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Inhibitory Effect of *Syzygium aromaticum* Extract on the Growth of *Pseudomonas aeruginosa* Clinical Isolates Associated with Urinary Tract Infections

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ABSTRACT

Background & Objective: *Syzygium aromaticum* is the scientific name for the aromatic flower buds of an Indonesian native tree from the Myrtaceae family. Cloves contain bioactive compounds such as caryophyllene, eugenol, and acetyl eugenol, which have been shown to possess various physiological properties, including antioxidant, antibacterial, analgesic, anti-inflammatory, anticancer, and anesthetic effects. The present study aims to examine the antibacterial effects of *S. aromaticum* extracts prepared using different solvents (methanol, ethanol, petroleum ether, n-hexane, and chloroform) against *P. aeruginosa*.

Materials & Methods: A total of 93 urine samples were collected from inpatients at Al-Sadder Hospital in Maysan, Iraq. The study specifically targeted urinary tract infections (UTIs) caused by Pseudomonas aeruginosa. The antibacterial activity of various *S. aromaticum* extracts (prepared using methanol, ethanol, petroleum ether, hexane, and chloroform) was evaluated against the isolates using the agar well diffusion method. The extracts were tested at concentrations of 62.5, 125, and 500 µg/mL and compared with a standard antibiotic. This investigation aimed to verify the antimicrobial potential of *S. aromaticum* against *P. aeruginosa*, with the findings highlighting its promise for medicinal applications.

Results: Phytochemical screening revealed the presence of tannins, flavonoids, terpenoids, alkaloids, phlobatannins, and saponins in the extracts. Among the tested extracts, the ethanol extract showed the lowest activity against *P. aeruginosa*, producing an inhibition zone of 24 mm at 500 µg/mL. The methanol extract exhibited the highest antibacterial activity, with an inhibition zone of 40 mm at 500 µg/mL. The *P. aeruginosa* isolates were sensitive to Gentamicin, Neomycin, Enrofloxacin, Imipenem, and Meropenem but resistant to Cefadroxil, Cephalexin, Ceftriaxone, Ciprofloxacin, and Sulfamethoxazole.

Conclusion: The results demonstrate notable antibacterial activity of *S. aromaticum* particularly its methanolic extract against *P. aeruginosa*. This potent activity is attributed to its diverse phytochemical composition. Overall, *S. aromaticum* shows potential as a natural source for developing novel antimicrobial agents capable of combating multidrug-resistant infections.

Keywords: *S. aromaticum*, Phytochemical Composition, *Pseudomonas aeruginosa*, Urinary Tract Infections

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1. Introduction

Pseudomonas aeruginosa is a Gram-negative bacterial pathogen responsible for a wide range of diseases, including pneumonia, infections in immunocompromised patients, and complications associated with cystic fibrosis (1). It is also a major cause of high-mortality infections in individuals with cystic fibrosis, neonatal infections, cancer, and severe burns (2, 3).

During infection, *P. aeruginosa* may adopt either a planktonic lifestyle (typical of acute infections) or a sessile, biofilm-associated lifestyle (common in chronic

infections). Several mechanisms contribute to its virulence, including cytotoxicity (hydrogen cyanide, exotoxin A, T3SS, pyocyanin), immune evasion (elastase, alkaline protease), antibiotic resistance (efflux pumps and modifying enzymes), iron acquisition (siderophores, proteases), biofilm formation (alginate, rhamnolipids), and motility (flagella, pili) (4).

The search for new antibacterial agents—including those derived from medicinal plants—has intensified in response to increasing antimicrobial resistance. Traditional medicine often relies on aromatic herbs to treat bacterial infections, and many plant-based preparations have shown promising therapeutic value (5, 6).

S. aromaticum consists of the dried flower buds of an evergreen tree belonging to the Myrtaceae family, widely grown in tropical regions. For centuries, it has been utilized in traditional medicine and cuisine, and its essential oil is used in perfumery, food flavoring, and herbal treatments. Historically, *S. aromaticum* has been associated with enhancing immunity and promoting resistance to disease. It continues to be used as an anesthetic, antiseptic, antiviral, antifungal, and antibacterial agent for various health conditions (7).

