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GC-MS Profiling and Antibacterial Activity of Ylang-Ylang Vine (*Artabotrys hexapetalus* (L.f.) Bhandari) Flower Extract

Waraporn Sutthisa[1]*, Chanittha Phimon[1], Chanthanat Thonglamol[1] and Nattaya Srisawad[2]

[1]Department of Biology, Faculty of Science, Mahasarakham University, Kantarawichai District, Mahasarakham Province 44150, Thailand.
[2]Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhon Pathom Province 73170, Thailand.

Abstract

The present study investigated the phytochemical composition and antibacterial activity of the hexane flower extract of *Artabotrys hexapetalus* (L.f.) Bhandari. The crude extract obtained from dried flowers was dark yellow, viscous, and possessed a characteristic floral odor, with an extraction yield of 20.78% (w/w). Gas chromatography-mass spectrometry (GC-MS) analysis detected 100 chromatographic peaks corresponding to 94 identified compounds, representing approximately 99.1% of the total peak area. The extract was predominantly composed of fatty acid derivatives (43.67%), followed by sesquiterpenes and oxygenated sesquiterpenes (31.09%), other identified constituents (18.22%), sterols and triterpenoids (5.57%), and monoterpenes (0.55%). The major constituents were 1H-cyclopropa[a] naphthalene derivative (10.08%), cyclohexane (9.50%), 9,12-octadecadienoic acid methyl ester (7.18%), hexadecanoic acid methyl ester (4.91%), and β-sitosterol (4.15%). The antibacterial activity of the extract was evaluated against representative Gram-positive and Gram-negative bacteria using the disc diffusion method. The extract inhibited the growth of all tested bacterial strains, with inhibition zones ranging from 6.33 ± 0.58 to 16.00 ± 1.73 mm. The strongest activity was observed against *Salmonella enterica* subsp. *enterica* serovar Typhi, followed by *Escherichia coli* ATCC 25922 and *Bacillus cereus*. However, the antibacterial activity was significantly lower than that of streptomycin ($P < 0.05$). The minimum inhibitory concentration (MIC) values ranged from 0.488 to 500 mg/mL, whereas the minimum bactericidal concentration (MBC) values were either 500 mg/mL or >500 mg/mL, depending on the bacterial strain. These findings demonstrate that the hexane flower extract of *A. hexapetalus* contains diverse bioactive phytochemicals, particularly fatty acid derivatives and sesquiterpenes, and exhibits broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The results provide a scientific basis for further studies on the isolation and characterization of antibacterial compounds from *A. hexapetalus*.

Keywords: *Artabotrys hexapetalus*, GC-MS Analysis, Hexane Extract, Sesquiterpenes, Fatty Acid Derivatives, Phytosterols, Antibacterial Activity

***Correspondence:** waraporn.s@msu.ac.th

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INTRODUCTION

Plant-derived metabolites have attracted considerable attention as valuable sources of bioactive compounds for pharmaceutical, agricultural, and food applications. Various classes of secondary metabolites, including terpenoids, phenolic compounds, alkaloids, and fatty acid derivatives, have been reported to possess a wide range of biological activities, such as antimicrobial, antioxidant, anti-inflammatory, and anticancer effects.[1,2] Owing to their structural diversity and biological potential, medicinal plants continue to be extensively investigated for the discovery of novel natural products.[3,4]

Artabotrys hexapetalus (L.f.) Bhandari is an aromatic flowering plant widely cultivated in tropical and subtropical regions.[5,6] The species is valued for its fragrant flowers and has long been used in traditional medicine and perfumery.[6] Previous phytochemical investigations have revealed that *A. hexapetalus* contains diverse bioactive constituents, including essential oil components, sesquiterpenoids, and other secondary metabolites.[5,6] Several of these constituents have been associated with antioxidant, antifungal, and other biological activities.[6] Despite its medicinal importance, information regarding the phytochemical composition and antibacterial activity of *A. hexapetalus* flower hexane extracts remains limited. Therefore, characterization of its phytochemical constituents and evaluation of its antibacterial potential are necessary to bridge this knowledge gap and provide scientific evidence supporting its future application as a natural source of antimicrobial compounds.

