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Research Article

In Vitro Antimicrobial Activity of Essential Oil Derived from Callistemon viminalis Aerial Part

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ABSTRACT

Background: Essential oils extracted from plants have been widely used in antimicrobial activity, particularly the Callistemon viminalis, with a high number of essential oils extracted.
Objectives: To identify the chemical composition of essential oil derived from Callistemon viminalis and evaluates its antimicrobial activity against selected bacterial and fungal strains.
Subjects and methods: During the study, the antimicrobial activity of different selected essential oils on some bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus, and Streptococcus pneumonia) and fungus (Candida albicans) was evaluated. The MicroScan WalkAway automated device was used to confirm the identification of the bacteria microorganisms and the germ tube and microscopy detection confirmed the fungus identity of specific morphological features after growth on corn meal agar, used in this study.
Results: the GC-MS analysis reveal that the chemical composition of the essential oil was contained Eucalyptol (41.17%) area, Viridiflorol (8.43%) area, Alpha-Pinene (4.53%) area, Alpha-Terpineol (4.53%) area and others. The essential oil shows activity against Staphylococcus aureus with inhibition zone diameter of 10 mm, and for Streptococcus pneumoniae, with ihibition zone of 22 mm.
Conclusion: The highest antimicrobial activity was against S. aureus and S. pneumonia of Gram-positive bacteria. The study result show that resistance from Gram-negative bacteria and resistance from fungus C. albicans to the oil. These varying results of bacterial suseptabilty may be based on the construction of the bacterial cell wall between Gram-positive and Gram-negative bacteria. The essential oil components detected with higher percent area in GC-MS from C. viminalis are Eucalyptol, alpha-Pinene, Viridiflorol, beta-Eudesmol, and alpha-Tocopherol.

Introduction

Medicinal plants are rich and generous source of healing power due to their fabulous natural bioactive compounds. Infectious diseases with various microbes' strains have developed their resistance to numerous types of drugs across the years of utilization, essential oil represent an important natural remedy by its known antimicrobial activity against wide spectrum of bacteria and fungi

(1,2). Essential oils are volatile, hydrophobic aromatic compounds, that plants produce in distinct parts for some various expected reasons, just like repellent or attractant to animals and insects, or may be to fight microbes, due to their strong antimicrobial activity, or just as wound healers for plant tissue abrasions and infections. Essential oils are largely employed in many applications that enhance our lives, they are widely used in industry, also in food and flavor production, pharmaceutical preparations, aromatherapy, and surely in essence and fragrance manufactory. Many essential oils have a glorious medicinal activity, for instance camphor, a decent emmenagogic and muscle relaxant, verbenone and pinene are effective expectorants and spasmolytics, and the interesting 1,8-cineole, a highly graded antifungal agent and inflammation reliever (3,4,5).

It is impressive that one essential oil may involve up to one hundred different chemical fragments; mostly terpenes, esters, aldehydes, ketones, alcohols, and phenols, they compose a unique mixture that is made up from different plant species. The aromatic components are derived from phenylpropane, a well-known amino acids precursor, besides terpinols, those important components that chemically derived from acetyl-coenzyme A (6).

Terpenoids are presented in 80% of plant essential oils, they describe the heterogeneous class of terpenes (double bonds containing compounds) and their oxygenated derivatives (7). Terpenes are compounds which derived from isoprene unit, this organic unit appears as a structure of five carbons with double bonds (8). Isoprene structure is shown in (Figure 1).

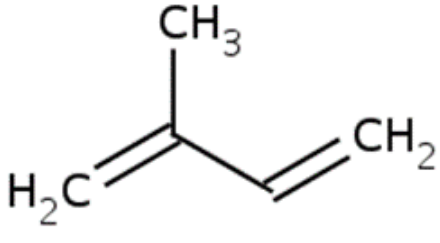

Figure. 1: Isoprene structure

Terpenes are divided according to the number of isoprene units, the monoterpene for example have two isoprene units, just like limonene and eucalyptol, while sesquiterpene involve 3 isoprene units, diterpenes have 4 units and so on. Phenylpropanoids is the aromatic part of essential oil, which may be a phenol like eugenol, or aldehyde like coniferyl aldehyde, or methoxy like anethole, or even alcohol, epoxy, or others (8).

