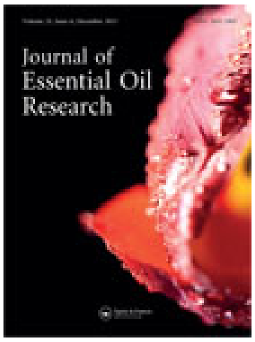

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Volatile oil composition of vegetative and reproductive parts of lemon-scented gum (*Eucalyptus citriodora* Hook.)

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Lemon-scented gum (*Eucalyptus citriodora* Hook.) is an important aromatic tree species, cultivated in different parts of the world for the production of citronellal-rich essential oil from its leaves. In present study, essential oils derived from leaves, flowers and fruits of the plant were investigated and compared in detail using gas chromatography/flame ionization detector (GC/FID) and GC/mass spectrometry (GC/MS). Altogether, sixty-nine constituents, representing 92.9–97.5% of the total oil compositions were identified. Leaf oil was represented by citronellal (86.0%), as the chief constituent. Flower oil was dominated by citronellal (34.9%), *α*-pinene (22.0%), citronellol (11.9%) and *β*-pinene (4.6%), while the fruit oil contained *α*-pinene (40.7%), *γ*-terpinene (11.8%), citronellal (7.1%) and *p*-cymene (6.5%) as major components.

Keywords: *Eucalyptus citriodora*; essential oil; GC/MS; citronellal; *α*-pinene

1. Introduction

Eucalyptus is a large genus that belongs to the family Myrtaceae, of evergreen trees and shrubs, containing about 700 species (1). Most of the *Eucalyptus* spp. are native to Australia and Tasmania (2); however, they have been successfully introduced worldwide and are being cultivated in many other countries, including India. The essential oil of *Eucalyptus* spp. finds uses in pharmaceutical, cosmetics and food industries (3–5). *Eucalyptus citriodora* Hook., commonly known as lemon-scented gum, is the most common species cultivated for the isolation of essential oil. It is used in anti-inflammatory and antipyretic remedies and against the symptoms of respiratory infections, such as colds, flu and sinus congestion (6). The essential oil from the leaves is purported to have number of biological activities, including antimicrobial (7), nematicidal (8), allelopathic (9) insecticidal (10) and antioxidant activities (11).

The essential oil of *E. citriodora* is mainly isolated from the leaves and hence its composition has been investigated by many researchers across the world (12–23). However, to the best of our knowledge, no attempt has ever been made to explore the essential oil composition of flowers and fruits of *E. citriodora* from anywhere and the composition from leaf oil from the foothills of north India. Therefore, in the present research, essential oils derived from leaves, flowers and fruits of the lemon-scented gum have been analyzed and compared.

2. Experimental

2.1. Plant material

Fresh leaves, flowers and fruits of the *E. citriodora* were collected from CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Pantnagar (Uttarakhand) in the last week of November (2011), when the plants were in flowering/fruiting stage. The experimental site is located between coordinates 29°N, 79.38°E, at an altitude of 243.84 m in foothills of north India. The plant material was authenticated at botany department of the centre by one of the author (CIMPANT-342).

2.2. Isolation of essential oil

Isolation of the essential oil from fresh leaves, flowers and fruits (crushed) was carried out by hydrodistillation in a Clevenger-type apparatus for 5 hours. Isolated oils were dried over anhydrous Na_2SO_4 and stored at 4°C prior to analysis.

2.3. Capillary GC/FID and GC/MS

Gas chromatography (GC) analysis of the essential oils was carried out on a Perkin–Elmer AutoSystem XL gas chromatograph or Nucon gas chromatograph model 5765, fitted with an Equity-5 capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm; Supelco Bellefonte, PA, USA) and flame ionization detector (FID). The oven column temperature ranged from 70° to 250°C, programmed at 3°C/minute, with initial and final

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Table 1. Comparative essential oil profiles of the different parts of *Eucalyptus citriodora* from India.

