RESEARCH PAPER

GC–MS based Phytochemical profiling of bioactive compounds and antibacterial activity of leaf extract of *Hibiscus arnottianus*

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ABSTRACT

Hibiscus arnottianus is a fragrant-flowered medicinal plant with potential therapeutic value. This study evaluated the phytochemical constituents and antibacterial activity of its leaf extracts prepared using methanol and petroleum ether. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, terpenoids, and phytosteroids. GC–MS analysis of the petroleum ether extract identified several compounds, including phytol, neophytadiene, squalene, and n-hexadecanoic acid, which are notable constituents for further investigation. The petroleum ether extract showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with maximum inhibition zones of 16.40 mm and 18.40 mm, respectively. The methanolic extract also exhibited activity, producing maximum inhibition zones of 16.29 mm against *S. aureus* and 18.38 mm against *E. coli*. These findings highlight the phytochemical diversity and potential antibacterial properties of *H. arnottianus* leaves.

Keywords: *Hibiscus arnottianus*, phytochemical screening, GC–MS, phytol, neophytadiene, antibacterial activity.

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INTRODUCTION

Over the years, humans have faced numerous diseases and health challenges, leading to the development of diverse approaches for their prevention and management (Picard and McEwen, 2018; Ferroni *et al.*, 2018). Among these approaches, medicinal plants have played an important role in the treatment of various ailments (Kruk *et al.*, 2019). Despite advances in modern medicine, interest in herbal remedies has increased, partly due to concerns regarding the adverse effects associated with conventional therapies (Akram *et al.*, 2021; Khalid *et al.*, 2022). Medicinal plants are also increasingly used as complementary and alternative therapies alongside conventional treatments (Kawamura and Muraoka, 2018). Medicinal plants continue to contribute significantly to healthcare, particularly in developing regions of Asia and Africa. They may serve as alternatives or complements to modern healthcare systems and remain closely associated with the social, cultural, and economic practices of local communities (Assefa *et al.*, 2024). Traditional medicine is based largely on knowledge and practices transmitted across generations and remains an important source of primary healthcare in many rural communities. Ethnomedicinal knowledge therefore provides a valuable basis for identifying plants with potential therapeutic applications (Khatoon *et al.*, 2018).

Plants are rich sources of biologically active secondary metabolites that can protect against oxidative damage and contribute to disease prevention (Ofoedu *et al.*, 2022). These compounds, commonly referred to as phytochemicals, include alkaloids, flavonoids, phenolics, terpenoids, steroids, lignans, saponins, and glycosides (Zahnit *et al.*, 2022). Phytochemicals have attracted considerable attention because of their nutraceutical and pharmacological properties, including antioxidant, antibacterial, antifungal, anti-inflammatory, antiallergic, chemopreventive, and neuroprotective activities (Palai *et al.*, 2021).

The genus *Hibiscus* has considerable cultural, ornamental, nutritional, and medicinal importance worldwide. Among its members, *Hibiscus arnottianus*, commonly known as Hawaiian

white hibiscus, is an evergreen shrub or small tree native to the moist and wet forests of the Hawaiian Islands. It is characterized by white, slightly fragrant flowers with a prominent pink to red staminal column and smooth, oval leaves. The species is also notable for its restricted geographical distribution and distinct subspecies, including the rare and endangered *H. arnottianus* subsp. *immaculatus* (Onyeukwu *et al.*, 2023).

Although *H. arnottianus* is recognized for its ornamental and traditional importance, information on the phytochemical composition and antibacterial potential of its leaves remains comparatively limited. Therefore, the present study was undertaken to investigate the phytochemical constituents, GC–MS profile, and antibacterial activity of petroleum ether and methanol leaf extracts of *H. arnottianus*. The study aimed to identify bioactive compounds present in the extracts and assess their potential antibacterial properties.

MATERIALS AND METHODS

2.1. Collection and identification of Plant Material

Hibiscus arnottianus plant leaves were collected from Chidambaram, Tamil Nadu, India. It was identified botanically with the help of Flora of Presidency.

