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GC-MS fingerprinting of wild *Cymbopogon flexuosus* (Nees ex Steud) Wats. essential oil

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Abstract
Wild plants play an essential role in the ecological and biological processes that are yet again significant to life. The essential oil of *Cymbopogon flexuosus* (Lemongrass) was collected from Bannerghatta, Bangalore, and isolated by hydro-distillation. The present research evaluates the chemical composition of essential oil of wild *Cymbopogon flexuosus* (Nees ex steud) Wats, growing in Bannerghatta, Bangalore, India. GC-MS analyses investigated the essential oil composition. The oil contains forty-eight compounds representing 98.107%. The major constituents were elemicin (33.616), δ-gurjunene (13.494), α-cadinol (8.385), β-caryophyllene (7.288), β-bourbonene (4.499), limonene (4.405), α-bergamontene (3.926), camphene (3.334), patchoulene (2.146), α-pinene (1.199), β-elemene (1.695) and germacrene (1.387). The major component Elemicin attributes as antibacterial, antifungal, anti-allergic, anti-proliferative, and anti-cholinergic.

Keywords: *Cymbopogon flexuosus*, wild, oil fingerprinting, elemicin, δ-gurjunene, morphology

Introduction

Wild plant species play a vital role in balancing the environment by providing stability to different natural processes of nature. They are associated mainly with humans for numerous emotional and social reasons. Based on the significance, we can categorize the wild plant species as economic importance, ecological importance, investigatory importance, and conservation of biological diversities. Wild plant products such as food, fibers, medicine, timber, etc. are of high economic value [1]. Biodiversity is the form of living with an ecosystem. Biodiversity plays a vital role in the function of climate. In terrestrial habitats, tropical areas are more affluent in species, whereas the Polar Regions are lesser in species. Wild plant conservation is the protection, preservation, and restoration of the endangered plant species. It represents all the wild plant species present in their natural habitats. Conservation of biodiversity is inevitable for the survival of humankind on earth [1].

Cymbopogon flexuosus, commonly called Lemongrass/East Indian lemongrass (syn. *C. travancorensis*). It is the primary source of essential oil having industrial-grade value. *C. flexuosus* from the family Poaceae and is a native plant to India. The plant grows in several countries such as Brazil, China, Congo, Indonesia, Republic of Malagasy, Sri Lanka, West Indies, Zambia, and other countries [2, 3]. The essential oil of *C. flexuosus* is considered superior in quality because of its 70% solubility in alcohol and high content of citral (neral + geranial) [3]. The identified compounds represent monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, phenylpropanoids, aliphatic, and nitrogenous compounds. *C. flexuosus* has several chemotypes *viz*., α-bisabolol type [4], geraniol type [5], geranyl acetate type [6], methyl eugenyl ether type [7], geraniol/citronellol/citral type [8], and geraniol/citronellyl acetate/geranyl acetate type [9], have been reported apart from its typical citral type [10].

During the floristic survey in Karnataka, we came across a wild taxon of *C. flexuosus* in the hills of Shri Champakadhama Swamy temple, Bannerghatta, which yielded essential oil with an unusual odor unlike most of the other oils obtained from various chemotypes of *C. flexuosus*. On critical detailed taxonomic study, the wild taxon *C. flexuosus* revealed many similarities with anatomical and morphological characteristics differing from those of typical *C. flexuosus* (Table 1). The plant has been identified as *C. flexuosus* by Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD), Bangalore. The taxon's live plant germplasm was maintained in Greenhouse, Department of Microbiology and Biotechnology, Bangalore University.

The present study reports on the chemical composition of wild *C. flexuosus* oil of a particular chemo ype.

Materials and methods

Collection of plant material

The wild *C. flexuosus* for the study has been collected from the hilly region of Shri Champakadhama Swamy temple, Bangalore, Karnataka. The plant material was identified by Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD), Bangalore, with the Accession number - SMPU/RARIMD/BNG/2019-20/230/RRCBI-mus 231.

Ecology

The wild *C. flexuosus* (Nees ex Steud.) Wats. Collected from Shri Champakadhama Swamy temple hill, Bangalore, India, has 260.51 km^2 (100.58 sq mi). The Latitude and

Longitude of the hill areas are 12.770132 and *77.567758, respectively* [11]. The hill showed a temperature of 27 ºC, Precipitation of 23% with Wind flow of 3 km/h, and an annual rainfall of 937mm ranging between 728mm and 1352mm. It shows an altitude of 865m and ranges between 700 and 1035m above mean sea level. The park's geology shows that the rocks are of the oldest formation revealing cryptocrystalline to coarse granites and complex gneiss.

