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Original Article

Phytochemical screening and evaluation of antibacterial and antifungal activities of *Callistemon viminalis* cultivated in Iraq

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Abstract

Callistemon viminalis, a member of the Myrtaceae family, is traditionally used to treat infections, respiratory, and gastrointestinal disorders. This study aimed to investigate the phytochemical profile and evaluate the antibacterial and antifungal activities of *C. viminalis* leaves cultivated in Iraq. Leaves were collected, dried, and extracted sequentially with hexane and 70% ethanol. Preliminary phytochemical screening revealed the presence of saponins, terpenoids, alkaloids, and flavonoids. The antimicrobial activity of the ethanol extract was assessed using the agar well diffusion method against three Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus sp.*), two Gram-negative bacteria (*Escherichia coli, Klebsiella sp.*), and one fungal strain (*Candida albicans*). The ethanol extract exhibited notable inhibitory effects, particularly against Gram-positive bacteria and *C. albicans*, with activity increasing in a concentration-dependent manner. GC-MS analysis of the hexane extract identified key bioactive compounds, including beta-sitosterol and vitamin E. These findings highlight the significant pharmacological potential of *C. viminalis* leaves and support their traditional use as a source of natural antimicrobial agents. Further studies are recommended to isolate and characterize the active constituents responsible for these effects.

Keywords: *Callistemon viminalis*, Antibacterial, Antifungal, Insecticidal, Phytochemistry.

1. Introduction

Due to their numerous therapeutic advantages and wide range of applications, herbal remedies have been used for centuries as essential components of traditional healthcare systems, particularly among indigenous populations who have relied on local plants for the prevention and treatment of various ailments. In addition to their historical significance, herbal medicines continue to play a vital role in the management of health and disease in many developing countries, where access to modern pharmaceuticals may be limited and natural remedies remain both accessible and culturally accepted [1]. The Myrtaceae family includes *Callistemon viminalis*, A shrub or small tree that is native to Western Australia, Queensland, and New South Wales. In traditional or old medicine, it's often employed to treat bronchitis, cough, gastrointestinal issues, pain and infectious infections. It's also utilised as an insecticidal agent. Significant antibacterial [2], antioxidant [3], antifungal [4], and insecticidal [5] properties are present in the plant. Phytochemical investigations have led to the identification of several major classes of bioactive compounds in medicinal plants, such as sterols, triterpenes, flavonoids, and essential oils. These groups are recognized for their diverse biological activities and contribute significantly to the therapeutic potential of many traditional herbal remedies [6].

As illustrated in Table 1, the essential oils extracted from the leaves and flowers of *C. viminalis* were found to contain several major constituents. Notably, eucalyptol was the predominant compound in both plant parts, accompanied by significant amounts of α-pinene, β-pinene, and α-terpineol. These findings highlight the chemical diversity of the essential oils and provide a basis for understanding their observed biological activities.

Therefore, this study aimed to conduct a comprehensive phytochemical screening of *C. viminalis* leaves cultivated in Iraq and to systematically evaluate their antibacterial and antifungal activities against a range of clinically relevant microorganisms. By identifying the major bioactive constituents and assessing their antimicrobial efficacy, this research seeks to provide scientific evidence supporting the traditional use of *C. viminalis* and to explore its potential as a natural source for the development of novel antimicrobial agents.

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Table 1. The essential oil content of *Callistemon viminalis*'s leaves and flowers [7].

Compound	Composition (%)		
	* RIcal	Leaves	Flowers
α-pinene	907	10.28	21.48
β-pinene	923	0.64	0.36
p-cymene	1000	0.45	1.05
Myrcene	926	0.20	--
α-terpinene	1019	--	0.26
γ-terpinene	1010	0.24	0.56
Terpinen-4-ol	1110	0.59	0.85
Eucalyptol	1003	84.60	61.47
Linalool	1088	0.20	0.55
α-Fenchol	1094	--	0.33
Isoporneol	1108	--	0.40
α-elemol	1483	--	1.80
Spathulenol	1481	--	2.27
β-selinene	1482	--	1.50
Viridiflorol	1479	--	0.90
α-terpineol	1114	2.59	2.79
β-eudesmol	1486	--	0.61
% identification		99.79	97.18

*"RIcal" refers to the calculated retention index of each compound, which is used to identify and compare the components detected in the essential oil by gas chromatography.

2. Materials and methods

2.1. Plant material collection

In November, *Callistemon viminalis* leaves were gathered from several locations in Iraq. After being sun-dried at room temperature and agitated every other day, they were then kept out of direct sunshine and heat in a glass box. The dried leaves weighed 50 grams when they were finally collected.

