pentanol que el n-butanol	[62]
CO y HC	1-octanol (10%, 20% y 30%) mezclado con diésel	La adición de 1-octanol mejora el BTE, pero aumenta el NOx	[63]
NOx	Mezcla de DME con biodiésel	Reducción del retardo de encendido	[64]
NOx	40% de diésel, 40% de biodiésel, 10% de aceite vegetal y 10% de propanol	Se tuvo el mayor BSFC y el menor NOx con un aumento de CO y HC	[65]
Varios	Biodiésel de aceite de girasol residual, diésel, n-pentanol y n-hexanol	La mezcla del n-hexanol con el biodiésel mejora los valores de BTE y BSFC	[66]
NOx	Residuos de aceite usado como biodiésel con propanol, n-butanol y pentanol	La mejor mezcla fue con pentanol	[23]
PM y hollín	Mezcla de DME con PODEn (polioximetilen dimetil ésteres)	Mejora la ignición y la calidad de pulverización; mejora el BTE, pero aumenta el NOx	[67]

biodiésel o diésel ayudan a reducir las emisiones de NOx, esto es debido a que el aditivo antioxidante tiene la capacidad de apagar los radicales libres durante la fase de combustión [58]. Uno de los métodos probados para mejorar el rendimiento y reducir el NO y el humo es agregar alcoholes como metanol, etanol, n-butanol y n-pentanol en mezclas de biodiésel o diésel [59]. En la Tabla 5 se muestra el desempeño del motor con mezclas de aceites con alcohol.

Raviteja *et al.* [68] estudiaron la mezcla de butanol enriquecido con hidrógeno y concluyeron que agregar hidrógeno reduce el consumo de combustible y además genera una disminución de emisiones. Diluir WPO (aceite de residuo de plástico) con alcohol, como etanol, metanol o butanol, tiene un beneficio adicional, ya que mejora significativamente las propiedades de almacenamiento y manejo de WPO [69]. Hellier *et al.* [70] investigaron el efecto de las cianobacterias para la obtención del geraniol en motor CI; los resultados mostraron que el uso de geraniol al 100% aumenta el retraso de ignición y aumenta el NO; sin embargo, si se mezcla con diésel reduce significativamente la emisión de NO, por lo que se llegó a la conclusión que el geraniol mejora su comportamiento de combustión con los aceites comestibles.

Propiedades del diesterol-BED

El bioetanol fue inicialmente considerado como un combustible alternativo para el motor SI debido a su mayor número de octanos; sin

embargo, en los últimos años, el bioetanol ha surgido como un combustible que puede mezclarse con diésel y biodiésel utilizando aditivos adecuados [71]. El etanol no es soluble en diésel y solo puede combinarse por medio del biodiésel que actúa como un intermediario; de hecho, el biodiésel actúa como un dispersante de partículas de etanol en el motor, creando así una mezcla estable. El etanol y el biodiésel son combustibles oxigenados que ayudan a mejorar la combustión. El etanol tiene un alto valor calorífico y baja densidad en comparación con el diésel puro y el biodiésel. Por otro lado, el biodiésel posee un bajo nivel calorífico, por lo que se compensa [72]. El biodiésel-etanol-diésel (BED) es una mezcla de biocombustibles y diésel que tiene el valor energético cercano a los combustibles fósiles, ya que cuenta con excelentes propiedades en cuanto a su lubricación, densidad y viscosidad y se considera un combustible ecológico [73]. También se conoce la mezcla de diésel-biodiésel-etanol por un nuevo término: diesterol [8]. Esta mezcla (biodiésel-etanol-diésel) da como resultado una mayor velocidad de calor, mayor retraso de encendido y más corta duración de la combustión [20]. En la Tabla 6 se analiza el rendimiento del motor con diesterol.

El etanol tiene baja viscosidad y buenas propiedades de flujo en frío; el biodiésel se puede mezclar con etanol para disminuir su viscosidad y aumentar su volatilidad y mejorar sus propiedades de flujo en frío [77]. Además, el mayor contenido de oxígeno en el etanol puede reducir aún más la emisión de PM (la materia particulada) en comparación con el biodiésel [20]. Se puede observar en la Tabla 7 la relación de emisiones en el motor aplicando diesterol.

Tabla 6. Rendimiento del diesterol-BED en comparación con el diésel puro.

Rendimiento del motor	Esto generó	Referencia
El BTE aumentó ligeramente	Una mayor tasa de combustión	[74]
El BTE fue inferior	Menor rendimiento que con el diésel puro	[75]
El BSFC aumentó	Sucedió cada vez que se incrementó el etanol	[76]

Tabla 7. Concentraciones de emisiones en el motor CI con el diesterol-BED en comparación con el diésel puro.

Emisión que reduce	La causa de esto	Referencia
NOx	La mezcla reduce la emisión	[75]
CO, NOx y humo	Con la mezcla se logra la reducción	[20]
PM y NOx	Con la mezcla tiene mayor tasa de liberación de calor	[78]
NOx y humo	Tiene reducciones de emisiones y no sufre cambios en la eficiencia térmica	[20]
CO y NOx	En alta velocidad se reduce el NOx y tiene mayor eficiencia térmica	[79]
HC, CO y hollín	El etanol es un oxidante que reduce el BSFC	[74]
NOx	Esto se debe al alto calor latente de evaporización del etanol, que reduce la temperatura de combustión.	[80]

La presencia del biodiésel en la mezcla de etanol y diésel hace aumentar el índice de cetano y mejora la calidad de autoignición de la mezcla de combustible en general; por otra parte, agregar etanol a la mezcla de biodiésel-diésel reduce la alta densidad y viscosidad del biodiésel, con lo cual se logran propiedades físicas y características de evaporación y de tamaño de gota más adecuadas en la mezcla del combustible [81], y además la mezcla diésel-biodiésel-etanol contiene un alto contenido de oxígeno que aumenta el rendimiento del motor y reduce las emisiones de escape [82]. La estabilidad, almacenamiento y transporte son los primeros inconvenientes de la mezcla diesterol, lo que dificulta su aplicación comercial; sin embargo, esto puede ser resuelto agregando iso-pentanol a la mezcla, lo cual la hace más estable y segura para su aplicación [83].

Mezclas de aceite con alcohol superior de cadena larga

La adición de bioalcoholes mejora la atomización del combustible, se logra así una mejor combustión y por lo tanto se reducen los depósitos de carbono que se pueden encontrar en el motor, como en los aros de pistón, paredes de cilindros, inyectores, etc [31]. El aceite de pirolisis de la madera y el n-butanol se pueden mezclar sin ninguna separación de gases, ya que el n-butanol puede servir como disolvente orgánico, el cual es capaz de disolver partículas sólidas existentes y suprimir la polimerización [69]. En la Tabla 8 se muestra el comportamiento del motor agregando mezclas de butanol con biodiésel.

De acuerdo con Lapuerta *et al.* [90], el taponamiento del filtro punto frío, punto de nube y punto de fluidez se reducen con el aumento del contenido del butanol en las mezclas de biodiésel-butanol [20]. Es posible utilizar hasta un 40% de butanol y un 45% de pentanol como componentes de mezcla con diésel para alimentar el combustible de los motores CI y en el caso de mezclas con biodiésel se puede lograr hasta un 20% de butanol y pentanol sin alteraciones en el motor CI [37]. Las mezclas de pentanol-biodiésel tienen viscosidades más bajas, mayor contenido de oxígeno y mayor calidad de ignición, lo que ayuda a lograr una mayor atomización

y combustión; las mezclas de pentanol tienen mayor estabilidad de la mezcla y no muestran separación de fases [24]. Es posible reemplazar hasta el 60% del diésel con biocombustibles (30% de biodiésel más 30% de pentanol) utilizando una estrategia de doble inyección [37]. En la Tabla 9 se muestran el rendimiento y las emisiones aplicando la mezcla de biodiésel con n-pentanol.

Tabla 8. Rendimiento y emisiones de la mezcla de biodiésel con butanol.

Emisiones que reducen	Forma de aplicar	La causa de esto	Referencia
Hollín y NOx	Se inyecta el butanol a mayor presión de inyección	La inyección a mayor presión es una técnica viable para reducir emisiones	[84]
NOx y humo	Se mezcla con aditivos	Se mezcla con aceite de cocina usado y tiene una mejora en la combustión	[85]
CO y humo	Mezcla homogénea biodiésel/butanol	Con la mezcla se genera un aumento en BSFC	[86]
PM	29% de butanol	Con la adición del butanol mejoran las propiedades de combustión	[87]
PM	20% de butanol	Se aumenta el BTE agregando butanol y se obtiene un buen rendimiento	[88]
NOx y humo	10% de butanol y 90% de diésel/WPO	La mezcla de butanol mejora el rendimiento del motor y reduce emisiones	[89]

Tabla 9. Rendimiento y emisiones aplicando una mezcla de biodiésel con n-pentanol.

Emisión que se reduce	Forma de aplicar	La causa de esto	Referencia
Humo	Se mezcló con varias condiciones de carga	La mezcla de aceites de cocina usados mejora el rendimiento, pero con aumento de HC	[91]
NOx	Se mezcló con un 20% de pentanol sin EGR	La mezcla de aceite de cocina de desecho y pentanol resulta en una mejor eficiencia de combustión	[92]
PM	Se mezcló homogéneamente pentanol con diésel	Con la mezcla se reducen las emisiones	[93]
NOx, HC y CO	Se compararon las mezclas pentanol/biodiésel y pentanol/diésel	Se redujo el BTE en la mezcla pentanol/biodiésel	[94]

Aplicación de bioalcohol como combustible piloto utilizando biogás como combustible principal

En un motor de doble combustible con biogás como combustible principal, el biogás se puede depositar en el cilindro de combustión a través de un puerto de admisión con aire, mientras que el alcohol servirá como la

fuelle de ignición, el cual se inyecta directamente en la combustión del cilindro (piloto) [95]; este combustible piloto puede estar conformado de diésel, biodiésel o éteres como el DME. El DME se mantiene con casi cero niveles de humo cuando es aplicado como combustible piloto, o bien se puede hacer en mezclas con biodiésel como lo señalan Paul *et al.* [96], quienes estudiaron la combustión dual de la mezcla de combustible piloto oxigenado y concluyeron que con un mayor porcentaje de biodiésel y etanol en el combustible piloto aumenta la eficiencia de combustión del motor y también disminuyen los NOx. En otro trabajo, Karabektas *et al.* [97] consideraron agregar el DEE como combustible piloto y encontraron que se genera una mejora en el BTE y en el BSFC; esto se debe al alto número de cetano de DEE y su contenido de oxígeno; por lo tanto, el proceso de combustión se mejora. El DEE se evapora rápidamente y se mezcla con el aire fácilmente; forma así una mezcla homogénea y además su alto calor de evaporación resulta en una temperatura más baja, y con esto se pueden reducir los NOx. En la Tabla 10 se presenta el éter como combustible piloto en un motor que tiene como combustible principal el biogás.