		Content (%)					Content (%)		
Compound	RI	I	II	III	Compound	RI	I	II	III
α-Thujene	928	–	0.6	0.1	α-Ylangene	1369	t	–	t
α-Pinene	932	0.1	22.0	40.7	Geranyl acetate	1378	0.2	t	–
Camphene	946	–	–	t	Isobutyl phenylacetate	1384	–	–	t
β-Pinene	972	0.4	4.6	2.0	β-Elemene	1394	0.1	–	–
Myrcene	992	0.1	t	–	(*Z*)-Jasmone	1396	0.1	0.1	0.1
α-Phellandrene	1002	0.1	0.1	0.1	(*E*)-Caryophyllene	1420	0.6	1.9	0.8
α-Terpinene	1018	–	0.1	0.3	*trans*-α-Bergamotene	1429	–	t	t
p-Cymene	1021	t	0.6	6.5	Aromadendrene	1435	–	t	0.2
Limonene	1024	0.2	1.7	2.4	*cis*-Muurola-3,5-diene	1449	–	–	0.5
1,8-Cineole	1026	–	1.4	0.5	α-Humulene	1454	0.1	t	0.1
(*Z*)-β-Ocimene	1030	–	–	0.1	Allo-aromadendrene	1460	–	t	0.1
(*E*)-β-Ocimene	1046	t	t	–	Viridiflorene	1497	1.2	0.4	0.6
Bergamal	1051	0.2	t	t	α-Muurolene	1500	–	0.1	0.4
γ-Terpinene	1054	t	2.7	11.8	*cis*-Calamenene	1531	0.1	0.1	1.1
Acetophenone	1063	–	–	t	*trans*-Cadina-1,4-diene	1535	0.1	–	t
m-Cymenene	1085	–	1.5	1.4	α-Calacorene	1548	–	–	0.2
Linalool	1096	0.3	0.1	0.6	Spathulenol	1578	0.1	0.1	0.5
Isopentyl 2-methyl butanoate	1099	–	–	t	Caryophyllene oxide	1584	0.1	0.4	1.4
p-1,3,8-Menthatriene	1104	–	–	t	β-Atlantol	1610	t	t	0.2
endo-Fenchol	1111	–	–	0.1	10-*epi*-γ-eudesmol[d]	1624	–	t	0.2
α-Campholenal	1123	0.1	0.1	t	γ-Eudesmol	1630	0.1	1.0	3.1
trans-Pinocarveol	1136	–	–	t	*epi*-α-Cadinol	1642	–	–	t
Isopulegol	1146	t	t	1.4	α-Muurolol	1645	–	–	t
Citronellal	1151	86.0	34.9	7.1	β-Eudesmol	1653	0.1	1.1	0.8
iso-Isopulegol	1154	t	1.0	0.6	α-Eudesmol	1655	t	0.9	0.9
Borneol	1168	t	t	0.1	*cis*-Calamenen-10-ol	1663	–	–	t
neoiso-Isopulegol	1169	0.3	0.2	0.1	*trans*-Calamenen-10-ol	1671	0.5	–	t
Terpinen-4-ol	1176	0.3	0.4	1.4	(2*E*,6*Z*)-Farnesol	1712	–	t	t
p-Cymen-8-ol	1184	–	–	t	Unidentified[a]	2036	–	0.5	0.3
α-Terpineol	1190	t	t	2.4	Unidentified[b]	2173	–	0.4	0.1
Myrtenal	1198	–	0.8	t	Unidentified[c]	2206	–	0.2	0.2
Verbenone	1207	–	t	t	Grouped components				
Citronellol	1224	4.5	11.9	1.5	Monoterpene hydrocarbons		0.9	31.8	57.5
Geraniol	1255	0.2	0.1	t	Oxygenated monoterpenes		93.1	53.9	16.3
cis-Myrtanol	1258	0.1	–	t	Sesquiterpene hydrocarbons		2.2	2.5	4.0
Geranial	1265	t	–	–	Oxygenated sesquiterpenes		0.4	3.5	7.1
Citronellyl formate	1271	t	–	t	Benzenoid compounds		0.6	2.1	7.9
trans-Verbenyl acetate	1290	t	–	t	Others		0.3	0.1	0.1
Citronellic acid	1316	0.3	–	0.3	Total identified (%)		97.5	93.9	92.9
Citronellyl acetate	1350	0.8	3.0	0.2	Essential oil yield (%)[e]		2.2	0.35	0.53
Eugenol	1353	0.1	t	–					

Notes: RI, retention index calculated on DB-5 column; I, leaf essential oil; II, flower essential oil; III, fruit essential oil; [a]m/e, 41(100%), 43, 55, 69, 81, 95, 123, 137, 177, 205, 247, 275, 318, 319; [b]m/e, 43(100%), 77, 93, 105, 119, 136, 196, 223, 252, 277, 281; [c]m/e, 43(100%), 77, 93, 105, 119, 136, 196, 207, 251, 281, 288; t, trace ($<0.05\%$); [d]tentative identification; [e]fresh weight basis.