2.2. Preparation of extracts

The leaves of *C. viminalis* were dried in the sunlight for about 5 days. Preliminary phytochemical tests were done to indicate the presence of active constituents in the leaves, including saponins, terpenoids, flavonoids, and alkaloids. Then, 50 grams of plant leaves were first extracted with 500 mL of hexane, followed by extraction with 500 mL of ethanol using the hot extraction method. Ethanol 70% extract with four different concentrations (100, 50, 25 and 12,5) was investigated using the agar well diffusion method to ascertain their antibacterial effectiveness against gram-positive, gram-negative, and fungal microorganisms.

2.3. Phytochemical screening

2.3.1. Test for saponins (foam test)

Fresh plant material was placed in a beaker, covered with water, and boiled for five minutes (Figure 1). After cooling, 1 ml of the decoction was transferred to a test tube, and 5 ml of distilled water was added. The mixture was shaken vigorously, and the formation of persistent foam indicated the presence of saponins [8].

2.3.2. Test for terpenoids (Salkowski test)

Chloroform (2 ml) and ethanolic leaf extract (5 ml) were combined, and 3 ml of sulfuric acid (concentrated) was then cautiously added. When terpenoids are present, the interface will appear reddish-brown [8].

2.3.3. Test for alkaloids

2.3.3.1. Mayer's test

Two milliliters of the plant's ethanolic extract were placed in a test tube, and a few drops of Mayer's reagent were added. The formation of a green precipitate was considered a positive indication of alkaloids.

2.3.3.2. Wagner's test

Two milliliters of the plant's ethanolic extract were mixed with a few drops of Wagner's reagent. The appearance of a brown or reddish precipitate indicated the presence of alkaloids.

2.3.4. Test for flavonoid

Two milliliters of the ethanolic plant extract were placed in a test tube, and three drops of sodium hydroxide solution were added. The mixture was observed for the development of a yellow color, which indicates a positive result for the presence of flavonoids [8].

2.4. Microorganisms and culture conditions

Three gram-positive bacteria (S. aureus, S. epidermidis, and Streptococcus sp.), one strain of fungus (Candida albicans), and two strains of gram-negative bacteria (*E. coli* and *Klebsiella* sp.) were investigated. The isolates were obtained from the Educational Laboratory of the College of Science / Al-Mustansiriyah University in Baghdad, Iraq. They were separated from various medical sources.

2.5. Antibacterial and antifungal assay

The agar well diffusion technique was used to measure the antibacterial activity of the 70% ethanolic extract of *C. viminalis* leaves, and pure cultures of all bacterial species were used. The examined bacterial species' stock cultures

were cultivated for 22 hours at 37 °C in Muller Hinton Broth (Merck, Germany) medium [9]. The McFarland turbid meter was used as a reference to adjust the final cell concentrations to 1.5 × 108 CFU/ml. After the media had solidified, wells were made in the seeded agar plates by inserting a cup borer (6 mm) into the agar. A volume of 100 µl of 70% ethanolic extract of *C. viminalis* leaves at concentrations of 100, 50, 25, and 12.5 mg/ml was dispensed into wells (6 mm diameter) prepared in the agar plates. Methanol was used as the negative control. The plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones formed around each well.

3. Results

3.1. Preliminary phytochemical study of *C. viminalis* leaves

The preliminary phytochemical tests of *C. viminalis* were performed to detect the presence of phytochemical constituents of the plant. Results of preliminary phytochemical testing are displayed in Table 2. As shown in Figure 1, the presence of saponins, terpenoids, alkaloids, and flavonoids was confirmed by qualitative assays.

3.1.1. Qualitative phytochemical tests

Qualitative phytochemical tests were conducted to screen for the presence of major bioactive groups. The foam test indicated the presence of saponins, while the Salkowski test confirmed terpenoids. Alkaloids were detected by both Mayer and Wagner tests, and flavonoids were identified by the sodium hydroxide test. All tests yielded positive results, as summarized in Table 2.

3.2. Antibacterial and antifungal activity of 70% ethanol extract

The antibacterial and antifungal activities of the 70% ethanol extract of C. *viminalis* leaves were evaluated using the agar well diffusion method. The extract was tested against three Gram-positive bacteria (S. aureus, S. epidermidis, Streptococcus sp.), two Gram-negative bacteria (*E. coli, Klebsiella* sp.), and one fungal strain (Candida albicans).