2.2. Preparation of plant crude extract

The plant leaves were sterilized using mild detergent and rinsed thoroughly in running tap water to remove specks of dirt and shade dried under room temperature for about 7 days and grounded to fine powder using blender and the fine coarse powder was stored in airtight container before extraction. Dried leaves were coarsely powdered and subjected to hot Soxhlet extraction with methanol and petroleum ether. The extract obtained was filtered and completely evaporated in a Petri plate. Then the extract was subjected to preliminary phytochemical screening to identify various phytochemical constituents.

2.3. Phytochemical screening studies of bioactive compounds

Test for Flavonoids

To 2 ml of plant extract, 1 ml of 2 N sodium hydroxide was added. The presence of yellow colour indicated the presence of flavonoids.

Test for Alkaloids

To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. The presence of green colour or white precipitate indicated the presence of alkaloids.

Test for Glycosides

2 ml of plant extract, 3 ml of chloroform and 10% ammonia solution were added. The formation of pink colour indicated the presence of glycosides.

Test for Terpenoids

To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulphuric acid was added carefully. The formation of red brown colour at the interface indicated the presence of terpenoids.

Test for Phenols

To 1 ml of the extract, 2ml of distilled water followed by a few drops of 10% ferric chloride was added. The formation of blue or green colour indicated the presence of phenols.

Phytosteroids

To 1 ml of plant extract equal volume of chloroform was added and subjected to a few drops of concentrated sulphuric acid where the appearance of a bluish-brown ring indicated the presence of phytosteroids.

2.4. GC-MS Analysis

GC-MS analysis of petroleum ether and methanol extract of *H. arnottianus* leaves was performed (Figure 4&5) on a GC/MSD Systems - 5975C Series GC/MSD System, fitted with an Agilent DB5MS capillary column (30 m×0.25 mm inner diameter, × 0.25 µm film thickness; maximum temperature, 350 °C). Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer line and ion source temperatures were all 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was programmed from 60 °C (hold for 2 min) to 280 °C at a rate of 3 °C/min. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 µL) were taken in a syringe and injected into injector with a split ratio 30:1. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The percentage composition of the crude extract constituents was expressed as a percentage by peak area. Based on GC retention time, chemical components in the crude extracts were identified and described. The mass spectrum was electronically compared to standards found in NIST mass spectrum libraries.

2.5. Anti-Bacterial Activity

The antibacterial activity of purified pigment was tested against the bacterial pathogens by well diffusion method. For this, bacterial pathogen were inoculated in nutrient broth and incubated for 12 hrs before antibacterial assay all the bacterial strains were individually spread on the Muller Hinton agar plates. Wells were made in the plates at 6mm by using cork borer. The different concentration 25µl, 50µl, 75µl, 100µl of fractions added on the wells and incubated

for 37◦C at 24 hrs. The assay was carried out in triplicates. The zone of incubation was measured in mm after the completion of the incubation period. All the data were statistically analyzed.

RESULTS AND DISCUSSION

In the present study leaf extracts of *H. arnottianus* were evaluated for phytochemicals analysis, GC-MS analysis and Antibacterial activity.

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening of *Hibiscus arnottianus* leaf extracts prepared using methanol and petroleum ether revealed the presence or absence of six major phytochemical groups (Table 1). Alkaloids, terpenoids, and phytosteroids were detected in both extracts. Flavonoids and phenols were present only in the petroleum ether extract, whereas glycosides were absent from both extracts. These findings indicate that the phytochemical composition of *H. arnottianus* leaves varies with the extraction solvent. While, phytochemicals encompass a wide range of chemical classes, including alkaloids, flavonoids, steroids/ triterpenoids, phenols, glycosides, tannins and more (Rajvanshy and Patel, 2020).

In contrast, secondary constituents are specialized metabolites that support the plant's defense and ecological interactions. These include alkaloids, flavonoids, terpenes, phenolics, lignans, plant steroids, curcumins, saponins, and glucosides (Zahnit *et al.,* 2022). Phytochemicals exhibit a wide range of pharmacological activities (Nazer *et al.,* 2019). They are known for their anti-inflammatory, antiplasmodic, anti-allergic, antioxidant, antibacterial, antifungal, chemopreventive, neuroprotective, hypotensive, and anti-aging effects (Khatoon *et al.,* 2018). These phytochemicals hold significant nutraceutical value and are considered bioactive components that contribute to health maintenance, forming a crucial link between the food and pharmaceutical industries (Palai *et al.,* 2021).

