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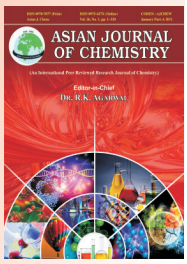

Chemical Variability of *Citrus maxima* Essential Oils from South India

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Essential oils from the peels and leaves of *Citrus maxima* collected from five different geographical locations in south India were isolated by hydrodistillation. *C. maxima* peel and leaf oils were analyzed by the gas chromatography-mass spectrometry, constituents were identified by mass spectral database search, linear retention index data and comparison of mass spectra with literature. Twenty-two to twenty-five constituents (98.00-99.55 %) were identified in *C. maxima* peel oils. Major groups of compounds in *C. maxima* peel oils were monoterpene hydrocarbons (78.66-90.09 %), oxygenated monoterpenes (3.71-8.83 %) and sesquiterpene hydrocarbons (3.86-10.51 %). limonene (66.85-84.08 %), β-pinene (1.57-9.14 %), geranyl acetate (1.09-3.58 %) and verbenone (0.27-3.00 %) were the major constituents in *C. maxima* peel oils. Twenty-nine to thirty-five constituents (97.90-99.15 %) were identified in *C. maxima* leaf oils. Monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes in *C. maxima* leaf oils were (14.01-84.82, 6.47-50.41, 4.25-17.60 and 3.10-30.00 %), respectively. Limonene contents in *C. maxima* leaf oils were 5.29 to 78.45 %, with highest in the pathanamthitta accession. Other major constituents in *C. maxima* leaf oils were α-pinene (t-21.09 %) and β-pinene (t-16.93 %). Limonene with sesquiterpenes and their oxygenated derivatives in relatively minor quantities are the constituents giving flavour and aroma to *C. maxima* peel and leaf oils. Only limited variability was observed between the chemical profiles of the five *C. maxima* accessions from south India.

Keywords: *Citrus maxima*, Chemical variability, Limonene, Geranyl acetate, Verbenone, α-Pinene, β-Pinene.

INTRODUCTION

Citrus maxima (J. Burman) Merrill (*Citrus grandis* (L.) Osbeck, Rutaceae) is a medium sized tree (5-15 m) native to Asia. Its leaves have small winged petioles. It is commonly known as shaddock or pomelo. In traditional medicine *C. maxima* fruit peel is used for cough, swelling and epilepsy[1]. *Citrus* species are well known sources of the essential oils[2,3]. *C. maxima* and other *Citrus* species have essential oil glands in their fruit peel, leaves and petals[4]. Chemical compositions of *Citrus* essential oils depend on variations in environmental conditions, collection period, isolation methods, dehydration procedure and storage conditions[5]. Examples of previous studies on chemical profiles of *C. maxima* volatile oils (collection location, plant part, distillation method, analysis techniques, major constituents): (i) Thailand, peel and flower, super critical carbon dioxide extraction, GC-MS, peel oil, limonene 93.74 %, myrcene 1.71 %, germacrene D 1.04 % and flower oil, limonene 86.2 %, (E)-β-ocimene 2.85 %, myrcene 1.77 %, geranial 1.17 %[6], (ii) Vietnam, peel, cold pressing method, GC, GC-MS, limonene 98.7 %. Nootkatone was found in trace amounts[7], (iii) Kenya, peel, cold pressing method, GC, GC-MS, limonene 91.10 %, β-caryophyllene 4.20 %, α-cubebene 2.00 %[2], (iv) China, peel, steam distillation, GC, GC-MS, limonene 62.48 %, anethole 9.50 %, nootkatone 5.60 %, linalool 2.30 %, β-caryophyllene 2.26 %[8], (v) Tunisia, peel (four cultivars), GC, GC-MS, limonene 92.52-97.3 %, β-pinene 1.37-1.82 % . The observed chemical variability between these four cultivars is mainly due to the influence of the different environmental factors[9], (vi) Japan, peel, cold pressing method, GC, GC-MS, limonene 87.07 %, α-terpinene 6.04 %, myrcene 1.81 %, α-pinene 1.13 %[10]. The quality and the odor of *C. maxima* volatile oils are influenced by their limonene contents. There are no systematic reports on the essential oil compositions of *C. maxima* from southern India. Here, we report the comparative chemical profiles of volatile oils isolated from the peels and leaves of *C. maxima* collected from five geographical locations in south India.

EXPERIMENTAL

C. maxima fruits (peels) and leaves were collected during the October to December 2009 from five locations

(i) Thiruvananthapuam, Kerala (CM-TH-1), (ii) Kottayam, Kerala (CM-KT-2), (iii) Pathanamthitta, Kerala (CM-PT-3), (iv) Alappuzha, Kerala (CM-AL-4) and (v) Kanyakumari, Tamil Nadu (CM-TN-5).