hold time of 2 minutes, using H_2 as carrier gas at 10 psi constant pressure, a split ratio of 1:35, an injection size of 0.03 μL neat, and injector and detector temperatures of 250° and 280°C, respectively. GC/mass spectrometry (GC/MS) analysis was carried out on a Perkin–Elmer AutoSystem XL GC interfaced with a Turbomass Quadrupole mass spectrometer fitted with an Equity-5 fused silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm; Supelco Bellefonte, PA, USA). The oven temperature programme was the same as described in capillary GC; injector, transfer line and source temperatures were 250°C; injection size 0.03 μL neat; split ratio 1:30; carrier gas He at 10 psi constant pressure; ionization energy 70 eV; mass scan range 40–450 amu.

2.4. *Identification of constituents*

Characterization was achieved on the basis of retention time and retention index, using a homologous series of *n*-alkanes (C_8–C_{30} hydrocarbons), co-injection with standards in a GC/FID capillary column (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data, 7th edition) and by comparing with the mass spectral literature data (24). The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

3. Results and discussion

The essential oil yields observed in fresh leaves, flowers and fruits of *E. citriodora* were 2.2%, 0.35% and 0.53%, respectively. The detailed essential oil compositions of the different parts of *E. citriodora* are summarized in Table 1. A total of sixty-nine constituents, representing 92.9–97.5% of the total oil compositions, were identified. GC/FID chromatograms of the leaves, flower and fruit essential oils with labeled major peaks are shown in Figures 1–3. Leaf oil was found to be dominated mainly by oxygenated monoterpenes (93.1%). The main constituents of the leaf oil were citronellal (86.0%), citronellol (4.5%), viridiflorene (1.2%), citronellyl acetate (0.8%) and (*E*)-caryophyllene (0.6%). Furthermore, the flower oil was characterized by high amounts of oxygenated monoterpenes (53.9%) and monoterpene hydrocarbons (31.8%). The major constituents of the flower oil were citronellal (34.9%), *α*-pinene (22.0%), citronellol (11.9%), *β*-pinene (4.6%), citronellyl acetate (3.0%), *γ*-terpinene (2.7%), (*E*)-caryophyllene (1.9%), limonene (1.7%), *m*-cymenene (1.5%), 1,8-cineole (1.4%), *β*-eudesmol (1.1%), iso-isopulegol (1.0%) and *γ*-eudesmol (1.0%). Moreover, the fruit oil was found to be rich in monoterpene hydrocarbons (57.5%), followed by oxygenated monoterpenes (16.3%), benzenoid compounds (7.9%) and oxygenated sesquiterpenes (7.1%). The individual major constituents of the fruit oil were *α*-pinene (40.7%), *γ*-terpinene (11.8%), citronellal (7.1%), *p*-cymene (6.5%), *γ*-eudesmol (3.1%), limonene (2.4%), *α*-terpineol (2.4%), *β*-pinene (2.0%), citronellol (1.5%), *m*-cymenene (1.4%), isopulegol (1.4%), terpinen-4-ol (1.4%), caryophyllene oxide (1.4%) and *cis*-calamenene (1.1%).

The essential oil composition of the leaves of *E. citriodora* has been reported by many researchers from different parts of the world. It was found that the citronellal content varied from 79.9% to 86.0% in the top, middle and lower leaves of *E. citriodora* grown in