Eugenol, the major volatile component of *S. aromaticum*, is responsible for its characteristic flavor and aroma (8). The flower buds also possess diverse pharmacological properties, including antioxidant, antibacterial, antifungal, anti-inflammatory, anticancer, and antidiabetic activities (9, 10). *S. aromaticum* extracts have shown antibacterial activity against several Gram-negative uropathogens (11).

In addition to tannins, vanillin, flavonoids, and triterpenoids, *S. aromaticum* buds contain 15–20% essential oil, primarily composed of eugenol, eugenol acetate, and caryophyllene. Their distinct aroma is further influenced by trace compounds such as methyl amyl ketone and methyl salicylate (12).

The present study aims to examine the antibacterial effects of *S. aromaticum* extracts prepared using different solvents (methanol, ethanol, petroleum ether, n-hexane, and chloroform) against *P. aeruginosa*. The extracts' efficacy was compared with standard antibiotics, and phytochemical screening was conducted to identify secondary metabolites associated with antibacterial activity. The findings may serve as a basis for further research on identifying, characterizing, and elucidating the mechanisms of active compounds.

2. Materials and Methods

2.1 Preparation of *S. aromaticum*

S. aromaticum buds was purchased from the local market in Maysan, Iraq. The identification and authentication of *S. aromaticum* was confirmed at the Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Misan, Maysan, Iraq.

At first, *S. aromaticum* were carefully washed and cleaned with tap water and then distilled water. Then, they were allowed to air dry. The plant material was finely powdered after drying (13).

2.2 Preparation of test samples and procedure

To identify phytoconstituents, the n-hexane, ethanol, petroleum ether, methanol, and chloroform extracts underwent preliminary phytochemical screening using the following procedure: 50 mL of n-hexane, ethanol, petroleum ether, methanol, and chloroform were added individually as solvents to separate conical flasks containing the test samples (5.0 g each). The mixtures were kept at room temperature for twenty-four hours. The crude clove extracts were then evaporated at 45 °C. After evaporation, the extracted material was stored at 4 °C in the dark. Before antibacterial testing, the extract was dissolved in 10% dimethyl sulfoxide (DMSO) to obtain the required concentrations (14, 15).

2.3 Clinical Bacteria Isolates

Urine samples were collected from patients admitted to the Intensive Care Unit (ICU) and the Burn Unit. The study focused exclusively on samples obtained from patients clinically diagnosed with urinary tract infections (UTIs) caused by *P. aeruginosa*. Samples containing other bacterial pathogens were excluded. The presence of *P. aeruginosa* was confirmed using standard microbiological identification procedures, including culture characteristics, Gram staining, and biochemical testing.

A total of fifteen *P. aeruginosa* isolates were recovered from ninety-three (93) culture-positive urine samples. Of these, eleven (11) isolates exhibiting a multidrug-resistant (MDR) phenotype—as determined by antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method—were selected for subsequent evaluation of the antibacterial activity of *S. aromaticum* extract.

To ensure the reliability and reproducibility of experimental outcomes, all agar well diffusion assays included a positive control (gentamicin 10 µg discs) and a negative control (Dimethyl Sulfoxide, DMSO). Following inoculation onto blood agar and MacConkey agar, all samples were incubated for 18 hours at 37°C. Plates were then incubated at 37°C for an additional 24 hours. Pure colonies obtained were subcultured onto nutrient agar. Identification of *P. aeruginosa* isolates was confirmed using morphological characteristics, Gram staining, conventional biochemical assays, and the VITEK automated system.

2.4 Biochemical tests

Conventional biochemical tests were performed to confirm the identity of the isolates, including Gram staining, urease production, oxidase test, lactose fermentation, motility test, catalase activity, and microscopic examination (16).