Callistemon viminalis composition:

Confidently rich in diverse types of secondary metabolites, Callistemon viminalis plant primarily involve essential oils as well as phenolic acids, tannins, phloroglucinol derivatives, flavonoids, and triterpenoids. Active molecules in the plant extraction show exceptional importance in nanoparticle drug development, medical and cosmetic aspirations. The plant is an ornamental tree native to Australia, and owns many therapeutic effects like anti-inflammatory, antimicrobial, larvicidal, and antidiabetic activity. In China it was traditionally used as cold remedy and to relive joint pain. The phloroglucinol component of the plant play a major role of biological effects in the genus of Callistemon which considered as aromatic plants rich in essential oils. (9,10)

Aim of the study:

To investigate the phytochemical constituents of the aerial part essential oil of Callistemon viminalis plant and to evaluate the in vitro antimicrobial activity of the essential oil derived from the plant on specific fungus and bacterial strains.

Subjects and Methods

Plant material:

The fresh aerial part of the C. viminalis was obtained in Iraq, Baghdad, from November 2020 to February 2021.The plant identified and authenticated by the Department of Biology / College of Science / University of Baghdad, after collection the plant was washed thoroughly, keeping in fridge to stay fresh, then extracted in the next day. The laboratory work was carried out in the laboratories of College of Pharmacy/ University of Baghdad under college approval and supervision.

Extraction method:

Hydrodistillation method was used, one hundred and seventy grams of Callistemon viminalis plant fresh aerial part was incised into smaller pieces and extracted by Clevenger-type apparatus using 1.7 liters distilled water as solvent for a time of about 6 hours. After that about 0.4 mL of essential oil was collected in 1.5 mL Eppendorf tube and kept in fridge until further analysis was applied (11,12).

Gas chromatography-mass spectrometry (GC-MS) analysis of essential oils

The GC-MS system Agilent (7820A) USA GC Mass Spectrometer at Ibn Al-Betar Research center/ Corporation for Research and Industrial Development/ Iraqi Ministry of Industrial and Minerals was used in the following conditions:

- Analytical Column: Agilent HP-5ms Ultra Inert (30 m length x 0.25 mm diameter x 0.25 µm inside diameter) (13).
- Injection volume:1 µL.

Bacterial and fungal strains:

The essential oil was tested against 6 reference strains, one fungal strain, Candida albicans ATCC 14053 reference strain. The bacterial strains included of 3 Gram-negative reference isolates (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Salmonella enteritidis ATCC 13076) and 2 Gram-positive reference isolates (Staphylococcus aureus ATCC 29213 and Streptococcus pneumonia ATCC 49619). The identity of the bacterial microorganisms used in this study was further confirmed by Microscan Walkaway automated instrument, while Candida albicans identity was confirmed by germ tube and by microscopy detection of distinctive morphological features after growth on corn meal agar.

Antimicrobial screening:

The antimicrobial activities of the essential oil to the different microorganisms were determined by Agar disc diffusion method and compared to activities of different antibiotics appropriate antibiotics

for each strain and based on The Clinical and Laboratory Standards Institute (CLSI) guidelines (14). For the Gram-negative strains, the following discs were used: Meropenem (10 µg), Imipenem (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Amikacin (30 µg) & cefepime (5 µg). For Gram-positive strains the following discs were used: Vancomycin (30 µg), Teicoplanin (30 µg), Erythromycin (15 µg), Levofloxacin (5 µg), and Trimethoprim/Sulfamethoxazole (25 µg). All the antibiotic discs were procured from Oxoid, Thermo Scientific, USA.

