Scientific research article / https://doi.org/10.15446/rcciquifa.v53n3.119241

Phytochemistry and antibacterial property of *Arisaema liemiana*

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Received: April 14, 2024
Corrected: June 18, 2024
Accepted: June 24, 2024

Summary

Introduction: *Arisaema liemiana* was newly identified as a new species for the flora of Vietnam in 2020. It was discovered in Takou Nature Reserve, Binh Thuan province, Vietnam, characterized by its height of 20-50 centimeters, deciduous nature, light yellow spathe limb, white spathe tube, and spadix appendix covered with filiform neuter structures. **Aim:** This study aimed to investigate the chemical components and antibacterial activity of the acetone extracts derived from the aerial parts and tubers of *A. liemiana*. **Methodology:** *A. liemiana* specimens were collected from Takou Mountain within Takou Nature Reserve, Binh Thuan Province, and subjected to acetone extraction. The chemical components of these extracts were analyzed using GC-MS, while the antibacterial activity was assessed through the agar disc diffusion method. **Results:** A total of 57 compounds were identified in the aerial part and tuber extracts of which 32 components were found in the first extract

while the latter one contained 34 constituents. The antibacterial effectiveness of the tuber acetone extracts was assessed against 10 bacterial strains, revealing diverse inhibition zones. Notably, *S. aureus* ATCC 25923 exhibited the largest inhibition zone diameter, followed by *B. cereus*, *V. parahaemolyticus*, *S. flexneri*, *P. aeruginosa*, *E. coli*, *L. monocytogenes*, *S. saprophyticus*, *S. typhimurium*, and *S. enteritidis*. **Conclusion:** The chemical composition and antibacterial properties of acetone extracts derived from *Arisaema liemiana* was investigated for the first time. The observed notable antibacterial efficacy suggests potential pharmacological implications of *A. liemiana*. Additionally, this research unveils broader applications of *A. liemiana* in herbal medicine.

Keywords: Aracae, *Arisaema liemiana*, GC-MS, antibacterial activity.

Resumen

Fitoquímica y propiedad antibacteriana de *Arisaema liemiana*

Introducción: *Arisaema liemiana* fue identificada recientemente como una nueva especie para la flora de Vietnam en 2020. Fue descubierta en la Reserva Natural de Takou, provincia de Binh Thuan, Vietnam, caracterizada por su altura de 20-50 centímetros, naturaleza caducifolia, extremidad de espata de color amarillo claro, tubo de espata blanco y apéndice del espádice cubierto de estructuras neutras filiformes. **Objetivo:** Este estudio tuvo como objetivo investigar los componentes químicos y la actividad antibacteriana de los extractos de acetona derivados de las partes aéreas y tubérculos de *A. liemiana*. **Metodología:** Los especímenes de *A. liemiana* se recolectaron de la montaña Takou dentro de la Reserva Natural de Takou, provincia de Binh Thuan, y se sometieron a extracción con acetona. Los componentes químicos de estos extractos se analizaron mediante GC-MS, mientras que la actividad antibacteriana se evaluó mediante el método de difusión en disco de agar. **Resultados:** Se identificaron 57 compuestos en los extractos de la parte aérea y del tubérculo, de los cuales 32 componentes se encontraron en el primer extracto, mientras que el último contenía 34 constituyentes. Se evaluó la eficacia antibacteriana de los extractos de acetona del tubérculo frente a 10 cepas bacterianas, lo que reveló diversas zonas de inhibición. Cabe destacar que *S. aureus* ATCC 25923 exhibió el mayor diámetro de zona de inhibición, seguido de *B. cereus*, *V. parahaemolyticus*, *S. flexneri*, *P. aeruginosa*, *E. coli*, *L. monocytogenes*, *S. saprophyticus*, *S. typhimurium* y *S. enteritidis*. **Conclusión:** Se investigó por primera vez la composición química y las propiedades

antibacterianas de los extractos de acetona derivados de *Arisaema liemiana*. La notable eficacia antibacteriana observada sugiere posibles implicaciones farmacológicas de *A. liemiana*. Además, esta investigación revela aplicaciones más amplias de *A. liemiana* en la medicina herbal.

Palabras clave: Aracae, *Arisaema liemiana*, GC-MS, actividad antibacteriana.

