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Evaluation of Polar and Non-Polar Fractions of Essential Oil from *Cymbopogon citratus* (DC.) Stapf

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Abstract: The essential oil of *Cymbopogon citratus* collected from Bangalore was fractionated into non-polar and polar fractions using silica column chromatography. The essential oil and the fractions were analyzed by GC and GC-MS. The main constituents of the essential oil were citral [neral (30.4%) + geranial (41.8%)], β- myrcene (8.8%) and geraniol (2.2%) along with traces of sesquiterpenes, aliphatic compounds and phenylpropanoids. GC analysis of the non-polar chromatographic fraction along with the β-myrcene standard showed that the non-polar fraction is rich in β-myrcene (≥93.87%) and the polar fraction contained the oxygenated terpenes viz., citral (neral+geranial), geraniol, linalool, isocitral as major constituents.

Keywords: *Cymbopogon citratus*, column chromatography, GC-MS, citral, β- myrcene

INTRODUCTION

Cymbopogon citratus (DC.) Stapf (*Andropogon citratus* DC.) popularly known as West Indian lemongrass belonging to the family Poaceae is a native of South India, Ceylon[1,2] and Malaysia[3]. It is largely cultivated in several countries particularly West Indies, China, Indonesia, Brazil, Congo, Republic of Malagasy, Sri Lanka, Zambia and other countries[4,5]. The lemon grass is used in fragrance and flavouring and for wide variety of ailments in folk medicine for treating nervous, gastrointestinal disturbances, fevers, hypertension, coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders[6-8]. The herb grows in dense clumps erupting from

tough bulbous base with a spread of about 1 meter wide and about 3 feet in height, a cultigen which rarely flowers. The leaves are bright green with sharp edges appearing similar to grasses. It flourishes well in fertile sandy soil with tropical climate receiving heavy rain.

The plant is grown principally for its qualities in perfumery and in medicine, qualities that derive from the fragrant oil that the grass yields, commercially known as 'West Indian Lemongrass oil'. According to the Indian Materia Medica, lemon grass oil is useful as carminative in flatulent and spasmodic affections of the bowels, colic, gastric irritability and is of great value in cholera with obstinate vomiting. Several investigations have demonstrated the antimicrobial[6-8], antioxidant[9], insecticidal and insect repellant properties[10,11]. The essential oil of *C. citratus* have been demonstrated for anticarcinogenic activities[12,13] , anti-inflammatory, vasorelaxing, diuretic and a valuable remedy in treating ringworm as local application[14]; lemongrass oil was claimed to have antihelmintic activity[15] .

The studies on essential oil composition of *C. citratus* reports citral, β-myrcene (characteristic and active ingredient of lemongrass oil), geraniol, geranyl acetate, piperitone, limonene, elemecin, monoterpene alcohols and sesquiterpenes as major constituents[16-25].The West Indian lemongrass oil is considered inferior to East Indian lemongrass oil (*C. flexuoses*) because the oil has low solubility in 70% alcohol and low citral content. The West Indian oil has tendency to polymerize due to high monoterpene β-myrcene (5-25%) content, which deteriorates the quality of oil[4]. Hence the rationale of the study was to increase the stability and long time storage ability (shelflife) of the West Indian essential oil by fractionating the β-myrcene component.

MATERIALS AND METHODS

Experimental Plant material: The plants were collected from Bangalore nursery and maintained at Department of Biotechnology, Bangalore University, Bangalore. The voucher specimen of the plant was authenticated and deposited at National Ayurveda Dietetics Research Institute, Bangalore (voucher specimen no RRCBI-Mus/06).

Isolation of essential oil: The shade-dried leaf material from the plant (200 g) was subjected to hydro-distillation in Clavenger apparatus[26] for 3 hrs. The pale yellow essential oil with strong, fresh citrus odor obtained were collected and dried over anhydrous Na_2SO_4 and stored in refrigerator until analyzed.

Fractionation of essential oil using column chromatography: The essential oil was fractionated into non-polar fraction using petroleum ether (AR 40-80ºC) and polar fraction using methanol (AR) on packed silica gel (size 60-120 mesh) column.

Standard: β-myrcene (Sigma-Aldrich)

Gas Chromatographic and Gas Chromatographic-Mass Spectral analysis: GC analysis of the oil sample, fractions and β-myrcene standard was performed on an Agilent Technologies gas chromatograph Model 6890N equipped with dual FID. A CPSil8CB 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^{o}C (2 min.) -280^{o}C at 10^{o}C per min. Injector and detector temperature were maintained at 250^{o}C and 280^{o}C respectively. Samples of 1 µl dissolved in hexane were injected using a split ratio of 10:1. GC-MS was done using the above GC interfaced with mass selective detector (Agilent technologies 5973). The same stationary phase as GC was used. Helium was used as the carrier gas. GC conditions same as above. Mass spectra were recorded in the EI mode at 70 eV in the m/z range of 30-450.