Each reference microorganism tested was prepared and spread on a solid agar medium (Mueller-Hinton agar) in Petri dishes. Filter paper discs (6 mm in diameter) were prepared and soaked in 8 µL of the essential oil and placed on the inoculated petri dishes, each plate inoculated with one reference strain. Candida albicans plate contained only one filter paper disc impregnated with the extracted oil. The plates were then incubated at 37 °C for 24 h. The diameters of the clear inhibition zones were measured in millimeters; measurement of overlapping zones was avoided (15,16,17).

Results

Phytochemical compounds:

Gas chromatography is the best technique to identify essential oils components specially when using mass spectrometer as detector connected to compounds data base software (18). The GC-MS chromatogram in (Figure 2). The database obtained from the GC-Mass device for essential oil extract demonstrate the presence of several compounds shown in the (Figures 3) and mentioned in the (Table 2).

Table 1: Components of essential oil analyzed by GC-MS

Name of compound	Retention time min	Area %	Molecular weight g/mol	Molecular formula
Alpha-Pinene	5.120	4.53%	136.23	$C_{10}H_{16}$
Eucalyptol	7.363	41.17%	154.25	$C_{10}H_{18}O$
Alpha-Terpineol	11.850	1.25%	154.25	$C_{10}H_{18}O$
Beta-Caryophyllene	14.759	0.87%	204.35	$C_{15}H_{24}$
Viridiflorol	17.629	8.43%	222.37	$C_{15}H_{26}O$
Ledol	17.629	8.43%	222.37	$C_{15}H_{26}O$
Epiglobulol	17.629	8.43%	222.37	$C_{15}H_{26}O$
Beta-Eudesmol	18.190	3.07%	222.37	$C_{15}H_{26}O$
Beta-Elemene	18.522	1.08%	204.35	$C_{15}H_{24}$
Gamma-Elemene	18.887	1.91%	204.35	$C_{15}H_{24}$
Gamma-Terpinene	25.657	0.45%	136.23	$C_{10}H_{16}$
3-Carene	25.657	0.45%	136.23	$C_{10}H_{16}$
Alpha-Tocopherol	29.394	3.10%	472.7	$C_{31}H_{52}O_3$