Meanwhile, antimicrobial resistance has emerged as a major global public health concern, reducing the effectiveness of conventional antibiotics and increasing the need for alternative antimicrobial agents. Foodborne and opportunistic pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella enterica*, remain important causes of human infections.[7,8] The increasing prevalence of antibiotic-resistant microorganisms has intensified efforts to identify natural products with antibacterial properties. Plant-derived metabolites, particularly terpenoids and fatty acid derivatives, have attracted considerable attention because of their antimicrobial potential against a wide range of pathogenic microorganisms.[9]

Therefore, the present study aimed to characterize the phytochemical constituents of the hexane flower extract of *A. hexapetalus* using GC-MS analysis and subsequently evaluate its antibacterial activity against selected Gram-positive and Gram-negative bacteria. In addition, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were determined to assess its antibacterial efficacy. The findings provide baseline information for future studies on the isolation, characterization, and application of bioactive compounds from *A. hexapetalus*.

MATERIALS AND METHODS

Preparation of *A. hexapetalus* flower extract

Fresh flowers of *A. hexapetalus* were collected and sun-dried under ambient conditions for 1-2 days until suitable for grinding. The dried material was then ground into a fine powder. The powdered material (100 g) was extracted with 900 ml of hexane (1:9, w/v) by maceration in a sealed container at room temperature for 7 days. The extract was initially filtered through muslin cloth to remove plant residues. To increase extraction efficiency, the plant residue was re-extracted twice following the same extraction conditions.[9] After filtration through Whatman No. 1 paper, solvent removal was performed under reduced pressure at 45 °C using a rotary evaporator. The extraction yield was calculated as follows:

$$\% \text{ yield} = [\text{weight of dried extract} / \text{initial dry weight of plant material}] \times 100$$

The obtained crude extract was transferred into sterile amber containers and kept at -20 °C prior to further analysis.

Gas chromatography–mass spectrometry (GC-MS) analysis

Chemical constituents of the hexane extract were characterized using a gas chromatography-mass spectrometry (GC-MS) system (Agilent Technologies, 8890 GC coupled with 5977C MSD). Prior to analysis, 50 mg of the extract was dissolved in 1 ml hexane and

passed through a 0.45 µm membrane filter. The GC-MS instrument was operated under electron impact (EI) ionization at 70 eV with an ion source temperature maintained at 230 °C. Mass spectra were recorded over a scan range of 35-550 amu. The oven program was initiated at 40 °C and maintained for 5 min, followed by heating to 200 °C at 8 °C/min, and subsequently increased to 280 °C at a rate of 5 °C/min with a final holding time of 20 min. The total analytical run time was 61 min. Compound identification was achieved by comparison of retention times and mass spectral data with entries in the Wiley and NIST libraries (version 2023).

Microorganisms

The bacterial strains used in this study included three Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, and *Staphylococcus aureus* DMST 20654) and four Gram-negative bacteria (*Escherichia coli*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, and *Salmonella enterica* subsp. *enterica* serovar Typhi). All strains were obtained from the Microbiology Laboratory, Department of Biology, Faculty of Science, Mahasarakham University, Thailand. Stock cultures were preserved in sterile 20% glycerol at -20 °C until further use.

Preparation of bacterial inoculum

Fresh bacterial cultures were prepared on nutrient agar (NA) and incubated at 37 °C for 18-24 hrs. Selected colonies were suspended in sterile normal saline to obtain a bacterial density equivalent to 0.5 McFarland standard (≈1.5×10^8 CFU/ml).

