

Studies on variation in essential oil composition from different accessions of *Cymbopogon citratus* (DC) Stapf

* Dr. KJ Thara Saraswathi, Vyshali P, Vidya Jagadeeshan

Department of Microbiology and Biotechnology Bangalore University, Jnana Bharathi campus, Bangalore, Karnataka, India

Abstract

Cymbopogon citratus (DC) Stapf belongs to the genus *Cymbopogon* (Poaceae) and are known to possess essential oil rich in Citral content. During the present investigation different accessions of *C. citratus* were collected from various locations of South India, assigned with different accession numbers and maintained at Departmental garden, Bangalore University, Bangalore for further studies. The essential oils were extracted by steam distillation and the essential oils were subjected to GC-MS analysis. A total of nine accessions (Cc1-Cc9) were selected for study and the total essential oil and Citral content were assessed. The Citral content was found to be highest in Cc5 accession. The study showed that there is variation existing with total percentage and composition of the essential oil among the accessions of *C. citratus* collected from various ecological locations.

Keywords: *cymbopogon citratus*, chemotype, essential oil, GC-MS studies

Introduction

The genus *Cymbopogon* is a major aromatic plant belonging to the family Poaceae. The *Cymbopogon citratus* (DC) Stapf is an important and oldest species of *Cymbopogon* and are commonly called as West Indian Lemon grass or Lemon grass. 'Citratus' in latin means 'steeped in citrus oil'. It is a tall, tufted, perennial grass with a short rhizome, which rarely flowers. *C. Citrates* is considered to be the most highly specialized grass tribe by many workers (Bews 1929 and Hartley 1950) [4, 13]. It is a well-defined monophyletic tribe under the genus *Cymbopogon* which exhibit morphological and adaptive variation due to hybridization, polyploidy etc. Introgression of various traits, intermittent mutations and selection through human intervention has lead to variations in chemotypic characters across geographical distribution of this species (Kuriakose 1995) [14]. It is reported to be present in both hexaploid ($2n=60$) and tetraploid ($2n=40$) state (Gupta 1965) [12]. The rich species diversity and high degree of endemism of this species also indicate that the group is actively evolving in the Western Ghats region.

At present *C. Citrates* exist as 'cultigen' (not known in wild form and presumably has originated from cultivation). The origin of *C. Citrates* is vague as there are many causes for the obscure history of the plant (Otto Stapf, 1906) [18]. The ancient history details the uses of this grass in southern India by natives of Madras (Browne, 1702) [5]. The *C. Citrates* is principally grown for its essential oil. It is one of the commercial sources of Citral apart from East Indian lemongrass oil obtained from *C. flexuosus*. The oil has strong lemon-like odour due to high percentage of Citral (over 70%) and thus had occupied prominent place in the perfume industry for several years. The oil serve as starting material for manufacture of ionones, producing vitamin-A. Due to the freshening note of oil it is used to scent soaps, cosmetics, detergents, insect repellent preparations as well as in beverage and non-beverage drinks (Bhattacharya 1977) [6].

Though extensive studies have been conducted on grasses all over the world (Soderstrom et al., 1986) [17] there is practically little work on *C. Citrates* species available in south India. Revisionary work on this plant is required for better understanding of this tribe (Claton and Renovoize 1986) [7]. Despite vast potential, the market for aromatic plants is still in the nascent stage, primarily because of low awareness among farming community (Purohit, 2004) [15] and with the advancement in organic chemistry; the synthetics have outnumbered the natural compounds due to limitations in their availability in sufficient quantities at a steady. There is need to develop proper agro-technologies for these crops to be able to compete in the international market.

During the present study, the *C. citratus* accessions collected from various ecological regions were subjected to essential oil analysis to study the variation in the content and composition of the oil for exploring them towards commercial utility.

Materials and Methods

Collection and maintenance of Germplasm

A total of nine accessions of *C. citratus* (Cc1 to Cc9) were collected from various regions of South India (Figure 1; Table 1). Plant samples were collected from Gudalur forest division, Nadugani (Coimbatore, TN), Yercaud hills (Salem, TN), local regions of Bangalore, Udupi, Puttur, Moodabidri, Mangalore (Karnataka) and from regions of Kasaragod and Kochi (Kerala) and were maintained at the Departmental garden, Bangalore University, Bangalore for further studies. The plant samples were taxonomically identified and deposited at the National Ayurveda Dietetics Research Institute, Bangalore [Ref RRCBI-Mus/06 (A-I)].