Resumo

Fitoquímica e propriedade antibacteriana de *Arisaema liemiana*

Introdução: *Arisaema liemiana* foi recentemente identificada como uma nova espécie para a flora do Vietnã em 2020. Foi descoberta na Reserva Natural de Takou, província de Binh Thuan, Vietnã, caracterizada por sua altura de 20-50 centímetros, natureza caducifólia, membro de espata amarelo claro, tubo de espata branco e apêndice de espádice coberto com estruturas neutras filiformes. **Objetivo:** Este estudo teve como objetivo investigar os componentes químicos e a atividade antibacteriana dos extratos de acetona derivados das partes aéreas e tubérculos de *A. liemiana*. **Metodologia:** Espécimes de *A. liemiana* foram coletados da Montanha Takou dentro da Reserva Natural de Takou, Província de Binh Thuan, e submetidos à extração de acetona. Os componentes químicos desses extratos foram analisados usando GC-MS, enquanto a atividade antibacteriana foi avaliada através do método de difusão em disco de ágar. **Resultados:** Um total de 57 compostos foram identificados na parte aérea e extratos de tubérculos, dos quais 32 componentes foram encontrados no primeiro extrato, enquanto o último continha 34 constituintes. A eficácia antibacteriana dos extratos de acetona de tubérculos foi avaliada contra 10 cepas bacterianas, revelando diversas zonas de inibição. Notavelmente, *S. aureus* ATCC 25923 exibiu o maior diâmetro de zona de inibição, seguido por *B. cereus*, *V. parahaemolyticus*, *S. flexneri*, *P. aeruginosa*, *E. coli*, *L. monocytogenes*, *S. saprophyticus*, *S. typhimurium* e *S. enteritidis*. **Conclusão:** A composição química e as propriedades antibacterianas dos extratos de acetona derivados de *Arisaema liemiana* foram investigadas pela primeira vez. A notável eficácia antibacteriana observada sugere potenciais implicações farmacológicas de *A. liemiana*. Além disso, esta pesquisa revela aplicações mais amplas de *A. liemiana* na medicina herbal.

Palavras-chave: Aracae, *Arisaema liemiana*, GC-MS, atividade antibacteriana.

Introduction

Arisaema Martius is one of a large genus belonging to Araceae family which includes 15 sections [1] and about 201 species [2] widely found in the tropical Asia, Himalayas, Australia and New Guinea [3-7]. About 25 species belonging to 6 sections have been recorded for the flora of Vietnam [2, 3, 7-11]. So many *Arisaema* members have been known as the medicinal plants. For example, *A. rhizomatum* is known for its traditional medicine to treat contusions, injuries from falls, strains. It is also used to dispel wind, quicken blood, free the flow of network vessels, etc. [11]. Apart from that, the tuber extracts of *A. amurense*, *A. asperatum*, *A. calcareum*, *A. serratum*, and *A. heterophyllum* were used as antitumor, analgesic, and pesticide agents [12]. In addition, studies provided the chemical compositions and pharmaceutical properties of the extracts obtained from various *Arisaema* plants [13-18].

Arisaema liemiana Luu, H.T.Van, H.C.Nguyen & V.D.Nguyen was first described as a new species for the flora of Vietnam in 2020 which the type specimens collected from Takou Nature Reserve, Binh Thuan province, Vietnam. *A. liemiana* is a deciduous herbs and the morphology of this species are characterized by having: 20-50 high deciduous, the light yellow spathe limb, white spathe tube, spadix appendix covered with filiform neuters [2]. To date, *A. liemiana* is a rare species and it is only found in the location where its type specimens were collected. Thus, the present study firstly investigated the chemical components and antibacterial activity of the acetone extracts from the aerial parts and tubers of *A. liemiana*.

Materials and methods

Plant

Arisaema liemiana was collected from Takou Mountain, Takou Nature Reserve, Binh Thuan province (Figure 1) where the type specimens of this species were discovered in the year 2020 [2].

Figure 1. *Arisaema liemiana* in its habitat

Bacterial strains

In this study, ten tested bacteria were used to identify the antibacterial activities of the acetone extracts of the studied species, including four *Gram-positive* bacteria (*Bacillus cereus* ATCC 11774, *Listeria monocytogenes* ATCC 19111, *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophyticus* BAA750), and six *Gram-negative* bacteria (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311, *Salmonella enteritidis* ATCC 13976, *Pseudomonas aeruginosa* ATCC 27853, *Shigella flexneri* ATCC 9199, *Vibrio parahaemolyticus* ATCC 17802).