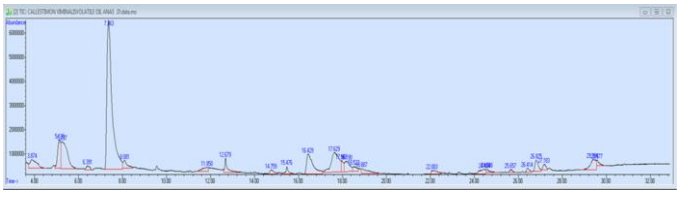

Figure. 2: The GC-MS chromatogram for essential oil extract

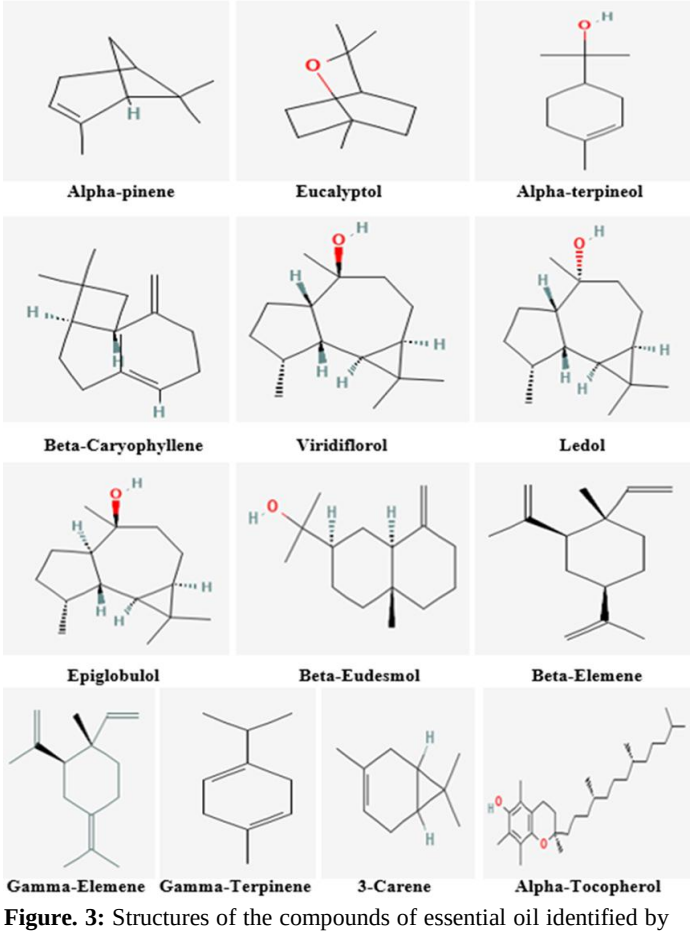

Figure. 3: Structures of the compounds of essential oil identified by GC-MS

In vitro evaluation of antimicrobial activity of essential oil extract

The study evaluates the antimicrobial activity of the essential oil aerial part extract from the plant, the antifungal evaluation was carried out without comparison with antifungal drug, while for antibacterial evaluation, a comparison with selected antibiotics that act against Gram-

positive strains and others act against Gram-negative strains was applied to provide evaluation with fair results. The antimicrobial activity results are shown in the (Figures 4), where essential oil disc appear in the center of the plates. The results and inhibition zones of essential oil and antibiotic discs are mentioned in (Table 3).

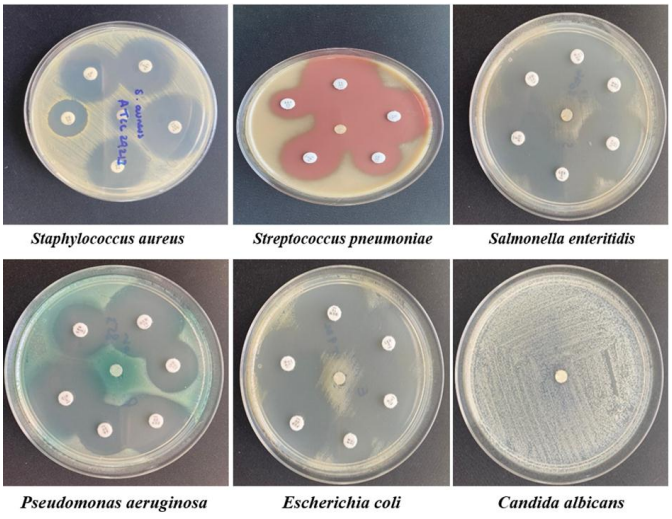

Figure 4. Essential oil antibacterial activity against bacterial and fungal strain

Table 2: Inhibition zone and results of essential oil antimicrobial activity and antibiotic discs

Bacterial/ Fungal strain	Antibiotic/ Antifungal drug name	Antibiotic inhibition zone (mm)	Essential oil Inhibition zone (mm)	Essential oil activity result
Staphylococcus aureus	Erythromycin	28 mm	10 mm	+
	Levofloxacin	29 mm		
	Co-Trimoxazole	32 mm		
	Tetracycline	28 mm		
	Vancomycin	18 mm		
Streptococcus pneumoniae	Erythromycin	33 mm	22 mm	+
	Levofloxacin	26 mm		
	Co-Trimoxazole	19 mm		
	Tetracycline	35 mm		
	Vancomycin	22 mm		
Salmonella enteritidis	Amikacin	23 mm	0 mm	-
	Levofloxacin	23 mm		
	Ciprofloxacin	30 mm		
	Imipenem	30 mm		
	Meropenem	33 mm		
	Cefepime	34 mm		
Pseudomonas aeruginosa	Amikacin	23 mm	0 mm	-
	Levofloxacin	24 mm		
	Ciprofloxacin	30mm		
	Imipenem	21 mm		
	Meropenem	34 mm		
Escherichia coli	Amikacin	22 mm	0 mm	-
	Levofloxacin	34 mm		
	Ciprofloxacin	33 mm		
	Imipenem	31 mm		
	Meropenem	32 mm		
	Cefepime	29 mm		
Candida albicans	none	none	0 mm	-