Essential oil extraction and analysis

1) Essential oil extraction: The shade-dried leaves of lemongrass were chopped into small (10cm long) pieces,

weighed and was subjected to hydro-distillation for 3 hrs using Clevenger's apparatus (Clevenger, 1928) [8]. The obtained oil was collected and dried over anhydrous Sodium sulphate and kept at 4°C until analysis. The essential oil yield was calculated on the basis of dry weight of the material (V/W) using the formula,

$$\text{Essential oil content} \left\{ \frac{V}{W} \right\} = a \times 100/b$$

Where, a = volume of the oil collected (ml)

b = weight of sample (g) taken.

2) Essential oil analysis by Gas Chromatography (GC-FID):

GC analysis of the oil samples was performed on an Agilent Technologies gas chromatograph Model 6890N equipped with dual FID. A CP Sil8CB column (30m x 0.25mm x 0.25 µm film thickness) coated with dimethylpolysiloxane with 5% diphenyl as the stationary phase. Helium was used as the carrier gas at a flow rate of 1 ml per min. (constant flow). Temperature programming was done from 50°C (2 min.) to 280°C at 10°C/min. Injector and detector temperature were maintained at 250°C and 280°C respectively. Samples of 1 µL dissolved in hexane were injected using a split ratio of 10:1.

Identification of compounds

Component identification was done by comparison of linear retention indices of GC peaks with those of standard compounds and literature (Adams, 2007) [1].

Results and Discussion

Aromatic plants are invaluable source and provide genetic material for upgrading the existing aromatic crops. The present investigation revealed that *C. Citrates* thrives in diverse habitat, broad altitudinal range of south India. They are grown in south Canara region (Udupi and Kasaragod) by indigenous population for their explicit usage in traditional medicine.

Hydro-distillation of the shade dried leaves of *C. citratus*

accessions collected from various regions gave essential oils with fresh grassy citrus odour, with the oil content ranging from 0.5-1% as shown in Table 2. All the accessions collected from Karnataka showed higher range of essential oil yield (0.6-1%) (Fig 2). The GC chromatograms of the essential oil studied showed chemical variation among the accessions (Fig 4-7). The major volatile compound Citral was found to be highest (72%) in the essential oil obtained from Cc5. The accessions collected from Tamil Nadu (Cc6 and Cc7) gave lower percentages of essential oil and Citral content (45-47%) and high levels of Geraniol (17%) (Figure3). The accessions from Kerala (Cc8 and Cc9) gave a medium percentage yield of essential oil (0.7%) when compared with the accessions collected from Karnataka and Tamil Nadu.

The broad range of variation in percentage and composition of the essential oil could be due to the diverse ecological conditions in which the accessions were collected. Hence, during the study considerable essential oil polymorphism has been found in the accessions, possibly due to their outstanding adaptability to a particular area in terms of climate, topography, soil and drainage. During the study on GC analysis of essential oils, it was established that the *C. citratus* accessions studied were of Citral chemotype (rich in Citral).

In the present scenario, the lemongrass oil is not been adequately explored and feasible business opportunities for essential oil industries are ignored. Unsustainable resource management due to complex environment and economic factors in essential oil trade has led to decline in lemongrass cultivation and in terms oil production. In view of this, newer approaches directed towards enhancing the quality of essential oil for up gradation to be used at commercial level to restore back the market is the need of the hour. For sustainable development of the species, conventional methods (cultivation by suckering) and biotechnological methods are required (in vitro culture techniques using cell and tissue culture) are required to harness the essential oil and its isolates for its economic usage.