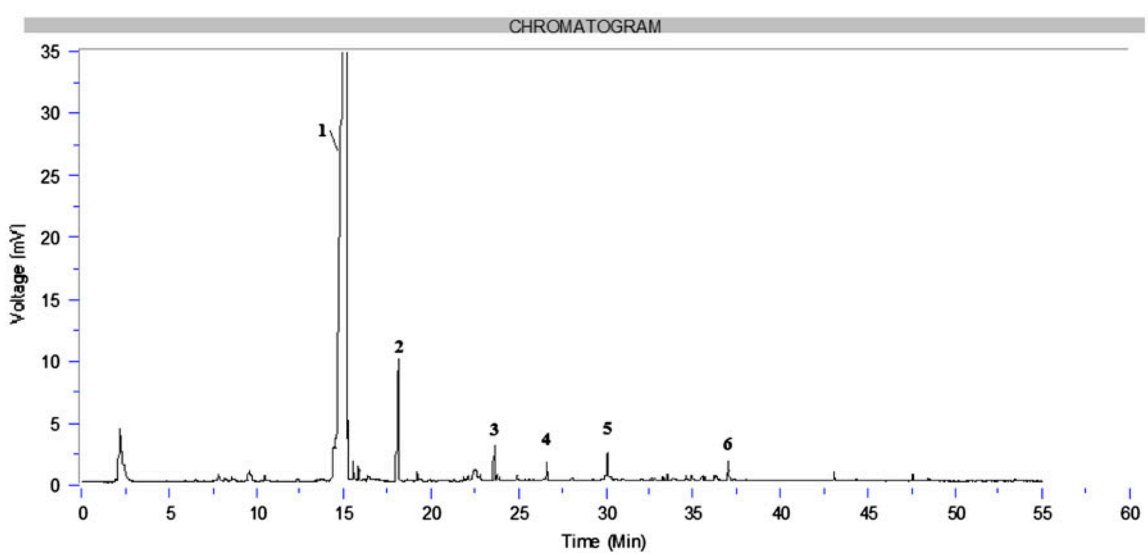

Figure 1. Chromatogram (gas chromatography/flame ionization detector) of *Eucalyptus citriodora* leaf essential oil (peaks; **1**: citronellal, **2**: citronellol, **3**: citronellyl acetate, **4**: (*E*)-caryophyllene, **5**: viridiflorene; **6**: *cis*-calamenen-10-ol. For other minor/trace constituents, please see Table 1.

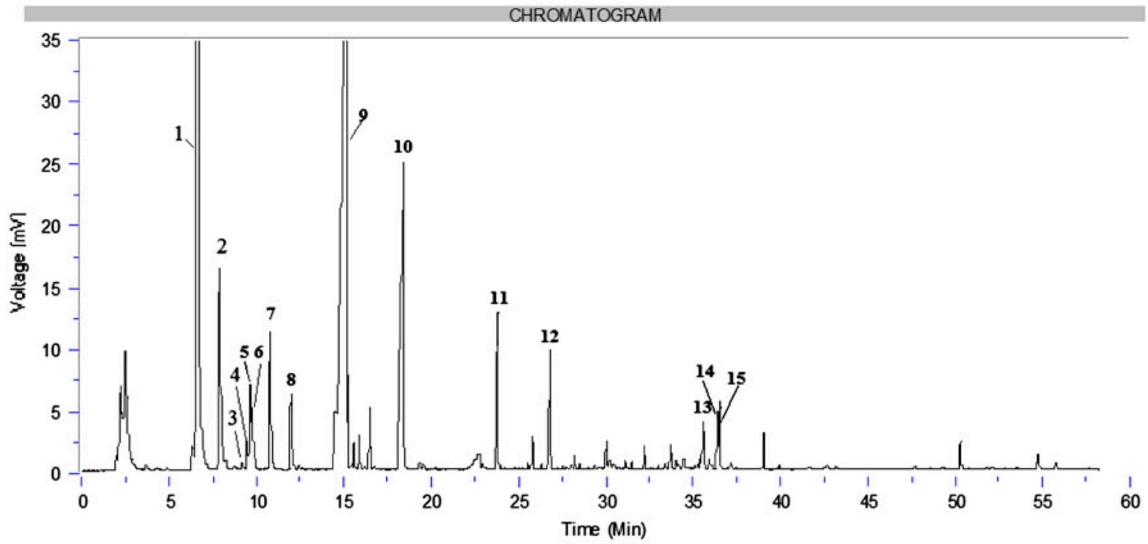

Figure 2. Chromatogram (gas chromatography/flame ionization detector) of *Eucalyptus citriodora* flower essential oil (peaks; **1**: α-pinene, **2**: β-pinene, **3**: α-terpinene; **4**: *p*-cymene, **5**: limonene, **6**: 1,8-cineole, **7**: γ-terpinene, **8**: *m*-cymenene, **9**: citronellal, **10**: citronellol, **11**: citronellyl acetate, **12**: (*E*)-caryophyllene, **13**: γ-eudesmol; **14**: β-eudesmol, **15**: α-eudesmol. For other minor/trace constituents, please see Table 1.

