

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *JASMINUM SAMBAC* (L.) AITON CULTIVATED IN IRAQ

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Abstract

Jasminum sambac (L.) Aiton (sambac jasmine) of the Oleaceae family is an evergreen perennial plant. It has been used medicinally for a very long time. Traditional medicine has long made use of the several phytochemicals found in Arabian jasmine. The antibacterial and chemical composition of Iraqi-grown *Jasminum sambac* leaves investigated. Alkaloids, terpenoids, flavonoids, and 70% ethanolic extract were used. The GC-MS analysis of the hexane extract revealed many bioactive components, such as vitamin E, stigmaterol, oleic acid, gamma-tocopherol, and alpha-tocopheryl acetate. In order to determine the efficacy against different types of bacteria and fungi antimicrobial experiments were conducted. Tests for antimicrobial action revealed that it effectively suppressed the growth of *Candida albicans* fungus, Gram-positive *Staphylococcus aureus* bacteria, and *Staphylococcus epidermidis*, but had no effect against *Klebsiella* sp. and Gram-negative *Escherichia coli*. Antimicrobial and antifungal efficacy varied with concentration. The results suggested that secondary metabolites of *Jasminum sambac* showed pharmaceutical-grade antibacterial activity. The development of new antimicrobial drugs requires more research for the identification of specific chemicals and the processes by which they work.

Key words: Botanical drug discovery; *Jasminum sambac*; Plant-derived antimicrobials

Introduction

Jasminum sambac (L.) Aiton (sambac jasmine), a perennial evergreen shrub of the Oleaceae family is famous as Arabian jasmine. Its native region is Asian subtropical and tropical regions, specifically India and Southeast Asia, and is grown a lot for its fragrant flowers (Mehraliyev *et al.*, 2021). Large number of people like it not only for its look, but also for its use in medicine and industry (Al-Snafi. 2024). There are different vernacular names in different countries for this species such as Philippines name of this flower is Sampaguita, Arabian jasmine in the United States, Moli in China, and Pikake in Hawaii (Chinese Academy of Sciences, 1959). *J. sambac* possess dark green ovate leaves and clusters of tiny, waxy, white salver form flowers. It has a strong smell as it contains volatile aromatic compounds (Ayuni *et al.*, 2024). The plant does well in warm weather compared to cold weather, thus it is not grown in colder areas (Mourya *et al.*, 2017). Phytochemical studies showed that *J. sambac* possessed many bioactive compounds, such as flavonoids (quercitrin and kaempferol derivatives), terpenoids, alkaloids, tannins, saponins, phytosterols, iridoids, polysaccharides, and organic acids such as oleanolic acid and dotriacontanoic acid (Sengar *et al.*, 2015). The fragrance, cosmetics, and

flavoring industries all use a lot of the essential oil, which has a lot of linalool, benzyl acetate, and cis-jasmone. The plant's polysaccharides and flavonoids are highly potent in fighting inflammation, changing the immune system, and protecting against free radicals (Rattan, 2023).

J. sambac has been used for long time in ethnomedicine for the treatment of a various ailments, like microbial infections, inflammation, skin conditions, ulcers, menstrual irregularities, fever, and hypertension (Table 1). These traditional uses have been substantiated by modern pharmacological studies demonstrating antibacterial, antifungal, antioxidant, anti-inflammatory, cytoprotective, and wound-healing activities (Sengar *et al.*, 2015) Particularly, the methanolic extracts, have shown cytotoxic effects against many human cancer cell lines, which determine a possible anticancer use (Ye *et al.*, 2015; Charoenying *et al.*, 2024).

J. sambac is an attractive prospect for the production of new medicinal substances because of its vast list of phytochemicals and large range of pharmacological effects. To be able to turn the traditional uses of it into evidence-based therapeutic applications, wider systematic investigations are needed. These studies should focus on the bioactive constituents, mechanisms of action, and optimal formulations of it (Khan *et al.*, 2021). The

chemical constituents and antibacterial action of *Jasminum sambac*, that is grown in Iraq, are the targets of this investigation. Phytochemical screening of the ethanolic extract, GC-MS component identification of the hexane extract, and antibacterial and antifungal properties testing of the extract of ethanol will be performed.