Table 1: Collection of accessions of *C. citratus* from South India

Karnataka	Tamil Nadu	Kerala
Udupi- Cc1	Nadugani, Coimbatore- Cc6	Kasaragod-Cc8
Mangalore- Cc2	Yercaud, Salem- Cc7	Kochi- Cc9
Moodabidri- Cc3		
Puttur- Cc4		
Bangalore- Cc5		

Table 2: Percentage of essential oil yield from the accessions of *C. citratus*

Sl. No.	Accessions	Essential oil yield (%)*
1.	Cc1	0.7±0.03a
2.	Cc2	0.65±0.1b
3.	Cc3	0.83±0.04c
4.	Cc4	0.72±0.02a
5.	Cc5	0.9±0.02d
6.	Cc6	0.7±0.02a
7.	Cc7	0.54±0.04e
8.	Cc8	0.7±0.01a
9.	Cc9	0.7±0.01a

*Data represented as mean±SD. Mean followed by different letter are significantly different (n=8, P<0.05 Tukey's HSD Test)

Table 3: Percentage and composition of essential oil from Cc5 accession

RI	Compound	Percentage
937	α - Pinene	Tr
986	6-Methyl 5-hepten-2-one	0.6
990	β -Myrcene	8.8
1026	p-Cymene	0.1
1030	Limonene	0.5
1038	(Z)- β -Ocimene	0.1
1048	(E)- β -Ocimene	Tr
1081	6-Camphenolone	0.7
1088	6,7-Myrcene epoxide	0.3
1098	Linalool	1.3
1103	2,2-Dimethyl 3,4-octadien-1-al	0.4
1111	epi-PhotoCitral B	0.1
1143	exo-IsoCitral	1.0
1151	Citronellal	0.3
1165	(Z)-IsoCitral	0.9
1174	Rosefuran epoxide	0.1
1184	(E)-IsoCitral	1.4
1204	Decanal	0.1
1229	Citronellol + Nerol	0.5
1259	Neral	30.4
1260	Geraniol	2.2
1280	Geranial	41.8
1298	2-Undecanone	0.1
1305	Geranyl formate	0.1
1344	6,7-Epoxynerali+2,4Octanediolti	0.6
1358	Neric acid	0.3
1362	Citronellyl acetate	0.1
1377	6,7-Epoxygeranialti	0.3
1385	Geranyl acetate	0.4
1387	Geranic acid	0.3
1392	β -Elemene	0.1
1419	(E)-Caryophyllene	Tr
1436	trans- α -Bergamotene	Tr
1442	α -Guaiene	0.1
1456	α -Humulene	Tr
1478	γ -Muurolene	Tr
1482	Germacrene D	Tr
1497	Tridecan-2-one	0.3
1500	α -Selinene	0.1
1513	γ -Cadinene	0.4
1525	δ -Cadinene	0.4
1572	Germacren-D-4-ol	Tr
1583	Caryophyllene oxide	0.3
1605	Humulene epoxide II	0.1
1617	Selin-6-en-4 α -ol	0.1
1643	α -Muurolol	0.1
1945	Hexadecanoic acid (Palmiticacid)	0.1
2100	Heneicosane	0.1
2200	Docosane	0.1
	Geranyl geranati	0.1
	Monoterpenes	9.5
	Oxygenated monoterpenes	84.0
	Sesquiterpenes and oxygenated sesquiterpenes	

RI = Retention Index on DB-5 column; nd = not detected; tr = trace (>0.05%); ti = tentative identification



Fig 1: Collection of *C. Citrus* accessions from South India

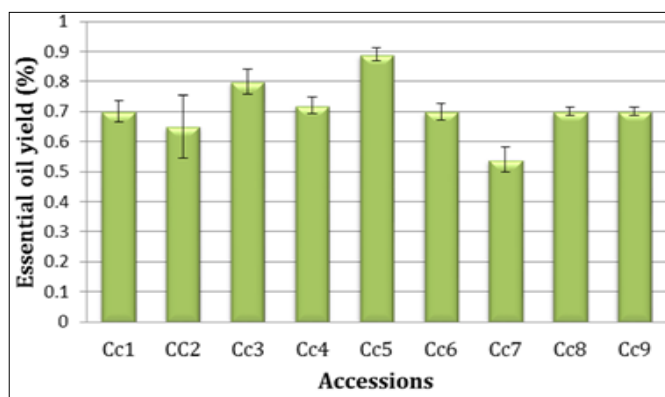


Fig 2: Graphical representation showing essential oil yield from accessions of *C. Citrus*

