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Volatile oil constituents of the leaves of *Eucalyptus citriodora* and influence on clinically isolated pathogenic microorganisms

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Abstract

The fresh leaves of *Eucalyptus citriodora* Hook. (Myrtaceae) of Delhi region yielded 1.27 % of a volatile oil which was analyzed by GC and GC-MS techniques. Fifteen components comprising 100 % of the total volatiles were identified. There were ten monoterpenes (88.8%) and three sesquiterpenes (10.7%). The main monoterpenes were 1, 8-cineole (44.1%), camphene (29.7%), α-pinene (8.4%) and linalyl acetate (4.4%). The major sesquiterpene was ledol present in 9.1%. There were two aliphatic hydrocarbons occurring in trace amounts. The volatile oil exhibited antibacterial and antifungal activities.

Keywords: *Eucalyptus citriodora*, Volatile oil composition, *E. coli*, Antimicrobial activity, Antifungal activity.

Introduction

Eucalyptus citriodora Hook. (syn. *Corymbia citriodora* Hook.), family Myrtaceae, is a native to Queensland, Australia, introduced into tropical India and in several other regions of the world.[1, 2] It is a tall, evergreen and graceful tree grown for production of essential oil, fuel wood, timbers and as a source of nectar in honey production. The leaves are intensely aromatic releasing a number of volatile terpenes into the environment. The essential oil of the leaves is a powerful antiseptic and is used all over the world as a respiratory decongestant, for relieving colds, coughs, bronchitis, flu, pneumonia, headache and sore throats.[2-5] It has disinfectant action and is applied externally to cure cuts and skin infections. It is inhaled to open blocked nasal passages. It is useful as gargles for sore throats and is taken internally for a wide range of complaints. The Eucalyptus oil is the starting material for the manufacture of citronellal and derived products. *E. citriodora* oil showed analgesic, anti-inflammatory, [6] antimicrobial, [4, 7-11] acricidal, [12] larvicidal, [13, 14] fungicial[15] and phytotoxic[16, 17] effects. The oil is mainly composed of citronellol, geranyl acetate, limonene and terpene-4-ol.[18] The present manuscript describes the isolation and analysis of the volatile oil of the leaves of *E. citriodora* of Delhi region and evaluation of antimicrobial activity.

Meterials and Methods

Plant material

The leaves of *E. citriodora* were collected from the campus of Jamia Hadard, New Delhi.

The plant material was authenticated by Prof. M.P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen No. PRL/JH/12/33 is retained in the herbarium of the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard.

Isolation of the volatile oil

The fresh leaves (1.8 kg) of *E. citriodora* were hydro distilled in all glass Clevenger apparatus according to the method recommended in the British Pharmacopoeia, 1988. The colorless volatile oil was dried over anhydrous sulphate and stored at 4°C in the dark. The yield was 1.27 % based on the weight of the fresh leaves.

GC analysis

Analytical GC was carried out by injection 01.µL of the leaf oil on a Varian 3300 gas chromatograph with FID detector fitted with silicone DB-I capillary column (30 m x 0.25 mm, film thickness 0.25 µm). GC operation condition split mode: carrier gas helium at a rate of 1.5 mL/min; temperature programmed, 80 - 225^0 C (40 C/min), injector temperature 280^oC and detector temperature 300^oC. Injection volume for all samples was 0.1 µl.

GC-MS analysis

GC-MS analysis was carried out by injection (0.1 µL) of the leaf oil on a QP-2000 instrument with a mass selective HP 597A detector fitted with Ulbon HR-1 capillary column (50 m x 0.25 mm, film thickness 0.25 µm). GC-MS operation condition split mode: carrier gas helium at a flow rate of 1.5 mL/min; temperature programme 70-225^0C (100 C/min), injector temperature 250^oC and detector temperature 280^oC. The mass spectrometry conditioned was as follows: ionization voltage, 70 eV; emission current, 40 mA; mass range 0–400 Da, ion source temperature, 200ºC.

