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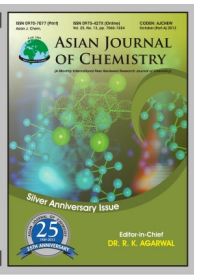

Comparative Chemical Profiles of *Citrus aurantifolia* Essential Oils from South India

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Essential oils isolated by hydrodistillation of the leaves and fruit peels of *Citrus aurantifolia* collected from five geographical locations in Kerala *viz*., Idukky, Thrivanthapuram, Pathanamthitta, Alappuzha and Tamil Nadu were analyzed by gas chromatography-mass spectrometry. Individual essential oil constituents were identified from their mass spectra, NIST database search, linear retention index data and comparison of spectra with literature. Twenty seven to thirty five constituents (99.98-100.03 %) were identified in *C. aurantifolia* peel oils whereas thirty five to forty constituents (94.59-100.02 %) were identified in its leaf oils. Monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons in peel oils were in the range 71.93-75.05, 10.44-13.81 and 13.24-17.63 %, respectively. Similarly, monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons in leaf oils were 18.66-29.66, 49.24-66.06 and 10.57-20.00 %, respectively. Limonene (37.8-41.93 %), β-pinene (12.78-15.39 %) and α-terpinene (8.02-12.36 %) were the major constituents in peel oils of *C. aurantifolia*. Limonene (17.33-26.14 %), perilla ketone (0-22.54 %), citronellyl formate (20.76-25.79 %), geranyl acetate (3.65-6.14 %) and (E)-caryophyllene (0-9.72 %) were the major constituents in *C. aurantifolia* leaf oils. Near similar terpenoid profiles were found in the volatile oils of *C. aurantifolia* collected from five geographical locations in south India.

Key Words: *C. auruntifolia*, Limonene, β-Pinene, α-Terpinene, Citronellyl formate, Geranyl acetate, Perilla ketone, (E)-Caryophyllene.

INTRODUCTION

Citrus fruits are commercially used worldwide in beverages, food products, fine perfumes and other cosmetic products, flavours, soaps and detergents, pharmaceuticals, toothpastes, air freshners and in traditional medicine[1]. Most important *Citrus* fruits are sweet orange, lime, lemon and grapefruit[2]. *Citrus auruntifolia* belongs to the family Rutaceae. It is an evergreen tree which is around 5 m tall with dense and irregular branches. Its twigs are armed with short, stiff, sharp spines. *C. auruntifolia* is native to northern India and is now cultivated throughout the tropics and subtropical areas such as USA, Mexico, Egypt and West Indies. It is commonly known as 'lime' and its fruits are widely used to flavour food in south-east Asia. Lime fruit drinks are also used for a variety of medicinal applications[3]. Its flavour and acid taste make it an additive in hot and spicy dishes[4]. Lime leaves and fruits have many medicinal uses.

Chemical profiles of volatile oils of the leaves and peels of *C. aurantifolia* from various geographical locations have been studied by gas chromatographic techniques[5,6]. Previous studies on *C. aurantifolia* leaf and peel oils (collection location, plant part/oil, analysis techniques, major constituents): (i) Banes, Cuba, commercial distilled lime oil, GC-MS, β-pinene 2.9 %, limonene 40.4 %, γ-terpinene 9.5 %, terpinolene 8.7 %, α-terpineol 12.7 %[7], (ii) Bangalore, India, peel oils at six ripening stages, GC, (dark green fruit) β-pinene 13.5 %, limonene 33.0 %, γ-terpinene 7.9 %, geraniol 7.3 %, geranial 3.6 %; (fully yellow fruits) β-pinene 23.8 %, limonene 47.0 %, γ-terpinene 9.9 %, geraniol 0.9 %, geranial 0.7 %[8]. Venkateshwarlu and Selvaraj[8] found a decrease in oxygenated monoterpenes and an increase in monoterpene hydrocarbons during fruit ripening, (iv) Bangalore, India, peel oils; capillary GC-FID, Co-GC; (dark green fruits) limonene 35.3 %, β-pinene 15.1 %, γ-terpinene 8.4 %, geraniol 5.9 %, neral 3.6 %, geranial 3.0 %; (yellow fruits) limonene 46.1 %, β-pinene 22.8 %, γ-terpinene 9.8 %, geraniol 1.2 %, neral 2.0 %, geranial 0.8 %; (ethylene treated yellow fruits) limonene 37.5 %, β-pinene 15.5 %, γ-terpinene 9.8 %, geraniol 1.8 %, neral 2.3 %, geranial 0.8 % and (acetylene treated yellow fruits) limonene 44.1 %, β-pinene 17.4 %, γ-terpinene 6.8 %, geraniol 4.4 %, neral 1.0 %, geranial 1.0 %[9], (v) Abidjan, Ivory Coast, leaf oil, GC, GC-MS, limonene 50.1 %, α-terpineol 14.2 %, β-pinene 6.6 %, γ-terpinene 6.4 %, terpinolene 2.8 %[10], (vi) Yaounde,