Table 1. Methanolic extract phytochemical study of *J. sambac* (Kalaiselvi & Kalaivani, 2011).

Secondary metabolites	Observation
Alkaloids	+
Flavonoids	+
Terpenoids	+
Carbohydrates	+
Proteins	+
Phenols	+
Tannins	+
Saponins	+
Glycosides	+
Steroids	-
Phytosterols	+
Gums and mucilages	-

Materials and Methods

Gathering the plant and verifying its identity: Fresh leaves of *J. sambac* were gathered in October 2024 from various plant nurseries in Baghdad, Iraq. The plant was taxonomically authenticated at the College of Pharmacy, Al-Esraa University, and a voucher specimen (Voucher No. JS-IRQ-2024) was deposited in the departmental herbarium for future reference. The plant leaves were cleaned, shade-dried at R.T., weighed, and kept in airtight containers until their usage (Fig. 1).

Preparation of extracts

Hexane extract: The dried powdered leaves were extracted with n-hexane. A rotary evaporator was used to concentrate the extract, which was then kept in sterile containers for further GC-MS analysis.

Ethanolic extract (70%): Following the hexane extraction by using Soxhlet apparatus, 500 ml of 70% aqueous ethanol was utilized to re-extract the residual residue by using Soxhlet apparatus. Following a process of drying and extract concentration using a rotary evaporator, the mixture was put in sterile Petri dishes covered with aerosol and foil. By mixing 100 mg of dried extract with 1 ml of ethanol solvent, a 100 ml/mg stock solution was gained. To perform antimicrobial testing, serial dilutions were produced at 50, 25, and 12.5 mg/ml concentrations.

Ethanolic extract phytochemical screening: Qualitative phytochemical analysis of the 70% ethanolic extract was performed using a standard method to find out various secondary metabolites:

Alkaloids: Mayer and Wagner tests. (Harborne, 1998).

Saponins: Test for foam (Kokate, 2001)

Terpenoids: Salkowski test (Evans, 2009)

Flavonoids: The extract was treated with conc. hydrochloric acid (alkaline reagent test) (Lakshmi *et al.*, 2024).

Analysis of hexane extract by GC-Mass: An Agilent 6200 GC-Mass system, which utilize an Agilent HP- five ms Ultra Inert column (30 meters × 250 μm × 0.25 μm), was utilized to analyze the n-hexane extract. Procedure parameters were: 1 μL injection volume, splitless mode, 7.07 psi intake pressure, and an oven temperature from range of 60°C to 325°C (Jian *et al.*, 2023). The chemical components were detected by matching the obtained mass spectra with Ibn Al-Baytar Center– Ministry of Science and Technology library data (Table 2).

Table 2. GC-Mass analysis conditions for the identification of the active constituents in n-hexane of leaves of *J. sambac* (Thuwaini, 2022).

Instrument	Gas chromatography: Agilent (6200) mass spectroscopy
Analytical column	Agilent HP-5ms Ultra Inert column (30 m length × 250 μm internal diameter × 0.25 μm film thickness)
Injection volume	1 μl
Inlet pressure	7.0699 psi.
Oven temperature	60–325°C
Injection type	Splitless.

Microorganisms and culture conditions: Antimicrobial evaluation was undergone at the College of Science, Al-Mustansiriyah University. Experimental creatures included:

- Gram-positive bacteria:** *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus* species.
- Gram-negative bacteria:** *Escherichia coli*, *Klebsiella* species.
- Fungal strain:** *Candida albicans*.

After 22 hours of incubation at 37°C, the bacterial cultures were maintained in Mueller-Hinton Broth (Wu *et al.*, 2021). Typical circumstances were utilized to cultivate the fungal cultures on Sabouraud Dextrose Agar.