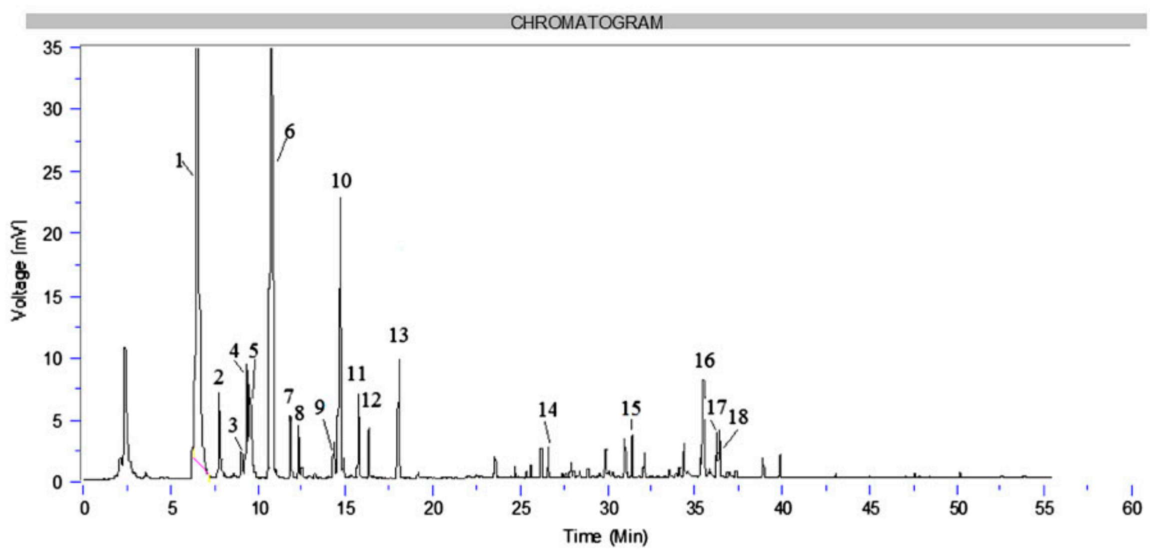

Figure 3. Chromatogram (gas chromatography/flame ionization detector) of *Eucalyptus citriodora* fruit essential oil (peaks; **1**: α-pinene, **2**: β-pinene, **3**: α-terpinene; **4**: *p*-cymene, **5**: limonene, **6**: γ-terpinene, **7**: *m*-cymenene, **8**: linalool, **9**: isopulegol, **10**: citronellal, **11**: terpinen-4-ol, **12**: α-terpineol, **13**: citronellol, **14**: (*E*)-caryophyllene, **15**: *cis*-calamenene, **16**: γ-eudesmol; **17**: β-eudesmol, **18**: α-eudesmol. For other minor/trace constituents, please see Table 1.

a sub-temperate region, India (15). However, the citronellal content varied from 69.7% to 87.4% in leaf oil during the year under subtropical conditions of India (13). Loumouamou et al. (18) reported leaf volatile oil composition from Congo, which having 57.1–75.4% citronellal. Mondello et al. (19) investigated leaf essential oil composition of this species growing in Bangladesh and found citronellal content up to 77.0% (19). Furthermore, the essential oils of *E. citriodora* of Benin origin contained 79.7% and 72.2% average citronellal in two locations, namely Calavi and Ketou, respectively (20). However, the leaf oil of N'Debougou origin contained 76.3% citronellal (21). Chalchat et al. (22) investigated the leaf essential oil of this plant from Mali and found 77.6–78.5% citronellal. Moreover, the leaf oil of *E. citriodora* from Ethiopia possessed 73.3%

citronellal (23). Thus, the leaf essential oil of *E. citriodora* grown in different countries was rich in citronellal and matched well with the leaf oil composition investigated in present study. However, the presence of higher citronellal content (86.0%) in Indian *E. citriodora* oil makes it superior over the other oils reported from different countries. Furthermore, the comparison of volatile oils of reproductive parts (flower and fruit oils) with vegetative parts (leaf oil) showed comprehensive differences, especially in the lower content of citronellal and dominance of monoterpene hydrocarbons in the former.

4. Conclusions

It is evident from the study that the volatile oil yield and composition of flowers and fruits are quite different from the oil yield and composition of the leaves of *E. citriodora*. Thus, the potential of flower and fruit of *E. citriodora* could be explored as a source of a new type of oil. Furthermore, the inclusion of reproductive parts with leaves during the commercial extraction of *E. citriodora* oil should be avoided, as it could deteriorate the quality of oil in term of citronellal content.

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