Table 1: Differences in morphological and anatomical characteristic between Krishna variety and Wild variety

Characteristic	Typical *C. flexuosus* (Var. Krishna)	Wild variety
Habit	Erect	Semi-erect
Height (cm)	250-275	245-280
	Color	
Leaf	Pale green	Dark green
Stem	Brownish at the base	Purplish at the base
Inflorescence	Greenish	Greenish brown
Inflorescence length	110-130	125-140
Culm color	Light brown	Dark red
Basal leaf sheath	Light green	Reddish-brown
Leaf-blade	Linear acuminate	Linear acuminate
Ligules	Coriaceous (2.5-3.5mm long)	Cretaceous to coriaceous (2-3mm long)
Spatheole	16-18mm long	18-20mm long
Peduncle	2.5-3mmlong	3-3.5mm long
Racemes	12-15mm long	15-20mm long
Awn length (cm)	1.1	1.2
Upper epidermis	Single-layer polygonal cells	Single-layer polygonal cells
Adaxial length (mean)	78±0.5µm	80.04±0.5µm
Abaxial length (mean)	82.05±0.6µm	85.33±0.6µm
Stomatal size (µm)		
Adaxial surface	21.06±0.2	22.14±0.2
Abaxial surface	12.02±0.6	15.02±0.3
Stomatal densities (mm^2)		
Adaxial	244.6±0.5	250.8±0.5
Abaxial	377.4±0.65	380.6±0.6

Isolation of essential oil

The essential oil was extracted from fresh leaves of wild *C. flexuosus* and air-dried using the hydro-distillation technique in the Clevenger-type apparatus for 6 h. The essential oil collected after distillation was pale yellow with a healthy, fresh citrus odor and was dried using anhydrous Na_2SO_4 to remove moisture and stored in sealed vials under refrigeration at -15 °C. The oil yield was calculated based on the material's fresh weight and dry weight (v/w) [12, 13].

GC-MS analysis

The essential oil (10µL) was reconstituted in 990 µL Methanol. The methanolic extracts (1 µl) were injected for GC-MS analysis. The oil constituents were identified by matching their 70ev mass spectra and linear temperature programmed retention indices with reference libraries [14, 15].

Table 2: Conditions for GC-MS analysis

Instrument used	Thermo Scientific GC Trace 1310 Equipped with Thermo Scientific MS TSQ 8000
Method type	Acquisition – General
MS transfer line temperature	300 °C
Ion source temperature	160 °C
Ionization mode	EI
Temperature program	Initial 60 °C hold for 2 min Ramp at 5 °C to 140 °C Ramp at 5 °C to 300 °C hold for 5 min
Flow rate	1ml/min
Carrier gas	Helium
Scan	50-600Da
Column used	Agilent DB 5MS (60 m × 0.25 mm)

Identification of compounds

Interpretation of the mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library based on linear retention indices. The significant compounds identified based on the NIST database match and relative concentration of major compounds are shown in Fig. 1 and summarized in Table 3.