Cameroon, fresh leaves, *C. aurantifolia* var Bearss, Mexican, 'Sans épines', GC-FID, GC-MS, limonene 53.9 % (Bearss), 43.5 % (Mexican), 51.9 % (Sans épines); nerol 1.4-3.9 %, neral 7.7-10.0 %, geraniol 1.3-4.6 %, geranial 10.9-12.6 %, geranyl acetate 2.0-3.2 %[11], (vii) Patoki, Lahore, Pakistan, peel oil, GC-MS, limonene 82.8 %, γ-terpinene 8.5 %[12]. (viii) Texas, USA, fruit oil, GC-MS, mature lime, d-limonene 30.1 %, d-dihydrocarvone 30.5 %, *m*-mentha-6,8-diene R(+) 9.3 %, α-terpineol 5.9 %[4]. As evident in these previous reports, limonene has been the major component in *C. aurantifolia* oils, but other major and minor constituents varied based on maturity of fruits and leaves, collection locations, oil isolation and analytical techniques[4,7-13]. So far there are no systematic reports on the chemical profiles of *C. aurantifolia* volatile oils from south India. This study describes the comparative chemical profiles of leaf and peel oils isolated from *C. aurantifolia* collected from five locations in south India.

EXPERIMENTAL

Plant samples: Fresh leaves and mature, ripened fruit peels of five accessions of *C. aurantifolia* were collected from October to December 2011 from five different locations, (*Citrus aurantifolia* collected from Idukki, Kerala (CA-ID-1), Thiruvanthapuram (CA-TH-2), Pathanamthitta (CA-PA-3), Alappuzha (CA-AL-4), Tamil Nadu (CA-TA-5), in the south Indian states of Kerala and Tamil Nadu.

Oil isolation and analysis: Essential oils from fresh leaves and peels of five accessions of *C. aurantifolia* were isolated separately by hydrodistillation for 5 h on a Clevenger apparatus. Pleasant smelling, transparent, yellow coloured oils were obtained from the leaves and peels of *C. aurantifolia*. CA-ID-1: leaves 200 g, oil yield 0.2 mL (0.1 %, v/w); peels 250 g, oil yield 1.5 mL (0.6 %, v/w); CA-TH-2: leaves 300 g, oil yield 0.3 mL (0.1 %, v/w); peels 450 g, oil yield 2.16 mL (0.48 %, v/w); CA-PA-3: leaves 275 g, oil yield 0.28 mL (0.1 %, v/w); peels 325 g, oil yield 1.20 mL (0.37 %, v/w); CA-AL-4: leaves 200 g, oil yield 0.2 mL (0.1 %, v/w); peels 250 g, oil yield 0.80 mL (0.32 %, v/w); CA-TA-5: leaves 350 g, oil yield 0.49 mL (0.14 %, v/w); peels 450 g, oil yield 2.07 mL (0.46 %, v/w). *C. aurantifolia* leaf and peel oils were stored at 4 ºC until further analysis.

GC-MS analysis: *C. aurantifolia* leaf and peel oils were analyzed by injecting 0.1 µL of each oil in split mode onto a Varian CP3800 GC-Saturn 2200 ion trap mass spectrometer fitted with a VF-5 (5 % phenyl 95 % dimethylpolysiloxane, non-polar, 30 m × 0.25 mm i.d., 0.25 µm film thickness) capillary column (Varian Inc., USA). GC-MS operation conditions: injector temperature 220 ºC; transfer line 240 ºC; detector temperature 250 ºC; oven temperature programme 60-246 ºC (3 ºC/min); carrier gas He 1.4 mL/min; mass spectra-electron impact (EI+) mode 70 eV and ion source temperature 240 ºC.

Identification of oil constituents: Linear retention indices of oil constituents were determined on the VF-5 column, using standard C5-C30 straight chain hydrocarbons (Aldrich Chemical Company, USA). Individual compounds in *C. aurantifolia* volatile oils were identified by NIIST database matching, comparison of mass spectra with Adam's database[13] and by comparison of their linear retention indices (Tables 1 and 2).