Oil isolation: Essential oils from fresh peels and leaves of five *C. maxima* accessions were isolated separately by hydrodistillation for 5 h on a Clevenger apparatus. Pleasant smelling, transparent, yellow coloured oils were obtained from the peels and leaves of *C. maxima*. CM-TH-1: peels 250 g, oil yield 1.7 mL (0.68 %, v/w); leaves 250 g, oil yield 0.2 mL (0.08 %, v/w); CM-KT-2: peels 400 g, oil yield 3.12 mL (0.78 %, v/w); leaves 280 g, oil yield 0.31 mL (0.11 %, v/w); CM-PT-3: peels 425 g, oil yield 1.50 mL (0.35 %, v/w); leaves 300 g, oil yield 0.31 mL (0.10 %, v/w); CM-AL-4: peels 450 g, oil yield 1.3 mL (0.28 %, v/w); leaves 320 g, oil yield 0.31 mL (0.09 %, v/w); CM-TN-5: peels 430 g, oil yield 1.9 mL (0.44 %, v/w); leaves 350 g, oil yield 0.46 mL (0.13 %, v/w). *C. maxima* leaf and peel oils were stored at 4 ºC until further analysis.

GC-MS analyses: GC-MS analyses of *C. maxima* peel and leaf oils were carried out by splitless injection of 1 µL of each oil on a Agilent 6890 gas chromatograph (Hewlett-Packard, USA) fitted with an HP-5 (5 % phenyl 95 % dimethyl polysiloxane, non-polar, 30 m × 0.32 mm i.d., 0.25 µm film thickness) capillary column, coupled with a Model 5973 mass detector. 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; ion source temperature 240 ºC.

Identification of oil constituents: Linear retention indices of oil constituents in Tables 1 and 2 were determined on the HP-5 column, using standard C_5-C_{30} straight chain hydrocarbons (Aldrich Chemical Company, USA). Individual compounds in *C. maxima* peel and leaf oils were identified by Wiley 275 L database matching, comparison of mass spectra with published data and by comparison of their LRIs[11] (Tables 1 and 2).

RESULTS AND DISCUSSION

Peels and leaves of five *C. maxima* accessions from south India on hydrodistillation yielded 0.28-0.78 and 0.08-0.13 % (v/w) of volatile oils, respectively. Both peel and leaf oils were pleasant smelling, yellow coloured and transparent. Twenty-two to twenty-five constituents (98.00-99.55 %) of *C. maxima* peel oils were identified by GC-MS analyses (Table-1). Monoterpene hydrocarbons (78.66-90.09 %) were the major class of compounds in all five accessions of *C. maxima* peel oils, followed by sesquiterpene hydrocarbons (3.86-10.51 %) and oxygenated monoterpenes (3.71-8.83 %). Limonene was the single major constituent in *C. maxima* peel oils (66.85-84.08 %) and it gives the pungent smell to these volatile oils. β-Pinene (5.15-9.14 %), geranyl acetate (1.09-3.58 %) and verbenone (0.27-3.00 %) were the other major terpenoids in

TABLE-1
COMPARATIVE CHEMICAL PROFILES OF THE PEEL ESSENTIAL OILS OF FIVE *Citrus maxima* ACCESSIONS FROM SOUTH INDIA

Constituent	LRI (cal.)	LRI (lit.)	CM-KT-1 (%)	CM-TH-2 (%)	CM-PT-3 (%)	CM-AL-4 (%)	CM-TN-5 (%)
β-Pinene	974	974	9.14	7.63	5.15	7.36	1.57
cis-meta-Mentha-2,8-diene	984	983	0.63	t	t	0.61	t
Limonene	1027	1024	66.85	81.42	84.08	80.11	79.78
β-Ocimene	1046	1044	t	t	0.86	t	t
γ-Terpinene	1054	1054	2.04	0.14	t	1.45	0.36
Linalool oxide	1066	1067	1.15	2.78	t	0.91	t
iso-Terpinolene	1081	1085	0.51	t.	0.24	0.25	t
Linalool	1091	1095	0.90	1.20	0.63	1.12	0.67
Terpinen-4-ol	1179	1174	0.18	0.35	–	t	0.28
α-Terpineol	1192	1186	0.55	–	0.46	t	0.84
Verbenone	1203	1204	3.00	0.44	0.27	0.64	2.00
Citronellol	1214	1223	0.91	t	0.33	0.37	0.69
m-Cumenol	1221	1224	0.37	0.52	0.82	0.11	0.59
Nerol	1224	1227	1.02	–	0.96	t	1.81
Neryl acetate	1351	1359	0.24	0.49	t	0.41	0.59
Isoledene	1360	1374	1.82	t	t	1.67	4.00
Geranyl acetate	1368	1379	3.58	2.12	3.12	2.01	1.09
β-Panasinsene	1376	1381	1.2	t	–	t	t
β-Elemene	1385	1389	0.29	0.36	1.06	0.48	0.57
iso-Caryophyllene	1400	1408	0.65	0.37	0.27	t	0.40
cis-Thujopsene	1426	1429	t	0.34	0.34	t	t
γ-Gurjunene	1476	1475	0.56	0.42	0.42	1.11	1.88
γ-Himachalene	1479	1481	1.36	–	t	0.40	t
δ-Cadinene	1522	1522	–	0.58	0.54	–	0.37
Germacrene B	1551	1559	1.05	t	–	0.20	0.84
Total number of constituents	–	–	31	32	28	30	30
Constituents identified, (%)	–	–	24, (98.00)	22, (99.28)	22, (99.55)	24, (99.21)	25, (98.33)
Monoterpene hydrocarbons (%)	–	–	78.66	89.25	90.09	89.53	81.71
Oxygenated monoterpenes (%)	–	–	8.83	5.84	3.71	5.82	8.56
Sesquiterpene hydrocarbons (%)	–	–	10.51	4.19	5.75	3.86	8.06

