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EVALAUATION OF ANTIMICROBIAL POTENTIAL OF FIELD DISTILLED AND WATER-SOLUBLE ESSENTIAL OILS OF *Cymbopogon flexuosus*

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Abstract- The essential oil from the shoot biomass of lemongrass (*Cymbopogon flexuosus*) obtained by field distillation and the water-soluble oil recovered from the distillation water (bye product of field distillation) employing different solvents and charcoal were analyzed by gas chromatography for their chemical compositions. The major compounds identified were (Z)-citral (1.96% - 34.8%), (E)-citral (12.32% - 42.07%), 6-methyl hept-5-en-2-one (1.93% - 32.89%) and geraniol (0.44% - 5.08%). The field distilled essential oil as well as the recovered water-soluble oils were evaluated for their antimicrobial activity against two gram-positive and two gram-negative bacteria and four pathogenic fungi by agar disc diffusion method. The minimum inhibitory concentration (MIC) values were determined by broth microdilution. All the samples exhibited antimicrobial activity against the tested microorganisms. Among the bacteria *Staphylococcus aureus* was the most sensitive. The water-soluble oil recovered by redistilling the hydrosol showed strong activity with zones of inhibition of 16 - 22 mm. Among all the tested fungi, *Aspergillus niger* was inhibited effectively with zones of inhibition of 11 - 17 mm.
Keywords- Antimicrobial activity, *Cymbopogon flexuosus*, Water-soluble oil, *Staphylococcus aureus*, *Aspergillus niger*

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Introduction

Cymbopogon flexuosus (Nees ex Steud) Wats. belonging to the family Poaceae is known as Cochin or East Indian or Malabar lemongrass. It is a perennial aromatic cum medicinal herb native to the Indian subcontinent. The essential oil of lemongrass is extracted from the shoot biomass through distillation. The essential oil is extensively used in perfumery, flavoring, pharmaceutical and aromatherapy industries. During the distillation process, a small hydrophilic portion of the essential oil gets disproportionally partitioned into the distillation water or hydrosol. Unless recovered, this water-soluble oil is lost as the distillation water is discarded. Redistillation of hydrosol or extraction with solvents or adsorbents like charcoal, yields water-soluble secondary or recovered oils. The compositions of recovered essential oils are different from their field distilled primary or commercial essential oils [18-22]. Hydrosols and water-soluble oils have national and international markets, therefore are considered as economically important products of the aromatic plants industry [15]. Hydrosols and water -soluble oils of many aromatic plants and spices exhibited antimicrobial property [12-14,25].

It is well known that bacteria and fungi are the causative organisms for several infectious diseases posing a great threat to human, animal and plant health. The indiscriminate use of synthetic antimicrobial medicines and commercially available antibiotics are leading to hypersensitivity reactions in sensitive persons. In addition, these microorganisms have developed resistance to many of the currently used medicines throwing challenges to the medical fraternity. It is therefore, desired and essential to develop effective, safe, natural methods for the treatment of infectious diseases. In recent years, essential oils which are extracted from wild or cultivated aromatic plants have received attention of researchers as potent bioactive compounds to combat several species of microorganisms [7,22,23].

Earlier studies demonstrated that lemongrass oil possesses anti-

cancer [24], analgesic, antiinflammatory [2], antibacterial [9] and antiviral [3] activities. To the best of our knowledge there have been no published reports on the antimicrobial activity of water-soluble secondary essential of *C. flexuosus*. The aim of the present study was to analyze the composition of field distilled and recovered oils of *C. flexuosus* and to evaluate their potential antimicrobial activity.

Materials and methods

Field distilled and recovered essential oils of lemongrass

Cymbopogon flexuosus cv. Cauvery was grown in the experimental farm of Central Institute of Medicinal and Aromatic Plants Research Center, Hyderabad, India following recommended agricultural practices [16,17]. The harvested shoot biomass was distilled in a field distillation unit. The essential oil (LG-1) and the distillation water (hydrosol) were collected separately. A sample (1000 ml) of the hydrosol was redistilled in Clevenger hydrodistillation apparatus [4] and the recovered essential oil was designated as LG-2. Five more samples of hydrosols (1000 ml each) were extracted separately with hexane, cyclohexane, dichloroethane, ethyl acetate and charcoal to isolate water-soluble oils designated as LG-3, LG-4, LG-5, LG-6 and LG-7, respectively.