Assay of antibacterial and antifungal activities: For the measurement of the efficacy against microbes, the agar well diffusion was utilized. Standardized microbiological suspensions were utilized to seed Mueller-Hinton Agar plates (for bacteria) and Sabouraud Dextrose Agar plates (for fungus), at 1.5×10^8 CFU/mL concentration (0.5 McFarland standard). Agar was punctured with wells of 6 mm diameter. After that, 100 μL of the ethanolic extract with doses of 100, 50, 25, 12.5, and 6.25 mg/mL was introduced. The negative control was methanol. At 37°C, the plates were maintained for full day to evaluate the antibacterial activity. The inhibition zones were determined in millimetres (Fig. 2).

Results and Discussion

Initial screening for phytochemical properties: Alkaloids, terpenoids, and flavonoids found in *J. sambac* leaves are shown in Table 3.

Table 3. Qualitative profile of phytochemicals in *J. sambac* leaves.

Test	Alkaloids	Saponins	Terpenoids	Flavonoids
Results	+	-	+	+

GC-Mass analysis of n-hexane extract: GC-Mass analysis (Fig. 3) of the n-hexane extract determine a large number of bioactive compounds with important medicinal properties (Table 4). Major constituents included alpha-tocopherol acetate, gamma-tocopherol O-methyl, oleic acid, stigmaterol, and vitamin E.



Fig. 1. Fresh and dried leaves of *J. sambac*.



Fig. 2. Qualitative profile of the Phytochemicals found in the leaf part of the plant.
1: Terpenoid test 2: alkaloid test

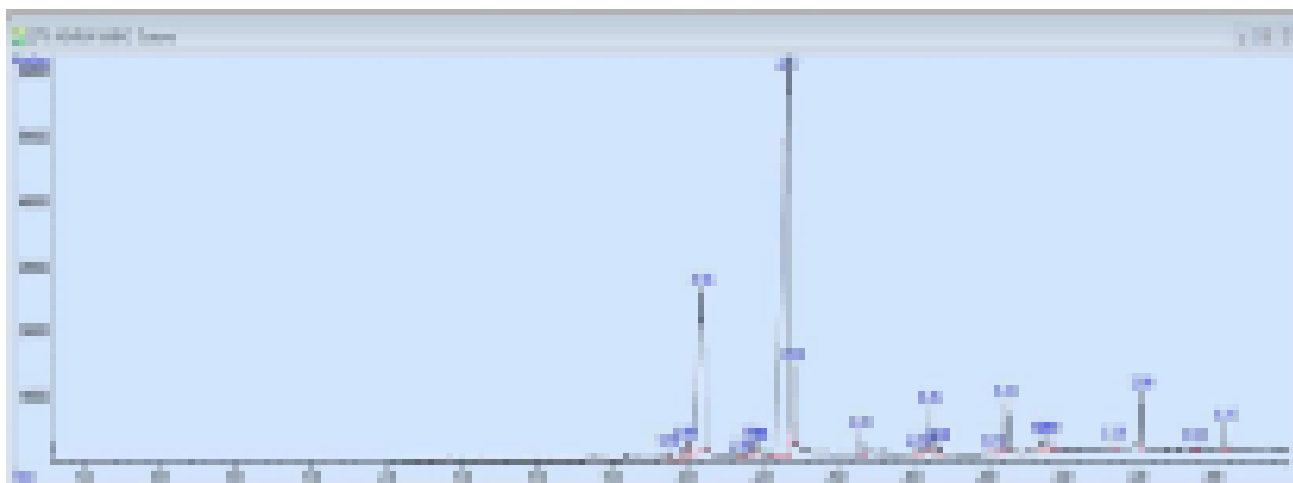
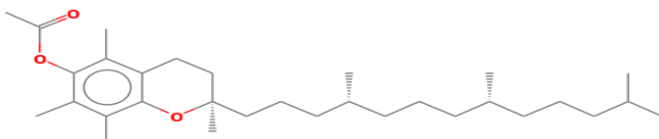
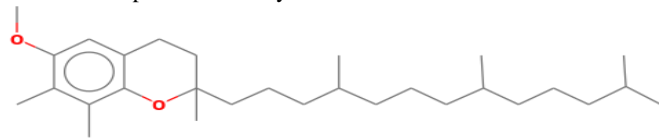
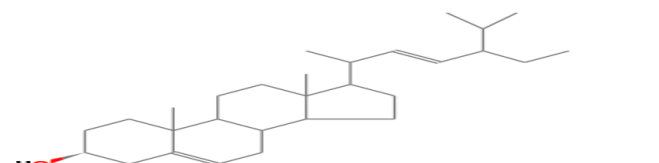
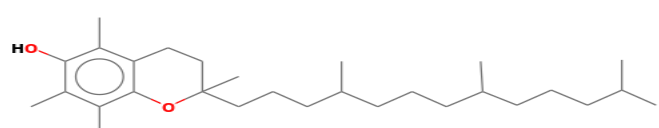