Table 1. Preliminary phytochemical screening of leaf extract of *Hibiscus arnottianus*

S.No.	Phytochemical Tests	Methanol leaf extract	Pet. ether leaf extract
1	Alkaloids	++	++
2	Flavonoids	-	+
3	Glycosides	-	-
4	Phenols	-	+
5	Terpenoids	+	+
6	Phytosteroids	++	+

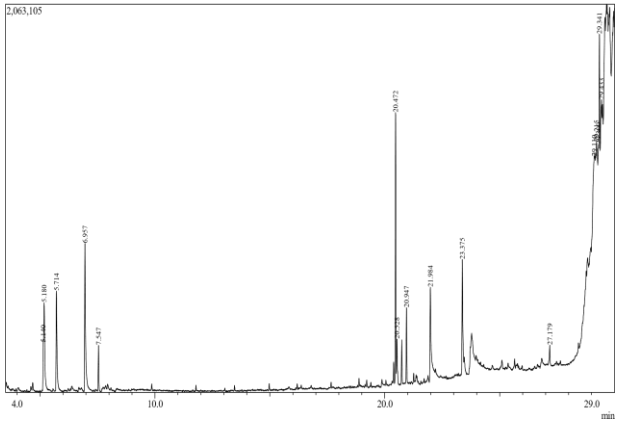
[++ Strongly Present; + Moderate Present; - Absent]

3.2. GC-MS analysis

The GCMS analysis of Petroleum ether leaf extract of *Hibiscus arnottianus.* shows the presence of following phytocompounds (Table. 2 & Figure 2) given by based on peak area such as 16.59 (Dodecanoic acid,1,2,3-propanetriyl ester), 12.37 (Neophytadiene), 10.41(3,3-Diethoxy-1-propyne), 7.95(Squalene), 6.71(2-Ethoxyethy 3- methylbutanoate), 6.66(n-Hexadecanoic acid), 6.50(Decanoic acid, 1,2,3-propanetriyl ester), 6.47(Phytol), 6.18(Valeric anhydride), 5.55(Dodecanoic acid,1,2,3-propanetriyl ester), 3.41(Neophytadiene), 2.85(Dodecanoic acid,1,2,3-propanetriyl ester), n2.53(3,7,11,15-Tetramethylhexadec-2-ene), 2.34(4,4-Dimethoxy-2-methyl-2-butanol), 2.26(Decane), 1.23(Bis(2-etthylhexyl)phthalate). The GCMS analysis of Methanol leaf extract of *Hibiscus arnottianus.* shows the presence of following phytocompounds (Table. 5) given by based on peak area such as 34.83(Dodecanoic acid,1.2.3-propanetriyl ester), 19.58(Dodecanoic acid,1,2,3-propanetriyl ester), 18.98(Dodecanoic acid,1,2,3-propanetriyl ester), 9.19(Lauric acid, 2-(hexadecyloxy)-3-propyl ester, 2.39(Phytol). Gas chromatography–mass spectrometry (GC–MS) identifies natural compounds such as limonene, squalene, and sesamin that exhibit strong cytotoxic effects on cancer cells, indicating their potential as alternative anticancer agents (Verma *et al.,* 2025).

The results from GC-MS analysis of the methanolic extract of the experimental plant show the presence of a variety of bioactive compounds (Sinha, 2017). Gas Chromatography–Mass Spectrometry (GC-MS) is a well-established and widely regarded analytical technique, often considered the "gold standard" for the analysis of volatile organic compounds (VOCs) in exhaled breath (De Lacy Costello *et al.,* 2014; Drabińska *et al.,* 2021). It provides high sensitivity, reproducibility, and the capability to identify and characterize unknown compounds, particularly when using high-resolution instruments (Sola-Martínez *et al.,* 2022; Sharma *et al.,* 2023).