Tabla 10. Emisiones y rendimiento en motor CI con la mezcla de biodiésel/biogás con DEE.

Emisión que reduce	Tipo de biodiésel/biogás	La causa de esto	Referencia
CO	WPO	Con WPO el BTE se incrementó (100% de DEE), pero aumentó el HC	[98]
NOx y humo	WPO	El BTE se incrementó (con 15% de DEE) y se tuvo una reducción de BSFC	[99]
NOx	WPO	El BTE se incrementó (con 10% de DEE)	[98]
CO, HC y NOx	Biodiésel/GNC	Un BTE mejorado con DEE en un combustible dual	[100]
CO	Biodiésel de soja	Mejora el BSFC (con 5% de DEE); sin embargo, si se agrega más del 40% de DEE, produce una tendencia de detonación	[49]
CO, HC y humo	Biodiésel/biogás	Produjo un aumento en BTE (con 6% de DEE) en la mezcla con biodiésel como combustible piloto	[49]
---	Gas acetileno	Se redujo el consumo de energía (con 10% de DEE)	[101]

Mezclas de bioalcohol con agua en motor diésel

Los bioalcoholes se pueden aplicar a los motores diésel mediante dos métodos: en modo de combustión dual y como emulsificación/microemulsificación [67]. A medida que aumenta el contenido de agua, la formación de NOx y el humo se reducen, pero el consumo de combustible, el CO y los HC se incrementan [102]. En el modo de emulsión (Figura 3) aumenta el BTE alrededor de un 6,6%; la razón de este aumento se debe a la eficiencia de combustión mejorada por una mejor atomización y evaporación del combustible que se produce como resultado de una microexplosión [56].

Algunos estudios confirman que los combustibles diésel/biodiésel se pueden mezclar con una baja relación de volumen de alcoholes para formar combustibles microemulsionados [67] (Figura 4). La microemulsión es un método utilizado para mezclar aceites vegetales o biodiésel con alcoholes y de esta manera reducir la viscosidad, y además sirve como disolvente (ejemplo, el butanol), debido a su mayor índice de cetano, menor riesgo de corrosión, menor polaridad y mayor valor calorífico [103]. Kannan *et al.* [104] estudiaron el efecto de las microemulsiones de diesterol-agua en motor diésel de un solo cilindro con diferentes cargas y una velocidad constante de 1500 rpm. Los resultados muestran que la microemulsión aumentó el BSFC y redujo el BTE, pero generó incrementos de CO, CO₂ y HC, aunque hubo reducciones significativas de NOx y humo. Otro estudio con resultados distintos lo presentan Mukhtar *et al.* [105]; ellos analizaron el efecto del etanol en la emulsión con diésel, en una emulsión tricombustible (etanol, biodiésel y diésel). Los resultados señalan que la tasa de liberación de calor y la presión dentro del cilindro de la emulsión tricombustible durante el período de retardo del encendido aumento en comparación al diésel. La tasa máxima de liberación de calor y la presión dentro del cilindro de las emulsiones tricombustibles mejoraron en comparación con las del diésel.

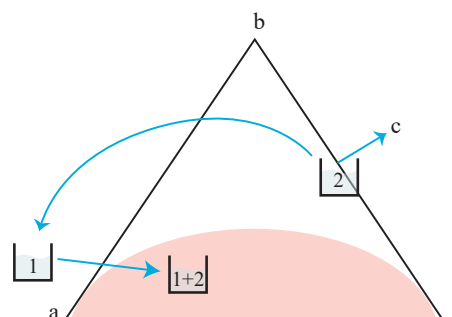


Figura 3. Emulsión con alcohol. a) Fase acuosa, b) surfactante + alcohol, c) mezcla autoemulsión, d) aceite + cosolvente. (Elaboración propia).

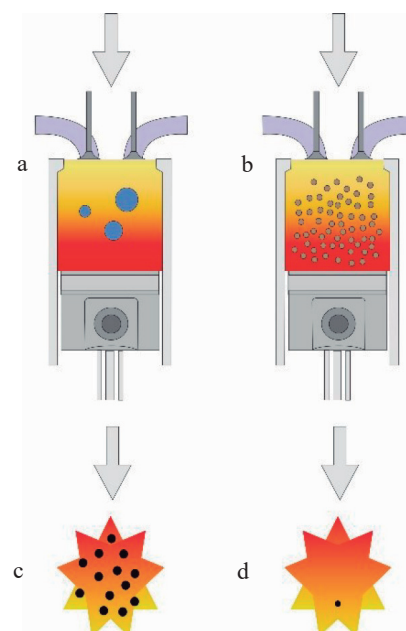


Figura 4. Comportamiento de emulsiones/microemulsiones. a) Motor sin emulsión, b) combustión con partículas, c) motor con emulsión, d) combustión limpia. (Elaboración propia).

Adición de nanopartículas con alcohol en motor CI

Las nanopartículas en las mezclas de combustible generan una oxidación debido a la alta relación del área de superficie-volumen y por su superficie reactiva más amplia, por lo que proporcionan mayor entalpía de combustión, con un aumento en la densidad energética [106]. Se ha demostrado que existe un gran potencial entre diversas mezclas como el n-butanol y BHT, con lo que se logra una mejor estabilidad a la oxidación y reducción de NOx [31]. Entre los estudios de mezclas de bioalcohol con nanopartículas destaca el de Venu *et al.* [107], en el cual investigaron el rendimiento y emisión de un motor diésel con una mezcla de diésel al 70%, biodiésel de aceite de Jatropha con 20%, etanol con 10% de combustible y con la adición de 25 ppm de nanopartículas de Al_2O_3 . Los resultados revelaron que la presión máxima del cilindro y el calor máximo de la tasa de liberación se alcanzaron más cerca de TDC (el punto muerto superior), y en el caso de las emisiones el HC, CO, NOx y la opacidad del humo se redujeron. Otro estudio destacable lo presentaron Shaafi *et al.* [108], quienes encontraron el efecto de las nanopartículas de alúmina, además de las mezclas de etanol e iso-propanol en un motor CI alimentado con biodiésel. El resultado mostró que la adición del etanol y la alúmina generó un aumento en la presión del cilindro de la tasa de liberación de calor. La temperatura de los gases de escape y el BTE aumentaron y además se redujeron las emisiones de CO y HC; sin embargo, los niveles de NOx aumentaron. Finalmente, otro estudio sobre este tema lo presentaron Selvan *et al.* [109], en el cual analizaron el efecto de incorporar nanopartículas de óxido de cerio y nanotubos de carbono en mezclas de diésel-biodiésel-etanol. Las nanopartículas de óxido de cerio actúan como catalizadores de suministro de oxígeno para oxidar el CO y reducir los óxidos de nitrógeno. La activación del óxido de cerio elimina los depósitos de carbono en el cilindro, lo que resulta en una reducción significativa de HC y emisiones de humo.

Conclusiones

Los alcoholes inferiores como combustible en los motores diésel solo pueden ser aplicados si se mezclan con biodiésel o con algún surfactante; en cambio, los alcoholes superiores son más adecuados para este tipo de motores. El n-butanol se destaca por su facilidad de desplazarse en las tuberías petroleras existentes; el pentanol por su parte tiene más estabilidad en la mezcla y no muestra separación de fases; si se logra tener una buena mezcla, se conseguirá una mejor atomización y combustión. Existe una gran variedad de alternativas de mezclas, entre las que se encuentran las emulsiones, las nanopartículas, los solventes, los aditivos oxigenados y antioxidantes, junto con las mezclas de los diferentes alcoholes además del biodiésel, que hacen que se tengan muchas opciones para generar valores en el desempeño del motor. Desafortunadamente, es necesario que se desarrolle una mayor aportación científica sobre la comparación de los efectos de los alcoholes inferiores (metanol y etanol) con los alcoholes superiores (butanol, pentanol y octanol) mediante análisis en el mismo motor bajo el mismo funcionamiento. Podemos asegurar que una de las claves de éxito para la aplicación de los biocombustibles es determinar la sinergia en la mezcla de alcoholes con otros compuestos que logren mejorar las condiciones del motor, a pesar de la variación que pueda generar el alcohol por las propiedades de su materia prima. Las flexibilidades en las propiedades de los alcoholes hacen que su mezcla con otras sustancias pueda ser efectiva, pero se recomienda emplear un intermediario que pueda servir para lograr una mezcla homogénea, por lo que los surfactantes pueden llegar a tener un protagonismo en el futuro. Por tal razón, este estudio recomienda su utilización. Una mezcla muy adecuada para la combustión puede ser el biodiésel con el pentanol, pero también puede ser con el n-octanol, y finalmente una mención especial al diesterol-BED. La posibilidad de elaborar una mezcla perfecta invita a seguir desarrollando investigaciones sobre esa línea.

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Biological potentialities and chemical composition of *Tarenaya aculeata* roots and stems

Abstract

Plant extracts are rich in secondary metabolites responsible for numerous biological activities. This study aimed to evaluate the antioxidant, antibacterial and photoprotective potentials, toxicity and chemical composition of extracts and fractions of stems and roots of *Tarenaya aculeata*. Phytochemical analyses were performed at qualitative and quantitative levels to evaluate the classes of secondary metabolites. The sun protection factor (SPF) and antioxidant potentials were determined spectrophotometrically, the antibacterial activity was tested against seven bacteria and the toxicity was evaluated using *Artemia salina* assay. Phytochemical screening revealed the presence of alkaloids, phenolic compounds, flavonoids, glycosides, tannins and saponins. The levels of phenolic compounds, tannins and alkaloids, SPF and antioxidant potentials showed greater results in the stem (SF) and root (RF) fractions in relation to the stem (SE) and root (RE) extracts. All samples exhibited a broad spectrum of antibacterial activity, with MIC values ranging from 31.25 to 250 $\mu\text{g mL}^{-1}$. SE, SF and RF caused mortality in *A. salina* larvae, with LC_{50} of 347.06, 34.71 and 85.39 $\mu\text{g mL}^{-1}$, respectively, whereas RE was non-toxic. Thus, *T. aculeata* is rich in bioactive secondary metabolites, although further studies will be needed to characterize its chemical constituents and investigate their adverse effects.

Keywords: Sojinha; Mussambê; photoprotective; antioxidant; antibacterial.