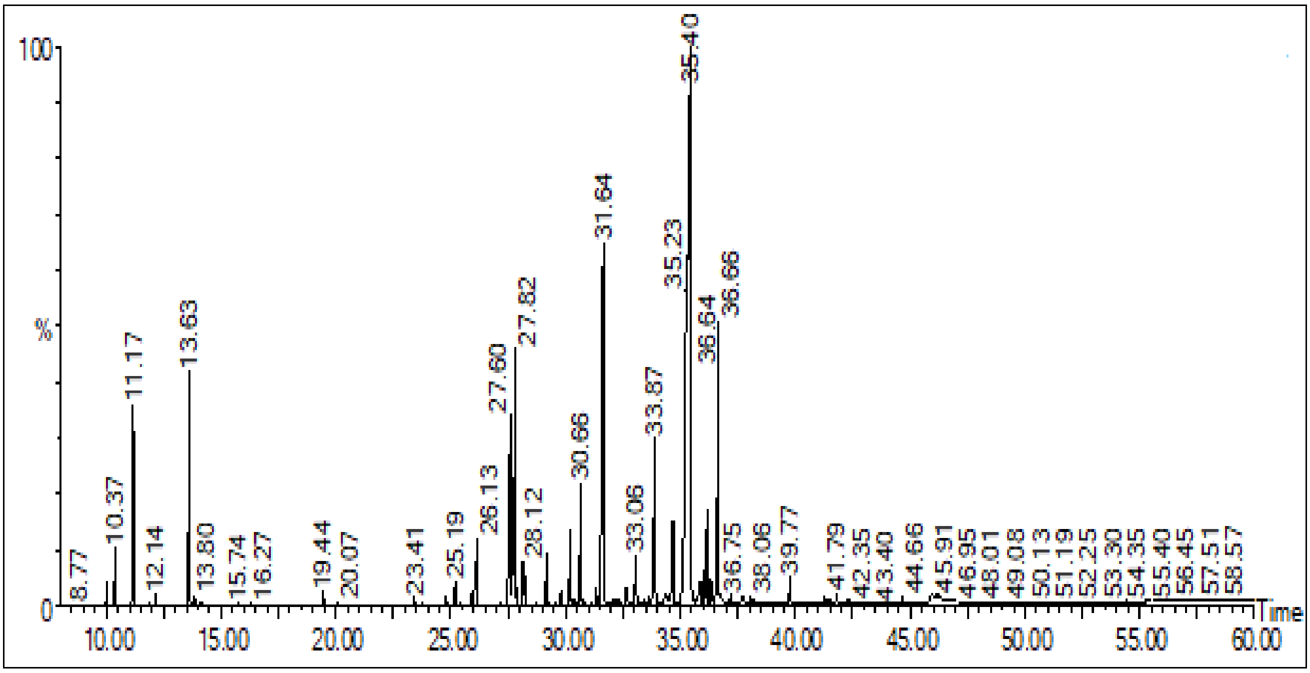

Fig 1: Chromatograph of wild *C. flexuosus* essential oil

Table 3: Chemical composition of wild *C. flexuosus* (Steud) Wats. Oil from Bannerghatta

Sl. No.	RT	Area %	Name
1	8.023	0.035	5,5-Dimethyl-3-cyclohexen-1-ol
2	8.774	0.027	Cyclohexane
3	10.009	0.351	D- (+) α-pinene
4	10.374	0.848	α-pinene
5	11.175	3.334	Camphene
6	11.880	0.046	β-phellandrene
7	12.140	0.151	β-pinene
8	13.631	4.405	Limonene
9	13.836	0.207	β-phellandrene
10	14.116	0.083	p-mentha-1,5,8-triene
11	16.267	0.033	Linalool
12	19.438	0.340	Isoborneol
13	20.073	0.048	α-terpineol
14	20.629	0.035	Citronellol
15	21.669	0.071	2H-Thiopyran, 3-hexyltetrahydro-, 1,1-dioxide
16	22.229	0.050	Geraniol
17	23.410	0.196	Bornyl acetate
18	23.790	0.045	α-cubebene
19	24.765	0.160	δ-muurolene
20	25.190	0.481	α-cubebene
21	25.375	0.053	α-bergamotene
22	25.891	0.212	β-bourbonene
23	26.131	1.695	β-elemene
24	27.146	0.049	Neryl-acetate
25	27.596	3.873	α-bergamotene
26	27.816	6.803	β-carophyllene
27	27.886	0.190	δ-cadinene
28	28.122	0.546	β-sesquiphellandrene
29	28.207	0.485	β-carophyllene
30	28.557	0.021	α-gurjunene
31	28.697	0.045	δ-muurolene
32	29.172	0.980	α-humulene
33	29.792	0.377	δ-cadinene
34	30.202	1.387	Germacrene
35	30.372	0.052	α-guaiene
36	30.663	2.202	δ-gurjunene
37	30.763	0.060	δ-cadinene
38	31.103	0.077	β-sesquiphellandrene