Extract preparation

The fresh aerial and tuber of *A. liemiana* were dried at 50°C and then ground into powder. Five hundred milliliters of 99% acetone solution (Thermo Fisher Scientific, USA) were used to soak 100 grams of the sample for for 48 hours at room temperature. The extract was obtained by filtration using Whatman paper and its residue was re-extracted two more times. The final filter was evaporated under vacuum condition at 45°C to remove acetone solution.

Gas Chromatography-Mass Spectrometry (GC-MS)

This method was performed using a TRACE™ 1310 Gas Chromatograph (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled with an ISQ 7000 Single Quadrupole Mass Spectrometer. A DB-5MS column (30 m × 0.25 mm × 0.25 μm) was utilized as the stationary phase, and Helium at a flow rate of 1.2 mL/min was used as

the carrier gas to determine the chemical composition present in the acetone extract. The sample was injected into the GC system using a sample splitting method, adopting a 30:1 split ratio, a non-split flow duration of 1 minute, at 250°C with a flow rate of 36 mL/min. The injection temperature was consistently held at 250 °C. The thermal program of the oven was designed to start at 80 °C, sustained for 5 minutes, followed by an increment of 20 °C/min until a terminal temperature of 280 °C was reached, which was then maintained for 10 minutes. Electron impact ionization was set at 70 eV and the source filament temperature at 250 °C. The mass scan range of the MS was 29-650 m/z with a scan rate of 2 scans per second.

Spectral analysis was conducted using Thermo Scientific Xcalibur software, which facilitated data generation. Chemical component identification within the extract was predicated on retention times, mass spectra, and the peak areas of the compounds, which were compared against the known spectra in the library (NIST library 2017). The percentage composition of the compounds was quantified based on the peak area of each compound relative to the total peak area of all compounds, multiplied by 100.

Antimicrobial methods

The agar disc diffusion method was utilized to assess the antibacterial activity of the samples [19]. Bacterial strains are cultivated in Mueller-Hinton Broth (MHB) until a turbidity of 0.5 McFarland standard was attained. A 0.1 mL sample of the bacterial suspension was inoculated onto a Petri dish containing Mueller-Hinton Agar (MHA) and spread uniformly. Paper discs were then placed onto the agar surface, and 10 µL of the sample solution was applied onto these discs. Gentamycin antibiotic discs (Nam Khoa, Vietnam) were used as positive controls, while a 10% acetone solution serves as the negative control. The Petri dishes are stored at 4 °C for 2 hours to allow the sample solution to permeate into the agar. The cultures were then incubated at 37 °C for 16-18 hours to evaluate the resistance of the extract against the bacterial strains. The diameter of the inhibition zone was measured after 16-18 hours. The antibacterial activity of the sample solutions against the bacterial strains was determined based on the diameter of the inhibition zones, deducting the diameter of the paper disc (6 mm), which will be the zone of bacterial inhibition of the research sample.

To evaluate the differences between the means of the experimental treatments, the analysis of variance (ANOVA) method, following the LSD test, was employed. Statgraphic Centurion software version 15.2 and Excel 2010 were used to calculate the mean and standard deviation among the measurements.

Results and discussion

Chemical compositions of acetone extracts from *A. liemiana* tuber and aerial part

The chemical constituents of the acetone extracts of the *A. liemiana* tuber and aerial part were shown in the Figure 2 and Table 1. The aerial part extract was mainly composed of 2-pentanone, 4-hydroxy-4-methyl- (12.62%), 2,3-butanediol (11.61%), n-hexadecanoic acid (8.09%), neophytadiene (8.87%), phytol (6.64%), 9(E),11(E)-conjugated linoleic acid (6.49%), linolenic acid (6.38%), 2,6-cresotaldehyde (4.93%), γ-sitosterol (4.42%), linolein, 2-mono-(4.14%), stigmasterol (3.95%). The major compounds in the tuber extract were consisted of 2-pentanone, 4-hydroxy-4-methyl- (21.95%), γ-sitosterol (13.91%), stigmasterol (12.08%), campesterol (10.72%), ascorbic acid 2,6-dihexadecanoate (7.67%), 9(E),11(E)-conjugated linoleic acid (6.89%), cis-vaccenic acid (4.28%).