Gas chromatography (GC)

The relative peak area percentages of the major constituents of essential oil samples were determined on Varian CP-3800 GC-FID (Varian Chromatography Systems, Middelburg, The Netherlands) equipped with Varian Galaxie chromatography data system (version 1.9 SP 1a), an electronic integrator and 100% dimethyl polysiloxane bonded phase Varian CP-Sil 5CB column (length = 50 m, internal diameter = 0.25 mm, film thickness = 0.25 µm). GC operating conditions were: injector temperature 250^{o}C; FID temperature 300^{o}C; carrier gas nitrogen; flow rate 0.5 ml/min and split injection with 1:20 split ratio. The column was initially held at 120^{o} C for 2 min, then heated to 240^{o}C at the rate of 8^{o}C/min and finally held at that temperature for 10 min. Essential oil samples (0.2 µl) were injected neat. Retention indices were generated with a standard solution of *n*-alkanes (C_6-C_{19}) (Sigma-Aldrich, St. Louis, USA). Relative percentages of the individual compounds were computed from the GC peak areas by normalization method without applying correction factors.

Identification of the essential oil constituents

The essential oil components were identified by comparison of their retention indices with published literature [1,5] and those stored in NIST/EPA/NIH (version 2.1) and Wiley (seventh edition) libraries.

Antibacterial activity

Two gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 10707) and two gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) obtained from the Department of Microbiology, Osmania University, Hyderabad were used for the present investigation. The bacteria were cultured and maintained in nutrient agar media. The antibacterial activity was carried out by agar disc diffusion technique [10,11]. The diluted bacterial strains were added to each petriplate containing 15 ml of sterile culture medium and uniformly spread with a sterilized spreader over the surface. Sterile filter paper discs (Whatmann No.3) were impregnated with different concentrations (20-100 µg/ml) of lemongrass samples. The discs treated with acetone served as control. The discs were placed on the inoculated plates and the plates were incubated at 37°C for 24 h after which the zones of inhibition were measured. All the tests were performed in quintuplicate. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of sample inhibiting the visible growth of each organism on the agar plate. The commercially available standard antibiotic, streptomycin was also tested as positive control under similar conditions against all the bacteria.

Antifungal activity

The fungi used were *Aspergillus niger* (ATCC 10553), *Fusarium oxysporum* (ATCC 10960), *Sclerotium rolfsii* (ATCC 15206) and *Rhizoctonia solani* (ATCC 16120). The fungal cultures were obtained from Mycology and Plant Pathology Laboratory, Department of Botany, Osmania University, Hyderabad, India and maintained in Potato Dextrose Agar medium. The paper disc agar diffusion method was followed in the present investigation for determining the antifungal activity of essential oil [10,11]. The sterile filter paper discs were thoroughly moistened with different concentrations of essential oil (20-100 µg/ml) and placed on the seeded agar plates. The discs moistened with ethanol served as negative control. The plates were incubated at 30°C for 48h and the zones of inhibition were measured. The minimum inhibitory concentration (MIC) necessary for complete inhibition of mycelial growth of the fungal strains was determined. Bifonazole was used as a positive control. All the tests were conducted in quintuplicate.

Statistical Analysis

The data were subjected to Student-Newman Kuels test for statistical analysis.

Results and discussion

The field distilled essential oil from the shoot biomass of *C. flexuosus* and the recovered essential oils from hydrosol were subjected to GC analysis and the results are summarized in Table-1. (Z)-Citral (1.96% - 34.8%), (E)-citral (12.32% - 42.07%), geraniol (0.44% - 5.08%) and 6-methyl hept-5-en-2-one (1.93% - 32.89%) were the major compounds. Field distilled essential oil showed predominance of (Z) and (E)-citral, while the recovered oils were richer in 6-methyl hept-5-en-2-one relative to the field distilled oil. Differences in composition between field distilled primary oil and water-soluble secondary/recovered oils were reported in many aromatic crops [18-22].

The antibacterial activity of the field distilled oil and the recovered oils was carried out against four bacterial species by disc diffusion method. Acetone, the negative control did not show any inhibiting activity. All the oil samples showed antibacterial activity as evidenced by their zones of inhibition (Table-2). Among the recovered oils, LG-2 at 100 µg/ml concentration inhibited the growth of *S. aureus and B. subtilis* with zones of inhibition of 21.7 and 19.6 mm, respectively. The inhibitory effect increased with increase in concentration of the oils from 20 to 100 µg/ml. The sensitivity of the bacterial species to the oil samples under investigation is in the following order *S. aureus* > *B. subtilis* > *E. coli* > *P. aerugino-*

sa. The inhibition zones observed in the present investigation were less than the inhibition zones of standard antibiotic. The minimum inhibitory concentrations are presented in Table-2. As evident from the Table, LG-2 sample inhibited complete visible growth of *S. aureus* at a minimum concentration of 250.5 μg/ml, while 256.4 μg/ml of LG-3 inhibited the growth of the bacterium. The MIC values for streptomycin ranged from 14.3 to 29.6 μg/ml.