Identification

The most constituents were identified by GC comparing their retention indices with those of authentic standard available in the laboratory or with the retention indices in close agreement with reference.[19] Further identification was achieved by GC/MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer data base using the NBS 54 KL and Wiley L-built libraries and with those reported in the literature.[19, 20]

Antimicrobial Activity

Test organisms and inoculums

Escherichia coli (NCTC-6571), *Staphylococcus aureus* (NCTC-10418) and *Bacillus subtilis* were obtained from the Division of Biotechnology, Faculty of Pharmacy, Jamia Hamdard, New Delhi. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans* were procured from the Institute of Genomics and Integrative Biology (CSIR), New Delhi.

Antimicrobial standard

Tetracycline solution with specific activity (50µg/ml) was prepared in DMSO solution (antibacterial). Fluconazole with specific activity (50µg/ml) was prepared in DMSO solution (antifungal).

Media

Dehydrated nutrient agar media was prepared in distilled deionized water. The media (g/100 ml) was composed of peptone (5.1 g), sodium chloride (5.0 g), beef extract (1.5 g), yeast extract (1.5 g) and agar (1.5 g).

Preparation of media

Dehydrated nutrient agar medium (28 g) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely. The conical flask containing the nutrient agar medium was plugged with the helpnon-absorbent cotton plug. Direct heating was avoided as it may lead to charring of the medium components and render it useless for the purpose.

Sterilization of media

The conical flask containing the nutrient agar medium was plugged with the help of a non-absorbent cotton plug. The mouth of the conical flask and the cotton bung were properly covered with aluminum foil. The medium was then sterilized by autoclaving at 15-lbs/in2 pressure for 20 minutes.

Preparation of organisms

The test organisms were maintained on slants of medium and transferred to a fresh slant once a week. The slants were incubated at 37^0C for 24 hours. Using 3 ml of saline solution, the organisms were washed from the agar slant on to a large agar surface (medium) and incubated for 24 hours at 37 ± 2^0C. The growth from the nutrient surface was washed using 50 ml of distilled water. A dilution factor

was determined which gave 25 % light transmission at 530 nm. The amount of suspension to be added to each 100 ml agar or nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

Temperature control

Thermostatic control is required in several stages of a microbial assay when culturing a micro-organism and preparing its inoculums and during inoculation in a plate assay.

Cup and plate method

A Previously liquefied and sterilized medium was poured in to plastic Petri-plates of 100 mm size. Required plates were prepared and kept for solidifying. Six holes were made in each plate with a stainless steel borer having 6 mm i.d. Different dilutions of the volatile oil of E. citriodora were made having concentration of 3 µl/ml, 5 µl/ml, 7 µl/ml and 9 µl/ml of solution. Tetracycline and fluconazole solutions were used as standards. The plates were labeled as Co (control), S (standard), X (*B. subtilis*), Y (*S. aureus*), Z (*E. coli*) with four or five different holes, labeled as 3, 5, 7 and 9 for different concentrations. All dilutions were made in dimethyl sulphoxide (DMSO) solvent, which were used in experiment. Co was used for Control and S was used for Standard. Micropipette was used to deliver the solutions into the holes. The plates were then left for standing for 1 h for proper diffusion of the drug solutions. They were incubated for about 24 h at 32 ± 2^{o}C. After 24 h the plates were examined and the diameter of zones of inhibition was accurately measured. Antifungal activity was determined against Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus and Candida albicans similar to antibacterial activity.