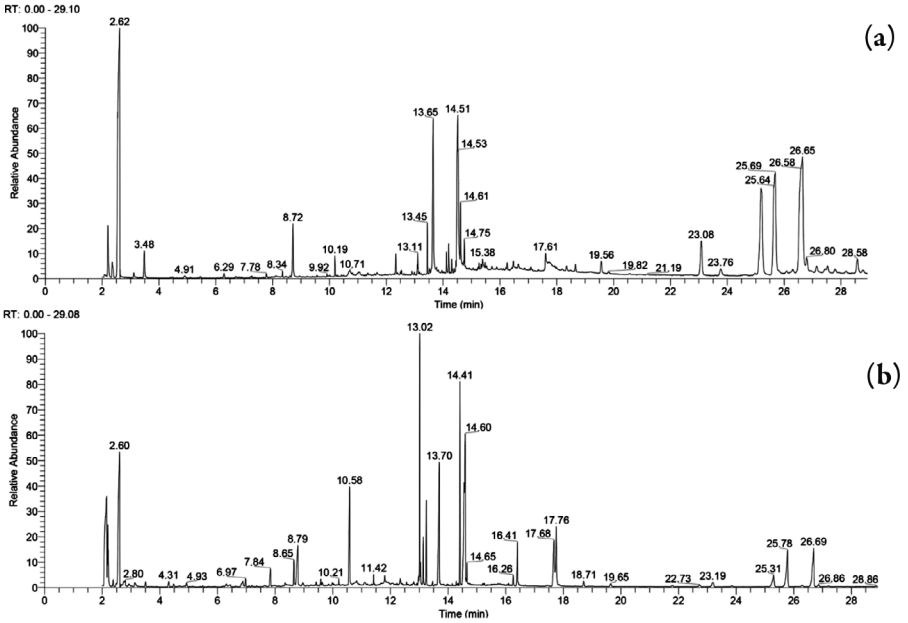

Figure 2. The GC chromatogram of acetone extracts from *A. liemiana* tuber (a) and aerial part (b)

The chemical compositions of the essential oils or the various solvent extracts obtained from the *Arisaema* plants using GC-MS technique were also reported by prior studies. For instance, the chemical compounds of the essential oils isolated from four organs like fruits, leaves, tubers and petioles of *A. amuremse* were also reported. Accordingly,

the fruit essential oil was found to be rich in hexadecanoic acid methyl ester (53.45%), 13-octadecenoate (7.82%), and (Z, Z)-9,12-octadecadienoic acid methyl ester (5.4%). The petiole oil contained 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (7.9%); 14-methylpentadecanoic acid methyl ester (7.7%); 1-hexadecanol acetate (4.05%) as the major compounds. The tuber essential oil was characterized by the prominence of 3-cyclohexyl-1-phenyl propane (14.86%), 2-pentadecanone (8.9%), perhydrofarnesyl acetone (6,10,14-trimethyl-2-pentadecanone (7.15%) whereas 6,10,14-trimethyl-2-pentadecanone (46.27%); hexadecanoic acid methyl ester (38.62%) and (Z) 9-octadecenoic acid methyl ester (12.27%) were the main components in the leaf oil [13].

In the case of the methanolic extract of *A. tortuosum* leaf, Garg *et al.* demonstrated the identification of a total of fifty-three phytochemicals. The initial compound eluted was nonane, which exhibited a retention time of 4.064 minutes within the leaf extract. Predominant peak areas were observed for 6,10,14-trimethyl-2-pentadecanone at 54.55% and phytol at 18.86% within the leaf extract. Other notable phytochemicals identified included 2-pentadecanone, 6,10,14-trimethyl-; phytol; benzene, 1,3-dimethyl-, etc. [20]. Moreover, the chemical components of the ethanolic extract, and its fractions like n-hexane and chloroform obtained from *A. tortuosum* leaf were also investigated. Accordingly, the ethanolic extract included 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (37.53%); hexadecanoic acid, methyl ester (20.57%) and 9,12-octadecadienoic acid, methyl ester (20.14%) as the major compounds. n-hexane fraction was found to be rich in phytol (16.90%); ethyl (9z,12z)-9,12-octadecadienoate (14.97%); hexadecanoic acid, ethyl ester (14.60%); dibutyl phthalate (19.33%); 1,2-benzenedicarboxylic acid, Bis(2- methylpropyl) ester (12.11%) and 1,2-benzenedicarboxylic acid (8.53%) [21].