Antibacterial activity assay (disc diffusion method)

The antibacterial activity of the extract was initially evaluated using the paper disc diffusion method as a preliminary screening assay. Therefore, a single extract concentration (500 mg/ml) was selected to assess antibacterial activity prior to quantitative determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).[10] Briefly, 100 µl of bacterial suspension was evenly spread onto Mueller-Hinton agar (MHA) plates using a sterile cotton swab, and the plates were allowed to dry for 3-5 min. Sterile paper discs were then placed on the agar surface, and 20 µl of the hexane extract (500 mg/ml) was applied to each disc. Streptomycin (3 mg/ml) and 10% dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively. Following incubation at 37 °C for 24 hrs, antibacterial activity was assessed by measuring the diameter of the inhibition zones, including the paper disc. All experiments were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was evaluated using a broth microdilution assay in sterile 96-well microplates. The extract was prepared by 2-fold serial dilution with sterile distilled water to obtain concentrations ranging from 500-0.488 mg/ml. Each well received 100 µl of diluted extract together with 100 µl of bacterial suspension. Mueller-Hinton broth (MHB) without extract served as the negative control, whereas streptomycin (3 mg/ml) was included as the positive control. Following incubation at 37 °C for 24 hrs, bacterial growth was monitored by measuring optical density at 600 nm using a microplate reader (ASYS UVM 340). The MIC value was recorded as the lowest concentration showing no visible bacterial growth.

Determination of Minimum Bactericidal Concentration (MBC)

Bactericidal activity was assessed by subculturing samples from MIC wells lacking visible growth onto nutrient agar (NA) plates. After incubation at 37 °C for 24 hrs, the MBC was defined as the lowest extract concentration at which no viable bacterial colonies were observed.

Statistical analysis

All experiments were carried out in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using IBM SPSS Statistics software licensed by Mahasarakham University. Differences between treatment groups were evaluated by one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test. Statistical significance was considered at $P < 0.05$.

Table 1. Chemical composition of *A. hexapetalus* flower extract as determined by GC-MS

No.	RT	Name	Formula	% Relative peak areas
1	3.164	dimethyl-2-Hydroxyglutarate	$C_7H_{12}O_5$	0.1
2	3.228	Hexane, 3,4-dimethyl	C_8H_{18}	2.17
3	3.317	Cyclohexane	C_6H_{12}	9.5
4	3.358	Butane, 2,2,3-trimethyl	C_7H_{16}	0.28
5	3.564	Pentane, 3,3-dimethyl	C_7H_{16}	0.12
6	3.623	Cyclohexane	C_6H_{12}	0.84
7	3.681	Cyclohexane	C_6H_{12}	4.37
8	3.728	Pentane, 2,3-dimethyl	C_7H_{16}	0.11
9	3.811	Cyclopentane, 1,3-dimethyl	C_7H_{14}	0.27
10	10.434	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	0.3
11	12.746	D-Limonene	$C_{10}H_{16}$	0.25
12	18.992	(1S,4S,4aS)-1-Isopropyl-4,7-dimethyl-1,2,3,4,4a,5-hexahydronaphthalene	$C_{15}H_{24}$	0.11
13	19.416	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta.,8S*)]	$C_{15}H_{24}$	0.17
14	19.516	Copaene	$C_{15}H_{24}$	1.72
15	19.716	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl	$C_{15}H_{24}$	0.5
16	20.057	1H-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]	$C_{15}H_{24}$	0.24
17	20.139	beta.-Cadinene	$C_{15}H_{24}$	1.06
18	20.286	Longifolene-(V4)	$C_{15}H_{24}$	3.9
19	20.528	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]	$C_{15}H_{24}$	10.08
20	20.733	1H-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]	$C_{15}H_{24}$	0.12
21	20.857	1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z	$C_{15}H_{24}$	1.04
22	20.927	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,[1R-(1.alpha.,7.beta.,8a.alpha.)]	$C_{15}H_{24}$	0.12
23	21.116	gamma.-Muurolene	$C_{15}H_{24}$	0.63
24	21.251	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.0 2,7]decane-rel	$C_{15}H_{24}$	0.3
25	21.392	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-,[4aR-(4a.alpha.,7.alpha.,8a.beta.)]	$C_{15}H_{24}$	0.22
26	21.48	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1.alpha.,4a.alpha.,8a.alpha.)	$C_{15}H_{24}$	0.76
27	21.586	beta.-Bisabolene	$C_{15}H_{24}$	0.11
28	21.727	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.beta.,8a.alpha.)	$C_{15}H_{24}$	0.44
29	21.786	delta.-Cadinene	$C_{15}H_{24}$	1.58
30	21.851	cis-Calamenene	$C_{15}H_{22}$	0.16
31	22.08	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methy lethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]	$C_{15}H_{24}$	0.12
32	22.457	Junipercamphor	$C_{15}H_{26}O$	0.22
33	22.522	1,7,7,7b-Tetramethyldecahydrocyclopropa[5,6]naphtho[1,8a-b] oxirene	$C_{15}H_{24}O$	0.28
34	22.645	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclopropa[a]naphthalen-7-ol	$C_{15}H_{26}O$	0.47
35	22.733	Spathulenol	$C_{15}H_{24}O$	0.66
36	22.845	Caryophyllene oxide	$C_{15}H_{24}O$	1.88