Figure 2. GCMS Chromatogram of Petroleum ether leaf extract of *H. arnottianus*



Peak#	R.Time	Area%	Name
1	5.140	2.34	4,4-Dimethoxy-2-methyl-2-butanol
2	5.180	6.18	Valeric anhydride
3	5.714	6.71	2-Ethoxyethyl 3-methylbutanoate
4	6.957	10.41	3,3-Diethoxy-1-propyne
5	7.547	2.26	Decane
6	20.472	12.37	Neophytadiene
7	20.528	2.53	3,7,11,15-Tetramethylhexadec-2-ene
8	20.947	3.41	Neophytadiene
9	21.984	6.66	n-Hexadecanoic acid
10	23.375	6.47	Phytol
11	27.179	1.23	Bis(2-ethylhexyl) phthalate
12	29.110	16.59	Dodecanoic acid, 1,2,3-propanetriyl ester
13	29.215	5.55	Dodecanoic acid, 1,2,3-propanetriyl ester
14	29.300	2.85	Dodecanoic acid, 1,2,3-propanetriyl ester
15	29.341	7.95	Squalene
16	29.433	6.50	Dodecanoic acid, 1,2,3-propanetriyl ester
		100.00	

Table 2. GCMS phyto-compounds of Petroleum ether leaf extract of *H. arnottianus*

GC-MS analysis of the plant's methanol extract revealed the presence of 15 bioactive compounds. Accessible compounds were displayed in Table 3 and Figure 3 along with retention time, area, and area percent, which show the presence of inositol, 1-deoxy-, trans-Sinapyl alcohol, (E)-4-(3-Hydroxyprop-1-en-1-yl)-2 methoxyphenol, brucine, etc. in the leaf of *Hibiscus* in the higher proportion. As shown in Table 3, the Inositol, 1-deoxy with 50.10 percentage area appears as the amplest chemical compound in the leaf of *Hibiscus*.

Hibiscus contains inositol in a significant amount, which is exclusively used to cure diabetes, nerve pain, panic disorder, high cholesterol, insomnia, cancer, depression, tumor (Bizzarri *et al.,* 2016). *Brucine* exhibits anti-inflammatory, anti-tumor, and analgesic effects (Qin, 2012). Hexadecanoic acid is commonly used in treating rheumatic symptoms (Aparna *et al.,* 2012), while 9,12-Octadecadienoic acid (Z,Z)- has potential in lipid-lowering therapies for atherosclerosis prevention (Wang *et al.,* 2020).

Fatty acids such as methyl palmitate and linoleic acid are effective in managing coronary heart disease (Modi *et al.,* 2021). Terpenoids offer diverse therapeutic benefits, including anti-mutagenic, anti-ulcer, hepatic-protective, antimicrobial, and diuretic activities, and include notable drugs like artemisinin and taxol (Wang *et al.,* 2025). GC-MS remains the reference method for analyzing volatile organic compounds, particularly in biomarker discovery during metabolomic studies (Bajo-Fernández *et al.,* 2024).

Figure 3. GCMS Chromatogram of methanol leaf extract of *Hibiscus arnottianus*

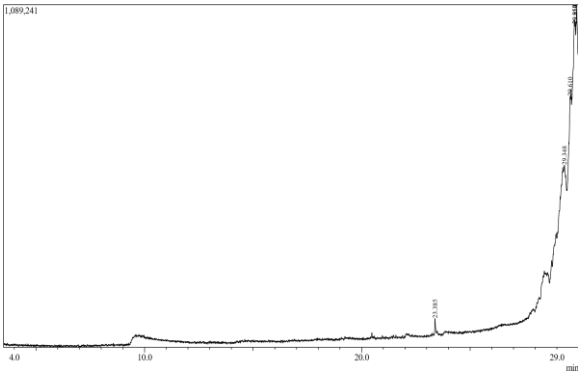


Table 3. GCMS of phyto-compounds present in methanol leaf extract of *H. arnottianus*

Peak#	R.Time	Area%	Height%	Name
1	23.385	2.39	4.73	Phytol
2	29.348	15.04	11.40	Dodecanoic acid, 1,2,3-propanetriyl ester
3	29.610	18.98	17.68	Dodecanoic acid, 1,2,3-propanetriyl ester
4	29.817	34.83	24.32	Dodecanoic acid, 1,2,3-propanetriyl ester
5	29.850	9.19	20.89	Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester
6	29.914	19.58	20.98	Dodecanoic acid, 1,2,3-propanetriyl ester
		100.00	100.00	