The antifungal activity of the essential oils was carried out using disc diffusion technique and the results are illustrated in Table-3. All the tested concentrations exhibited good activity against the four species of fungi at 100 μg/ml concentration. Among the fungi, the growth of *A. niger* was inhibited with zones of inhibition of 16.7 and 11.9 mm by LG-2 and LG-3, respectively. The sensitivity of the organisms were in the following order: *A. niger* > *F. oxysporum* > *R. solani* > *S. rolfsii*. The minimum inhibitory concentrations are presented in Table-3. The standard antifungal agent showed the highest antifungal activity against all the fungi. The MIC of Bifonazole ranged from 18.3 to 38.4 μg/ml. LG-2 sample inhibited the growth of *A. niger* at 252.6 μg/ml, while LG-3 sample achieved complete inhibition at 267.7 μg/ml. The variation in sensitivity is due to varying permeability of the mycelial and spore walls of different fungi.

The results obtained in the present study revealed that gram-positive bacteria are more sensitive than gram-negative bacteria which are in contrast with published reports [6, 26]. The mild activity against gram-negative bacteria was ascribed to the presence of an outer membrane in these bacteria [8, 27]. The antimicrobial properties of the field distilled and recovered essential oil samples of lemongrass are attributed to the presence of large amounts of monoterpenes.

Conclusion

These findings suggest that lemongrass essential oil has promising antimicrobial property, but needs further detailed investigations against large number of microorganisms.

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Table 1- Major compounds (%) of field distilled and recovered essential oils of Cymbopogon flexuosus

S. No.	Compound	Retention index	LG-1	LG-2	LG-3	LG-4	LG-5	LG-6	LG-7
1	6-methyl hept-5-en-2-one	967	1.93	32.89	12.63	13.23	11.16	7.28	14.35
3	(Z)-Citral	1224	34.18	26.69	32.94	1.96	33.13	29.35	16.48
4	Geraniol	1234	1.08	1.87	1.27	5.08	0.44	1.36	2.73
5	(E)-Citral	1250	42.07	24.94	33.94	12.32	24.92	33.48	25.42

LG-1: Field distilled essential oil
LG-2: Recovered oil obtained through redistillation of hydrosol
LG-3: Oil isolated from hydrosol through hexane extraction
LG-4: Oil isolated from hydrosol through cyclohexane extraction
LG-5: Oil isolated from hydrosol through dichloroethane extraction
LG-6: Oil isolated from hydrosol through ethyl acetate extraction
LG-7: Oil isolated from hydrosol through charcoal extraction

Table 2- Antibacterial activity of C. flexuosus essential oil and water-soluble oil samples