Potencialidades biológicas y composición química de raíces y tallos de *Tarenaya aculeata*

Resumen

Los extractos de plantas son ricos en metabolitos secundarios responsables de numerosas actividades biológicas. Este estudio tuvo como objetivo evaluar el potencial antioxidante, antibacteriano y fotoprotector, además de la toxicidad y la composición química de extractos y fracciones de tallos y raíces de *Tarenaya aculeata*. Se realizaron análisis fitoquímicos a nivel cualitativo y cuantitativo para evaluar las clases de metabolitos secundarios. El factor de protección solar (SPF) y los potenciales antioxidantes se determinaron espectrofotométricamente, la actividad antibacteriana se probó contra siete bacterias y la toxicidad se evaluó mediante el ensayo con *Artemia salina*. El tamizaje fitoquímico reveló la presencia de alcaloides, compuestos fenólicos, flavonoides, glucósidos, taninos y saponinas. Los niveles de compuestos fenólicos, taninos y alcaloides, SPF y potencial antioxidante mostraron mayores resultados en las fracciones de tallo (SF) y raíz (RF) en relación a los extractos de tallo (SE) y raíz (RE). Todas las muestras exhibieron actividad antibacteriana de amplio espectro, con valores de MIC que oscilaron entre 31,25 y 250 $\mu\text{g mL}^{-1}$. SE, SF y RF causaron mortalidad en larvas de *A. salina*, con CL_{50} de 347,06; 34,71 y 85,39 $\mu\text{g mL}^{-1}$, respectivamente, mientras que RE no fue tóxico. Por tanto, *T. aculeata* es rica en metabolitos secundarios bioactivos, aunque se necesitarán más estudios para caracterizar sus componentes químicos e investigar sus efectos adversos.

Palabras clave: Sojinha; Mussambê; fotoprotector; antioxidante; antibacteriana.

Potencialidades biológicas e composição química de raízes e caules de *Tarenaya aculeata*

Resumo

Extratos vegetais são ricos em metabólitos secundários responsáveis por inúmeras atividades biológicas. Este estudo teve como objetivo avaliar os potenciais antioxidante, antibacteriano e fotoprotetor, toxicidade e composição química de extratos e frações de caules e raízes de *Tarenaya aculeata*. Análises fitoquímicas foram realizadas em níveis qualitativo e quantitativo para avaliar as classes de metabólitos secundários. Fator de proteção solar (FPS) e potenciais antioxidantes foram determinados espectrofotometricamente, a atividade antibacteriana testada contra sete bactérias e a toxicidade avaliada pelo ensaio com *Artemia salina*. A triagem fitoquímica revelou a presença de alcaloides, compostos fenólicos, flavonoides, glicosídeos, taninos e saponinas. Os teores de compostos fenólicos, taninos e alcaloides, FPS e potencial antioxidante apresentaram maiores resultados nas frações caule (SF) e raiz (RF) em relação aos extratos de caule (SE) e raiz (RE). Todas as amostras exibiram atividade antibacteriana de amplo espectro, com valores de MIC variando de 31,25 a 250 $\mu\text{g mL}^{-1}$. SE, SF e RF causaram mortalidade em larvas de *A. salina*, com LC_{50} de 347,06, 34,71 e 85,39 $\mu\text{g mL}^{-1}$, respectivamente, enquanto RE não foi tóxico. Portanto, *T. aculeata* é rico em metabólitos secundários bioativos, embora mais estudos sejam necessários para caracterizar seus constituintes químicos e investigar seus efeitos adversos.

Palavras-chave: Sojinha; Mussambê; fotoprotetor; antioxidante; antibacteriana.



Introduction

The use of medicinal plants to treat various diseases is a common practice in Latin American countries. In this sense, a significant increase in Latin American scientific production has been observed in recent years in order to encourage the use of standardized herbal medicines that have proven quality, safety and efficacy [1]. Among the hundreds of families studied, the *Cleomaceae* family stands out for having numerous biological activities, such as anti-inflammatory, antinociceptive, antibacterial, fever, malaria and diabetes [2].

Tarenaya aculeata is a species belonging to the *Cleomaceae* family, and there are very few studies in the literature that prove its effectiveness. In the literature, its popular use is mainly reported in the treatment of fever and inflammation [3, 4], but there are reports of its use also for the treatment of other disorders, such as diabetes, cholesterol and high blood pressure [5]. Tea from the whole plant is normally administered [3, 5], but there are also reports on its use for bathing or with respect to tea from the root or the whole plant [3-5].

Fever is caused by several factors, one of the known causes being the inflammatory process [6]. The inflammatory response consists of an innate system of cellular responses elicited after an injury, which may arise from exposure to heat or cold, schema/reperfusion, blunt trauma, as well as due to the action of infectious organisms [7]. Therefore, evaluating the antibacterial activity of this plant and possible photoprotective effects may be related to the antipyretic and anti-inflammatory capacity of this plant.

Another widely evaluated biological activity in medicinal plants is the antioxidant activity, and as this is the first screening to contemplate the biological potential of *T. aculeata*, it will be contemplated in our study. The evaluation of antioxidant studies is important because many diseases are caused due to oxidative stress, and medicinal plants are rich in secondary metabolites and can act as free radical scavengers, avoiding numerous diseases, such as cancer, Alzheimer's, heart, kidney and liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders and aging [8].

The assessment of photoprotective activity has gained importance among researchers in recent times, mainly due to the number of cases of skin cancer that has been increasing worldwide, caused mainly by ultraviolet radiation [9]. The exposure to this specific wavelength range interacts with unprotected living tissue and causes biochemical and physiological changes, which can result in sunburn, premature aging and increased risk of skin cancer [10]. In this sense, plant extracts have proven to be a great alternative to be incorporated into sunscreens, which become an important strategy in order to prevent the negative damage caused due to overexposure [11].

Since antimicrobial resistance has become one of the main threats to public health in recent years, research directed at discovering new compounds that can act as antimicrobial agents has also been strongly encouraged. Antibiotic resistance compromises the ability of the human immune system to fight infectious diseases and in this sense, plant-based antimicrobial studies have received attention due to their potential for fighting diseases caused by microorganisms (bacteria, fungi, protozoa and viruses), and for their ability to act as anti-infective agents with few side effects [12, 13].

However, and due to the various biological activities, that can be found in a plant, a study is also necessary for the evaluation of the possible toxic effects present in the extract. In this sense, the lethality assay involving *Artemia salina* constitutes a good alternative for preliminary evaluation, since it is a quick, convenient and low-cost test [14]. *A. salina* is a small halophilic invertebrate belonging to the Crustacean class that has been widely used in applied toxicology research [15].

Chemical characterization studies of plant extracts are essential, as they allow a more rational exploration of Brazilian flora. Secondary metabolites are responsible for numerous biological activities in plants [9, 16]. Thus, and due to the possibility of this plant serving as a natural source of effective compounds, the objective of this study is to carry out a phytochemical screening, a study of the content of alkaloids, phenolic compounds and tannins as well as the antioxidant, photoprotective and antibacterial activities of *T. aculeata* and the toxicity against *A. salina*.

Materials and methods

Plant material and extraction

Stems and roots of *T. aculeata* were collected in the municipality of Dourados, MS, Brazil. The plant was identified by a botanist specialising in the *Tarenaya* genus, Dr. Raimundo Luciano Soares Neto, Federal University of Paraíba. An exsiccate (DDMS 7618) of the plant species was deposited at the DDMS Herbarium, Universidade Federal da Grande Dourados- MS. Registration number of the SISGEN (AD26007).

The plant materials were dried in an oven with air circulation at 50 °C for 72 h and ground using a Wiley mill (Marconi, Brazil). Their respective extracts were prepared by the decoction method. The decoctions were prepared at 2% (w/v) by immersing the plant material in boiling water for 20 min, in an open system, followed by an approximately 10 min wait before performing the filtration. Subsequently, the extract was filtered and a portion was separately frozen and lyophilised (Martin Christ, Alpha 1-2 LD plus, Germany), with a pressure of 0.045 mbar and a temperature of -42 °C, in order to obtain the stem extracts (SE) and root extracts (RE), while another portion of each extract was used to obtain the methanolic stem (SF) and root (RF) fractions. The yields obtained for the stem and root extracts were 15 and 8%, respectively, in relation to the plant dried, and were determined according to eq (1).

$$\% \text{ yield} = \frac{\text{Mass of the extractly philised}}{\text{Initial weight of the plant dried}} \times 100 \quad (1)$$

In order to eliminate water-soluble compounds and obtain enriched fractions, the remaining aqueous solutions were individually subjected to a solid phase extraction procedure using Amberlite XAD-2 resin, as per the methodology described by Yung An et al. [17] with modifications. In short, a glass column was packed with 100 g of XAD-2 resin (chromatographic bed dimensions: 25 cm h × 3 cm Ø), initially conditioned in distilled water. Then, the hydrophobic resin was, successively, loaded with the sample (two L of one of the decoctions), washed with 700 mL of distilled water and the adsorbed compounds eluted with 700 mL of methanol. Finally, the methanolic solutions thereby obtained were concentrated to dryness in a fume hood with exhaustion, and the yield of the methanolic fractions was SF: 6% and RF: 9%, in relation to aqueous extracts, respectively, and were determined according to eq (2):

$$\% \text{ yield} = \frac{\text{Mass of the fraction dried}}{\text{Initial weight of the extract}} \times 100 \quad (2)$$

Chemical composition

Phytochemical screening

All samples were tested for the presence of phytochemicals. The alkaloid screening was performed using the methodology described by Silva et al. [18]. Similarly, the presence of phenolic compounds, flavonoids, glycosides, saponins and tannins in the extracts and fractions was also evidenced by means of qualitative phytochemical tests, which were performed as described by Guterres et al. [19].

Analysis of the alkaloid contents

The analysis of the total alkaloid content was determined according to the methodology described by Oliveira et al. [20]. For this, two g of plant

material was reacted with eight mL of 10% acetic acid, prepared in ethanol, for four h, filtered and precipitated with concentrated ammonium hydroxide. The solution was allowed to stand and filtered with dilute ammonium hydroxide. The residue obtained was then dried and its composition was determined according to eq (3):

$$\% \text{alkaloid} = \frac{\text{Final sample weight}}{\text{Initial weight of the plant material}} \times 100 \quad (3)$$

Determination of phenolic and tannin contents

Stock aqueous solutions at 1.000 µg mL⁻¹ of SE, SF, RE and RF were prepared and used for the quantification of total phenolic compounds and tannins. All analyses were performed in triplicate.

The content of the first group was determined using the Folin-Ciocalteu colourimetric method [21]. Briefly, 100 µL of the stock solution was mixed with the Folin-Ciocalteu reagent, distilled water and 20% sodium carbonate and incubated in a dark environment at 23±2 °C for two h. The absorbance was recorded at 760 nm wavelength, using a UV/Vis spectrophotometer (Global Trade Technology, Brazil). This process was carried out for each sample. The analytical curve was prepared with gallic acid at concentrations between 10 and 100 µg mL⁻¹. The results were expressed as mg of gallic acid per g of dried extract or fraction (mg GAE g⁻¹).

The total tannin content was determined using the Folin-Denis spectrophotometric method [22]. The sample (two mL) was mixed with two mL of Folin-Denis reagent and two mL of 8% sodium carbonate. The mixture was left to react for two h and then the absorbance was read at 725 nm. This process was undertaken for each sample. Standard solutions of tannic acid in the concentration ranging from 0.5 to 150 µg mL⁻¹ were used to plot an analytical curve. Results were expressed as mg of tannic acid equivalent per g of dried extract or fraction (mg TAE g⁻¹).