Table 1. Cont...

No.	RT	Name	Formula	% Relative peak areas
37	22.886	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]	$C_{15}H_{26}O$	1.04
38	23.169	2-Naphthalenemethanol,decahydro-.alpha.,.alpha.,4atrimethyl-8-methylene-,[2R-(2.alpha.,4a.alpha.,8a.beta.)]	$C_{15}H_{26}O$	0.21
39	23.245	Junicedranol	$C_{15}H_{26}O$	0.67
40	23.521	alpha.-Isonootkatol	$C_{15}H_{24}O$	0.16
41	23.657	alpha.-cadinol	$C_{15}H_{26}O$	0.51
42	23.721	1-Naphthalenol,1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R(1.alpha.,4.beta.,4a.beta.,8a.beta.)]	$C_{15}H_{26}O$	0.16
43	23.857	(4aS,9aR)-3,5,5,9-Tetramethyl-2,4a,5,6,7,9a-hexahydro-1H-benzo [7]annulene	$C_{15}H_{24}$	0.42
44	24.074	14-Hydroxycaryophyllene	$C_{15}H_{24}O$	0.55
45	24.157	exo-Nootkatol	$C_{15}H_{24}O$	0.12
46	24.698	Methyl tetradecanoate	$C_{15}H_{30}O_2$	0.12
47	25.363	(E)-3-((4S,7R,7aR)-3,7-Dimethyl-2,4,5,6,7,7ahexahydro-1H-inden-4-yl)-2-methylacrylaldehyde	$C_{15}H_{22}O_2$	0.2
48	26.098	Cedran-diol, 8S,13	$C_{15}H_{26}O_2$	0.16
49	27.275	Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	0.37
50	27.586	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	4.91
51	28.145	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.98
52	28.251	Palmitoleic acid ethyl ester	$C_{18}H_{34}O_2$	0.15
53	28.58	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	1.68
54	29.062	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	0.16
55	30.144	9,12-Octadecadienoic acid (Z,Z),methyl ester	$C_{19}H_{36}O_2$	7.18
56	30.233	9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)	$C_{19}H_{34}O_2$	3.92
57	30.303	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	1.25
58	30.615	Methyl stearate	$C_{19}H_{38}O_2$	1.09
59	30.765	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	2.61
60	30.815	Oleic Acid	$C_{18}H_{34}O_2$	1.25
61	30.898	Hexadecanoic acid, 2-methylpropyl ester	$C_{20}H_{40}O_2$	1.09
62	31.15	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	2.65
63	31.239	Ethyl Oleate	$C_{20}H_{38}O_2$	1.39
64	31.333	Ethyl Oleate	$C_{20}H_{38}O_2$	0.38
65	31.58	Isopropyl linoleate	$C_{21}H_{38}O_2$	0.19
66	31.65	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	0.46
67	33.05	1-Methylbutyl hexadecanoate	$C_{21}H_{42}O_2$	0.22
68	33.18	1-Methylbutyl hexadecanoate	$C_{21}H_{42}O$	0.17
69	33.321	Palmitic anhydride	$C_{32}H_{62}O_3$	0.71
70	33.486	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	0.62
71	33.586	i-Propyl 9-octadecenoate (Z)	$C_{21}H_{40}O_2$	0.24
72	33.727	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	0.4
73	35.044	Pentyl linoleate	$C_{23}H_{42}O_2$	0.45
74	35.621	Isopropyl linoleate	$C_{21}H_{38}O_2$	0.63
75	35.75	Isopropyl linoleate	$C_{21}H_{38}O_2$	1.12
76	35.865	9,12,15-Octadecatrienoic acid, 1-methylethylester, (Z,Z,Z)-	$C_{21}H_{36}O_2$	0.44
77	35.938	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	0.75
78	36.033	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{48}O_2$	0.34
79	36.356	Octan-2-yl palmitate	$C_{24}H_{48}O_2$	0.27
80	36.797	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	0.31
81	37.615	Isopropyl linoleate	$C_{21}H_{38}O_2$	0.31