Identification of compounds: The essential oil components were identified by comparison of linear retention indices of GC peaks obtained using alkanes (C8-C25) with those of compounds reported in literature [27] and by comparison of the mass spectra of the peaks with those of compounds from literature[27] and those stored in NIST library. Peak area percentages were computed from GC peak areas without using correction factors.

RESULTS

The percentage essential oil distilled from leaf herbage of the plant was 0.7% (dry wt basis). GC and GC-MS analyses of the Clavenger distilled essential oil was found to be rich in monoterpenes (9.5%), oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes (84%), aliphatic compounds and phenylpropanoids. Citral [neral (30.4%) + geranial (41.8%)], β- myrcene (8.8%) and geraniol (2.2%) were found as a major component of the oil. **Figure 1** represents the gas chromatogram of the essential oil. The essential oil of *C. citratus* was separated into two major fractions (non-polar and polar) using silica column chromatography. The fractions recovered from column chromatography were subjected to GC and GC-MS analyses. GC analysis of non-polar chromatographic fraction along with β-myrcene standard showed that the non-polar fraction was rich in β-myrcene (≥93.87%) constituent whereas the polar chromatographic fraction contained oxygenated terpenes viz., citral (neral+geranial), geraniol, linalool, isocitral as major constituents. **Figure 2, 3, 4** represents the gas chromatogram of the fractions and standard β-myrcene.