3.2.1. Agar well diffusion assay results

The results of the agar well diffusion assay are presented in Table 3 and Figure 2. The 70% ethanol extract exhibited concentration-dependent inhibitory effects against all tested Gram-positive bacteria and Candida albicans,

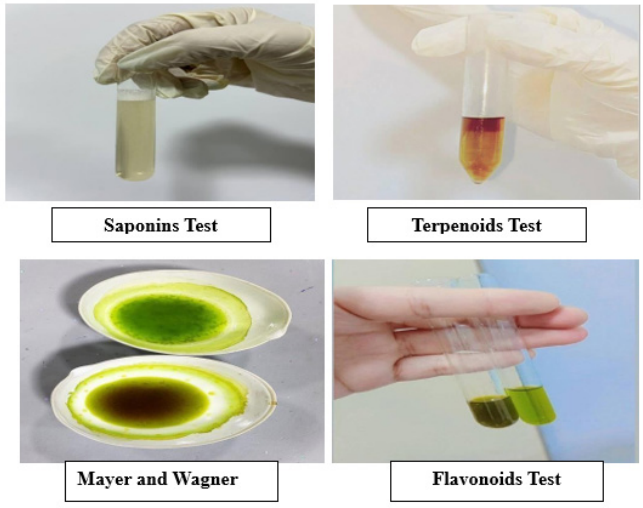

Fig. 1. Results of preliminary phytochemical study of *Callistemon viminalis* leaves.

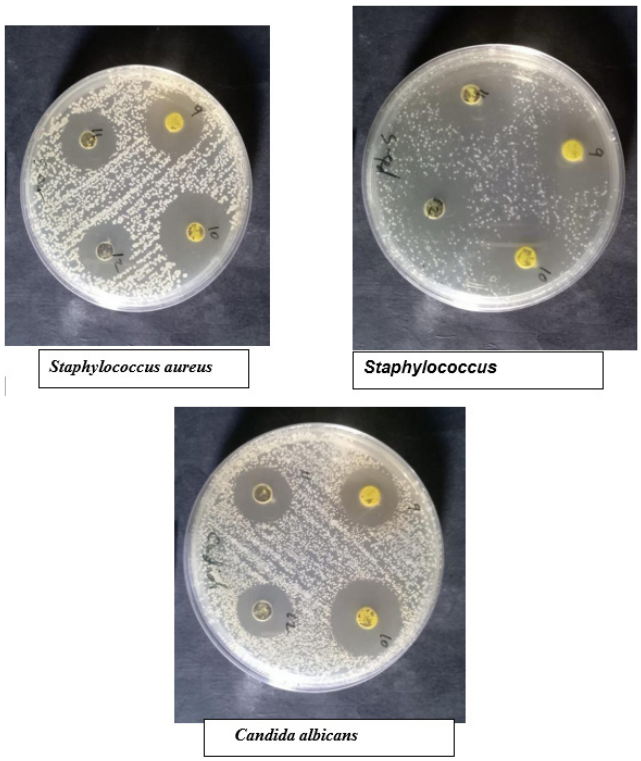

Fig. 2. Anti-bacterial and antifungal assay of aqueous ethanol 70% extract of the leaves of *Callistemon viminalis* cultivated in *Iraq* by agar well diffusion method

Table 2. Qualitative profile of the phytochemicals found in the leaves of the plant.

Test	Saponins	Terpenoids	Alkaloids	Flavonoids
Result	+	+	+	+

Table 3. Antibacterial and antifungal activities of 70% ethanol extract of *Callistemon viminalis* leaves: inhibition zone diameters (mm) at different concentrations determined by agar well diffusion method.

Microorganism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus aureus	12	10	11	9
Staphylococcus epidermidis	27	24	22	20
Streptococcus sp.	27	25	24	20
Escherichia coli	12	12	0	0
Klebsiella sp.	0	0	0	0
Candida albicans	25	21	19	17

while the activity against Gram-negative bacteria was limited. The largest inhibition zones were observed for *S. epidermidis* and *Streptococcus* sp., indicating strong antibacterial activity against these strains.

Table 3 presents the inhibition zone diameters (in millimeters) produced by various concentrations (100, 50, 25, and 12.5 mg/ml) of 70% ethanol extract of *C. viminalis* leaves against selected bacterial and fungal strains using the agar well diffusion assay. The extract exhibited strong, concentration-dependent inhibitory effects against Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*, and *Streptococcus* sp.) and the fungus Candida albicans, while its activity against Gram-negative *Escherichia coli* was moderate and no inhibition was observed for Klebsiella sp. at lower concentrations.