1. To perform the alkaloid test, 0.5 g of extract is weighed and mixed with 1 mL of 2N HCl and 9 mL of distilled water. The mixture is heated in a water bath for two minutes, cooled, and filtered. The filtrate is then used for reactions with Mayer's, Bouchardat's, and Dragendorff's reagents. For Mayer's reagent, three drops of *S. aromaticum* leaf extract and two drops of Mayer's reagent are added to a test tube. The appearance of a white or yellow precipitate indicates the presence of alkaloids. For Bouchardat's reagent, three drops of *S. aromaticum* leaf extract and two drops of the reagent are mixed. A brown to black precipitate indicates alkaloids. For Dragendorff's reagent, three drops of extract and two drops of Dragendorff's reagent are combined. Formation of orange to red or brick-red precipitates confirms the presence of alkaloids. The sample is considered positive for alkaloids if at least two of the three reagents give a positive reaction.

2. For the flavonoid test, 10 mL of hot water is added to a test tube containing the thick S. aromaticum extract. The mixture is boiled and filtered while still hot. To 5 mL of filtrate, 0.1 g of Mg powder, 1 mL of HCl, and 2 mL of amyl alcohol are added. After shaking, the mixture is allowed to separate. The presence of flavonoids is indicated by a yellow, orange, or red coloration in the amyl alcohol layer.

3. A thick extract of *S. aromaticum* is placed in a test tube, and hot water is added. The tube is shaken vigorously for 15 minutes. After adding one drop of 2N HCl, persistent foam lasting approximately 10 minutes indicates the presence of saponins.

4. A thick *S. aromaticum* leaf extract is mixed with 10 mL of distilled water and filtered. Distilled water is then added to the filtrate until it becomes colorless. One to two drops of iron (III) chloride reagent are added to 2 mL of the filtrate. A dark blue or blackish-green coloration indicates the presence of tannins.

5. For the triterpenoid test, 30 mg of extract is treated with 5 drops of glacial acetic acid and 2 drops of concentrated sulfuric acid. A blue or green color indicates the presence of steroids, while a red or purple color indicates the presence of triterpenoids.

6. To test for phlobatannins, 500 µL of the clove plant extract is heated with 0.5 mL of 1% aqueous HCl. The formation of a red precipitate confirms the presence of phlobatannins.

7. For the steroid test, 0.5 g of extract is treated with 2 mL of acetic anhydride, followed by the careful addition of 2 mL of sulfuric acid along the wall of the test tube. A color change from violet to blue-green indicates the presence of steroids.

2.5 Stock and Standard Concentrations of *S. aromaticum* Extract

The stock solution consisted of crude extract at 100 mg/mL^{-1} DMSO (20mg/50ml) was used to prepare concentrations of 62.5 mg/mL^{-1}, 125 mg/mL, and 500 mg/mL^{-1} for antibacterial testing.

2.6 Antibacterial Activity of Plant Extracts

The isolates were re-cultured on Mueller–Hinton agar, and antibacterial activity was evaluated using the agar well diffusion method. A 0.5 McFarland suspension (≈108 CFU/mL) was prepared and spread on the agar. Wells of 5 mm diameter were filled with 50 µL of extract. Gentamicin served as the reference antibiotic. Plates were incubated at 35°C ± 2°C for 18–24 hours and inhibition zones were measured. The germs collected were re-isolated on Muller-Hinton Agar for Pseudomonas. The tested extract solutions were prepared. Antibiotic susceptibility testing was performed using the agar well diffusion method described by Karou et al **(17)**. Active extracts were initially identified starting at a high concentration through a presumptive test. A microbial suspension of 0.5 McFarland ($\approx 10^8$ CFU/mL) was used. Densitometer turbidity measurements were performed to quantify the suspension. The inoculum was added to culture media prepared according to standard procedures. Sterility and fertility tests were conducted prior to use to assess the quality of the media. Following inoculation of the medium, sterile hollow punches were used to create wells with a diameter of 5 mm. Fifty milliliters of extract at concentrations of 62.5 mg/mL, 125 mg/mL, and 500 mg/mL were added to each well. The reference antibiotic was 10 mg of gentamicin solution for Pseudomonas. The Petri dishes were incubated for 18 to 24 hours at 35˚C ± 2˚C after 30 minutes of pre-diffusion at room temperature. Diameters of microbial growth inhibition zones were measured using an electronic reading chart.

2.7 Antibiotic resistance

The agar well diffusion method was used to examine the antibacterial activity of five extracts of *C.zeylanicum* bark **(18)**.

