Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(6): 270-273

Received: 06-09-2016
Accepted: 07-10-2016

Vijender Singh
School of Pharmacy, Sharda University, Plot No. 32-34, Knowledge Park – III, Greater Noida - 201306, Uttar Pradesh, India

Mohammed Ali
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India

Shahnaz Sultana
(1) Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India
(2) Present address: College of Pharmacy, Jajan University, Jajan, Saudi Arabia

Correspondence
Mohammed Ali
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India

Analysis and antimicrobial activity of essential oil of the leaves of *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson

Vijender Singh, Mohammed Ali and Shahnaz Sultana

Abstract
Cymbopogon flexuosus (Nees ex Steud.) W. Watson (Poaceae), known as Lemongrass, yields an essential oil which is used to treat headache, abdominal and muscle pains, stress, anxiety, irritability, insomnia, drowsiness and hair loss. Hydro-distillation of the fresh leaves (1.5 kg) gave a pale yellow essential oil (0.5%). The GLC and GC-MS analysis of the oil indicated that it was composed of large amount of monoterpenes (93.4%). Out of the thirteen monoterpenes, there were five alcohols (54.4%), four aldehydes (31.7%), one ester (0.7%) and three hydrocarbons (6.7%). The predominant monoterpenes characterized were geraniol (51.7%), geranial (29.0%), *α*-pinene (4.8%), citronellal (1.3%), citronellol (1.2%), myrcene (1.1%) and *trans*-verbenol (1.0%). Among four identified sesquiterpenes (6.5%), spathulenol (2.9%) and *β*-selinene (2.0%) were the main constituents. The significant antimicrobial activities were observed with the essential oil and ethanolic extract of the leaves against *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger.*

Keywords: *Cymbopogon flexuosus,* leaves, essential oil, geraniol, geranial, antimicrobial activity

1. Introduction
Cymbopogon flexuosus (Nees ex Steud.) W. Watson; syn. *C. travancorensis* Bor; *Andropogon flexuosus* Nees ex Steud.; *A. ampliflorus* Steud. (Poaceae), known as Cochin grass, Lemongrass or Malabar grass, is a perennial grass native to India, Sri Lanka, Burma and Thailand. It was introduced in India about a century back and is now commercially cultivated along western Ghats of Maharashtra and Kerala, in Karnataka, Tamil Nadu, foot-hills of Arunachal Pradesh and Sikkim. Apart from India, lemongrass is also cultivated on large scale in Australia, Thailand, Europe, Mexico, Dominica, Haiti, Madagascar, Indonesia and China [1]. Lemongrass is a tall, perennial sedge with dense fascicles of leaves from a short rhizome; culm stout, erect, up to 1.8 meter high; leaves long, glaucous, green, linear tapering upwards and along the margins; ligule very short; sheaths terete, those of the barren shoots widened and tightly clasping at the base, others narrow and separating, with flowering inflorescence in a long spike.

Lemongrass is used for treating digestive tract spasms, stomach ache, hypertension, convulsions, pain, cough, rheumatism, period cramps, ringworm, athlete's foot, toothache, migraine, fever, common cold, vomiting, exhaustion, to kill germs and as a mild astringent. The plant and its essential oil are applied to the skin to relieve headache and abdominal and muscle pains. The oil is inhaled in aromatherapy to alleviate muscle ache. It works as an air freshener and deodorizer, especially when blended with other essential oils like geranium or bergamot. It repels insects such as mosquitoes and ants due to its high citral and geraniol contents. Its aroma helps to relieve stress, anxiety, irritability and insomnia and to prevent drowsiness and hair loss. Lemon grass oil has a strong lemon-like odour due to high percentage of citral and is used for scenting of soaps, deodorants, shampoos, lotions, tonics detergents and insect repellent preparations. Citral is utilized in perfumery, cosmetics, beverages and is a starting material for manufacture of ionones, which produce vitamin A. The citral rich oil has germicidal, medicinal and flavouring properties. Lemongrass oil is analgesic, antiseptic, carminative, astringent, febrifuge, fungicidal, bactericidal nd antidepressant [2].

The main reported components of lemongrass oil were limonene, myrcene, citral, citronellal, citronellol, terpineol, methyl heptenone, dipentene, geraniol, geranial, geranyl acetate, neral, nerol and farnesol [3-5]. These compounds possess antifungal, antiseptic, insecticidal and counterirritant properties.

Citral is antiseptic and suppresses the growth of bacteria and fungi [4, 6]. Lemongrass oil's quality is generally determined by its citral content. The essential oil components are greatly influenced by genetic, environmental and geographical conditions [1, 7-9]. The aim of this study was to analyze chemical composition and to evaluate antimicrobial activity of the essential oil and ethanolic extract of the leaves of *Cymbopogon flexuosus* collected from the western Uttar Pradesh state if India.