4. Discussion

The present study demonstrates the significant antimicrobial potential of *Callistemon viminalis* leaves cultivated in Iraq. Phytochemical screening confirmed the presence of saponins, terpenoids, alkaloids, and flavonoids, all of which are known for their diverse pharmacological activities, including antimicrobial effects. This is consistent with previous reports on Callistemon species, which have attributed their therapeutic properties to these bioactive constituents [10].

The agar well diffusion assay revealed a concentration-dependent inhibitory effect of the 70% ethanolic extract against all tested microorganisms. The extract displayed pronounced activity against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus* sp.) and the fungal strain Candida albicans, while the effect on Gram-negative bacteria (*Escherichia coli* and *Klebsiella* sp.) was less marked. This observation aligns with the general trend that Gram-negative bacteria are more resistant to plant-derived antimicrobials, likely due to their outer membrane acting as a barrier to the penetration of many compounds [11, 12].

The strong efficacy against Gram-positive bacteria and fungi may be attributed to the high content of flavonoids and terpenoids, which have been shown to disrupt microbial cell walls and inhibit essential cellular processes [10, 13]. Saponins and alkaloids may further enhance the antimicrobial spectrum by increasing membrane permeability and inhibiting key microbial enzymes [10].

GC-MS analysis of the hexane extract identified major constituents such as beta-sitosterol and vitamin E, both of which possess documented antimicrobial and antioxidant properties (2,7). Eucalyptol, which was found in high abundance in the essential oil profile (Table 1), is another compound with well-established antibacterial and antifungal activities (1,2,7). The predominance of eucalyptol in the leaves may partly explain the potent bioactivity observed in this study [7, 11, 14].

Our findings are in agreement with studies conducted in other regions, such as India and South Africa, which also reported significant inhibition of bacterial and fungal pathogens by *C. viminalis* extracts [1, 2, 4]. However, the phytochemical composition and relative abundance of active constituents can vary depending on geographic origin, environmental factors, and extraction methods, which may influence the observed bioactivity [1].

The observed antimicrobial effects, particularly against clinically relevant Gram-positive bacteria and Candida albicans, highlight the potential of *C. viminalis* as a source of novel antimicrobial agents. This is especially relevant in the context of rising antibiotic resistance, where plant-derived compounds offer promising alternatives or adjuncts to conventional therapies. Furthermore, the presence of multiple classes of bioactive compounds suggests possible synergistic interactions, which could enhance the overall antimicrobial efficacy and reduce the likelihood of resistance development [6, 10].

In summary, these results validate the traditional use of *C. viminalis* in treating infectious diseases and underscore its potential for further development as a natural antimicrobial agent. Future research should focus on isolating and characterizing the individual active constituents, elucidating their mechanisms of action, and evaluating their efficacy in in vivo models.

Callistemon viminalis leaves cultivated in Iraq exhibit a rich phytochemical profile, including saponins, terpenoids, alkaloids, and flavonoids, which are associated with notable antibacterial and antifungal activities. The 70% ethanolic extract demonstrated strong, concentration-dependent inhibition against Gram-positive bacteria and Candida albicans, while activity against Gram-negative bacteria was limited, reflecting the typical resistance patterns of these organisms. These findings reinforce the traditional medicinal use of *C. viminalis* and highlight its potential as a source of natural antimicrobial agents. The identification of key bioactive compounds such as beta-sitosterol, vitamin E, and eucalyptol further supports the pharmacological value of this species. However, the study was limited to in vitro assays, and the precise mechanisms of action and in vivo efficacy remain to be elucidated. Further research should focus on isolating individual active constituents, exploring synergistic effects, and evaluating safety and therapeutic potential in clinical settings. The results contribute valuable evidence to the field of medicinal plant research and suggest that *C. viminalis* could play a role in the development of alternative antimicrobial therapies, particularly in the context of rising antibiotic resistance.

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Author contributions

Zaineb Aziz Ali: Conceptualization, methodology, investigation, writing—original draft. Fatimah Ahmed Challoob AL-Khuzaee: Data curation, formal analysis, writing—review & editing. Yasser Kadhim Hashem Al-Zwaini: Supervision, project administration. Mustafa Mohammed Albassam, Rwaieda Adil Muhsen, Balqess Hisham Salih, Mohammed Hasan Kadhim, Sumia Samer Tayeh: Investigation, resources, validation. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Ethical approval

Not applicable. This study did not involve human participants or animals.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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