Discussion

The chemical composition of the extracted essential oil reveals the presence of eucalyptol with higher percent area in GC-MS, beside alpha-Pinene and Alpha-terpineol were also important components, this result is match with previous studies (19,20), while Viridiflorol and its isomers appear in higher percent area than alpha-Pinene and alpha-Terpineol relative to the studies, which may indicate the different concentration of compounds present in Iraqi cultivated plant. Viridiflorol is an interesting natural compound show significant anticancer activity against cancer cells from lung, brain, and breast according to a novel study (21), also antioxidant, anti-inflammatory, and anti-mycobacterium activity were observed in recent study (22). The difference in plant composition and concentration of natural compounds depends on several factors such as soil, moisture, and latitude; Iraqi soil mostly appears as sandy, with most central and south parts having low altitude areas, also, the salinity is considered high in soil (23,24). Such climate and soil type may make the variation in the plant composition results.

The components of plant essential oil have a wide range of therapeutic effects, Alpha-Pinene

Eucalyptol, Alpha-Terpineol, beta and gamma- Elemene, beta-Eudismole, and 3-Carene reveal obvious anticancer activity, also Alpha-Pinene, Eucalyptol and gamma-Terpinene own important anti-inflammatory potency, one example is preventative effect revealed by Eucalyptol against ilium inflammation, while the antimicrobial activity for the oil may come from the major component, Eucalyptol (1,8 Cineol), beside alpha-Terpineol, 3-Carene, and β-Caryophyllene (25,26,27,28,29).

According to the study results, Callistemon viminalis essential oils have a good antimicrobial activity against Gram-positive bacteria, while no antifungal activity observed. The increase of essential oil quantity or using alcoholic diluent with different oil concentration not only the concentrate oil or increase the duration of exposure to oil may reveal or enhance the well-known inhibitory effect over fungal growth for the plant essential oil as in the studies (30,31).

The results show good antibacterial activity of the oil against Gram-positive bacteria, especially, on Streptococcus pneumoniae strain with inhibition zone comparable to that of vancomycin antibiotic drug, while the Gram-negative bacterial strains, Salmonella enteritidis, Pseudomonas aeruginosa, and Escherichia coli were not susceptible to the essential oil. The Gram-negative strains resistance may be explained by the structure of Gram-negative bacteria outer membrane that contains hydrophilic lipopolysaccharides, this surface could act as barrier for the penetration of hydrophobic compounds and macromolecules (32,33).

Conclusion

In the current study, the antimicrobial effect of essential oils extracted from C. viminalis was investigated against S. aureus, S. pneumonia, S. enteritidis, P. aureginosa, E. coli, and C. albicans. The only antimicrobial activity was against S. aureus and S. pneumonia of Gram-positive bacteria. Whereas the resistance from Gram-negative bacteria were found in S. enteritidis, P. aeruginosa, E. coli, and resistance from fungus C. albicans. These varying results may be based on the construction of the bacterial cell wall between Gram-positive and Gram-negative bacteria. The essential oil components detected with higher percent area in GC-MS from C. viminalis are Eucalyptol, alpha-Pinene, Viridiflorol, beta-Eudesmol,

and alpha-Tocopherol. Plant essential oil has very important therapeutic effects, while the major oil component, Eucalyptol is already used in antibacterial lozenges and aromatherapy.

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Conflict of Interest
No conflict of interest

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