RESULTS AND DISCUSSION

Oil yields in *C. aurantifolia* leaves and peels ranged from 0.10 to 0.14 %, (v/w) and 0.32-0.60 %, (v/w), respectively. In *C. aurantifolia* leaf oils from Idukki (CA-ID-1), Thiruvananthapuram (CA-TH-2), Pathanamthitta (CA-PA-3), Alappuzha (CA-AL-4) and Tamil Nadu (CA-TA-5) 35 to 40 constituents (94.59-100.02 %) were identified by GC-MS analysis (Table-1). Monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons in leaf oils were 18.66-29.66, 49.24-66.06 and 10.57-20.00 %, respectively. Limonene (17.33-26.14 %), perilla ketone (0-22.54 %), citronellyl formate (20.76-25.79 %), geranyl acetate (3.65-6.14 %) and (E)-caryophyllene (0-9.72 %) were the major terpenoid constituents in *C. aurantifolia* leaf oils. Nerol (0-2.89 %), neral (0-11.08 %) and neryl acetate (0-1.79 %) were also found in the leaf oils.

In *C. aurantifolia* peel oils from the five geographical locations, 27 to 35 components (99.98-100.03 %) were identified by GC-MS analysis (Table-2). Monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons in peel oils were in the range 71.93-75.05, 10.44-13.81 and 13.24-17.63 %, respectively. Limonene (37.8-41.93 %), α-terpinene (8.02-12.36 %), β-pinene (12.78-15.39 %), δ-cymene (2.06-6.00 %) and *cis*-β-guaiene (0-4.43 %) were the major constituents in *C. aurantifolia* peel oils. Nerol (0-1.31 %), citronellol (0-0.13 %), citronet (0-2.12 %), neral (0-2.28 %) and geraniol (0-1.79 %) were also found in the peel oils.

C. aurantifolia peel oils from south India, with higher oil yields compared to leaf oils, are good sources of limonene (37.8-41.93 %). Limonene is a common additive in cosmetic products. It is also used in food products, medicines and as an insecticide. *C. aurantifolia* leaf oils showed relatively low contents of limonene (17.33-26.14 %). Citronellyl formate (20.76-25.79 %) and perilla ketone (0-22.54 %) were two major constituents in leaf oils. Perilla ketone is a toxic terpenoid molecule which could prevent herbivore and microbial attack on *C. aurantifolia* leaves[14]. Perilla ketone was not found in *C. aurantifolia* peel oils. Again the contents of citronellyl formate in *C. aurantifolia* peel oils were very low (0-2.79 %) compared to leaf oils (20.76-25.79 %). Monoterpene hydrocarbons were high in peel oils (71.93-75.05 %) compared to leaf oils (18.66-29.66 %). This is compensated by oxygenated monoterpenes in *C. aurantifolia* peel (10.44-13.81 %) and leaf (49.24-66.06 %) oils.

Nerol is the *cis*-isomer of geraniol and both of them have a sweet rose-like odour. Geranial and neral are aldehydes of geraniol and nerol. Citronellol is dihydrogeraniol and citronellal is its aldehyde. Neral is the primary source of sweet lemon odour in citrus oils. The volatile terpenoid mix in *C. aurantifolia* peel oils with limonene (37.8-41.93 %), α-terpinene (8.02-12.36 %), β-pinene (12.78-15.39 %), δ-cymene (2.06-6.00 %), *cis*-β-guaiene (0-4.43 %) as major components and geraniol (0-1.79 %), nerol (0-1.31 %), neral (0-2.28 %), geranial (0-2.82 %), citronellol (0-0.13 %), citronet (0-2.12 %) as minor constituents makes them a potential candidates for perfumery, nutrition and medicinal applications[15].