Antioxidant activity against the 2,2-diphenyl-1-picrylhydrazyl radical

The antioxidant activity of the extracts and fractions were evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical method [23]. For this, a stock solution of SE, SF, RE and RF was prepared at a concentration of 500 µg mL⁻¹ in a 50% water/methanol solution and, from there, dilutions at concentrations of 240, 120, 60 and 30 µg mL⁻¹ were prepared. To assess the antioxidant potential, two mL of a methanolic DPPH 0.004% solution was added to 100 µL of the stock solution to be tested, and also to each of its dilutions. The samples were incubated in a dark environment and left to react for 30 min at a controlled temperature (25 ± 1 °C). Absorbance was recorded at a wavelength of 517 nm. A 50% water/methanol solution was used as a blank.

The results were expressed in inhibitory concentration (IC₅₀), that is the concentration of the sample required to scavenge 50% of DPPH free radicals [24] and for this, a graph was plotted, considering the Y axis the concentration of the extracts (µg mL⁻¹) and the X axis the percentage of inhibition activity (%). All tests were performed in triplicate.

Photoprotective potential

The analysis of the photoprotective potential was determined by means of a spectral scan of the extracts, the calculation of the critical wavelength and the SPF value. For the analyses of the photoprotective potential, extracts and fractions of stems and roots were used, at a concentration of 200 µg mL⁻¹, obtained from the dilution of stock solutions. The exploratory scans of the solutions were recorded at wavelengths between 200 and 600 nm, with a 1 nm interval, using a UV/Vis spectrophotometer. To calculate

the critical wavelength (λ_c), the intervals from 290 nm to 400 nm were selected and the graph area was calculated and integrated, using the Origin Pro 2018 software version 9.5. The wavelength was equivalent to 90% of the spectrum area [25, 26].

To determine the SPF of the solutions, the samples were read in a UV/Vis spectrophotometer between 290 and 320 nm, with an interval of 5 nm. As a blank of the analysis, distilled water is used for the extracts and fraction samples. The absorbances obtained were substituted in the eq (4), as follows [27]:

$$\text{SPF}_{\text{spectrophotometric}} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda) \quad (4)$$

Where CF= correction factor of 10; EE= Erythral effects spectrum; I= Solar intensity spectrum and ABS= absorbance of sunscreen sample. The values of EE x I are constant values that were determined by Sayre et al. [28] and are presented on Table 1:

Table 1. Normalized product function determined by Sayre et al. [28] used to calculate the SPF value.

Wavelength (λ nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

EE: Erythral effect spectrum; I: Solar intensity spectrum.

Antibacterial activity

The determination of the minimum inhibitory concentrations (MIC) of the SE, SF, RE and RF were ascertained according to the methodology described by Bernardi et al. [29]. For this, seven bacterial strains were tested, four of which were Gram-positive (*Enterococcus faecalis* ATCC29212; *Staphylococcus epidermidis* ATCC12228; *Staphylococcus aureus* ATCC 25232 and *Staphylococcus saprophyticus* ATCC15305) and three Gram-negative (*Burkholderia cepacia* NEWP 0059; *Escherichia coli* ATCC 38731 and *Pseudomonas aeruginosa* ATCC27853).

Microplates containing 96 wells were used to carry out the assay. They were all filled with 100 µL of Mueller Hinton broth containing different concentrations of the SE, SF, RE and RF (15.62-1000 µg mL⁻¹) and the same volume of bacterial saline solution (0.9%) in Mueller Hinton broth. The microplate was incubated at 36 ± 1 °C for 24 h. Tetracycline (15 µg mL⁻¹) was used as a positive control and distilled water as a negative control. After incubation, the MIC was determined using a spectrophotometer. All analyses were performed in triplicate.

Toxicity test

The toxicity test was performed based on the proposal put forth by Meyer et al. [14], with adaptations. To carry out the toxicity test on *A. salina*, a stock solution was prepared at a concentration of 1500 µg mL⁻¹, and dilutions at concentrations of 0; 0.5; 1.0; 5.0; 25; 125; 250; 500 and 1000 µg mL⁻¹. 10-mL of each of the solutions was transferred to graduated test tubes and then 10 nauplii of *A. salina* were added. To perform the toxicity test, the *A. salina* cysts had been previously incubated for a period of 48 h in a 3%

sea salt solution at room temperature and under 100 W light and constant aeration.

After 24 h of exposure, a count of live and dead nauplii was performed, and all those that showed any type of movement when observed near a light source were considered alive. A control was prepared containing only saline water and *A. salina*. To calculate the mean lethal concentrations (LC_{50}), a graph was plotted considering the percentage of dead nauplii (%) by the extract concentration ($\mu\text{g mL}^{-1}$), obtaining an equation of the straight line for each of the tested extracts and fractions, and the respective values of a and b. All tests were performed in triplicate.

Statistic analysis

Statistical analyses were carried out only to evaluate the levels of phenolic compounds and tannins present in the extracts and fractions of *T. aculeata*. For this, these samples were subjected to one-way analysis of variance (ANOVA) and a posteriori Tucker test. The level of significance of the analyses was considered when $p < 0.05$. These analyses were performed using the free software Bioestat 5.0 [30].

Results

Phytochemical screening

According to Table 2, *T. aculeata* has a great variety of phytochemicals. Alkaloids, phenolic compounds, flavonoids, glycosides and tannins were found in all extracts and fractions, whereas saponins were detected only in the SE and the RE (Table 2). In general, the classification in relation to the intensity showed that the fractions present the highest levels of secondary metabolites, especially the SF (Table 2).

Table 2. Phytochemical screening of *T. aculeata* extracts and fractions.

	SE	SF	RE	RF
Alkaloids	+	+++	+	++
Phenolic compounds	+	+++	+	++
Flavonoids	++	+++	+	+
Glycosides	+	+++	+	++
Saponins	+	-	+	-
Tannins	+	++	+	+

- negative; + positive – low intense; ++ positive medium intense; +++ positive strong intense. SE: Stem extract; SF: Stem fraction; RE: Root extract; RF: Root fraction

Analyses of the alkaloids, tannins and phenolic compounds

T. aculeata stem presented 7.65% of alkaloids, a value higher than the root (1.10%), in the plant material. The content of phenolic compounds and tannins are shown on Table 3. The content of phenolic compounds evidenced a statistical difference between extracts and fractions ($p < 0.05$), when the same part of the plant was used. Statistical similarity was visualized between the SE and the RE ($p > 0.05$), showing that these plant parts have similar compound contents when extracted using the same extraction method. The tannin content, however, presented statistical similarity ($p > 0.05$) among all the analyzed samples, not showing chemical differentiation regarding the tannin analysis, in the extracts and/or fractions.

Table 3. Contents of phenolic compounds and tannins in *T. aculeata* extracts and fractions.

Extract	Phenolic compounds (mg GAE g ⁻¹)	Tannins (mg TAE g ⁻¹)
SE	54.13 ± 2.52 ^a	44.51 ± 0.84 ^a
SF	312.80 ± 2.77 ^b	44.07 ± 3.84 ^a
RE	41.47 ± 8.57 ^a	44.55 ± 0.83 ^a
RF	248.80 ± 2.04 ^c	43.96 ± 3.58 ^a

Values expressed as mean ± standard deviation. SE: Stem Extract; SF: Stem fraction; RE: Root extract; RF: Root fraction. GAE: Gallic acid equivalent; TAE: Tannic acid equivalent. Averages followed by different letters differ by Tukey test.

Evaluation of the antioxidant, photoprotective and antibacterial activities

Considering the free radical-scavenging capacity using the DPPH reagent, and according to Reynertson et al. [31], the antioxidant activity of a plant can be classified into groups, being considered active (values below 50 $\mu\text{g mL}^{-1}$), moderately active (values between 50 and 100 $\mu\text{g mL}^{-1}$), slightly active (values between 100 and 200 $\mu\text{g mL}^{-1}$) and inactive (values > 200 $\mu\text{g mL}^{-1}$). The fractionation of the samples affected the results of the antioxidant analysis. Thus, the SF were considered active and the RF moderately active, whereas the SE and the RE were considered inactive (Table 4). The use of the XAD-2 resin caused an increase in the levels of compounds that resulted in a greater antioxidant effect present in the evaluated fractions.

Table 4. Antioxidant and photoprotective potentials, critical wavelength and toxicity assessment of *T. aculeata* extracts and fractions.

	IC ₅₀ ($\mu\text{g mL}^{-1}$)	SPF value	λ_c (nm)	LC ₅₀ in <i>Artemia salina</i> ($\mu\text{g mL}^{-1}$)
SE	347.06 ± 0.01	2.39 ± 0.20	364.67 ± 3.05	602.58 ± 3.99
SF	34.71 ± 0.67	16.58 ± 0.06	370.67 ± 0.58	569.58 ± 11.67
RE	> 500	1.49 ± 0.08	350.00 ± 1.00	> 1500
RF	85.39 ± 3.08	17.73 ± 0.03	371.67 ± 0.58	289.23 ± 8.87

Values expressed as ± standard deviation. SE: Stem extract; SF: Stem fraction; RE: Root extract; RF: Root fraction. IC₅₀: Inhibitory concentration; SPF: Sun protection factor; λ_c : Critical wavelength; LC₅₀: Lethal concentration.

The results of the molecular absorption analysis (Figure 1) showed that the extracts and fractions present UV absorption. The RF showed the highest absorption values for both UVB and UVA regions, followed by the SF. The SPF value and critical wavelength results are contained on Table 4.

According to Machado et al. [32], when plant extracts present MIC below 10 $\mu\text{g mL}^{-1}$, the antibacterial activity is considered excellent; good when it varies from 10 to 100 $\mu\text{g mL}^{-1}$, moderate from 100 to 500 $\mu\text{g mL}^{-1}$, weak when it varies from 500 to 1000 $\mu\text{g mL}^{-1}$ and inactive for extracts above 1000 $\mu\text{g mL}^{-1}$. Thus, the tested extracts and fractions showed good to moderate antimicrobial activity against all Gram-positive and Gram-negative bacteria tested (MIC < 1000 $\mu\text{g mL}^{-1}$), with broad-spectrum action being evidenced for all extracts and fractions (Table 5).

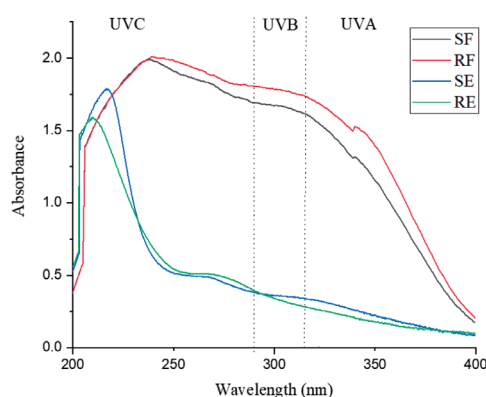


Figure 1. Scanning spectra (from 200 to 400 nm) of extracts and fractions of *T. aculeata*.