Fig. 3. Soxhlet extraction of n-hexane from leaf parts and its GC-chromatogram.

Table 4. Key components determined in *J. sambac* leaves using an n-hexane extraction method.

Compound	Retention time (min)	Similarity index	Reported biological activity
Alpha-Tocopheryl Acetate 	32.099	91	Antioxidant, skin protection, wound healing, immune support
Gamma-Tocopherol O-Methyl 	32.099	96	Antioxidant, anti-inflammatory, anti-aging, cognitive support
Stigmasterol 	33.510	98	Cholesterol-lowering, antioxidant, anti-inflammatory, anticancer
Vitamin E 	32.099	97	Antioxidant, skin hydration, cardiovascular and eye health

Antimicrobial and antifungal activities of the ethanolic extract: The aqueous ethanol (70%) extract showed concentration-dependent antimicrobial activity against the tested microorganisms (Table 5).

Table 5. Inhibition zones (mm) of *J. sambac* leaf ethanolic extract against tested microorganisms (Agar well diffusion method).

Organism	Concentration (mg/mL)	Inhibition zone (mm)
<i>Staphylococcus aureus</i>	100	–
	50	12
	25	14
	12.5	38
<i>Staphylococcus epidermidis</i>	100	–
	50	12
	25	–
	12.5	18
<i>Klebsiella</i> sp.	100	–
	50	–
	25	–
	12.5	12
<i>Escherichia coli</i>	100	–
	50	–
	25	–
	12.5	–
<i>Candida albicans</i>	100	–
	50	12
	25	14
	12.5	36

Discussion

Phytochemical screening of Iraqi-grown *J. sambac* ethanolic extract reveals a wide array of secondary metabolites, including glycosides, alkaloids, tannins, flavonoids, phenols, and saponins.

The qualitative analysis of *J. sambac* leaves showed the presence of alkaloids, terpenoids, and flavonoids, while there were no saponins.

These results corroborate those of previous research that has isolated *J. sambac*'s primary phytoconstituents, which include alkaloids, flavonoids, and terpenoids. These constituents have well-known pharmacological properties, including antibacterial and antioxidant actions, which might explain why *J. sambac* has been utilized traditionally in folk medicine (Lakshmi *et al.*, 2024). The antioxidant and antibacterial properties of flavonoids and terpenoids are well known, whereas alkaloids show a wide spectrum of pharmacological activity. The large proportion of vitamin E derivatives (alpha- and gamma-tocopherol) shows a strong antioxidant activity, while oleic acid and stigmasterol contribute to anti-inflammatory and antimicrobial effects.

The determination of these metabolites aligned with findings from studies performed on *J. sambac* grown in other regions, although slight variations in phytochemical profiles may be resulted from the variations in environmental conditions, soil nature, and cultivation practices in Iraq (Lin *et al.*, 2016).