TABLE-1
COMPARATIVE CHEMICAL PROFILES OF *C. aurantifolia* LEAF OILS FROM FIVE GEOGRAPHICAL LOCATIONS IN SOUTH INDIA

Constituents	LRI (cal.)	LRI (lit.)	CA-ID-1 (%)	CA-TH-2 (%)	CA-PA-3 (%)	CA-AL-4 (%)	CA-TA-5 (%)
α-Pinene	928	932	t	t	–	t	0.12
trans-Isolimonene	981	980	t	0.3	–	–	t
Sabinene	967	969	0.24	–	0.37	t	t
β-Pinene	984	974	0.55	0.51	0.42	–	0.32
cis-meta-Mentha-2,8-diene	981	983	0.81	–	–	t	0.67
Myrcene	984	988	–	t	t	0.28	t
β-Carene	989	1001	0.77	t	1.75	t	t
ρ-Cymene	1022	1044	0.61	–	–	t	–
Limonene	1025	1024	23.9	20.88	20.40	17.33	26.14
(Z)-β-ocimene	1031	1032	0.48	1.42	–	t	0.40
E-β-Ocimene	1042	1044	1.75	t	t	1.05	1.71
γ-Terpinene	1054	1054	0.55	t	t	t	–
Terpinolene	1099	1086	0.99	1.15	1.14	t	1.20
Linalool	1099	1095	–	–	t	0.91	–
cis-β-Terpinolene	1140	1140	t	t	–	–	2.01
Citronellal	1149	1148	2.23	2.31	0.65	t	0.69
neo-3-Thujanol	1159	1149	0.86	0.90	1.03	–	–
Umbellulone	1168	1167	–	–	–	t	1.08
(E)-Isocitral	1177	1177	1.05	1.07	t	1.32	t
α-Terpineol	1184	1186	–	t	1.02	–	t
Verbanol	1195	1197	0.29	t	t	t	–
Nerol	1224	1227	2.34	2.04	1.96	2.89	–
Neral	1229	1235	0.77	–	–	t	11.08
Perilla ketone	1238	1245	18.29	22.54	21.6	22.47	–
cis-Myrtanol	1250	1250	3.15	3.05	2.90	3.58	3.68
Citronellyl formate	1269	1271	20.76	25.45	24.62	25.79	22.47
Linalool acetate	1270	1272	t	–	t	t	–
δ-Elemene	1328	1335	t	t	0.86	0.87	t
α-Terpinyl acetate	1346	1346	–	t	–	t	1.23
Neryl acetate	1354	1359	1.79	1.41	0.91	0.75	–
Geranyl acetate	1374	1379	5.40	6.14	3.97	3.65	5.80
β-Patchoulene	1374	1379	t	t	–	t	1.39
β-Cubebene	1381	1387	–	–	1.46	–	t
β-Elemene	1383	1389	0.98	0.82	0.39	1.39	t
β-Longipinene	1402	1400	–	t	–	t	10.26
Longifolene	1406	1407	t	–	0.57	–	t
(E)-Caryophyllene	1414	1417	5.09	6.11	7.62	9.72	–
cis-Thujopsene	1425	1429	0.50	t	1.25	0.65	t
α-Humelene	1448	1452	0.78	t	0.88	1.45	t
γ-Gurjunene	1474	1475	t	–	0.47	1.26	0.50
Germacrene D	1474	1484	0.87	t	2.61	t	–
γ-Himachalene	1489	1508	0.45	–	1.17	t	t
cis-β-Guaiene	1489	1492	t	t	t	–	2.44
trans-β-Guaiene	1491	1502	–	t	t	t	1.40
Viridiflorene	1498	1496	2.44	2.42	t	3.14	t
γ-Patchoulene	1500	1502	t	t	–	t	t
Germacrene A	1501	1508	t	1.22	t	1.52	t
Silphiperfol-5-en-3-ol A	1551	1557	t	t	–	t	–
Total number constituents	–	–	48	50	48	49	48
Constituents identified, (%)	–	–	40, 98.69	37, 99.74	35, 100.02	40, 100.02	36, 94.59
Monoterpenes hydrocarbons (%)	–	–	29.66	23.11	22.94	18.66	29.36
Oxygenated monoterpenes (%)	–	–	57.92	66.06	59.8	61.36	49.24
Sesquiterpenes (%)	–	–	11.11	10.57	17.28	20.00	15.99

CA-ID-1: *Citrus aurantifolia* collected from Idukki, Kerala; CA-TH-2: Thrivanthapuram, CA-PA-3: Pathanamthitta; CA-AL-4: Alappuzha, CA-TA-5: Tamil Nadu; t = < 0.05 %; LRI (cal.): Linear retention index (calculated); LRI(lit.): Linear retention index (literature).