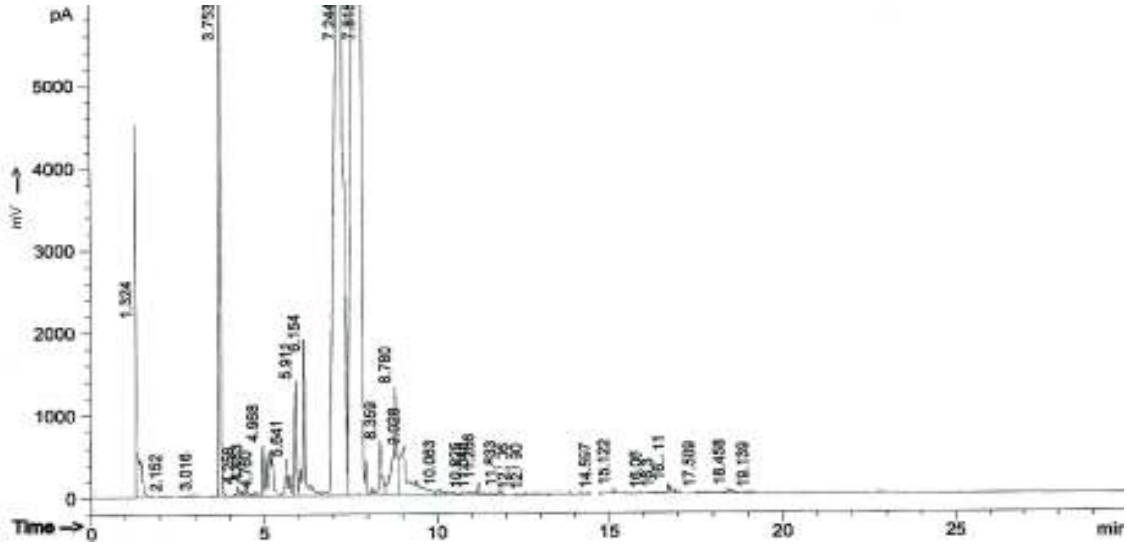

Fig 1: Chromatogram of essential oil

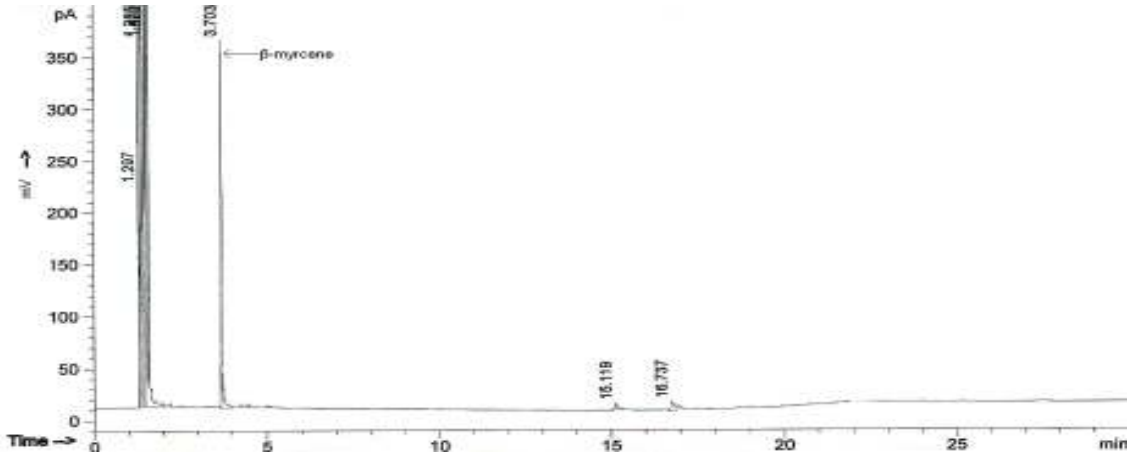

Fig 2: Chromatogram of non-polar fraction of essential oil

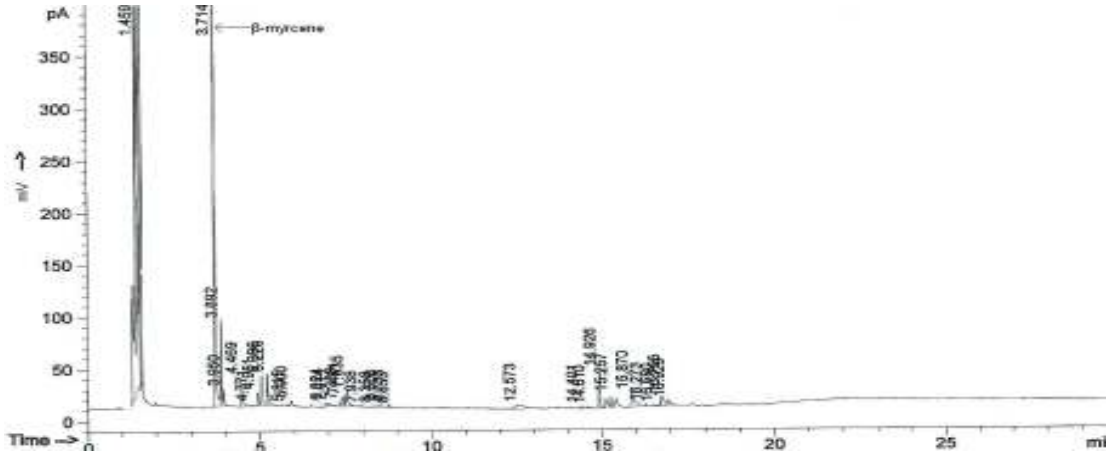

Fig 3: Chromatogram of β- myrcene (Std)

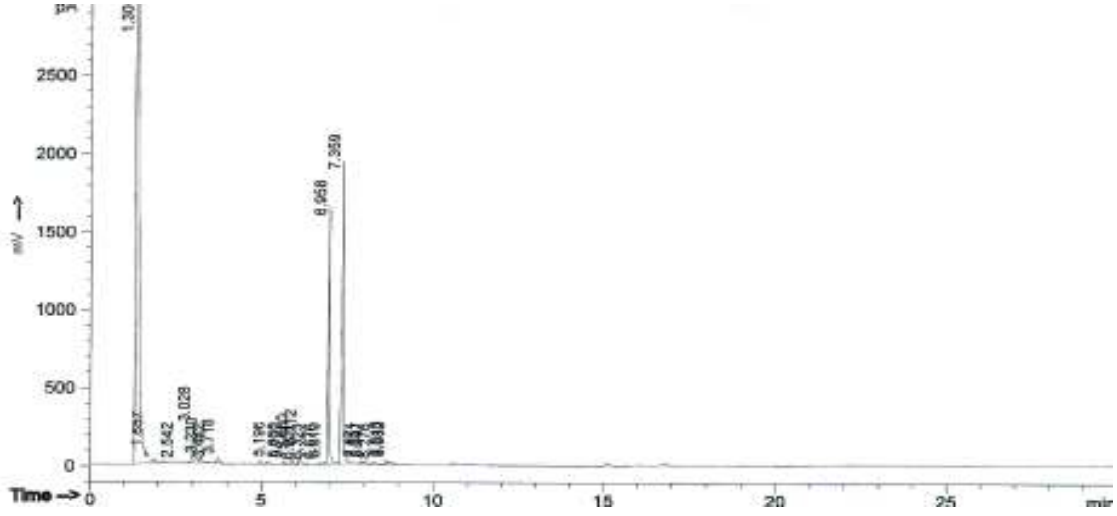

Fig 4: Chromatogram of polar fraction of essential oil

DISCUSSION

Column chromatography is a valid method for fractionation of monoterpenoids. Isocratic elutions of monoterpenoids with solvents such as pentane, petroleum ether, hexane or gradient elution with mixture of solvents with increasing polarity lead to successive isolation[28]. Terpenoids are lipid-soluble compounds and are extracted with petroleum ether. The results obtained showed that column chromatography using petroleum ether is efficient for separation of β-myrcene fraction from the essential oil which avoids deterioration of essential oil quality. The non-polar fraction (β-myrcene rich) can be utilized for commercial applications which serve as intermediate for production of large volume aroma and flavor chemicals[29-32].

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