Table 1. The chemical compounds of acetone extracts of *A. liemiana* aerial part and tuber

RT	Compounds	Relative peak area (%)	
		Aerial part	Tuber
2.15	2,3-Butanediol	11.61	-
2.21	3-Penten-2-one, 4-methyl-	-	2.00
2.36	2-Pentanone, 4-hydroxy-	-	0.79
2.60	2-Pentanone, 4-hydroxy-4-methyl-	12.62	21.95
2.80	L-Lactic acid	0.45	-
3.12	2-Ethyl-trans-2-butenal	-	0.24
3.48	2-Heptanol, acetate	-	1.16
4.31	2,5-Hexanedione, 3,4-dihydroxy-3,4-dimethyl-	0.40	-

(Continued)

Table 1. *Continuation.*

RT	Compounds	Relative peak area (%)	
		Aerial part	Tuber
4.91	1,3-Dioxane, 2-methyl-	-	0.26
4.93	2-Acetoxyisobutyryl chloride	0.36	-
5.45	Tetraethylene glycol, diacetate	-	0.12
6.29	Benzeneacetaldehyde	-	0.21
6.70	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	-	0.15
6.97	Pyrazine, tetramethyl-	0.41	-
7.84	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1.07	0.23
8.34	2-Pentanol, 2,4-dimethyl-	-	0.19
8.65	Coumaran	1.03	-
8.79	5-Hydroxymethylfurfural	3.75	2.28
9.58	2-Methoxy-4-vinylphenol	0.41	
10.19	1,6-Octadiene, 2,6-dimethyl-	-	0.60
10.58	2,6-Cresotaldehyde	4.93	-
11.42	Phenol, 4-ethenyl-2,6-dimethoxy	0.45	-
12.33	13-Methyltetradecanal	-	0.70
12.53	Tetradecanoic acid	-	0.24
13.02	Neophytadiene	8.87	-
13.04	3,7,11,15-Tetramethylhexadec-2-ene	0.56	-
13.09	Pentadecanoic acid	-	0.27
13.11	(E)-Hexadec-2-enal	-	0.49
13.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3.71	-
13.47	Hexadecanoic acid, methyl ester	0.26	-
13.65	Ascorbic acid 2,6-dihexadecanoate	-	7.67
13.70	n-Hexadecanoic acid	8.09	-
14.12	Heptadecanoic acid	-	0.67
14.20	Verticiol	-	0.79
14.30	8,11-Octadecadienoic acid, methyl ester	-	0.49
14.41	Phytol	6.44	-
14.53	cis-Vaccenic acid	-	4.28
14.56	9(E),11(E)-Conjugated linoleic acid	6.49	6.89
14.60	Linolenic acid	6.38	-
14.65	Octadecanoic acid	0.71	1.94
15.38	2-cis-9-Octadecenyloxyethanol	-	0.45

(Continued)

Table 1. *Continuation.*

RT	Compounds	Relative peak area (%)	
		Aerial part	Tuber
16.26	Heptacos-1-ene	0.58	-
17.61	7-Methyl-Z-tetradecen-1-ol acetate	-	0.66
17.68	Linolein, 2-mono-	4.14	-
17.76	Butyl 9,12,15-octadecatrienoate	3.80	-
18.34	E,E,Z-1,3,12-Nonadecatriene-5,14-dio	-	0.22
18.71	Squalene	0.51	-
19.56	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	-	0.62
19.65	17-Pentatriacontene	0.41	-
21.79	γ-Tocopherol	0.31	-
22.73	17-Pentatriacontene	0.37	-
23.19	Vitamin E	0.81	2.74
25.31	Campesterol	1.58	10.72
25.78	Stigmasterol	3.95	12.08
26.69	γ-Sitosterol	4.42	13.91
26.80	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	-	0.76
28.58	α-Tocopheryl acetate	-	0.89
	Total	**99.88**	**97.66**

Antibacterial activity of acetone extracts from *A. liemiana* tuber and aerial part

Overall, the acetone extract from *A. liemiana* tuber displayed activity against all the tested bacteria whereas these strains were not sensitive to the aerial part extract (Table 2). Accordingly, the tuber extract showed the strongest antibacterial effects against *S. aureus* ATCC 25923 with the inhibition zone diameter of 7.7 mm, followed by *B. cereus* (6.2 mm), *V. parahaemolyticus* (5.3 mm), *S. flexneri* (4.8 mm), *P. aeruginosa* (4.7 mm), *E. coli* (4.7 mm), *L. monocytogenes* (4.3 mm), *S. saprophyticus* (4.2 mm), *S. typhimurium* (2.3 mm), *S. enteritidis* (1.8 mm).