Table 5. Minimum Inhibitory Concentration (MIC) of *T. aculeata* extracts and fractions.

Microorganism	MIC ($\mu\text{g mL}^{-1}$)			
	SE	SF	RE	RF
<i>Burkholderia cepacia</i> ⁻	250	125	250	125
<i>Enterococcus faecalis</i> ⁺	250	125	250	125
<i>Escherichia coli</i> ⁻	125	62.5	125	62.5
<i>Pseudomonas aeruginosa</i> ⁻	125	31.25	125	31.25
<i>Staphylococcus epidermidis</i> ⁺	250	125	250	125
<i>Staphylococcus aureus</i> ⁺	250	62.5	250	62.5
<i>Staphylococcus saprophyticus</i> ⁺	500	250	500	250

+ gram-positive bacteria; - gram-negative bacteria; **SE**: Stem extract; **SF**: Stem fraction; **RE**: Root extract; **RF**: Root Fraction

Toxicity test

In relation to the toxicity test the classifications were based on those proposed by Sandoval et al. [33] and can be classified as non-toxic (LC_{50} greater than $1000 \mu\text{g mL}^{-1}$), low toxic (concentrations between 500 and $1000 \mu\text{g mL}^{-1}$), moderate toxicity (LC_{50} between 100 and $500 \mu\text{g mL}^{-1}$) and high toxicity (below than $100 \mu\text{g mL}^{-1}$). Thus, the RE can be classified as non-toxic, the SE and the SF are of low toxicity and the RF was the only sample to be considered of medium toxicity (Table 4).

Discussion

Several studies have already reported the presence of alkaloids, phenolic compounds, flavonoids, glycosides, saponins and tannins in *Tarenaya* species, being common in the *Cleomaceae* family [2, 34]. Phenolic compounds were identified and quantified by high performance liquid chromatography (HPLC) in the ethanolic and aqueous extracts of *Tarenaya spinosa*, a species very similar to *T. aculeata* [35]. Alkaloids, glycosides, steroids, flavonoids, saponins and tannins were identified in the methanolic extracts of *Cleome viscosa* leaves [36]. Species of the genus *Cleome* present a wide range of

chemical compounds that are an excellent option for the treatment of diseases in humans [37]. Silva et al. [18], when studying four types of organic extracts of leaves and roots of *T. spinosa*, found a broad-spectrum of antimicrobial activity in all analysed extracts and related the activity to the presence of the evaluated phytochemicals, especially flavonoids, terpenoids and saponins. Alkaloids, phenolic compounds and tannins were quantified using standard procedures. *T. aculeata* stem presented contents of alkaloids higher than the root. Alkaloids are bioactive nitrogen-containing compounds that occur naturally in plants and have been studied by numerous research groups due to the wide range of pharmaceutical activities reported in various plants, such as analgesic, muscle relaxant, antioxidant. However, they can be toxic in large amounts [38, 39].

SF showed the highest content of phenolic compounds ($312.80 \pm 2.77 \text{ mg AGE g}^{-1}$), followed by RF ($248.80 \pm 2.04 \text{ mg ATE g}^{-1}$), which allows us to verify that the processing of the aqueous extracts to remove water-soluble compounds was effective, resulting in an increase in the levels of phenolic compounds when compared to those of the respective extracts (Table 3). However, the tannin levels did not show significant differences between all analyzed samples, ranging from 43.96 to $44.55 \text{ mg ATE g}^{-1}$ (Table 3). Tannins are polyphenolic compounds known for their high molecular weight and, although multiple biological properties are attributed to them, they have been more extensively studied for their *in vitro* antioxidant and antimicrobial activities [40].

The phenolic compounds are a group of compounds naturally present in most plants, and these are characterized by having one or more aromatic rings containing one or more hydroxyl groups. This class of bioactive compound has been shown to exhibit, for example, antioxidant, antidiabetic, anti-inflammatory, antiallergic, antiviral, anticancer and antimicrobial activities [41, 42]. In the species of the genus *Tarenaya*, promising antioxidant activity results were found by Bezerra et al. [43], its activity justified by the presence of phenolic compounds present in the aqueous and ethanolic extracts of *T. spinosa* leaves. In another study, carried out by Silva et al. [38], organic extracts of stems and roots were evaluated, and their antimicrobial activity justified by some metabolites, including flavonoids, a specific class of phenolic compounds that have already been strongly indicated to have antimicrobial activity.

The best results of the methanolic fractions in the analysis performed in relation of the extract can be attributed to the use of Ambertlite XAD-2 resin. This resin technique promotes a concentration of compounds in the fraction [44]. In this study this technique proved to be efficient to increase the levels of phenolic compounds in fractions obtained of the aqueous extracts of *T. aculeata*. This result corroborates studies in the literature that establish that this technique has been shown to be efficient for the sample preparation, isolation and purification of these constituents [45].

Antioxidant activity has already been observed for *Tarenaya* species. Bezerra et al. [43] evaluated the antioxidant activity of aqueous and ethanolic extracts of *T. spinosa* leaves, with IC_{50} of 377.7 and $445.8 \mu\text{g mL}^{-1}$ (Table 4), respectively and attributed this property to the phenolic compounds present in the extracts.

All extracts and fractions showed UVA and UVB absorption, indicating that both extracts and fractions of *T. aculeata* species are promising as a UV filter (Figure 1). The presence of chemical compounds such as flavonoids, alkaloids and phenolic compounds are associated with the ability of plant extracts to absorb UV radiation in several studies [46]. Flavonoids are polyphenols containing two aromatic rings that present two absorption ranges in the UV region, specifically between 240 and 280 nm and between 300 and 550 nm [47, 48].

The resolution RDC No. 30 of June 1, 2012 by ANVISA determines that the photoprotectors must have a minimum SPF of six and a critical wavelength greater than 370 nm for commercial use and that the products must be classified into different categories, according to the calculated SPF value, which can be low (6.0-14.9), medium (15.0-29.9) and high (30.0-50.0) protection, in addition to very high solar protection (greater than 50.0 and less than 100). However, to be considered as a multifunctional product, it needs to have a minimum SPF of two [25]. Among all the extracts and fractions tested, the RF presented the highest SPF value

(17.73), followed by the SF (16.58). However, the SE (2.39) can be used in multifunctional formulations, (SPF greater than two) and being classified as low protection products (SPF between 6.0 and 14.9), indicated for skins with little sensitivity to sunburn (Table 4).

The calculation of the critical wavelength allows associating how much protection the sample has against UVA radiation, since the wavelength calculation takes into account the area of the graph relative to 90% of the spectrum area, between 290 and 400 nm [26, 49]. The ANVISA requires the minimum value for its use as a sunscreen and therefore, the RE did not present the minimum wavelength value necessary for its use as a photoprotective product [25]. However, the fractions can be considered for future studies related to incorporations in photoprotection as well as the SE products, for multifunctional products.

Thus, combined with the fact that both extracts and fractions absorb UV radiation, and the presence of phenolic compounds and alkaloids that may explain the absorption values found, the SPF values and the critical wavelength evaluated, we can conclude that RF and SF are promising for use in photoprotective formulations in order to achieve a broad spectrum of protection against ultraviolet radiation, and the SE in multifunctional formulations.

The SE and RE showed moderate antibacterial activity (ranging from 125 to 500 $\mu\text{g mL}^{-1}$), while the SF and RF showed good to moderate activities (MIC ranging from 62.5 to 250 $\mu\text{g mL}^{-1}$). The antibacterial activities were considered good against the Gram-negative bacteria *Pseudomonas aeruginosa*, (MIC: 31.25 for the SF and 31.25 for the RF), *Escherichia coli* (MIC: 62.5, for both the SF and RF) and the Gram-positive bacterium *Staphylococcus aureus* (MIC: 62.5, for both the SF and RF) (Table 5).

The presence of secondary metabolites found in our studies may justify the results of the antibacterial activity obtained in our study. Phenolic compounds and extracts rich in such secondary metabolites can be excellent inhibitors of pathogenic and spoilage bacteria of food origin [50], alkaloids have an underlying structure, which is capable of enabling the development of antibiotics with a wide range of action [51] and tannins are mainly present in the stems of trees and plants, acting in defense against pathogens, reason that may justify the large number of studies related to its antimicrobial activity [40].

Previous studies have already reported antimicrobial activity for species of the *Tarenaya* genus. The results obtained for the analysis of antimicrobial activity corroborated those obtained by Nascimento et. al. [52], in which the ethanolic extracts of *T. aculeata* showed antimicrobial activity against bacteria and fungi. In addition, aqueous extract of *T. spinosa* leaves showed a MIC value of 512 $\mu\text{g mL}^{-1}$ against *Staphylococcus aureus*, indicating relevant antibacterial activity [18].

The hydroalcoholic extract of *T. aculeata* leaves has already been evaluated in another study involving *A. salina*, however, it was classified as highly toxic [53]. In another study involving another species of the same genus, *T. spinosa* showed moderate toxicity (LC_{50} : 150 $\mu\text{g mL}^{-1}$) for hydroalcoholic root extracts [54]. This corroborates the results obtained in our study, presenting the same classification range as the stem fraction (moderate toxicity).

Despite being classified as low to medium toxicity, the extracts/fractions SE, SF and RF showed antitumor potential. This is due to the fact that some studies already present the *A. salina* test as an excellent indicator of biological activity. The extract is considered active when it presents LC_{50} to 1000 $\mu\text{g mL}^{-1}$ [55]. The genus *Tarenaya* does not present studies related to antitumor activity so far, however, the Cleomaceae family stands out for presenting some studies in this regard [34, 56]. Hence, more robust studies with the purpose of evaluating the antitumor properties of these strata can be considered in future studies.

The extracts and fractions showed promising antimicrobial agents. In addition, associated with the SPF values found, it can serve as an indication of a multifunctional product formulated from fractions and the SE (except for the RE, with a SPF less than two). In the cosmetics industry, formulations with antimicrobial activity can significantly decrease the use of synthetic preservatives, resulting in a product with less toxic effects and greater stability [46, 57].

Conclusions

T. aculeata can biosynthesize alkaloids, phenolic compounds, flavonoids, glycosides, tannins and saponins, and extracts and fractions of stems and roots of this plant were found to have photoprotective, antioxidant and antibacterial potentials. The best photoprotection results were attributed to the SF and RF, while the SE can be indicated as a multifunctional product. The SF and RF showed the best results for antioxidant and antibacterial potentials, confirming that the use of polymeric adsorbent resins such as XAD-2 for the elimination of water-soluble compounds is an efficient strategy to access enriched fractions in bioactive natural products. The RE was considered non-toxic, while SE and SF were of low toxicity and RF was considered of moderate toxicity.