Table 1. Cont...

No.	RT	Name	Formula	% Relative peak areas
82	37.709	Isopropyl linoleate	$C_{21}H_{38}O_2$	0.14
83	38.285	Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	0.14
84	38.815	10-Hydroxy-5,9,12,13,16-pentamethyl-4-oxatricyclo[10.3.1.03,5] hexadeca-1(15),8-dien-2-one	$C_{20}H_{30}O_2$	0.3
85	38.856	9,12-Octadecadienoic acid (Z,Z)-, octyl ester	$C_{26}H_{48}O_2$	0.42
86	39.738	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	0.36
87	41.515	(9Z,12Z)-Phenethyloctadeca-9,12-dienoate	$C_{26}H_{40}O_2$	0.23
88	42.209	Pentacosane	$C_{25}H_{52}$	0.11
89	44.685	gamma.-Tocopherol	$C_{28}H_{48}O_2$	0.15
90	48.567	Campesterol	$C_{28}H_{48}O$	0.68
91	49.302	Stigmasterol	$C_{29}H_{48}O$	0.24
92	51.149	gamma.-Sitosterol	$C_{29}H_{50}O$	4.15
93	55.496	beta.-Sitosterol, propionate	$C_{32}H_{54}O_2$	0.29
94	55.932	9,19-Cyclolanostan-3-ol,24-methylene-,(3.beta.)-	$C_{31}H_{52}O$	0.21

Chemical group	Total relative peak area (%)
Fatty acid derivatives	43.67
Sesquiterpenes and oxygenated sesquiterpenes	31.09
Sterols and triterpenoids	5.57
Monoterpenes	0.55
Other constituents (hydrocarbons, unknowns, miscellaneous compounds, tocopherol, etc.)	18.22

RESULTS

Extraction yield, physicochemical characteristics and GC-MS chemical profiling

The extraction yield of *A. hexapetalus* flower hexane extract was 20.78% (w/w), which may be attributed to the efficiency of hexane in extracting lipophilic constituents such as fatty acids, sterols, and terpenoids. The crude extract exhibited a dark yellow color, viscous texture, and characteristic pungent odor, which are typically associated with non-polar phytochemical constituents. The dark coloration may be related to lipid-soluble pigments and oxidized terpenoid derivatives, whereas the viscous nature suggests the presence of fatty acids, fatty acid esters, and other hydrophobic compounds. The characteristic odor is likely due to volatile terpenoid and sesquiterpene constituents. GC-MS analysis of the hexane extract of *A. hexapetalus* flowers revealed a complex phytochemical profile, as presented in Table 1 and Figure. A total of 100 chromatographic peaks were detected, corresponding to 94 identified compounds. The chromatogram showed several prominent peaks distributed across different retention time regions. As illustrated in Figure, a cluster of peaks between 19 and 24 min represented sesquiterpenes and oxygenated sesquiterpenes, including copaene, longifolene, cadinene derivatives, spathulenol, and caryophyllene oxide. The most abundant sesquiterpene-related compound was 1H-cyclopropa[a]naphthalene derivative (RT 20.53 min), accounting for 10.08% of the total peak area. Peaks observed between 27 and 36 min were mainly associated with fatty acid derivatives, including hexadecanoic acid methyl ester (4.91%), 9,12-octadecadienoic acid methyl ester (7.18%), and 9,12,15-octadecatrienoic acid methyl ester (3.92%). In the later retention time region (48-56 min), sterols and triterpenoids such as γ-sitosterol, campesterol, and stigmasterol were