Prior reports demonstrated that members of the genus *Arisaema* grown in some Asian countries were used in the ethnopharmacology as an antimicrobial agent. For instance, in traditional medicine in India and China, *A. consanguineum* was extensively used as the antifungal and antibacteral medications [21]. Furthermore, *A. jacquemontii* was traditionally used as a folk remedy to treat microbial illness In Bhutan, India, Nepal, and Pakistan [22]. Similarly, In Chinese and Indian traditional medicine, *A. flavum* was also used as bacterial efficacy [23]. In addition, studies also provided the antibacterial activities of different solvent extracts obtained from the *Arisaema* species. For instance,

the ethanolic extract of the *Arisaema tortuosum* leaf had an inhibitory effect on *B. subtilis* and *S. typhimurium* whereas two its fractions, n-hexane and chloroform was active against *S. aureus* and *E. coli*, respectively [24]. Furthermore, the different extracts from *A. jacquemontii*, acetone, chloroform, distilled water, and methanol, was found to be effective against so many bacterial strains, including *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Shigella dysenteriae*, *Lysteria monocytogenes* and *Staphylococcus aureus* [25]. Similarly, the ethanol and methanol extracts from *A. jacquemontii* displayed activity against some bacterial and fungal strains, including *Micrococcus luteus* and *Aspergillus flavus* [17]. The methanolic extract of *A. jacquemontii* root was also reported to have an inhibitory effect on many microorganisms, such as *Proteus mirabilis*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella enteritidis*, *Micrococcus luteus*, *Enterobacter cloacae*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pasteurella multocida* [26].

Table 2. The antibacterial activity of acetone extracts from *A. liemiana* tuber

Bacterial strains	Inhibition zone diameter (mm)		
	Tuber	Gentamicine	Negative control
Bacillus cereus ATCC 11774	6.2 ± 0.6[a]	18.7 ± 0.8[b]	-
Escherichia coli ATCC 25922	4.7 ± 0.3[a]	14.8 ± 0.3[b]	-
Listeria monocytogenes ATCC 19111	4.3 ± 0.3[a]	6.3 ± 0.6[b]	-
Pseudomonas aeruginosa ATCC 27853	4.7 ± 1.2[a]	24.7 ± 0.6[b]	-
Staphylococcus aureus ATCC 25923	7.7 ± 0.6[a]	25.2 ± 1.0[b]	-
Staphylococcus saprophyticus BAA750	4.2 ± 0.3[a]	23.7 ± 0.6[b]	-
Salmonella enteritidis ATCC 13976	1.8 ± 1.3[a]	12.3 ± 0.3[b]	-
Salmonella typhimurium ATCC 13311	2.3 ± 0.6[a]	14.8 ± 0.8[b]	-
Shigella flexneri ATCC 9199	4.8 ± 0.8[a]	18.2 ± 1.0[b]	-
Vibrio parahaemolyticus ATCC 17802	5.3 ± 0.6[a]	12.3 ± 0.3[b]	-

Different lowercase letters within a row indicate significant differences in the inhibition zone diameter (± SD) (mm) between acetone extracts of Tuber and Gentamicin (LSD test: $P < 0.05$)

Conclusion

This research provides insights into the recently identified plant species *Arisaema liemiana* found in the flora of Vietnam in Takou Nature Reserve, Binh Thuan province. By examining acetone extracts from both aerial parts and tubers, the study investigated the chemical composition and antibacterial effects of *A. liemiana*. GC-MS analysis facilitated the identification of the chemical constituents, while evaluation against 10 bacterial strains revealed significant antibacterial activity of the tuber acetone extract. These findings enhance the understanding the pharmacological potential of *A. liemiana* and underscore its importance in medicinal and ecological research. Further

investigations are warranted to elucidate the mechanisms underlying its antibacterial properties and explore its broader pharmacological applications.

Acknowledgements

This research is supported by Industrial University of Ho Chi Minh City, Vietnam. The authors would like to thank MSc. Nguyen Thi Hong Van, officials of Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City for her collaboration.

Compliance with ethical standards

Conflict of interest: The authors have reported no conflicts of interest

Ethics approval: Not applicable.

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

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How to cite this article

D.H. Truong, T.V. Pham, N.H. Ngo, N. Nguyen-Phi, H.T. Luu, T.T. Le, Q.H. Nguyen, H.T. Van, Phytochemistry and antibacterial property of *Arisaema liemiana*, *Rev. Colomb. Cienc. Quim. Farm.*, **53**(3), 890-904 (2024). Doi: https://doi.org/10.15446/rcciquifa.v53n3.119241