In addition to its low toxicity, the SE, because of its biological profile, also stood out in this research as a promising sample for the development of multifunctional products. However, a detailed chemical characterization and comprehensive toxicological studies will still be needed in order to better understand the medicinal potential of *T. aculeata* decoctions, as well as whether there are risks when consuming them.

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Nuevas tecnologías de adsorción y factores de influencia para iones Cd (II) y Pb (II) – una revisión

Resumen

La contaminación por metales pesados es un problema de salud pública y ambiental; la contaminación por plomo (Pb) y cadmio (Cd) causa graves daños a la salud humana y a los ecosistemas, especialmente a los acuáticos. En vista de este problema, el estudio tiene como objetivo buscar en la literatura investigaciones relevantes sobre tecnologías de adsorción para iones Cd (II) y Pb (II), en aguas superficiales y efluentes industriales. La búsqueda de trabajos se realizó en las bases de datos Scopus, Web of Science, CAB, ScienceDirect e Engineering Village, utilizando los términos “Tecnologías de adsorción”, “Cd (II)”, “Pb (II)”, “Adsorción iónica”, “Medios de influencia” y “Adsorción de Cd (II) y Pb (II)”, y analizados con la ayuda del software Rayyan y el Microsoft Excel. El análisis de los estudios mostró una extensa diversidad de materiales con capacidad adsorbente, como nanocompuestos, aplicación de microorganismos y biomásas vegetales. Entre los materiales adsorbentes destacó el uso de bacterias del género *Bacillus* sp. y la aplicación de quitosano libre o asociado; los nanocompuestos basados en quitosano mostraron una capacidad de adsorción de hasta el 98% para el ion Pb (II). El uso del macrófito *Potamogeton malaianus* permite obtener altos resultados en la adsorción de cadmio en agua; por medio de la fitorremediación la adsorción se produce rápidamente (2 h) y logra un equilibrio dinámico en menos de 72 h. La adsorción media para iones Cd (II) es del 94%, con una capacidad de adsorción en equilibrio de 6,29-6,97 mg/kg. Entre los medios de influencia, el pH, la temperatura, la dosis de adsorbente, el tiempo de contacto y la concentración de iones son los principales factores que interfieren en la actividad adsorbente.

Palabras clave: contaminación por metales pesados; tratamiento ambiental; tecnologías de adsorción; revisión.

New adsorption technologies and influence factors for ions Cd (II) and Pb (II) – A review

Abstract

Heavy metal contamination is a public and environmental health problem, lead (Pb) and cadmium (Cd) contamination cause serious damage to human health and ecosystems, especially aquatic. In view of this problem, the study aimed to search the literature for relevant research on adsorption technologies for Cd (II) and Pb (II) ions, in surface water and industrial effluents. The search for works took place in the databases Scopus, Web of Science, CAB, ScienceDirect and Engineering Village, using the terms “Adsorptive Technologies”, “Cd (II)”, “Pb (II)”, “Ion adsorption”, “Means of influence” and “Adsorption of Cd (II) and Pb (II)”, and analyzed with the help of Rayyan and Microsoft Excel software. The analysis of the studies showed an extensive diversity of materials with adsorbent capacity, such as nanocomposites, application of microorganism, and plant biomasses. Among the adsorbent materials highlighted the use of bacteria of the genus *Bacillus* sp. and application of chitosan freely or associated, nanocomposites based on chitosan showed adsorptive capacity of up to 98% for Pb ion (II). The use of the macrophyte *Potamogeton malaianus* allows to obtain high results in the adsorption of cadmium in water, through phytoremediation, adsorption occurs quickly (2 h) and achieves a dynamic equilibrium in less than 72 h. The average adsorption for Cd (II) ions is 94%, with an equilibrium adsorption capacity of 6.29-6.97 mg/kg. Among the means of influence, pH, temperature, adsorbent dosage, contact time and ion concentration are the main factors that interfere in adsorbent activity.

Keywords: Contamination by heavy metals; environmental treatment; adsorptive technologies; review.

Novas tecnologias de adsorção e fatores de influência para íons Cd (II) e Pb (II) – uma revisão

Resumo

A contaminação por metais pesados é um problema de saúde pública e ambiental, contaminação por chumbo (Pb) e cadmio (Cd) provocam graves danos à saúde humana e aos ecossistemas, principalmente aquático. Em vista dessa problemática, o estudo objetivou buscar na literatura pesquisas relevantes sobre tecnologias de adsorção para íons Cd (II) e Pb (II), em águas superficiais e efluentes industriais. A busca por trabalhos se deu nas bases de dados Scopus, Web of Science, CAB, ScienceDirect e Engineering Village, com uso dos termos “Tecnologias adsorptivas”, “Cd (II)”, “Pb (II)”, “Adsorção de íons”, “Meios de influência” e “Adsorção de Cd (II) e Pb (II)”, e analisados com auxílio do software Rayyan e Microsoft Excel. A análise dos trabalhos demonstrou uma extensa diversidade de materiais com capacidade adsorvente, como nanocompósitos, aplicação de microrganismo e biomassas vegetais. Entre os materiais adsorventes de destacou a utilização de bactérias do gênero *Bacillus* sp. e aplicação de quitosano de forma livre ou associada, nanocompósitos a base de quitosano demonstraram capacidade adsorptiva de até 98% para íon Pb (II). O uso da macrófita *Potamogeton malaianus* permite obter altos resultados na adsorção de cádmio em água, através da fitorremediação, a adsorção ocorre rapidamente (2 h) e atinge um equilíbrio dinâmico em menos de 72 h. A adsorção média para íons Cd (II) é de 94%, com uma capacidade de adsorção de equilíbrio de 6,29-6,97 mg/kg. Entre os meios de influência, o pH, temperatura, dosagem do adsorbente, tempo de contato e concentração de íons são os principais fatores que interferem na atividade adsorbente.

Palavras-chave : Contaminação por metais pesados; tratamento ambiental; tecnologias adsorptivas; revisão.



Introducción

En las últimas décadas es motivo de preocupación que los recursos acuáticos corran un mayor riesgo de contaminación por contaminantes como los derivados de hidrocarburos, los desechos orgánicos y los metales pesados [1]. El desalojo de metales pesados de las industrias y las zonas urbanas aumenta constantemente y causa daños al medio ambiente y a la salud humana [2]. Los contaminantes inorgánicos se introducen diariamente en el medio ambiente a causa de actividades naturales como el vulcanismo, la erosión del lecho rocoso, el transporte atmosférico y la liberación por plantas y acciones antrópicas, debido a la industrialización, la actividad de exploración y la gestión de residuos [3], [4].

Entre los principales metales contaminantes en el suelo y el agua se encuentran el cadmio (Cd) y el plomo (Pb). El cadmio es uno de los elementos más tóxicos para el medio ambiente y la salud humana [5]. En medio acuoso, el cadmio ocurre comúnmente como el ion Cd (II), el cual es movilizado en condiciones ácidas. Las directrices de la Organización Mundial de la Salud (OMS) para la calidad del agua potable restringen las dosis altas de cadmio como un valor de referencia de 3 µg/L de Cd; las dosis altas de cadmio se asocian con trastornos del metabolismo de la glucosa, cáncer de mama y pulmón, infarto cerebral e insuficiencia cardíaca [6], [7].

Entre los metales pesados, los iones metálicos Pb (II) se consideran uno de los más altamente tóxicos para el medio ambiente y los humanos, porque estos iones no se biodegradan y se acumulan en los ecosistemas [8], [9]. Múltiples fuentes pueden proporcionar liberación de Pb en el medio ambiente, como gasolina con plomo, pinturas, baterías, cerámica y desechos electrónicos destacados, que son fuentes de contaminación ambiental potencial [10]. La OMS tiene como valor de referencia para el plomo en el agua potable 10 µg/L [11]. Las dosis continuas de plomo pueden causar presión arterial sistólica en adultos e impactos en el aprendizaje de los niños [12], enfermedades cardiovasculares y provocar la muerte [13].

En vista de los impactos sobre el medio ambiente y la salud humana, varias investigaciones han vuelto a estudiar tecnologías para la adsorción de plomo y cadmio, principalmente sus iones 2+, porque son la forma más encontrada en los recursos hídricos y el suelo. Algunos estudios reportan la actividad adsorbente del carbón activado de *Cocos nucifera* contra iones 2+ de Pb, Cd, Ni, Mg y otros iones metálicos, libremente formados o asociados con conjuntos microbianos y ácidos orgánicos [14]-[16].

Además de las características del adsorbente elegido, factores como el tipo de adsorción, los niveles de concentración de contaminantes, la dosis del adsorbente, el pH, la temperatura y otras características fisicoquímicas pueden interferir con el proceso de adsorción de iones metálicos. Visualizando la complejidad de las tecnologías de adsorción y el daño causado por los iones Cd (II) y Pb (II), el presente estudio tiene como objetivo describir nuevas tecnologías adsorbentes y factores que pueden ayudar o dificultar la adsorción de estos iones, mediante un estudio bibliográfico sobre bases de trabajo relevantes dentro del tema en la última década.

Materiales y métodos

Para llevar a cabo el estudio, se optó por realizar una revisión bibliográfica relacionada con las tecnologías descritas en la literatura con potencial de adsorción frente a iones Cd (II) y Pb (II), con el fin de recopilar información relevante, como capacidad, tiempo, curva de adsorción y aspectos fisicoquímicos que pueden influir en el proceso de adsorción. Al llevar a cabo la revisión, se abordaron los procedimientos y normas de Prisma (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*).

Procedimiento de búsqueda de artículos

Las bases de datos se utilizaron para buscar artículos de fuentes de investigación primarias, incluyendo Scopus, Web of Science, CAB, ScienceDirect e Engineering Village, y se accedió a las bases de datos a través del Portal de Periódicos Capes – MEC (Coordinación de Perfeccionamiento del Personal de Educación Superior, una agencia vinculada al Ministerio de Educación de Brasil). Se analizaron artículos publicados en los últimos 10 años (2013-2023); con el objetivo de integrar la revisión, la elección del período sirve como instrumento para analizar las tecnologías más recientes aplicadas a la adsorción de iones Cd (II) y Pb (II) en aguas superficiales y efluentes industriales. Para la búsqueda en las fuentes de investigación se utilizaron los términos “Tecnologías de adsorción”, “Cd (II)”, “Pb (II)”, “Adsorción iónica”, “Medios de influencia” y “Adsorción de Cd (II) y Pb (II)”, junto con los operadores booleanos *AND* y *OR*; los términos fueron buscados en portugués, inglés y español.

Análisis de estudios

Los resultados obtenidos en las bases de datos tuvieron su RIS (Reference Manager) exportado y añadido al *software* libre Rayyan (<https://www.rayyan.ai/>) [17]; para la selección de artículos dentro del tema del estudio propuesto y la eliminación de duplicados, en caso de estudios duplicados, se dio preferencia a la inclusión del estudio encontrado por el primero, independientemente de la base de datos indexada. Se incluyeron estudios disponibles en su totalidad, dentro del tema de interés, en cualquier idioma. Se excluyeron los estudios incompletos, como el tema de investigación, tesis, disertaciones, monografías, libros y capítulos de libros, carta al editor y resúmenes de eventos.