Organism		Concentration (µg/ml)							
		20	40	60	80	100	MIC (µg/ml)	Streptomycin	
								DD[a]	MIC
E. coli	LG-1	0	1.0 ± 0.14	1.3 ± 0.15	2.3 ± 0.22	2.8 ± 0.17	548.2 ± 4.92	20.6 ± 1.13	24.7 ± 0.17
	LG-2	3.4 ± 0.15	6.3 ± 0.17	10.8 ± 0.36	14.6 ± 0.38	17.5 ± 0.43	294.5 ± 3.27		
	LG-3	1.8 ± 0.13	3.2 ± 0.23	4.3 ± 0.21	6.6 ± 0.25	8.3 ± 0.30	297.2 ± 2.11		
	LG-4	0	1.0 ± 0.12	1.8 ± 0.18	3.7 ± 0.23	6.9 ± 0.24	298.7 ± 2.23		
	LG-5	0	1.1 ± 0.14	1.6 ± 0.16	3.4 ± 0.20	5.8 ± 0.25	312.8 ± 2.45		
	LG-6	0	1.0 ± o.13	1.8 ± 0.15	4.1 ± 0.23	7.3 ± 0.28	300.2 ± 2.29		
	LG-7	0	0	2.3 ± 0.13	4.8 ± 0.24	7.1 ± 0.26	302.6 ± 2.37		
P. aeruginosa	L.G-1 0		0	1.0 ± 0.11	2.1 ± 0.15	2.5 ± 0.18	568.3 ± 4.76	22.2 ± 1.09	29.6 ± 0.26
	LG-2	2.5 ± 0.10	4.6 ± 0.11	8.3 ± 0.19	12.7 ± 0.32	16.2 ± 0.45	299.4 ± 4.76		
	LG-3	1.0 ± 0.13	2.8 ± 0.22	3.8 ± 0.27	5.9 ± 0.25	8.1 ± 0.32	303.5 ± 3.18		
	LG-4	0	0	1.2 ± 0.12	3.2 ± 0.20	6.2 ± 0.28	305.6 ± 3.21		
	LG-5	0	0	1.4 ± 0.15	3.0 ± 0.22	5.4 ± 0.28	322.2 ± 3.29		
	LG-6	0	0	1.6 ± 0	3.5 ± 0.23	6.9 ± 0.30	304.8 ± 3.33		
	LG-7	0	0	2.1 ± 0.14	4.2 ± 0.21	6.5 ± 0.29	305.7 ± 3.46		
S. aureus	LG-1	1.0 ± 0.21	1.5 ± 0.18	2.1 ± 0.24	3.6 ± 0.29	4.2 ± 0.32	521.5 ± 2.98	28.4 ± 1.02	12.4 ± 0.22
	LG-2	5.2 ± 0.15	8.1 ± 0.23	14.3 ± 0.32	17.1 ± 0.28	21.7 ± 0.39	250.5 ± 3.89		
	LG-3	3.1 ± 0.13	4.7 ± 0.19	6.2 ± 0.24	8.3 ± 0.25	10.8 ± 0.30	256.4 ± 2.78		
	LG-4	1.0 ± 0.14	2.1 ± 0.18	4.1 ± 0.22	5.6 ± 0.21	8.2 ± 0.33	271.3 ± 2.94		
	LG-5	1.2 ± 0.15	2.3 ± 0.19	3.2 ± 0.20	5.1 ± 0.23	7.3 ± 0.26	301.3 ± 2.99		
	LG-6	0	1.2 ± 0.11	3.3 ± 0.17	6.2 ± 0.31	8.1 ± 0.29	264.8 ± 2.56		
	LG-7	0	1.0 ± 0.09	3.6 ± 0.18	6.7 ± 0.27	8.3 ± 0.34	259.1 ± 2.77		
B. subtilis	LG-1	0	1.1 ± 0.13	1.5 ± 0.14	2.8 ± 0.19	3.4 ± 0.21	535.2 ± 3.68	25.7 ± 1.26	16.8 ± 0.18
	LG-2	3.8 ± 0.22	7.2 ± 0.25	12.6 ± 0.41	15.7 ± 0.38	19.6 ± 0.37	257.3 ± 2.98		
	LG-3	2.2 ± 0.15	3.9 ± 0.20	4.9 ± 0.24	7.1 ± 0.29	9.3 ± 0.32	261.4 ± 3.02		
	LG-4	1.0 ± 0.11	1.6 ± 0.16	2.9 ± 0.20	4.4 ± 0.25	7.5 ± 0.36	273.5 ± 2.93		
	LG-5	1.0 ± 0.15	1.4 ± 0.13	2.3 ± 0.22	3.9 ± 0.22	6.2 ± 0.29	300.8 ± 3.35		
	LG-6	0	1.0 ± 0.17	2.4 ± 0.19	4.8 ± 0.27	7.6 ± 0.30	268.7 ± 3.41		
	LG-7	0	0	2.7 ± 0.18	5.3 ± 0.30	7.7 ± 0.32	263.1 ± 2.98		

DD[a] = diameter of zone of inhibition calculated at 5 µg/ml; Mean diameter of zone of inhibition in mm (mean ± S.E)

Table 3- Antifungal activity of field distilled and recovered essential oils of C. flexuosus