2. Material and Methods

2.1 Plant Material

The fresh leaves of *C. flexuosus* were collected from Neha nursery, Greater Noida, western Uttar Pradesh. The plant material was identified by Prof. M.P Sharma, Department of Botany, Jamia Hamdard. A voucher specimen is preserved in the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

2.2 Preparation of Alcoholic Extract

A coarse powder of the dried leaves (10 g) of *C. flexuosus* was extracted with ethanol (35 ml) in a Soxhlet apparatus exhaustively. The ethanolic extract was dried under reduced pressure to get dark brown semisolid mass (1.1 g). It was stored at 4 °C prior to testing.

2.3 Isolation of Essential Oil

The chopped fresh leaves (1.5 kg) were added to deionized water (1.5 L) and subjected to hydro-distillation in a Clevenger apparatus for 4 h in accordance with the British Pharmacopoeia specification [10]. The essential oil was evaporated together with water vapour and finally collected in a condenser. The upper phase that contained the essential oil was separated from the lower one. The yield of essential oil obtained was 0.5% *v/w*. The pale yellow essential oil was collected in a graduated tube. It was dried over anhydrous sodium sulphate and stored at 4 ^{0}C in the dark until analysis.

2.4 GC Analysis

Analytical GC was carried out on a Varion 3300 Gas Chromatograph fitted with a silicone DB-1 capillary column (30 m x 0.25 mm i.d.), film thickness 0.25 µm, carrier gas nitrogen, flow rate 1.5 ml/min., split mode ratio was 1:25. The oven temperature was programmed from 50 °C (after 2 min) to 240 °C at 5 °C/min with column pressure of 155.1 kPa and the final temperature was held for 10 min. Injector and detector temperatures were 250 ^{0}C and 270 ^{0}C, respectively. The GC instrument was equipped with a flame ionization detector (FID). An aliquot (0.5 µL of the diluted oil) was injected into the GC. Component separation was achieved following a linear temperature programmed from 60 – 230 °C at a rate of 3 °C/min and then held at 230 °C for 9 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas. A homologous series of *n*-alkanes were run under the same conditions for determination of retention indices.

2.5 GC-MS Analysis

GC-MS analysis of the oils was performed on a Hewlett Packard HP 6890 Gas Chromatography interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a Ulbon HR-1 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70- 240 °C at the rate of 5 °C/min. Oven temperature was programmed as 50 °C for 1 min and subsequently held isothermal for 2 min. injector port: 250 °C, detector: 280 °C, split ratio 1:50, volume injected: 1µL of the oil. The recording was performed at 70 eV, scan time was 1.5; mass range 40-750 amu. Software adopted to handle mass spectra and chromatograph was a Chem station. Helium was used as a carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. An amount of 1.0 µL of the diluted oil in hexane was injected into the GC/MS instrument.

2.6 Identification of Components

The individual essential oil components were identified by comparing their retention indices (RI) either with those of authentic compounds available in author's laboratory or with those of literature in close agreement to the RI [11]. Further identification was made by comparison of fragmentation pattern of the mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, Wiley L-built libraries and with those published in the literature [12,13].

2.7 Antimicrobial Activity

The antimicrobial activities of essential oil and dried alcoholic extract of *C. flexuosus* fresh leaves were performed in the Department of Microbiology, R V Northland Institute, Greater Noida. The identification of microbial strains was based on morphological, cultural and biochemical tests. *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger* were used to evaluate antimicrobial activity of the essential oil. Identification of each culture was done by conventional methods. Pure chloramphenicol and ketoconazole (Ranbaxy Ltd., Gurgaon) 50 mcg/ml were used as standards for comparison of antimicrobial activities.

2.8 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).

2.9 Preparation of Media

All media were prepared in distilled water by dissolving agar (15 g), peptone (5 g), beef extract (1.5 g), dextrose (1 g), sodium chloride (3.5 g), dipotassium-hydrogen phosphate (3.68 g) and potassium dihydrogen phosphate (1.32 g), and pH of the media was adjusted between 6.95-7.05. 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 help of a non-absorbent cotton plug. Direct heating was avoided as it may lead to charring of the medium components and render it useless for the purpose.

2.10 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 an aluminium foil. The medium was then sterilized at 121 ^{0}C by autoclaving at 15 lbs/in^2 pressure for 30 minutes.

2.11 Preparation of Standard Solution

For the preparation of standard solution, pure chloramphenicol and ketoconazole were dissolved in dimethyl sulphoxide for comparison of antibacterial and antifungal activities, respectively. The concentration of both standard drug solutions was 10 mg./ ml.