Los principales resultados obtenidos con el análisis de los artículos fueron dispuestos en una hoja de cálculo de Microsoft Excel para ser utilizados en la preparación del estudio. A fin de seleccionar los artículos que incluyen la revisión, inicialmente se excluyeron los estudios fuera del marco temporal; después de eliminar los duplicados en el *software* Rayyan, los estudios fueron seleccionados por medio de la lectura de resúmenes y palabras clave, luego de ser seleccionados nuevamente mediante la lectura completa, lo que resultó en la selección de los artículos que forman parte de la versión final de la revisión. Para la proyección de fórmulas y estructuras químicas, se utilizó el *software* ChemDraw 17.0.

Resultados y discusión

En total se encontraron 1172 artículos, 612 en Scopus, 104 en Web of Science, 91 en CAB, 348 en ScienceDirect y 17 en Engineering Village; 831 artículos fueron excluidos porque no estaban en el marco de tiempo descrito para el estudio; 341 artículos pasaron para análisis y lectura duplicados. Usando el *software* Rayyan, se excluyeron 167 artículos porque estaban duplicados; la tasa de duplicación es alta debido a que las bases de datos Scopus y ScienceDirect son administradas por Elsevier y ambas agregan algunas de las mismas revistas del editor. Se leyeron a fondo los títulos, resúmenes y palabras clave de 174 estudios, y se excluyeron 111 artículos porque no estaban dentro del tema, porque informaban métodos de adsorción mecánica o porque trabajaban con otros iones que no son objeto del presente estudio. Se leyeron 63 artículos completos y se seleccionaron 9 artículos para componer la revisión final, gracias a que estaban dentro de todos los criterios de inclusión propuestos. La base de datos Scopus tuvo la mayor cantidad de artículos incluidos en la versión final de la revisión, con 4 artículos, seguida de ScienceDirect con 3, CAB con 1 y Web of Science con 1. En Engineering Village no se seleccionaron artículos.

Con el análisis de los estudios encontrados en las bases de datos, se identificaron aspectos de los principales materiales adsorbentes y se dispusieron para la Tabla 1.

Tabla 1. Materiales adsorbentes más citados y factores de influencia.

Autor, año	Iones	Material adsorbente y dosaje	Factores influyentes	Matriz acuosa	Referencia
Todorava <i>et al.</i> , 2019	Cd (II) y Pb (II)	<i>Bacillus cereus</i> (2 g/L)	pH, temperatura y dosificación	Aguas superficiales	[18]
Park <i>et al.</i> , 2019	Cd (II) y Pb (II)	Posos de café (0,1 g/L)	pH	Efluentes industriales	[19]
Mathivaran <i>et al.</i> , 2021	Cd (II) y Pb (II)	<i>Bacillus cereus</i> (25 mg/L)	pH y dosificación de adsorbentes	Aguas superficiales	[20]
Igberase <i>et al.</i> , 2020	Cd (II) y Pb (II)	Quitosano (3,9 mmol/L)	Concentración inicial y velocidad de agitación	Aguas superficiales	[21]
Mohapatra <i>et al.</i> , 2019	Cd (II) y Pb (II)	Posos de café (1 g/L)	Tiempo de inmersión, fuerza iónica y pH	Efluentes industriales	[22]
Akhtar <i>et al.</i> , 2022	Cd (II) y Pb (II)	<i>Bacillus cereus</i> (10 mg/L)	Concentración de solución y pH	Aguas superficiales	[23]
Quiroga-Flores <i>et al.</i> , 2020	Cd (II)	Nanotubos de silicato-titanato en gránulos de quitosano (0,56 mg/L)	pH	Aguas superficiales	[24]
Zhang <i>et al.</i> , 2022	Cd (II) y Pb (II)	Quitosano/relaves amino oro (20 mg/L)	Dosis de adsorbente, tiempo de contacto	Aguas superficiales	[25]
Chwastowski <i>et al.</i> , 2020	Cd (II) y Pb (II)	Posos de café (0,5 g/L)	pH, dosis de adsorbente y tiempo de contacto	Aguas superficiales	[26]

Adsorción de iones Pb (II) y Cd (II) utilizando productos naturales

La literatura retrata diferentes especies vegetales con actividad adsorbente para iones Pb (II) y Cd (II), como para otros iones; se demostró la acción adsorbente del polvo foliar, cáscaras, raíces y tallo frente a iones metálicos en medio acuoso, así como la biomasa de diversas plantas, algas y microalgas, con la aplicación en diferentes medios, como la fitorremediación.

Varios estudios reportan la acción adsorbente de varias plantas y sus diferentes partes contra iones Pb (II) y Cd (II); se identificó la acción adsorbente de cáscaras de papa (*Solanum tuberosum*) [27], hojas de espinillo de Cristo (*Ziziphus spina-christi*) [28], cáscaras de rambutón (*Nephelium lappaceum* Linn.), maracuyá (*Passiflora laurifolia* Linn.), pitaya (*Hylocereus costaricensis*) [29], biomasa de phytolaca (*Phytolacca americana* Linn.) [30], entre otras varias especies vegetales.

Al-Onazi *et al.* [31] verificaron la acción de iones de adsorción de Cd (II) y Pb (II) con el uso de carbón activado de cáscaras de *Punica granatum* (granada) y cáscaras de *Phoenix dactylifera* (dátil). Las cáscaras fueron molidas y tamizadas para obtener polvo fino con un promedio de $<120 \mu\text{m}$, para la activación H_3PO_4 y ZnCl_2 en una proporción de 1:1 durante 24 h. A fin de calcular la adsorción de Cd (II) y Pb (II), evaluaron en un rango de pH de 2,5 – 7 con 100 mg/L de concentración de metal, tiempo de contacto de 90 min y dosis de adsorbente de 0,5 g/L para granada y 1 g/L para dátil. Los resultados demuestran la capacidad de adsorción de los carbonos evaluados; la tasa máxima de eliminación para Pb (II) fue del 91,1% para el carbón activado de granada (CAR) y del 88,8% para el carbón activado con dátil (CAT) a pH 6. La tasa máxima de eliminación para Cd (II) fue del 92,5% para CAR y del 87,3% para CAT a pH 6,5. Debido al proceso de protonación y la formación de óxidos hidratados protonados, las dosis de adsorbente son ineficientes en soluciones de pH bajo (1,3).

Un estudio demostró que el uso del macrófito *Potamogeton malaianus* permite obtener altos resultados en la adsorción de cadmio y plomo en agua; por medio de la fitorremediación, la adsorción se produce rápidamente (2 h) y logra un equilibrio dinámico en menos de 72 h. La adsorción media para los iones Pb (II) y Cd (II) es del 94%, con una capacidad de adsorción en equilibrio de 6,29-6,97 mg/kg. El estudio también reveló que los iones metálicos se concentran en hojas (12,44-38,15 mg/kg) y raíces (10,32-26,10 mg/kg); la espectroscopia infrarroja muestra que la acción $C = N$ puede estar involucrada en la capacidad de adsorción de la especie [32].

El uso de carbón activado de cáscaras de melón (*Cucumis melo*) con un tamaño entre 75 y 125 μm actúa como material adsorbente de iones Cd (II) y Pb (II), así como Cr (IV) y Ni (II). Todas las pruebas fueron a la concentración de 250 mg de adsorbente en 100 mL de solución acuosa a concentraciones iniciales (100, 200, 300, 400 mg/L). Las curvas de adsorción mostraron que la concentración de 100 mg/L es la más eficiente en la eliminación de iones Pb (II) y Cd (II) en todos los períodos evaluados (40, 80, 120, 160, 200 min); para ambos iones fue posible la adsorción de 100 mg después de 200 min [33]. Otro estudio que utilizó carbón activado de cáscaras de melón también retrató la actividad adsorbente contra los iones de plomo. La tasa de adsorción alcanza el 94,88% para los iones Pb (II), mientras que la isoterma de adsorción ajustada al modelo de Langmuir alcanzó 0,0830 mg/g. La espectroscopia de energía dispersiva de rayos X (EDX) relaciona el intercambio iónico y la microprecipitación como los principales mecanismos relacionados con la adsorción [34].

La optimización de la adsorción de iones Pb (II) y Cd (II) con el uso de carbón activado de vainas de *Acacia senegal* en solución ternaria acuosa es un método viable. La actividad adsorbente puede estar relacionada con la presencia de grupos funcionales -OH y -C = C-, que fueron identificados por espectroscopia infrarroja por transformada de Fourier, además de la diversidad de tamaños de poro de carbón, identificados con la ayuda de microscopía electrónica de transmisión. El carbón activado con H_3PO_4 mostró una tasa de adsorción de 98,54 y 99,96% para Cd y Pb, respectivamente, a una concentración de 145,06 y 63,83 mg/L, y un tiempo de contacto de 8 min [35].

Investigadores describen que la biomasa de múltiples especies actúa como material adsorbente. Phaenark *et al.* [36] informan que el bagazo de caña de azúcar y la pluma de maíz tienen acción adsorbente para los iones Pb (II) y Cd (II) en agua contaminada; sufren influencia directa del pH y la dosificación en la eficiencia de eliminación. Con el aumento de la dosis de Pb (II) y Cd (II) la eliminación también aumentó, en relación con el pH, las soluciones de Pb (II) con pH 7, y presentan menores tasas de adsorción con un pH óptimo entre 5 y 6, con una tasa de hasta el 98%; para soluciones de Cd (II) el pH no muestra influencia en la curva de adsorción.

Adsorción iónica Pb (II) y Cd (II) con hongos y bacterias

Investigadores visualizan en bacterias y hongos nuevas tecnologías para la biorremediación de diversos compuestos contaminantes del medio ambiente. El uso de bacterias y hongos ya es conocido en la biorremediación de metales pesados y otros productos químicos en el suelo. En el caso de la adsorción de iones metálicos en soluciones acuosas, se vio en la literatura que el género *Bacillus* sp. es ampliamente estudiado como tecnología adsorbente.

Wang y Sun [37] estudiaron la capacidad adsorbente de una especie mutante de *Bacillus subtilis* (B38), que en una investigación anterior ya había presentado una resistencia cuatro veces mayor que la de las soluciones de cadmio [38]. Los estudios de Wang y Sun utilizaron concentraciones de 56 mg/L de Cd y 104 mg/L de Pb, preparadas con agua destilada y concentración de 5×10^9 células/mL, 2-100 mL/L de material adsorbente, en un rango de pH 3 a 9. *Bacillus subtilis* (B38) tiene afinidad por los iones metálicos catiónicos y presenta tasas de sorción de equilibrio de 210,6 y 420,9 mg/g para Cd (II) y Pb (II), respectivamente, lo que muestra a la especie mutante como un biosorbente prometedor.