GC–MS analysis of the hexane extract gives a more in-depth chemical fingerprint, determining the presence of large number of volatile and non-polar bioactive compounds. Many of these detected constituents, as terpenes, fatty acids, and aromatic compounds, have been previously detected to possess antimicrobial, anti-inflammatory, and antioxidant activities (Lakshmi *et al.*, 2024). Plant phytochemicals such as

flavonoids, tannins, terpenes, and phenolic acids can show antimicrobial, anti-inflammatory, and antioxidant effects. They inhibit microbes by damaging the cell membrane, blocking key enzymes, and interfering with DNA/RNA processes. These compounds also reduce inflammation by suppressing NF- κ B signaling and lowering cytokines like TNF- α and IL-1 β . In addition, they act as antioxidants by neutralizing free radicals and preventing lipid peroxidation. Through these combined actions, plant extracts help protect cells from infection, inflammation, and oxidative stress (Pecorini *et al.*, 2022.).

The differences in compound abundance and diversity between the ethanolic and hexane extracts shows the influence of solvent polarity on extraction efficiency, with ethanol preferring the extraction of polar phytochemicals and hexane targeting non-polar constituents (Fontana *et al.*, 2024).

Its efficacy as a natural antibacterial agent was proven by the inhibitory effects of the ethanolic extract against various bacterial and fungal strains, as evidenced in the antimicrobial evaluation. Older studies have determined that extracts from *J. sambac* have broad-spectrum antibacterial activity, which is in line with our current findings (Sengar *et al.*, 2015). *Staphylococcus aureus* and *Candida albicans* exhibit higher inhibition at lower doses (12.5 mg/mL), with *S. aureus* having the biggest inhibition zone (38 mm) and *C. albicans* having 36 mm at the same dosage. *S. epidermidis* demonstrated no inhibition at values over 12.5 mg/mL, with a minor inhibition of 18 mm. All concentrations of *Klebsiella sp.* and *Escherichia coli* exhibited no inhibition, with the exception of *Escherichia coli* at 12.5 mg/mL (12 mm).

Phytochemicals such as flavonoids, tannins, and phenolic compounds have a synergistic effect to disrupt microbial cell membranes, inhibit enzyme function, and reduce nucleic acid production. Thus, they possess antibacterial potential. It is possible that differences in the composition of the cell wall, metabolic pathways, and mechanisms of the resistance account for the determined differences in the sensitivity between the tested microbes (Sengar *et al.*, 2015).

The extract had a low potency on Gram-negative bacteria (*E. coli*, *Klebsiella sp.*), but it had a strong potency on Gram-positive bacteria (*S. aureus*, *S. epidermidis*) and *Candida albicans*. According to (Wu *et al.*, 2021), flavonoids such as quercetin and kaempferol may destruct bacterial membranes and prevent important enzymes. On the other hand, alkaloids can prevent the replication of the DNA and protein synthesis. The prevention of the synthesis of ergosterol, which weakens fungal membranes, and the generation of ROS (reactive oxygen species), which harm fungal protein, lipids, and DNA, might be resulted from the antifungal action.

Overall, the research lends credence to *J. sambac*'s ethnopharmacological significance and determines its promise as a repository for bioactive chemicals for future pharmaceutical research and development. Antimicrobial evaluation, GC-MS profiling, and phytochemical screening all of them work together to show its whole chemical make-up and biological action (Rizal & Razak, 2024). With the prevalence of microbes on the rise, it is imperative that researchers isolate and characterize the active ingredients, demonstrate how they act, and investigate their potential uses in the production of new antimicrobials.

Conclusions

This research determines that *J. sambac* leaves that are grown in Iraq possess a variety of phytochemicals, involving the terpenoids, alkaloids, flavonoids and phenols, many of them possess antibacterial and antioxidant actions. Multiple bioactive substances, involving alpha-tocopheryl acetate, oleic acid, gamma-tocopherol, and stigmaterol, were detected by GC-MS analysis of the hexane extract. These components have recognized medicinal potential.

Regardless of its little effect against Gram-negative bacteria, the ethanolic extract possess a potent concentration-dependent antimicrobial activity, particularly against two types of Gram-positive bacteria *S. aureus* and *S. epidermidis* and also the yeast *C. albicans*. It appears from the results that the synergistic effect of *J. sambac*'s secondary metabolites may disrupt the critical cellular processes and destructs microbial membranes, is mainly responsible for its antibacterial potency.

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Conflict of Interest: The authors declare no conflict of interest.

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