Table 2. Antibacterial activity of *A. hexapetalus* flower hexane extract against pathogenic bacteria

Bacteria	Inhibition zone (mm)	
	A. hexapetalus extract (500 mg/ml)	Streptomycin (3 mg/ml)
B. cereus	11.67 ± 0.58^{bAB}	31.33 ± 2.31^{aB}
S. aureus	7.33 ± 0.58^{bAB}	33.33 ± 1.53^{aAB}
S. aureus DMST 20654	7.00 ± 0.00^{bAB}	41.33 ± 1.53^{aA}
E. coli	6.33 ± 0.58^{bB}	35.67 ± 2.89^{aAB}
E. coli ATCC 25922	14.33 ± 1.15^{bAB}	33.67 ± 0.58^{aAB}
P. aeruginosa	7.00 ± 0.00^{bAB}	40.33 ± 0.58^{aAB}
S. enterica subsp. *enterica* serovar Typhi	16.00 ± 1.73^{bA}	41.67 ± 1.53^{aA}

Values are expressed as mean ± standard deviation (SD). Different lowercase letters (a,b) within the same row indicate significant differences between treatments, while different uppercase letters (A,B) within the same column indicate significant differences among bacterial strains, as determined by the Least Significant Difference (LSD) test at $P < 0.05$

detected. Overall, the chromatographic pattern was consistent with the predominance of fatty acid derivatives (43.67%) and sesquiterpenes (31.09%) in the hexane extract.

Antibacterial activity of *A. hexapetalus* flower hexane extract

The antibacterial activity of the *A. hexapetalus* flower hexane extract against selected pathogenic bacteria is presented in Table 2. The extract exhibited inhibitory effects against all tested bacterial strains, with inhibition zones ranging from 6.33 ± 0.58 to 16.00 ± 1.73 mm. The largest inhibition zone was observed against *S. enterica* subsp. *enterica* serovar Typhi (16.00 ± 1.73 mm), followed by *E. coli* ATCC 25922 (14.33 ± 1.15 mm) and *B. cereus* (11.67 ± 0.58 mm). In comparison, streptomycin (3 mg/ml) exhibited significantly greater antibacterial activity against all tested bacteria, with inhibition zones ranging from 31.33 ± 2.31 to 41.67 ± 1.53 mm ($P < 0.05$). No inhibition was observed for the negative control (10% DMSO). The extract exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria; however, its activity was significantly lower than that of streptomycin.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the *A. hexapetalus* flower hexane extract against selected pathogenic bacteria