TABLE-2
COMPARATIVE CHEMICAL PROFILES OF *C. aurantifolia* PEEL OILS FROM FIVE GEOGRAPHICAL LOCATIONS IN SOUTH INDIA

Constituents	LRI (cal.)	LRI (lit.)	CA-ID-1 (%)	CA-TH-2 (%)	CA-PA-3 (%)	CA-AL-4 (%)	CA-TA-5 (%)
α-Thujene	927	924	0.38	t	t	0.38	1.24
α-Pinene	928	932	1.85	1.39	2.02	1.86	2.46
Sabinene	968	969	1.73	2.45	2.70	1.74	t
β-Pinene	974	974	12.78	14.3	15.39	12.87	13.99
cis-meta-Mentha-2,8-diene	984	983	0.84	0.81	t	0.84	0.82
δ-3-Carene	1011	1008	0.67	0.05	t	0.67	t
δ-Cymene	1022	1020	2.06	3.58	6.00	2.07	3.98
Limonene	1027	1024	40.00	41.62	37.8	40.25	41.93
α-Terpinene	1054	1054	12.29	10.59	8.02	12.36	10.63
ρ-Mentha-2-4(8)-diene	1081	1085	1.12	1.19	t	1.17	1.04
trans-Sabinene hydrate	1099	1098	0.76	0.98	–	0.77	t
Terpinen-4-ol	1179	1174	1.17	1.23	t	1.18	1.03
α-Terpineol	1192	1186	t	2.02	2.26	t	t
Cumenol	1203	1196	t	t	–	2.64	–
cis-Caranone	1203	1200	1.42	–	t	1.03	2.03
Linalool formate	1221	1214	0.52	–	1.3	–	–
Nerol	1221	1227	1.04	1.31	–	t	1.31
Citronellol	1224	1223	–	–	0.13	t	–
Citronet	1234	1234	–	1.71	2.12	–	t
Neral	1236	1235	2.27	–	–	2.28	1.72
Geraniol	1247	1249	t	1.75	1.79	–	–
cis-Piperitone epoxide	1250	1250	1.81	–	t	1.82	1.77
Geranial	1264	1264	–	1.76	2.82	t	–
Citronellyl formate	1267	1271	2.77	–	0.02	2.79	1.77
δ-Elemene	1328	1335	0.47	t	t	–	–
Limonene aldehyde	1328	1326	0.46	–	t	0.47	t
Longipinene	1354	1350	0.55	–	t	0.56	t
β-Patchoulene	1383	1379	t	–	–	0.49	0.52
E-Caryophyllene	1411	1417	0.49	3.18	–	–	t
α-Gurjunene	1412	1409	1.53	t	3.37	1.54	3.28
cis-Thujopsene	1426	1429	1.66	2.63	3.28	1.67	2.65
α-.Himachalene	1448	1449	t	t	–	t	t
γ-Gurjunene	1474	1475	0.99	t	4.80	1.00	–
β-Himachalene	1498	1500	t	3.63	–	t	t
cis-β-Guaiene	1499	1492	4.40	–	t	4.43	3.73
Patchoulene	1500	1502	t	–	5.56	t	t
trans-β-Guaiene	1502	1502	4.00	3.80	t	3.11	4.11
δ-Cadinene`	1522	1522	–	–	0.62	–	t
Germacrene B	1551	1559	t	t	–	t	–
Total number constituents	–	–	42	40	38	40	39
Constituents identified, (%)	–	–	35, 100.03	27, 99.98	30, 100.00	33, 99.99	31, 100.01
Monoterpene hydrocarbons (%)	–	–	72.6	74.79	71.93	73.04	75.05
Oxygenated monoterpenes (%)	–	–	13.81	11.95	10.44	13.68	10.67
Sesquiterpenes (%)	–	–	13.62	13.24	17.63	13.27	14.29

CA-ID-1: *Citrus aurantifolia* collected from Idukki, Kerala; CA-TH-2: Thrivanthapuram, CA-PA-3: Pathanamthitta; CA-AL-4: Alappuzha, CA-TA-5: Tamil Nadu; t = < 0.05 %; LRI(cal.): Linear retention index (calculated); LRI(lit.): Linear retention index (literature).

Conclusion

Leaf and peel oils of *C. aurantifolia* from five different locations in Kerala and Tamil Nadu showed near similar chemical profiles with limonene (leaf oil: 17.33-26.14 %; peel oil: 37.8-41.93 %) as their common major constituent. Perilla ketone (18.29-22.54 %) and citronellyl formate (20.76-25.79 %) were major constituents in most leaf oils. Perilla ketone was not found in *C. aurantifolia* peel oils and citronellyl formate (0.02-2.79 %) was very low in peel oils. Geranyl acetate (3.65-6.14 %) and (E)-caryophyllene (5.09-9.72 %) were the other two major terpenoids in *C. aurantifolia* leaf oils. Geranyl acetate was not found in peel oils and (E)-caryophyllene (0-3.18 %) was low. The terpenoid profiles of *C. aurantifolia* leaf and peel oils were different, indicating their different roles. The terpenoid composition in *C. aurantifolia* peel oils leads to its use in beverages, food products, perfumes, cosmetics, flavours, soaps and detergents, pharmaceuticals, toothpastes, air freshners and in traditional medicine.

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