Organism		Concentration (µg/ml)							
		20	40	60	80	100	MIC (µg/ml)	Streptomycin	
								DD[a]	MIC
A. niger	LG-1	2.1 ± 0.14	2.4 ± 0.23	3.2 ± 0.27	4.4 ± 0.32	5.5 ± 0.32	431.4 ± 4.54	27.7 ± 1.23	18.3 ± 0.56
	LG-2	5.3 ± 0.24	8.4 ± 0.27	9.8 ± 0.25	12.6 ± 0.33	16.7 ± 0.47	252.6 ± 3.27		
	LG-3	3.4 ± 0.25	5.1 ± 0.32	7.5 ± 0.33	8.7 ± 0.37	10.3 ± 0.36	267.7 ± 3.22		
	LG-4	2.8 ± 0.21	3.7 ± 0.24	6.2 ± 0.28	8.1 ± 0.36	9.8 ± 0.31	271.3 ± 3.12		
	LG-5	2.4 ± 0.20	3.1 ± 0.23	4.6 ± 0.19	7.4 ± 0.33	9.2 ± 0.34	274.6 ± 3.31		
	LG-6	1.8 ± 0.17	2.8 ± 0.27	4.1 ± 0.21	6.8 ± 0.32	8.7 ± 0.37	281.3 ± 2.39		
	LG-7	1.4 ± 0.14	2.2 ± 0.22	3.5 ± 0.26	5.9 ± 0.32	8.2 ± 0.38	285.4 ± 3.23		
F. oxysporum	L.G-1	1.6 ± 0.18	2.1 ± 0.13	2.9 ± 0.23	3.2 ± 0.31	4.7 ± 0.28	450.3 ± 3.15	25.2 ± 1.10	25.6 ± 0.48
	LG-2	3.9 ± 0.10	6.6 ± 0.11	8.3 ± 0.19	11.2 ± 0.22	14.1 ± 0.41	266.4 ± 4.76		
	LG-3	3.1 ± 0.21	4.4 ± 0.26	7.1 ± 0.23	7.9 ± 0.26	9.2 ± 0.34	272.3 ± 3.44		
	LG-4	2.3 ± 0.23	3.1 ± 0.20	5.6 ± 0.22	7.7 ± 0.30	9.3 ± 0.32	278.2 ± 3.12		
	LG-5	2.1 ± 0.19	2.6 ± 0.22	4.1 ± 0.24	6.9 ± 0.31	8.8 ± 0.33	285.6 ± 3.22		
	LG-6	1.4 ± 0.12	2.2 ± 0.24	3.7 ± 0.26	5.9 ± 0.33	8.3 ± 0.31	290.7 ± 2.98		
	LG-7	1.0 ± 0.13	1.7 ± 0.23	2.8 ± 0.21	4.7 ± 0.27	7.8 ± 0.35	294.2 ± 3.35		
R. solani	LG-1	1.1 ± 0.14	1.8 ± 0.18	2.1 ± 0.24	2.6 ± 0.29	3.6 ± 0.32	462.5 ± 2.98	22.8 ± 1.18	34.3 ± 0.51
	LG-2	3.2 ± 0.17	5.8 ± 0.20	7.4 ± 0.28	10.1 ± 0.25	12.4 ± 0.35	270.3 ± 3.89		
	LG-3	2.7 ± 0.19	3.8 ± 0.25	6.2 ± 0.27	7.1 ± 0.32	8.5 ± 0.33	276.1 ± 3.37		
	LG-4	1.8 ± 0.17	2.4 ± 0.22	4.7 ± 0.24	6.8 ± 0.27	8.1 ± 0.31	283.4 ± 3.16		
	LG-5	1.5 ± 0.12	1.9 ± 0.15	3.4 ± 0.26	6.2 ± 0.24	7.9 ± 0.37	289.7 ± 2.96		
	LG-6	0	1.6 ± 0.18	2.6 ± 0.22	5.1 ± 0.28	7.8 ± 0.29	293.6 ± 3.07		
	LG-7	0	0	2.1 ± 0.23	4.2 ± 0.29	7.4 ± 0.36	297.3 ± 3.25		
S. rolfsii	LG-1	0	1.2 ± 0.17	1.9 ± 0.16	2.2 ± 0.23	2.9 ± 0.27	476.2 ± 3.68	20.9 ± 1.21	38.4 ± 0.47
	LG-2	2.5 ± 0.22	4.9 ± 0.27	6.3 ± 0.29	9.4 ± 0.30	11.2 ± 0.38	277.8 ± 2.98		
	LG-3	2.2 ± 0.26	3.1 ± 0.21	5.6 ± 0.24	6.6 ± 0.28	8.1 ± 0.33	281.3 ± 3.04		
	LG-4	1.4 ± 0.11	1.8 ± 0.17	4.2 ± 0.31	6.2 ± 0.32	7.6 ± 0.34	287.9 ± 3.24		
	LG-5	0	0	2.2 ± 0.21	5.4 ± 0.25	7.4 ± 0.32	291.6 ± 3.31		
	LG-6	0	0	2.1 ± 0.27	4.8 ± 0.32	7.1 ± 0.39	296.3 ± 3.51		
	LG-7	0	0	1.8 ± 0.22	3.7 ± 0.37	6.9 --± 0.33	300.4 ± 3.46		

DD[a] = diameter of zone of inhibition calculated at 5 µg/ml; Mean diameter of zone of inhibition in mm (mean ± S.E)