2.12 Preparation of Test Solutions
The essential oil (0.1% v/v, 0.5% v/v, 1% v/v) and dried alcoholic extract (5.0% w/w) were dissolved in dimethyl sulfoxide (DMSO) for anti-microbial activity.

2.13 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 37^0 C 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 ^{0}C. 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. The anomeric bacteria were cultured as mentioned in the literature [14].

2.14 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.

2.15 Cup and Plate Method
The antibacterial activities of the ethanolic extract and leaf essential oil were studied against four microorganisms, viz., *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger.* The dried ethanolic extract was dissolved in ethanol. Pure chloramphenicol and ketoconazole, 50 c mg/ml each, were used as standard for comparison of antimicrobial activity. The antimicrobial activities were screened by the agar well diffusion method. Nutrient agar plates were swabbed with the respective broth culture of the organisms and kept for 15 minutes in laminar chamber for absorption to take place. Wells were made in agar plates using a sterile cork borer and 10µl of different concentrations of extracts were added to different wells. Excess amounts of the suspensions were drained and discarded properly with the help of a filter paper strip. Wells of 6 mm in diameter and 2 cm apart were pricked in the culture media using sterile cork borers. Equal volumes of the test samples and standards were administered to fullness in each well. 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. The plates were incubated at 37± 2 ^{0}C for bacteria and 25 ± 2 ^{0}C for fungus for 24 hours. The diameters of the zone of inhibitions were measured accurately in millimeter. The tests were performed in triplicate. The observations are tabulated in Table – 2.

3. Results
Hydro-distillation of the leaves of *C. flexuosus* yielded 0.5% pale yellowish essential oils. The composition of the oil is tabulated in Table 1. The constituents are listed in order of their elution on the Ulbon HR-1 column. A total 16 components were identified. The significant antimicrobial activities were observed with the essential oil and ethanolic extract of the leaves against *S. aureus, E. coli, C. albicans* and *A. niger.* The dried ethanolic extract was dissolved in ethanol. The zone of inhibitions of the microorganisms were compared with standard samples of chloramphenicol and ketoconazole (Table 2).

4. Discussion
The components of the oil, the percentage of each constituent and their RI values are summarised in Table -1. The constituents are arranged in order to GLC and GC-MS elution on silicon DB-1 and Ulbon HR-1fused silica columns, respectively. Analysis of the oil by GC-MS resulted in the identification of eighteen components. The oil was composed of large amount of monoterpenes (93.4%). Out of the thirteen monoterpenes, there were five alcohols (54.4%), four aldehydes (31.7%), one ester (0.7%) and three hydrocarbons (6.7%). The predominant monoterpene characterized was geraniol (51.7%) followed by geranial (29.0%), *α*-pinene (4.8%), citronellal (1.3%), citronellol (1.2%), myrcene (1.1%) and *trans*-verbenol (1.0%). Among four identified sesquiterpenes (6.5%), there were two hydrocarbons (2.2%) and two alcohols (3.7%) in which spathulenol (2.9%) was the predominant sesquiterpene followed by *β*-selinene (2.0%), (E,E)-farnesol (0.8%) and *β*-caryophyllene (0.2%). There was one benzoic ester derivative occurring in trace amount (0.1%).
Antimicrobial activities of dried alcoholic extract and different concentrations of the essential oil of *C. flexuosus* fresh leaves from Greater Noida, U.P. were summarized in Table 2. The maximum antibacterial activity was shown with 1% v/v of the volatile oil on *S. aureus* (15.3 mm) followed by *E. coli* (12.8 mm), and maximum antifungal activity was shown by 1% v/v of the volatile oil against *C. albicans* (12.5 mm) and *A. niger* (10.2 mm).

5. Conclusion
The leaf essential oil of *C. flexuosus* was composed prominently of monoterpenes (93.4%) including geraniol (51.7%), geranial (29.0%), *α*-pinene (4.8%), citronellal (1.3%), citronellol (1.2%), myrcene (1.1%) and *trans*-verbenol (1.0%). The main sesquiterpenes (6.5%) were spathulenol (2.9%) and *β*-selinene (2.0%). The leaf essential oil and ethanolic extract exhibited antimicrobial activity against *Staphylococcus aureus* (15.3 mm), *Escherichia coli* (12.8 mm), *Candida albicans* (12.5 mm) and *Aspergillus niger* (10.2 mm).

6. Acknowledgments
The authors are thankful to the Head, Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute (CSIR), Lucknow for recording GC-MS analysis of the essential oil.