Otra especie del género *Bacillus* sp. también mostró buenos resultados en la adsorción de Cd (II). En labiomasa muerta de *Bacillus laterosporus* (MTCC 1628), la capacidad máxima de adsorción se midió a partir del modelo de Langmuir, definido por la ec. (1):

$$R_L = \frac{1}{1 + bC_0} \quad (1)$$

C_0 es la concentración inicial de vehículos metálicos (mg/L) y b la constante de Langmuir (mg/L). Las isothermas de adsorción muestran la eficiencia a todas las temperaturas evaluadas para la adsorción de Cd (II), por biomasa de *B. laterosporus*, con tasas de 84,03, 85,47 y 86,20 mg/g, a 20, 30 y 40 °C, respectivamente. Los resultados revelan que el aumento de la temperatura influye en la adsorción de cadmio por la biomasa de *B. laterosporus* [39].

La aplicación de bacterias mutantes o recombinantes ha sido un campo de estudio para la adsorción de iones metálicos. Jia *et al.* [40] utilizaron la especie bacteriana gramnegativa *Rhodospseudomonas palustris* recombinante. El plásmido pSCMT (Kana) se utilizó para la expresión de metalotioneína (MT) llamada ShMT, originada del cangrejo de agua dulce *Sinopotamon honanensis*. Mediante electroporación del plásmido se obtuvo *R. palustris*/pSCMT recombinante. *R. palustris*/pSCMT alcanzó el balance de biosorción después de 30 min; luego de alcanzar el equilibrio los niveles permanecieron estables. *R. palustris*/pSCMT mostró una capacidad máxima de adsorción de 225,19 mg/g para Cd (II); comparando las tasas de adsorción de *R. palustris*/pSCMT y la de *R. palustris* salvaje, se observa que la cepa recombinante es más eficiente, ya que la cepa salvaje mostró una capacidad máxima de adsorción para iones cadmio de 147,48 mg/g.

Los hongos también han sido una alternativa para la adsorción de iones metálicos en el agua y el suelo. Las especies fúngicas se utilizan de forma recombinante, junto con una biomasa o de forma aislada. Varios hongos demostraron ser viables en la adsorción de iones Pb y Cd; entre ellos se encuentran *Aspergillus flavus* (TISTR 3637), *Aspergillus gracilis* (TISTR 3638), *Aspergillus penicillioides* (sp. 1) (TISTR 3639), *Aspergillus penicillioides* (sp. 2) (TISTR 3640), *Aspergillus restricted* (3641) y

Sterigmatomyces halophilus (TISTRA 5926), con tasas de eliminación entre el 50 y el 93% para los iones de cadmio y entre el 40 y el 87% para los iones de plomo [41].

La especie *Trametes pubescens* (MB 89) probó la adsorción de iones metálicos de cadmio; las esporas se inocularon inicialmente en solución tampón de citrato de fosfato al 20%, pH 4,5 y resistencia al cadmio investigada a través del crecimiento radial en medio de agar dextrosa de papa. Las pruebas de adsorción se realizaron en Erlenmeyer de 250 mL llenos con 100 mL de medio líquido y diferentes concentraciones de Cd (II) (0, 5, 10, 50 y 100 mg/L). Para calcular las tasas de adsorción de Cd (II) se utilizó la ecuación de Xu *et al.* [42], Ec. (2):

$$q_e = \frac{V(C_i - C_e)}{W} \quad (2)$$

q_e es la adsorción de Cd (mg Cd/kg biomasa); V es el valor de la solución metálica en mL; C_i y C_e son las concentraciones iniciales y de equilibrio de cadmio en la solución, en mg/L; y W el peso del micelio seco en g. La eliminación de Cd por *T. pubescens* en solución acuosa viable, a una concentración de 100 mg/L de adsorción, fue de 6,565 mg/g, la más alta reportada en el estudio. La tasa máxima de adsorción fue del 53,13%, para la concentración inicial de 10 mg/L de Cd [43].

La especie fúngico-macroscópica *Pleurotus ostreatus*, probada por Kocaoba y Arisoy [44] contra iones metálicos de plomo y cadmio, fue una de las variedades fúngicas que mostró acción adsorbente. En soluciones de 2,5 - 25 µg de Cd (II) y Pb (II) en 100 mL, se evaluó la actividad adsorbente de la biomasa de *P. ostreatus* en una columna de corriente descendente. En la menor cantidad de material adsorbente (0,1 g) ya ha presentado recuperación de ambos iones por encima del 80%, con el aumento de la cantidad de adsorbente, consiguiendo un equilibrio en la dosis de 0,3 g con una tasa de recuperación del 100%.

Adsorción por nanocompuestos químicos y asociados

Los nanocompuestos son estructuras en las que al menos una partícula tiene un tamaño nanométrico. Su uso ha sido generalizado porque tiene una mayor eficiencia en comparación con los materiales compuestos. La literatura describe nanocompuestos de $\text{Fe}_3\text{O}_4/\text{MnO}_2$ [45], quitosano con ácido polimetacrílico reticulado y nanotubo de haloisita (Cs-PMA/HNT) [46], nanocompuestos híbridos de hidroxifosfato e hidroxisulfato férrico asociados con el hongo *Aspergillus niger* (n-HFP + n-HFS)@An [47], como algunos entre la diversidad de nanocompuestos que pueden actuar como material adsorbente de iones Cd (II) y Pb (II).

Un nanocompuesto superparamagnético basado en el poli (*N*-vinilpirrolidona-co-anhídrido maleico) enrejado (PNVPMA) en reacción con ácido 3-aminobenzoico se identificó como un agente modificador de reticulación. A través de un sistema discontinuo, se probó la capacidad de adsorción del nuevo nanocompuesto contra Pb (II) y Cd (II), y se observó la influencia de múltiples factores en la adsorción, incluido el pH de la solución y la dosificación del nanocompuesto. Los resultados de las isoterms de adsorción son similares a las isoterms de adsorción de Langmuir; el nanocompuesto presentó una capacidad máxima de adsorción de 312,5 y 32,78 mg/g para iones de plomo y cadmio, respectivamente [48].

Nanocompuesto LH-MH a base de Polyphedan (lignina hidrolítica conifera comercial) y solución de hidróxido de sodio obtenida por agitación magnética fue evaluado por Ponomarev *et al.* [49] como material adsorbente, en soluciones madre catiónicas de concentración de 0,1 mol/L y 2 g/L de solución de Cd y Pb, respectivamente a pH 6,5. La capacidad de adsorción (q_{exp}) fue determinada por la ec. (3): v

$$q_{\text{exp}} = \frac{C_i - C_e}{m} V \quad (3)$$

C_i es la concentración inicial de cationes (mmol/L), C_e la concentración de cationes después de la eliminación (mmol/L), m la masa del material granular (mg) y V el volumen de la solución (mL). El pH interfiere activamente en la adsorción de plomo y cadmio; debido a la interacción electrostática del pH por debajo de 6,7 para lignina y 10,6 para LH-MH, favorece la adsorción de iones metálicos. LH-MH muestra mayor capacidad de adsorción para iones Pb (II), con una tasa de adsorción de 1,24 mmol/g, atribuida a su menor hidratación, que ayuda en la fijación en la superficie. Para los iones Cd (II), LH-MH mostró una tasa menor de 0,94 mmol/g.

El polisacárido de quitosano (Figura 1) es uno de los compuestos más identificados para la preparación de material adsorbente de iones Cd y Pb. Se preparó y evaluó un nuevo nanocompuesto basado en quitosano, llamado Gd/SrFe@SBCs, mediante microscopía electrónica de barrido por emisión de campo (FE-SEM), espectro dispersivo de energía de rayos X (EDS) y espectroscopía infrarroja por transformada de Fourier (FT-IR) y, posteriormente, se evaluó la capacidad de adsorción. El estudio relaciona la capacidad de adsorción con la estructura química del quitosano, lo que facilita la captura de iones metálicos; debido a su estructura permiten la porosidad del elemento, visualizado en microscopía electrónica de barrido, que puede fijar los iones metálicos y eliminarlos de la superficie acuosa.

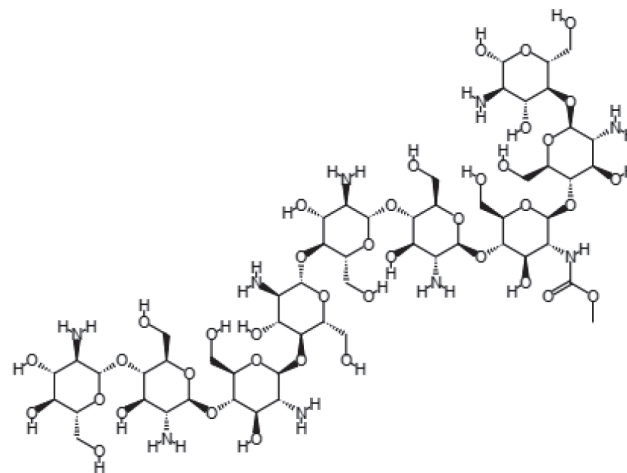


Figura 1. Estructura química del quitosano ($\text{C}_{36}\text{H}_{103}\text{N}_9\text{O}_{39}$).

Gd/SrFe@SBCs ensayado en la adsorción de Iones Pb (II) y Cd (II) en solución acuosa presentó eficiencia del 98% para ion Pb (II) y del 78% para ion Cd (II); en las mejores condiciones la capacidad máxima de adsorción alcanza valores de 146,2 y 183,7 mg/g para Pb (II) y Cd (II), respectivamente [50].

Conclusiones

Con el análisis de los artículos que componen el estudio fue posible observar una diversidad de nuevos compuestos que se utilizan como materia adsorbente de iones Pb (II) y Cd (II). Los estudios dentro de este tema son relevantes para discutir e investigar nuevas tecnologías para el tratamiento de suelos y aguas contaminadas con metales pesados. Diferentes estudios informan de la posible capacidad adsorbente de estos materiales frente a otros iones metálicos, como níquel, manganeso, zinc y aluminio. Deberían realizarse más estudios para reforzar el tema y buscar nuevas tecnologías alternativas para la adsorción de iones de plomo y cadmio.

Las altas tasas de adsorción de plomo y cadmio en aguas superficiales y efluentes industriales, como las de Acacia senegal (98,54 y 99,96% para Cd y Pb), granada (91,1 y 92,5% para Cd y Pb) y dátil (88,8 y 87,3% para Cd y Pb), demuestran que la biomasa vegetal puede ser utilizada como alternativa en la eliminación de iones de plomo y cadmio.

Este artículo contribuye a la profundización y desarrollo de nuevas reflexiones sobre la adsorción de iones 2+ de plomo y cadmio, con el fin de proporcionar una base bibliográfica de apoyo científico para otros estudios dentro del área.

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