39	31.328	0.274	δ-cadinene
40	31.638	11.134	δ-gurjunene
41	31.818	0.030	β-bourbonene
42	32.008	0.123	δ-muurolene
43	32.173	0.095	Tricycloundecene
44	32.283	0.081	α-ionene
45	32.633	0.603	Nerolidol
46	33.064	1.342	α-elemol
47	33.314	0.039	Biscyclodecene
48	33.444	0.067	Patchoulene
49	33.634	0.129	Geranyl linalool
50	33.874	4.257	β-bourbonene
51	34.089	0.026	Cadalatriene
52	34.324	0.143	Longifolene
53	34.374	0.158	δ-gurjunene
54	34.664	1.638	Patchoulene
55	35.024	0.207	Cubenol
56	35.420	33.424	Elemicin
57	35.855	0.592	α-selinene
58	36.025	0.366	Patchoulene
59	36.150	1.531	α-cadinol
60	36.315	0.352	α-cadinol
61	36.420	0.130	α-farnesene
62	36.660	6.502	α-cadinol
63	36.750	0.035	Patchoulene
64	37.175	0.134	α-selinene
65	37.320	0.045	Polyquaternium
66	37.660	0.202	Longifolol
67	37.751	0.192	Elemicin
68	38.056	0.116	α-selinene
69	38.236	0.094	Adamantane
70	38.726	0.036	Aromadendrene
71	39.316	0.076	Octadecenamide
72	39.501	0.028	α-farnesene
73	39.606	0.032	Aromadendrene oxide
74	39.766	0.568	Mesityl oxide
75	39.946	0.025	Longifolol
76	40.822	0.066	Aromadendrene oxide
77	41.012	0.058	Aromadendrene oxide
78	41.277	0.185	Methenolone
79	41.482	0.074	Unknown
80	41.792	0.208	Delta-cadinene
81	42.097	0.047	Pentadecanoic acid
82	42.272	0.074	Boronia butenal
83	42.347	0.021	Vinblastine
84	42.677	0.022	Retinal
85	42.898	0.028	Kojic acid
86	43.563	0.040	Patchoulene
87	43.763	0.042	Retinal
88	43.988	0.028	Retinal
89	44.268	0.028	Triethanolamine
90	44.658	0.195	Mangiferin
91	45.489	0.064	Oleyl alcohol
92	45.944	0.548	Oleyl alcohol
93	46.169	1.312	Oleyl alcohol
94	47.044	0.154	Octadecenal
95	47.269	0.175	Octadecenal
96	47.765	0.041	Octadecenal
97	47.940	0.036	Octadecenal
98	48.265	0.037	Octadecenal
99	48.515	0.021	Octadecenal
100	51.721	0.027	Octadecenal

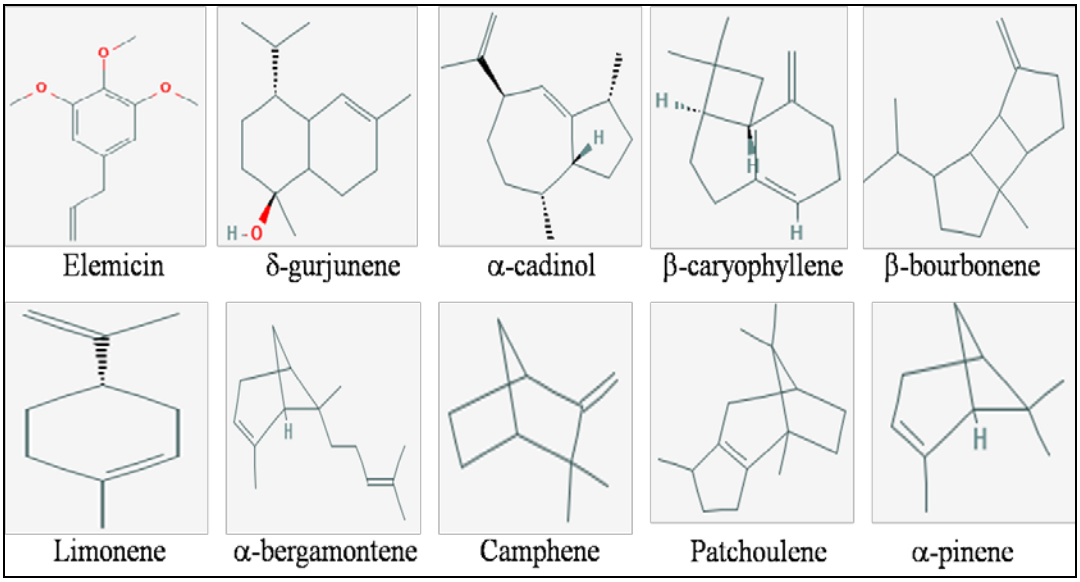

Fig 2: Structure of top 10 compounds present in essential oil

Results and Discussion

Biodiversity fulfills the basic needs of humans, such as shelter, food, and medicine, and maintains the ecosystem. It enhances the soil, purifies water, protects us from flood and storm, and regulates climate. Hence, it is crucial to consider the endangered or threatened species of plants as it provides our necessary human and capital development expenses. Therefore we are destined to protect our wild plant species (Chetram Meena 2019) [1].