Antibiotic susceptibility testing was performed using the agar well diffusion method. Tested antibiotics included Gentamicin (10 mg), Neomycin (30mg), Meropenem (10 mg), Imipenem (10 mg), Cephalexin (30mg), Cefadroxil (30 mg), Ceftriaxone (30 mg), Enrofloxacin (5 mg), Ciprofloxacin (5mg), and Sulfamethoxazole (25 mg) **(19, 20)**.

2.8 Statistical Analysis

All experiments were performed in triplicate (n = 3). Data were expressed as mean ± SD. One-way ANOVA followed by Tukey's post hoc test was used to determine significant differences ($p < 0.05$). Statistical analyses were conducted using SPSS version 22.

3. Result

3.1 phytochemical composition of *S. aromaticum*

Based on initial phytochemical analysis, the extract of *S. aromaticum* buds in the current study contain alkaloids, flavonoids, terpenoids, tannins, phlobotannin, steroids, and saponins.

3.2 Sensitivity test for antibiotics of *P. aeruginosa*

The antibiotic susceptibility results of the clinical isolates are summarized in Table 2. Most isolates showed high resistance rates to the tested antibiotics, with only a limited number remaining sensitive. The presence of multidrug-resistant isolates was evident.

3.3 Antibacterial activity of *S. aromaticum* extract against isolates of *P. aeruginosa*

The inhibitory action of *S. aromaticum* extract is determined using three different concentrations of the extract: 62.5 $mg.mL^{-1}$, 125 $mg.mL^{-1}$, and 500 $mg.mL^{-1}$. Five extracts of Ceylon *S. aromaticum* are used against *P. aeruginosa*, including chloroform, hexane, petroleum ether, methanol, and ethanol. The findings demonstrat that the methanol extract had the largest inhibition range against *P. aeruginosa*, measuring 40 mm at a concentration of 500 mg/ml^{-1}. Petroleum ether came in second with an inhibition range of 33 mm at the same concentration, while the ethanol extract had the smallest inhibition range (Table 3).

Table 1. Phytochemical composition of *S. aromaticum*.

Phytochemical tests	***S. aromaticum* extracts**				
	Ethanol	**n-hexane**	**Methanol**	**petroleum ether**	**chloroform**
Alkaloids	-	+	+	-	+
Flavonoids	-	-	+	+	+
Saponins	-	+	+	-	+
Steroids	+	-	+	-	+
Tannins	+	+	+	-	-
Phlobotannins	+	-	+	+	-
Terpenoids	-	+	+	-	+

Table 2. Antibiotic sensitivity test of *P. aeruginosa*.

Antibiotics	**Clinical Isolate**														
	p1	**p2**	**p3**	**p4**	**p5**	**p6**	**p7**	**p8**	**p9**	**p10**	**p11**	**p12**	**p13**	**p14**	**p15**
Cefadroxil (30 mg)	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
Cephalexin (30mg)	R	R	R	R	R	S	R	R	R	R	R	S	S	R	R
Ceftriaxone (30 mg)	R	R	R	R	R	R	R	R	S	R	R	R	S	S	S
Ciprofloxacin (5 mg)	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R
Enrofloxacin (5 mg)	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S
Sulfamethoxazole (25 mg)	R	R	R	R	R	R	R	R	R	S	R	R	S	S	R
Neomycin (30 mg)	R	R	R	R	S	R	R	S	S	R	R	S	S	R	S
Imipinem (10mg)	R	R	R	R	R	S	S	R	R	R	S	S	R	S	S
Meropenem(10 mg)	R	R	R	R	S	R	R	S	R	R	R	S	S	S	S

Table 3. Inhibitory effect of *S. aromaticum* extracts.

Concentration (mg/mL)	Ethanol extract	n-Hexane extract	Methanol extract	Petroleum ether extract	Chloroform extract
62.5	15.0 ± 0.5^{a}	15.0 ± 0.4^{a}	31.0 ± 0.6^{b}	27.0 ± 0.5^{c}	27.0 ± 0.5^{c}
125	22.5 ± 0.6^{a}	22.5 ± 0.5^{a}	32.0 ± 0.6^{b}	30.0 ± 0.5^{b}	28.5 ± 0.5^{b}
500	24.0 ± 0.5^{b}	25.5 ± 0.6^{b}	40.0 ± 0.7^{a}	33.0 ± 0.6^{b}	30.0 ± 0.5^{b}

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences among extracts at the same concentration ($p < 0.05$, one-way ANOVA followed by Tukey's test).