Results and Discussion

The components of the volatile oil of the leaves of *E. citriodora*, their retention indices and percentage are listed in Table -1. The constituents are arranged in the order of their elution on Ulbon HR-1 capillary column. Analysis of the oil by GC-MS led to identification of fifteen components comprising 100% of the total volatile oil. The oil was characterized by a large amount of monoterpene constituents (88.8%) with α-pinene, camphene, 1, 8-cineole and linalyl acetate type. All the twelve components present in the oil were positively identified. There were nine monoterpenes including four hydrocarbons (39.4%), three alcohols (44.9%) and one each aldehyde (0.1%) and ester (4.4%). The predominant hydrocarbons were camphene (29.7%) and α-pinene (8.2%). The main monoterpene alcohol was 1, 8-cineole (44.1%). Linalyl acetate was present in 4.4% yield. There were three sesquiterpenes, comprising 10.7% of the sample. The major sesquiterpene was ledol present in 9.2%. The volatile oil components, viz. β-pinene, cis-sabinene, citronellol, p-cymene-8-ol, myrtenol and trans-caryophyllene occurred in trace amounts. Only one aromatic compound, p-cymene-8-ol and two aliphatic hydrocarbons, viz. n-eicosane and n-heneicosane were detected in the volatile oil. The chemical composition of the volatile oil of Delhi region was entirely different from the earlier reported oil constituents. Generally citronellal (52-88 %) along with geraniol, citronellol, cetronellyl acetate and isopulegol were the predominant components of the E. citriodora oil21-24. However, oxygenated compounds and hydrocarbons in Cuban Eucalyptus leaf oil21 and 6-octadecanal (77%) in Nigerian Eucalyptus leaf oil of Pachim Vihar (New Delhi) is consisted of α-pinene (38.6 %), β-pinene (25.7%), sabinene (19.6%), α-thujene (11.9%) and ten aliphatic constituents25.

The volatile oil of the *E. citriodora* leaves was examined for antibacterial activity against *E. coli*, *S. aureus* and *B. subtilis* and antifungal activity against *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Candida albican*. It showed significant antimicrobial and antifungal activity in comparison to standard, Tetracycline and Fluconazole (Fig 1 and Fig 2). The observations were recorded in the Tables 2 and 3.

Table 1: Percentage composition of volatile oil of *E. citriodora* fresh leaves

	Components	**Refractive indices**	**Percentage**
1	α-Thujene	921	1.1
2	*α*-Pinene	925	8.2
3	Camphene	939	29.7
4	β-Pinene	964	0.3
5	1,8-Cineole	1016	44.1
6	*cis*-Sabinene hydrate	1045	0.1
7	Citronellal	1136	0.1
8	*p*-Cymene-8-ol	1167	0.7
9	Myrtenol	1169	0.1
10	Linalyl acetate	1240	4.4
11	α-Cadinene	1403	0.5
12	*trans*-Caryophyllene	1502	1.0
13	Ledol	1580	9.2
14	*n*-Eicosane	2003	0.3
15	*n*-Heneicosane	2105	0.2

Table 2: Antibacterial activity of *E. citriodora* leaf volatile oil

Sample conc. (µl/ml)	**Zone of inhibition (mm) S. *aureus***	**Zone of inhibition (mm) *E. coli***	**Zone of inhibition (mm) *B. subtilis***
3 (A)	00	00	00
5 (B)	00	12	00
7 (C)	00	14	12
9 (D)	14	14	13
50 (Co)	---	---	---
50 (S)	12	18	27

Table 3: Antifungal activity of *E. citriodora* leaf oil

Sample conc. (µl/ml)	Zone of inhibition (mm) *A. niger*	Zone of inhibition (mm) *A. fumigates*	Zone of inhibition (mm) *A. flavus*	Zone of inhibition (mm) *C. albican*
3 (A)	11	12	15	15
5 (B)	23	14	11	16
7 (C)	19	16	17	15
9 (D)	33	38	28	13
50 (Co)	---	----	---	---
50 (S)	23	13	15	31