Table 1: Chemical Composition of essential of the leaves of *Cymbopogon flexuosus*

S. No.	Components	RI	% Area (GC-MS)	Method
1	*α*- Pinene	925	4.8	A,B
2	Myrcene	991	1.1	A,B
3	Limonene	1031	0.8	A
4	Citronellal	1153	1.3	B
5	*trans*-Verbenol	1162	1.0	B
6	Terpene-4-ol	1165	0.5	B
7	*α*-Terpineol	1169	0.1	B
8	Citronellol	1228	1.2	A,B
9	Geraniol	1237	51.7	A,B
10	Neral	1240	0.6	A.B
11	Citral	1242	0.8	B
12	Geranial	1260	29.0	A,B
13	Geranyl acetate	1383	0.7	B
14	*β*- Caryophyllene	1405	0.2	B
15	2-Methyl propyl benzoate	1410	0.6	A,B
16	Spathulenol	1436	2.9	B
17	*β*-Selinene	1449	2.0	A,B
18	(E,E)-Farnesol	1722	0.8	B

RI= Retention Index on ULBON HR-1; A = GC-MS Analysis; B = GLC Analysis

Table 2: Anti-microbial activity of the essential oil and ethanolic extract of the leaves of *Cymbopogon flexuosus*

S. No.	Test Organism	Zone of Inhibition in mm[a]					
		Conc. of Essential Oil			Dried ethanolic extract 5.0%w/v	Standard Chloramphenicol (0.1 mg/ml)	Standard Ketoconazole (0.1 mg/ml)
		0.1%v/v	0.5%v/v	1.0%v/v			
1	*Staphylococcus aureus*	9.2	12.8	15.3	10.2	18.2	-
2	*Escherichia coli*	7.8	10.2	12.8	9.8	14.8	-
3	*Candida albicans*	7.5	9.8	12.5	9.0	14.2	15.0
4	*Aspergillus niger*	6.8	8.2	10.2	7.5	13.5	14.2

[a] An average of triplicate; Chloramphenicol - against all micro-organism ; Ketoconazole - against fungal strains only.

7. References

1. Ganjewala D, Kumari A, Khan KH. Ontogenic and developmental changes in essential oil content and compositions in *Cymbopogon flexuosus* cultivars. In: Prasad BN, Lazer Mathew, editor. Recent Advance in Biotechnology. New Delhi, India: Excel India Publishers, 2008, 82-92.
2. Ganjewala D, Gupta AK. Lemongrass (*Cymbopogon flexuosus* Steud.) Wats essential oil: overview and biological activities. In Govil JN, Bhattacharya S, ed., Recent Progress in Medicinal *Plants.* Essential oils II, Studium Press LLC. 2013; 37:235-271.
3. Nath, SC, Saha BN, Bordoloi DN, Mathur RK, Leclercq PA. The chemical composition of the essential oil of *Cymbopogon flexuosus* (Steud) Wats. growing in Northeast India. J Essen. Oil Res. 1994; 6(1):85-87.
4. Ganjewala D. Cymbopogon essential oils: Chemical compositions and bioactivities. Internat J Ess Oil Therap. 2009; 3:56-65.
5. Avoseh O, Oyedeji O, Rungqu P, Nkeh-Chungag B, Oyedeji A. *Cymbopogon* Species; Ethnopharmacology, Phytochemistry and the Pharmacological Importance. Molecules. 2015; 20:7438-7453.
6. Ganjewala D, Mittal R, Gupta AK, Premlatha M, Dawar R. Antibacterial properties of lemongrass (*Cymbopogon flexuosus* Steud) Wats essential oils in single form and combination of honey against drug resistant pathogenic bacteria. J Biol Active Prod Nat. 2014; 4:278-285.
7. Khanuja SPS, Shasany AK, Pawar A, Lal RK, Darokar MP, Naqvi AA *et al*. Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon* Spreng. (Poaceae). Biochem Syst Ecol. 2005; 33:171-186.
8. Gupta AK, Ganjewala D. A study on developmental changes in essential oil content and composition in *Cymbopogon flexuosus* cultivar *suvarna*. Acta Biologica Szegediensis. 2015; 59(2):119-125.
9. Ganjewala D, Luthra R. Geranyl acetate esterase controls and regulates the level of geraniol in lemongrass (*Cymbopogon flexuosus* Nees ex Steud.) mutant cv. GRL-1 leaves. Z Naturforsch C. 2009; 64:251-259.
10. Anonymous. British Pharmacopoeia, Part II. (HMSO, London). 1988, 109-110.
11. Adams RP. Identification of Essential Oil Component by Gas Chromatography/ Mass Spectrometry, (4th ed.). IL: Allured Publishing Co: Carol Stream, 2007.
12. Ali M. Techniques in terpenoid identification. Delhi; Birla Publications, 2001.
13. Linstrom PJ, Mallard WG. NIST Standard Reference Database, Number 69, National Institute of Standard and technology, Gaithersburg MD, 20899. 2012. http://webbook.nist.gov.
14. Brook I. Collection and transportation of specimens in anaerobic infections. J. Fam. Pract. 1982; 4:775-779.