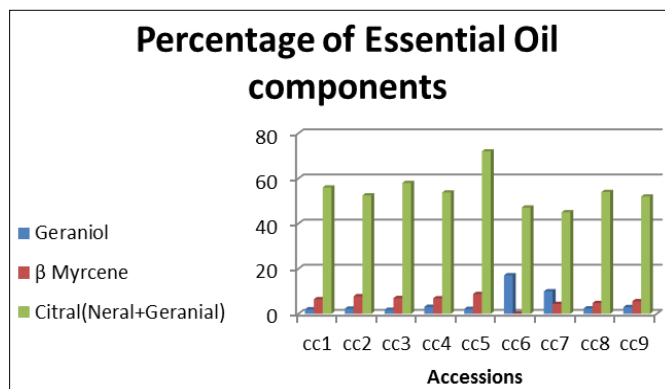


Fig 3: Percentage of major components of essential oil in accessions of *C. citratus*

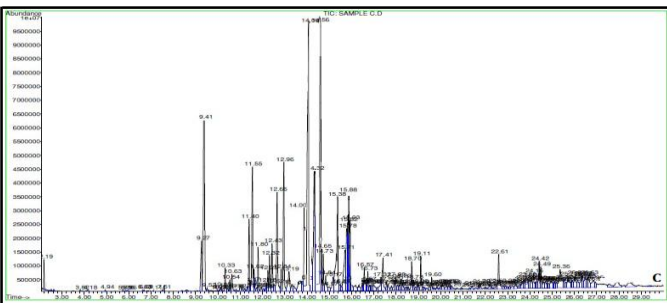
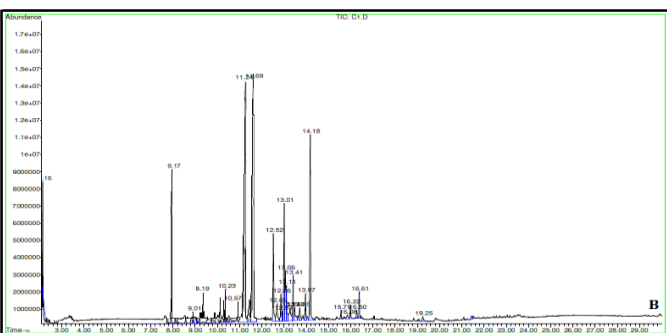
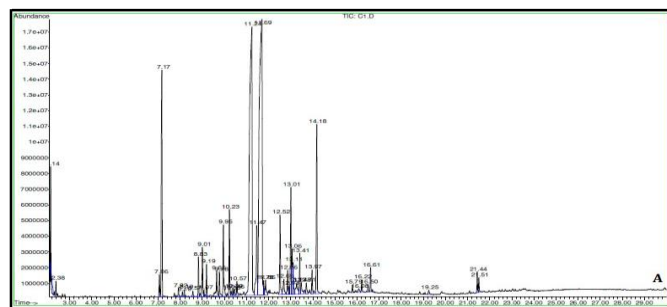


Fig 4: Gas Chromatogram of essential oil from accessions of *C. citratus* (Karnataka) A-Cc1, B-Cc2, C-Cc3

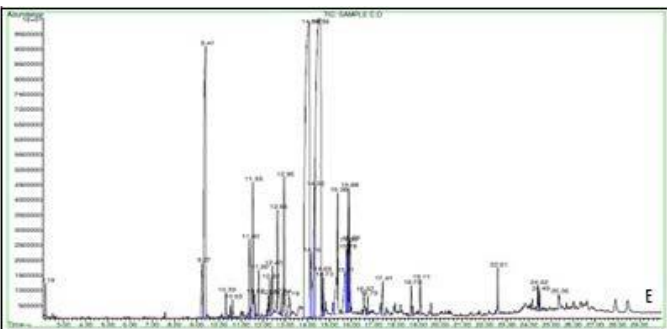
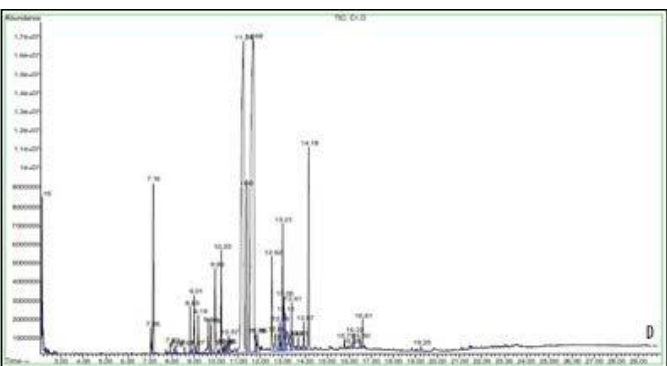