3.3. Antibacterial Activity

Antibacterial activity of petroleum ether and methanolic leaf extract *Hibiscus arnottianus* shows the greatest zone of inhibition recorded at higher concentration at 100 µg/mm (Figure 4 &Table 6). The crude petroleum ether extract showed the highest activity against *Staphylococcus aureus* such as 16.35±0.13, 15.59±0.18, 16.40±0.23 and 17.48±0.30 mm, *E. coli* shows the 18.40±0.14, 11.63±0.17, 14.67±0.22 and 17.74±0.27 at 25, 50, 75 and 100 µL respectively. Methanolic leaf extract shows the greatest zone of inhibition recorded at higher concentration at 100 µg/mm (Figure 5) against *Staphylococcus aureus* such as 16.29±0.12, 13.47±0.16, 14.42±0.20 and 15.43±0.28 and *Escherichia coli* shows as 18.38±0.13, 16.22±0.15, 17.59±0.19 and 13.60±0.26 mm at 25, 50, 75 and 100 µL respectively.

Recent years many researchers focus on antibacterial activity of *Hibiscus* Similarly, A study reported that silver nanoparticles synthesized from the stem of *Hibiscus* possess good antibacterial activity against the bacteria *Pseudomonas aeruginosa* found in the patient suffering from burn injury. Various bacterial strains such as *S. typhi, K. Pneumoniae, E. coli, S. aeruginosa* and other bacteria have been tested against extracts of *T. cordifolia* and showed potential anti-bacterial activity by either inhibiting their growth or mitigating the very existence of these bacteria (Narayanan *et al.,* 2011).

An active chemical compound that has been found from the stem of *T. cordifolia* as reported, found to be effective against bacteria like *E. faecalis* and *B. subtilis* and fungus like *T. Simii* and *T. rubrum* (Duraipandiyan *et al.,* 2012). The stem and leaves of this plant showed maximum inhibitory activity against the clinical urinary pathogens such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Tiwari *et al.,* 2018). Similarly, the methanolic extract of *Tinospora cordifolia* has demonstrated significant antimicrobial activity (Narayanan *et al.,* 2011). Its antibacterial properties have been bioassayed against various pathogens, showing zones of inhibition as follows: *E. coli* (24 mm), *S. aureus* (22 mm), *K. pneumoniae* (Narayanan *et al.,* 2011), *P. vulgaris* (20 mm), *S. typhi* (26 mm), *S. flexneri* (18 mm), *S. typhi* (16 mm), *S. typhimurium* (22 mm), *P. aeruginosa* (23 mm), *E. aeruginosa* (20 mm), and *E. aerogenes* (24 mm) (Jayachandran *et al.,* 2003; Ambekar *et al.,* 2009). Additionally, *T. cordifolia* extract has been reported to inhibit bacterial growth and enhance both the phagocytic and intracellular killing capacities of neutrophils in mice (Sengupta *et al.,* 2009).

Figure. 4 Anti-bacterial activity of petroleum ether leaf extract of *H. arnottianus*

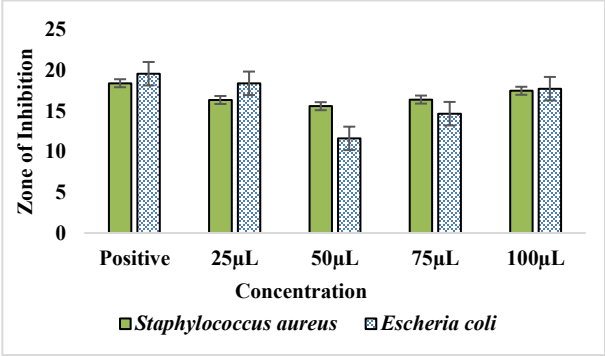
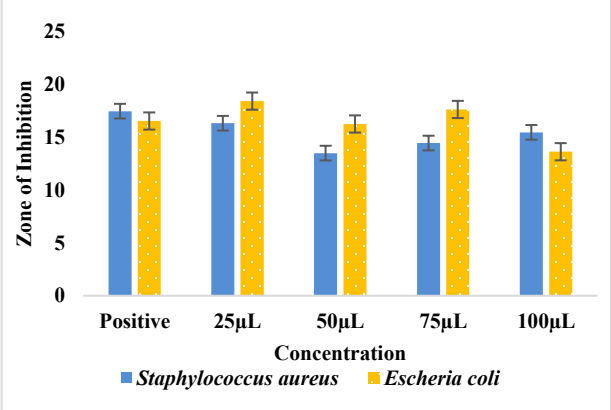