4. Discussion

The medicinal value of plants arises from the bioactive chemical compounds they contain, each of which has specific physicochemical effects on the human body Al-Behadili and Faraj (15) and, Batiha et al (9). Phytochemicals exhibit diverse biological activities that may play a role in preventing chronic diseases such as heart disease and stroke. For instance, saponins protect against hypercholesterolemia, while steroids and terpenoids have been shown to exert analgesic effects on the central nervous system. The importance of alkaloids, saponins, and tannins any of which contribute to the action of existing antibiotics—is increasingly recognized by researchers (21).

Flavonoids, a widespread group of phenolic secondary metabolites, are present in the leaves, stems, roots, flowers, and seeds of nearly all plant families. These compounds can exert antibacterial activity by disrupting the cytoplasmic membrane, inhibiting nucleic acid synthesis, and slowing cellular energy metabolism, making them important natural antimicrobial agents (22). The present study demonstrates that *P. aeruginosa* was sensitive to Gentamicin, Neomycin, Meropenem, and Enrofloxacin; however, it exhibited resistance to several other antibiotics tested. *P. aeruginosa* remains a major global health concern due to its ability to resist numerous antibiotics, including imipenem, which is often reserved as a last-line therapeutic option (23).

Given the increasing antibiotic resistance observed in *P. aeruginosa*, the development of new antimicrobial agents and the prudent use of existing antibiotics are essential (9, 24). Findings from this study, along with previous research, indicate that *S. aromaticum* possesses significant antibacterial potential. The results show that the methanolic extract exhibited the greatest inhibitory effect against *P. aeruginosa,* whereas the ethanolic extract displayed the lowest. Previous studies show that *S. aromaticum* extract contains numerous components, predominantly flavonoids and phenolic compounds (25).

Flavonoids play an important role as antimicrobial agents in plants. Serving as part of the plant's natural defense system, they help protect against microbial infections (26). Many plant-derived flavonoids demonstrate antibacterial effects distinct from those of conventional antibiotics, suggesting their potential use as complementary therapeutic agents (27). Additionally, it is proposed that flavonoid DNA intercalation, which inhibits the synthesis of bacterial nucleic acids, is the mechanism behind the antibacterial properties of flavonoids. The chemical properties of phenolic compounds, notably their aromatic structure and numerous hydroxylgroups capable of donating electrons or hydrogen atomsto neutralize free radicals and other reactive oxygen species (ROS), which are linked to their beneficial bioactivities (15, 28). Furthermore, phenolic compounds exert antibacterial affects by altering virulence factors, genetic regulatory elements, the ability to disrupt biofilms, and the structure and function of membranes (29).

5. Conclusion

Medicinal plants, particularly *S. aromaticum*, hold significant importance in light of the declining effectiveness of many conventional antimicrobial agents. Therefore, exploring new therapeutic alternatives for the treatment of UTIs is essential. The findings of this study highlight the promising antibacterial activity of *S. aromaticum*, especially its methanolic extract, against *P. aeruginosa*. However, isolating and characterizing the active compounds within *S. aromaticum* remains a critical challenge that must be addressed to enable their potential use in biomedical applications.

6. Declarations

6.1 Acknowledgments

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6.2 Ethical Considerations

The study protocol was approved by the Institutional Review Board (IRB) of University of Misan, Iraq and Ethical code: (No.853-25-5-2025).

6.3 Authors' Contributions

Conceptualization, supervision, funding acquisitionand resources: Diana Basim and Zahraa Qasim;Methodology Diana Basim and Zahraa Qasim and Zainab Jabbar; Datacollection:Zainab Jabbar; Data analysis: All authors. All authors reviewed, edited, and approved the final version of the manuscript.

6.4 Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

6.5 Fund or Financial Support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

6.7 Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

7. Publisher's Note

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