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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OIL OF INDIAN *CALLISTEMON LANCEOLATUS* LEAVES

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ABSTRACT

The chemical composition of essential oil from the leaves of *Callistemon lanceolatus*, obtained by conventional hydrodistillation, was analysed by GC–MS. 1,8–Cineole (41.5%), β–pinene (4.2%), α–pinene (4.1%), α–terpineol (7.3%) and limonene (6.0%) were found to be the major components. Further, the essential oil was studied for antibacterial and antifungal activities. It was observed that the oil showed significant activity against *Staphylococcus aureus* and moderate activity against other gram positive, gram negative bacteria and mold type fungi.

Key words: *Callistemon lanceolatus*, Essential oil, Antimicrobial activity

INTRODUCTION

Callistemon lanceolatus (family: Myrtaceae) is an ornamental plant, commonly known as bottlebrush, indigenous to Australia, available in the prominent gardens of India. It is an evergreen shrub, grows to a height of 3.7–4.3 m. Nine species of *Callistemon* are available in the major national botanical gardens, of which *C. lanceolatus* are found on the grounds of our institute. Leaves are entire, lanceolate in shape and bipinnate and oppositely arranged, length 130 mm and width 12 mm and in the form of long dense bottlebrush cluster. Each cluster is produced in bunches of up to 11 brushes. Stems are woody. Leaves are with thin elongate lamina. Flowers are with bisexual and regular. Stamens are in bundle and ovary is inferior. *C. lanceolatus* is reported to have insecticidal, antifeedant, growth inhibitory[1,2], antiviral[3], analgesic and antiinflammatory[4] and antithrombin activities[5]. *C. lanceolatus* is reported to contain triterpenoids, ursolic acid, oleanolic acid[6–8], phluoroglucinol derivatives, myrtucommulone A, alkenol and c–methylated flavones[9]. The present study was undertaken to demonstrate the chemical composition of essential oil of *C. lanceolatus* leaves and activity against various pathogenic gram positive and gram negative bacteria and pathogenic fungi.

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EXPERIMENTAL

Collection of plant material: Leaves of *Callistemon lanceolatus* are collected from medicinal plant garden of Padmabhushan Dr. B. V. Raju Foundation Campus, Bhimavaram in November, 2002. The plant material was identified and authenticated taxonomically by National Botanical Research Institute (NBRI), Lucknow and voucher specimen was deposited in the Herbarium of Shri Vishnu College of Pharmacy for the future reference.

Extraction of essential oil : *C. lanceolatus* leaves (500 g) were subjected to hydrodistillation in a Clevenger apparatus for 4.5 hours. In different batches of distillation, the yield of the oil varied from 0.65 – 0.8 percent on fresh weight basis. The oil is colourless with a characteristic aroma having the specific gravity 0.9074 at 29.5°. Optical rotations were measured using a JASCO DIP 181 polarimeter and it is + 6.9°. Refractive index is 1.4604 at 30° as determined using an Abbe's refractometer.

Table 1. Chemical composition of a typical *C. lanceolatus* leaf oil

Constituents	Method of identification	Percentage
α–Pinene	A, B	4.1
β–Pinene	A, B	4.2
α–Thujene	A, B	3.0
δ–3–Carene	A, B	1.3
α–Phellandrene	A, B	3.2
Limonene	A, B	6.0
1,8–Cineole	A, B	41.5
Terpinolene	A, B	1.0
Terpinen–4–ol	A, B	5.8
Linalool	A, B	1.5
α–Terpineol	A, B	7.3
β–Caryophyllene	A, B	1.0
β–Sesquiphellandrene	B	0.5
Eremophilene	B	0.7
Aromadendrene	B	0.5
α–Humulene	B	0.8
Unidentified compounds	–	17.6

A: retention time; B: MS

Phytochemical examination of the essential oil: The oil was subjected to gas chromatographic examination on a gas chromatograph equipped with thermal conductivity detector and a stainless steel column (6' x 1/4") packed with C 22 firebrick (42–60) having 30% coating of carbowax 1000. The apparatus was run with two different isothermal temperatures of

160°C and 100°C[10,11]. It revealed the presence of 17 components in the oil, the major constituents were 1,8–cineole (41.5%), β–pinene (4.2%), α–pinene (4.1%), α–terpineol (7.3%), limonene (6.0%) (Table 1).

Microbial strains: All microorganisms, except *Escherichia coli,* were obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India, whereas *E. coli* was obtained from Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Gram positive bacteria: *Bacillus subtilis* (NCIM 2063), *B. pumilus* (NCIM 2327), *Staphylococcus aureus* (NCIM 2127), *Streptococcus faecalis* (NCIM 5025); **Gram negative bacteria:** *E. coli* (enteropathogen), *Proteus vulgaris* (NCIB 8067), *Pseudomonas aeruginosa* (NCIM 8162); **Mold type fungi:** *Aspergillus niger* (NCIM 616) and *Rhizopus oligosporus* (NCIM 1215).

Antimicrobial activity: Antibacterial and antifungal activity of the essential oil by microdilution susceptibility test was used for the determination of maximum inhibitory dilution (MID)[12,13]. Nutrient broth and Saboraud liquid medium were used. The microbial inhibitory activities were studied by cup plate diffusion method.

RESULTS AND DISCUSSION

The results of antibacterial and antifungal activities are given in Table 2. The appraisal of obtained results showed that the essential oil possess potent antibacterial activity against

Table 2. Antimicrobial activities of essential oil of *C. lanceolatus* against standard organisms

Test Organisms	MID*	Inhibition Zone Diameter (mm)**			% Inhibition
		Oil (30 µg/mL)	Streptomycin (30 µg/mL)	Nystatin (20 IU/mL)	
Gram Positive Bacteria					
Bacillus subtilis	1/60	16	22	–	72.72
Bacillus pumilus	1/60	16	22	–	72.72
Staphylococcus aureus	1/100	22	24	–	91.66
Streptococcus faecalis	1/80	17	24	–	70.83
Gram Negative Bacteria					
Escherchia coli	1/80	17	22	–	77.27
Pseudomonas aeruginosa	1/50	15	24	–	62.50
Proteus vulgaris	1/60	16	22	–	72.72
Mold type Fungi					
Rhizopus oligosporus	1/80	18	–	22	81.81
Aspergillus niger	1/60	16	–	24	66.66

*MID: Maximum inhibitory dilution of the essential oil; ** Inhibition zone diameter values are the mean of the three replicates. 50 µL of solution was applied to each well.

Staphylococcus aureus (91.66%) and significant antifungal activity against *Rhizopus oligosporus* (81.81%). Results also indicate that the oil is moderatively active against various other pathogenic gram positive bacteria, gram negative bacteria and mold type fungi. Out of the reported components, terpinen–4–ol and α–terpeniol possess antiseptic activity and α–pinene possesses insecticidal activities[14]. Hence, these components may be responsible for antimicrobial activities of the essential oil of *C. lanceolatus*.

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