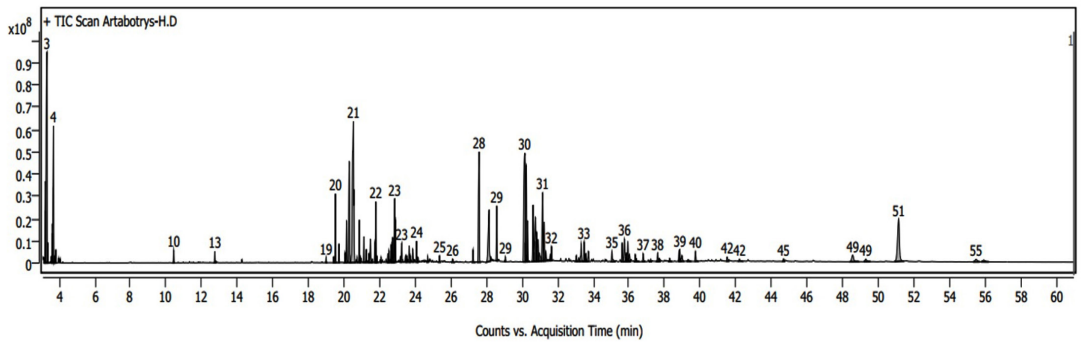

Figure. GC-MS chromatogram of the hexane extract of *A. hexapetalus* flowers showing the major chromatographic peaks corresponding to sesquiterpenes, fatty acid derivatives, and sterols identified in Table 3

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of hexane extract from *A. hexapetalus* flowers against pathogenic bacteria

Bacteria	MIC (mg/ml)	MBC (mg/ml)
Bacillus cereus	7.812	500
Staphylococcus aureus	250	>500
S. aureus DMST 20654	500	500
Escherichia coli	500	>500
E. coli ATCC 25922	0.488	>500
Pseudomonas aeruginosa	250	500
S. enterica subsp. *enterica* serovar Typhi	500	500

>500 indicates that no bactericidal activity was observed within the tested concentration range (0.488-500 mg/mL)

are presented in Table 3. MIC values ranged from 0.488-500 mg/ml. The lowest MIC value was observed for *E. coli* ATCC 25922 (0.488 mg/ml), followed by *B. cereus* (7.812 mg/ml). *S. aureus* and *P. aeruginosa* exhibited MIC values of 250 mg/ml, whereas MIC values of 500 mg/ml were recorded for *S. aureus* DMST 20654, *E. coli*, and *S. enterica* subsp. *enterica* serovar Typhi. Regarding bactericidal activity, the extract exhibited MBC values of 500 mg/ml against *B. cereus*, *S. aureus* DMST 20654, *P. aeruginosa*, and *S. enterica* subsp. *enterica* serovar Typhi. However, no bactericidal effect was observed against *S. aureus*, *E. coli*, and *E. coli* ATCC 25922 at the tested concentrations (>500 mg/ml). The antibacterial activity of the extract varied among the tested bacterial strains. For several strains, MBC values were higher than the corresponding MIC values, indicating that higher extract concentrations were required to achieve bactericidal effects than to inhibit bacterial growth.

DISCUSSION

The crude hexane extract of *A. hexapetalus* flowers exhibited a dark yellow color, viscous texture, and characteristic pungent odor. These physicochemical characteristics are typically associated with extracts enriched in lipophilic constituents such as terpenoids, fatty acids, and other non-polar metabolites efficiently extracted by hexane.[11,12] The dark yellow coloration may be attributed to lipid-soluble pigments, including carotenoids and oxidized terpenoid derivatives, whereas the viscous consistency suggests the presence of fatty acids, fatty acid esters, and other hydrophobic compounds.[13,14] The characteristic odor is likely associated with volatile terpenoids and sesquiterpenes. GC-MS analysis further supported these observations by confirming a chemically diverse profile composed mainly of sesquiterpenes, oxygenated sesquiterpenes, fatty acid derivatives, and phytosterols (Table 1). The predominance of hydrophobic constituents is also consistent with the insolubility of the extract in water and may contribute to antibacterial activity through interactions with bacterial cell membranes and disruption of membrane integrity.[15,16]

The extraction yield of the hexane extract was 20.78% (w/w), which may be attributed to the efficiency of hexane in solubilizing abundant lipophilic constituents in the flower matrix, including fatty acids, sterols, and other non-polar compounds identified by GC-MS analysis. Extraction yield is known to be influenced by solvent polarity, plant matrix composition, and extraction conditions.[11,17]