Figure. 5 Anti-bacterial activity of methanol leaf extract of *H. arnottianus*



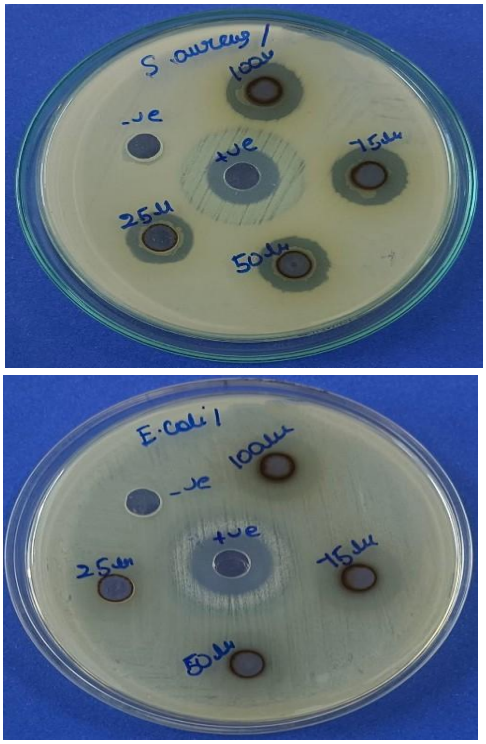


Figure. 6 Anti-bacterial activity of Petroleum ether leaf extract in *Hibiscus arnottianus*

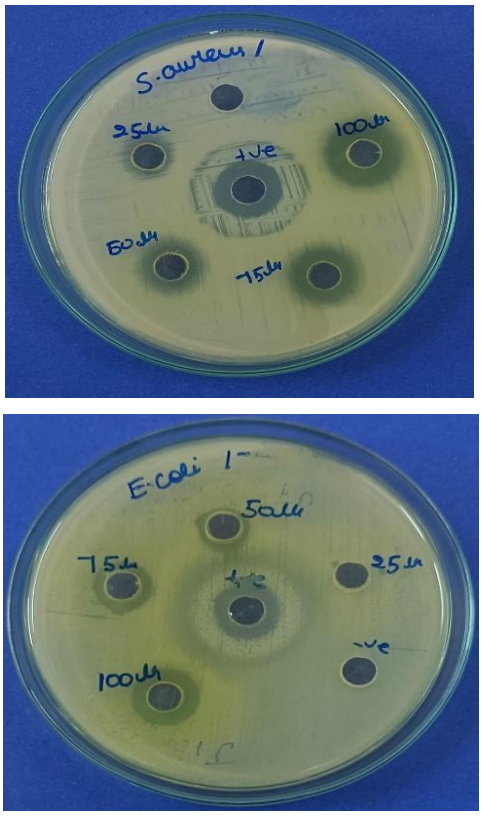


Figure. 7 Anti-bacterial activity of Methanol leaf extract of *Hibiscus arnottianus*

CONCLUSION

The present study highlights the phytochemical composition and antibacterial potential of leaf extracts of *Hibiscus arnottianus*. Preliminary phytochemical screening using methanol and petroleum ether extracts provided an initial assessment of the plant's chemical constituents. GC–MS analysis of the petroleum ether extract tentatively identified several compounds, including n-hexadecanoic acid, phytol, neophytadiene, squalene, and fatty acid esters. These compounds may contribute to the biological properties of the extract, although their individual activities require further investigation. The antibacterial evaluation demonstrated inhibitory activity against both *Staphylococcus aureus* and *Escherichia coli* in the tested extracts. Finally this findings suggest that *H. arnottianus* leaves may serve as a potential source of bioactive compounds with antibacterial properties. Further studies involving the isolation and structural confirmation of individual compounds, determination of minimum inhibitory concentrations (MICs), and evaluation of their safety and mechanisms of action are necessary to establish their potential applications in the development of plant-based antibacterial agents.

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