Cymbopogon is a perennial grass that is spread across the world. Several investigations have revealed many significant bioactivities such as antimicrobial, allelopathic, anthelmintic, anti-inflammatory, anticancer, antioxidant, insect and mosquito repellent of lemongrass extract, essential oil, Citral and Citral derived compounds.

The wild variant of *C. flexuosus* studied was found to be quite distinct in its anatomical, morphological and essential oil composition from the typical citral chemotype. The difference in its morphological and anatomical characters was reflected in essential composition showing 100 compounds representing 98.107% of oil. The oil content was found to be 0.6 % (w/w) yield on a fresh weight basis was almost colorless liquid with a pleasant odor. Forty-eight constituents representing 94.906 % and 1.341 others of total oil were identified as a result of the GC-MS examination of the oil (Table 3).

Elemicin is found to be the major constituents with, 33.616%. Elemicin is widely used in the perfumery industry and is used as a starting material for the synthesis of mescaline, an important alkaloid. Moreover, it can also be used in confectionery, condiments, non-alcoholic beverages, etc. The other constituents were δ-gurjunene (13.494), α-cadinol (8.385), β-caryophyllene (7.288), β-bourbonene (4.499), limonene (4.405), α-bergamontene (3.926), camphene (3.334), patchoulane (2.146), α-pinene (1.199), β-elemene (1.695) and germacrene (1.387). Elemicin has been reported previously as a major component grown in North Eastern India does not contain citral. The GC-MS analysis of the essential oil showed the presence of elemicin (53.0 %), limonene (11.6 %), β-ocimine (7.6 %) and camphene (4.1 %) (Kamini *et al*., 2011). The other species of Cymbopogon commonly known as lemongrass are used in different parts of the world. The chemical composition of citratus is quite similar to *C. flexuosus.* The chemical constituents of fresh plant of *C. citratus* are geranial (50.2 %), neral (31.5 %), myrcene (1.6 %), geraniol (1.4 %), linalool (0.8 %), undecan-2-one (0.7 %) and 6- methyl hept-5-en-2-one (0.5%). EOs of the Cymbopogon spp. are mainly composed of cyclic and acyclic monoterpenes like citral (3,7-dimethyl-2, 6-octadienal; a mixture of two isomer geranial and neral), geraniol, citronellol, citronellal, linalool, elemol, 1, 8-cineole, limonene, b-carophyllene, methy lheptenone, geranyl acetate and geranyl formate. The above observed variability in the essential oils grown in wild *C. flexuosus* produced at geographically diverse locations is indicative of wide genetic differences showing significant differences in composition of essential oils between genotypes of different regions.

Conclusions

Extracted wild essential oils of *Cymbopogon flexuosus* owes to their specific aromatic and medicinal properties. They are widely used as flavor, fragrance, perfumery, cosmetics and pharmaceuticals. Several years of tremendous investigations are made on pharmacological and medicinal significance of the lemongrass EO and its constituents. Wild lemongrass has diverse origin and its essential oil mainly consists of monoterpene fractions, with large proportion of citral and geraniol. Inspite of enormous commercial significance of the lemongrass EO minimal work has been done so far towards understanding of the wild EO biosynthesis and its regulation. Major research work has been done in family members of Asteraceae, Lamiaceae, Myrtaceae, Pinaceae and Rutaceae in areas of monoterpene biosynthesis and regulations. The most significant discovery was the elucidation of the MEP pathway and characterization and cloning of enzymes/genes of this pathway. Unfortunately, the wild genus of Cymbopogon remains uncertain from the benefits of the recent advancement to improve EO yield and quality. Though, lot of progress has been made towards understanding the biological activities of essential oil. It is very important to understand the biosynthesis of essential oil and its molecular regulatory processes in order to manipulate the oil yield and quality due to its high commercial value for essential oil. Over the past decades, the essential oil has been recognized for its bioactivities such as antimicrobial, anti protozoan, anti-inflammatory and chemo-preventive properties. To conclude, we believe in investigating more wild Cymbopogon species to understand the undisturbed gene pool. This makes us understand the preliminary knowledge of the essential oil biosynthesis's developmental and seasonal regulation and to gain a vast understanding of their bioactive potential. Hence, this would help for develop lemongrass as a model system to understand the biosynthesis of essential oil and its regulation in depth.

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