GC-MS profiling revealed 100 chromatographic peaks, of which 94 compounds were identified. The extract was predominantly composed of sesquiterpenes, fatty acid derivatives, and phytosterols, which is consistent with extraction using a non-polar solvent. Sesquiterpenes such as longifolene, cadinene derivatives, and murolene, as well as oxygenated derivatives including caryophyllene oxide and spathulenol, have been widely reported to exhibit antimicrobial activity, primarily through disruption of microbial membranes and interference with essential cellular functions.[18,19] Fatty acid methyl esters, such as methyl palmitate and methyl linoleate, have also been associated with antimicrobial effects via membrane destabilization and metabolic disruption.[20] In addition, phytosterols including gamma-sitosterol, campesterol, and stigmasterol may contribute to the overall biological activity, potentially through additive or synergistic interactions with other bioactive constituents.[21]

The antibacterial assay demonstrated that the extract exhibited inhibitory activity against all tested bacterial strains, although with lower potency compared to streptomycin.

The extract showed broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Notably, relatively stronger inhibition was observed against Gram-negative bacteria, including *E. coli* ATCC 25922 and *S. enterica* serovar Typhi, despite the presence of an outer membrane that typically limits antimicrobial penetration. This suggests that lipophilic constituents in the extract may interact with and destabilize bacterial membrane structures.[22] The observed antibacterial activity is likely attributable to terpenoids and fatty acid derivatives identified by GC-MS, which are known to exert antimicrobial effects through membrane disruption, increased permeability, leakage of intracellular components, and interference with metabolic processes.[6,18] However, the lower activity of the crude extract compared to standard antibiotics is expected due to the presence of complex mixtures in which active compounds occur at relatively low concentrations.[23]

MIC and MBC results further demonstrated variability in antibacterial susceptibility among tested bacterial strains. The lowest MIC observed for *E. coli* ATCC 25922 indicates higher susceptibility, whereas higher MIC values in other strains may be associated with intrinsic or acquired resistance mechanisms, including reduced membrane permeability, efflux pump activity, and enzymatic detoxification.[24] Overall, the relatively high MIC and MBC values, together with the absence of bactericidal effects in some strains, indicate that the extract primarily exhibits bacteriostatic activity. This finding is consistent with previous reports showing that many plant-derived crude extracts tend to inhibit bacterial growth rather than induce cell death.[25-27] The relatively high MIC and MBC values may also be attributed to the crude nature of the extract, where active compounds are diluted by inactive constituents. Fractionation and purification could enhance antibacterial potency by concentrating bioactive molecules and removing interfering components.[23]

Overall, the antibacterial activity observed in this study is likely the result of combined effects of multiple phytochemical classes, including fatty acid derivatives, terpenoids, and sterol-like compounds identified by GC-MS analysis. These compounds may act synergistically or additively to disrupt bacterial cell membranes and interfere with essential cellular processes. However, no experimental evaluation of synergistic interactions was conducted in this study. Therefore, further studies are required to isolate and characterize individual active compounds, evaluate their interaction effects, and elucidate detailed mechanisms of action against both Gram-positive and Gram-negative bacteria. Such investigations would support the potential application of *A. hexapetalus* flower extract as a natural antimicrobial agent.

CONCLUSION

The hexane extract of *A. hexapetalus* flowers exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. The extract showed more pronounced inhibitory effects against *Salmonella enterica* serovar Typhi and *Escherichia coli* ATCC 25922 compared with other tested strains, although its activity was lower than that of streptomycin. MIC and MBC analyses indicated that the extract primarily exerted bacteriostatic rather than bactericidal effects. GC-MS analysis indicated the presence of bioactive constituents, mainly sesquiterpenes, fatty acid derivatives, and phytosterols, which have been reported to possess antimicrobial properties. These findings are consistent with previous reports on *A. hexapetalus* and related *Artocarpus* species, which have demonstrated various biological activities including antimicrobial, antioxidant, and anti-inflammatory effects. Overall, this study highlights the potential of *A. hexapetalus* flower extract as a natural source of antimicrobial compounds. However, further studies are required to isolate active constituents, confirm their bioactivities, and elucidate their mechanisms of action for potential applications in pharmaceutical and food systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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