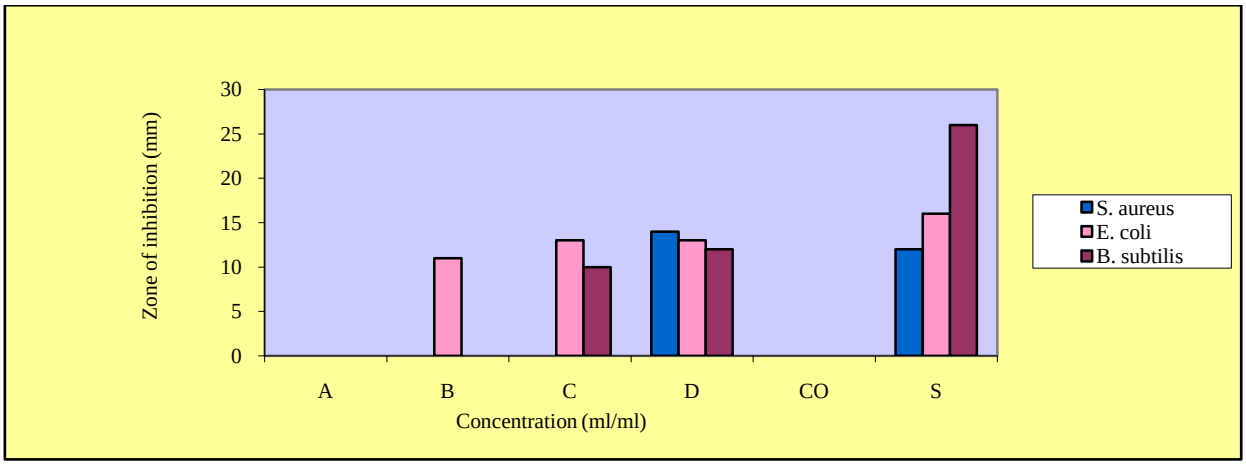

Figure 1: Antibacterial activity of *E. citriodora* leaf volatile oil

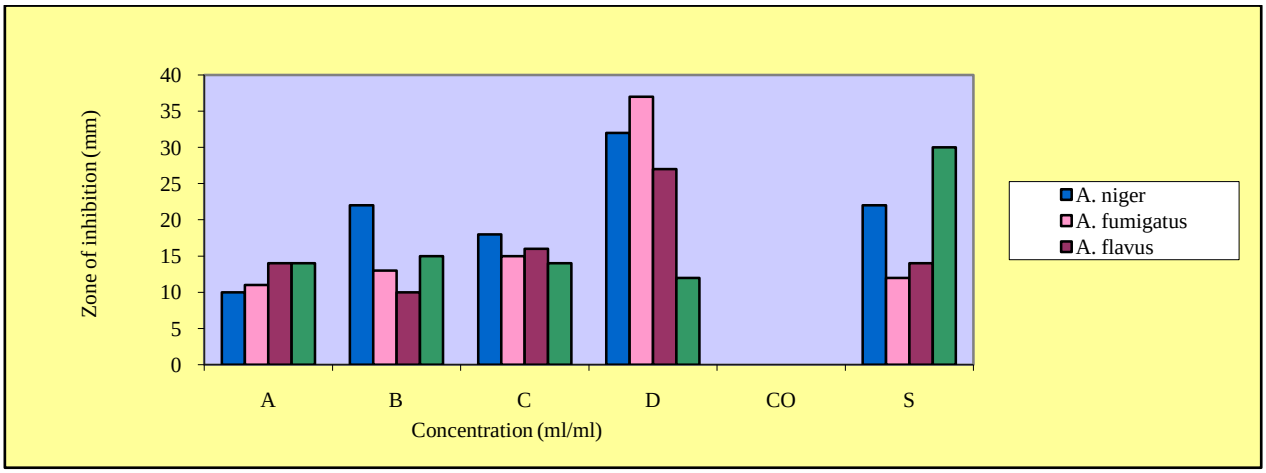

Figure 2: Antifungal activity of *E. citriodora* leaf volatile oil

Conclusion

The volatile oil of *E. citriodora* was consisted mainly of monoterpenes (88.8%) and the predominent constituent was 1, 8-cineole (44.1%) followed by camphene (29.7%), ledol (9.2%) and α-pinene (8.2%). It showed significant antimicrobial activity.

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Conflict of Interest

Authors have no conflict of interest.

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