Fig 5: Gas Chromatogram of essential oil from *C. citratus* accessions (Karnataka) D-Cc4 and E-Cc5

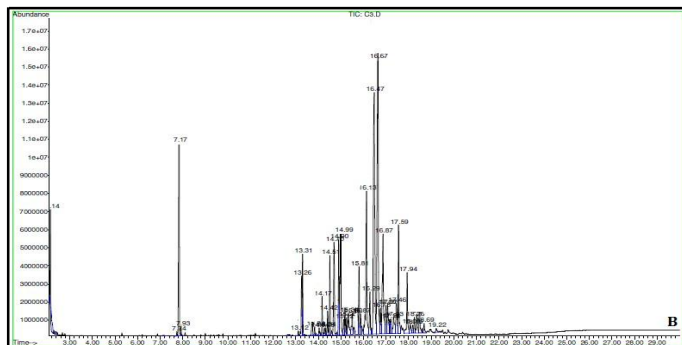
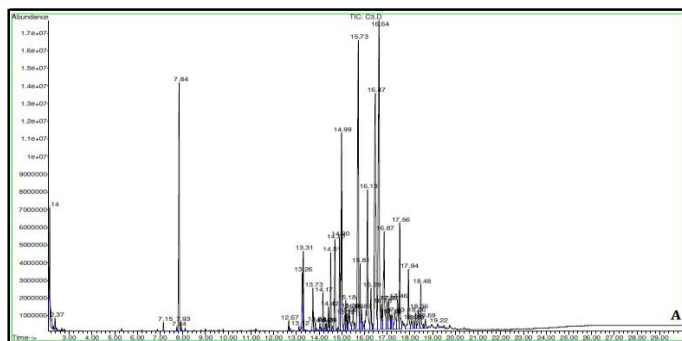


Fig 6: Gas Chromatogram of essential oil from accessions of *C. citratus* (Tamil Nadu) A-Cc6 and B-Cc7

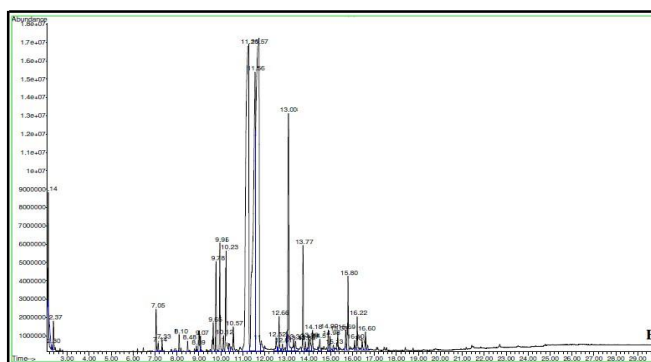
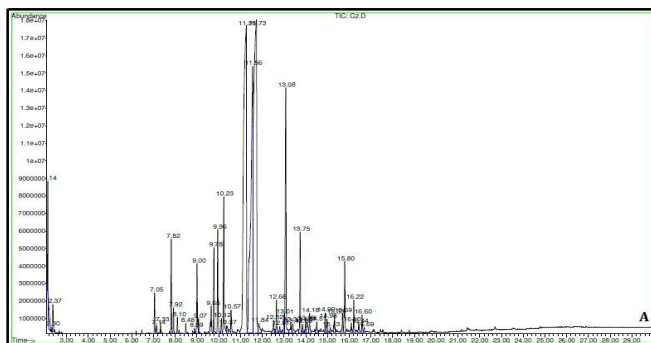


Fig 7: Gas Chromatogram of essential oil from accessions of *C. citratus* (Kerala) A-Cc8 and B-Cc9

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