TABLE-2
COMPARATIVE CHEMICAL PROFILES OF THE LEAF ESSENTIAL OILS OF FIVE *Citrus maxima* ACCESSIONS FROM SOUTH INDIA

Constituent	LRI (cal.)	LRI (lit.)	CM-TH-1 (%)	CM-KT-2 (%)	CM-PT-3 (%)	CM-AL-4 (%)	CM-TN-5 (%)
α-Pinene	928	932	t	1.32	6.37	1.09	21.09
β-Pinene	984	974	9.91	4.01	t	16.93	t
Myrcene	989	988	–	0.42	–	t	5.14
Limonene	1025	1024	11.71	5.95	78.45	5.29	7.72
(Z)-β-ocimene	1031	1032	t	2.31	–	3.18	t
α-Terpinene	1010	1014	t	t	0.14	–	1.67
Linalool	1099	1095	4.38	1.19	1.34	2.16	1.68
Citronellal	1137	1148	1.99	0.38	t	t	0.48
Terpinen-4-ol	1169	1174	t	–	0.72	t	1.05
E-isocitral	1177	1177	4.66	2.07	t	–	2.18
Citronellol	1213	1223	3.08	12.91	t	5.34	2.99
cis-Carveol	1221	1226	t	1.48	0.24	t	t
Nerol	1224	1227	2.16	3.51	0.22	3.2	1.64
Neral	1238	1235	t	3.13	–	t	t
Geranial	1262	1264	5.21	1.89	1.63	8.21	5.01
cis-Myrtanol	1250	1250	t	3.13	–	t	1.55
Dimethoxy-Z-citral	1301	1316	5.07	5.65	0.27	0.64	t
Citronellyl acetate	1341	1350	1.88	t	–	0.99	–
Neryl acetate	1354	1359	4.73	8.61	0.42	2.50	5.73
Geranyl acetate	1374	1379	16.41	6.46	1.49	7.46	t
β-Elemene	1383	1389	t	1.99	0.42	t	–
β-Caryophyllene	1414	1417	2.81	11.06	1.65	10.03	3.16
α-Guaiene	1433	1437	3.72	1.68	0.19	1.95	4.67
α-Himachalene	1447	1449	–	1.00	t	t	1.76
α-Patchoulene	1454	1454	t	1.87	1.99	2.94	0.87
(E,E)-α-Farnesene	1494	1505	t	t	0.31	0.48	–
α-Cadinene	1529	1537	t	t	t	t	0.76
Silphiperfol-5-en-3-ol A	1551	1557	t	0.64	t	0.75	0.57
Spathulenol	1571	1577	12.17	5.71	t	18.88	18.82
Ledol	1599	1602	t	0.11	–	–	0.71
Caryophyllene oxide	1587	1582	2.89	3.04	t	1.44	1.5
epi-α-Cadinol	1632	1638	1.96	1.24	t	1.77	t
z-8-hydroxy-Linalool	1621	1619	–	1.00	–	1.00	0.71
α-Cadinol	1645	1652	3.72	4.14	t	4.03	7.69
Nootkatone	1804	1806	t	–	2.79	–	t
Total number of constituents	–	–	41	40	32	37	37
Constituents identified, (%)	–	–	34, (98.46)	35, (97.90)	29, (98.64)	32, (98.26)	34, (99.15)
Monoterpenes hydrocarbons (%)	–	–	21.62	14.01	84.82	26.49	33.95
Oxygenated monoterpenes (%)	–	–	49.57	50.41	6.47	30.50	23.98
Sesquiterpene hydrocarbons (%)	–	–	6.53	17.60	4.25	14. 92	11.22
Oxygenated sesquiterpenes (%)	–	–	20.74	14.88	3.10	26.35	30.00

C. maxima peel oils (Table-1). Isoledene (4.00 %) was a major component in Kanyakumari peel oil, but it is relatively low (trace-1.82 %) in rest of the *C. maxima* accessions.

Twenty-nine to thirty-four (97.90-99.15 %) constituents in *C. maxima* leaf oils were identified by GC-MS (Table-2). Monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes in *C. maxima* leaf oils were in the range 14.01-84.82, 6.47-50.41, 4.25-17.60 and 3.10-30.00 %, respectively. Monoterpene hydrocarbons were relatively low in most *C. maxima* leaf oils compared to peel oils. Limonene content was the highest (78.45 %) in Pathanamthitta leaf oil, but its content in the rest of the *C. maxima* leaf oils were very low (5.29-11.71 %). Spathulenol (trace-18.88 %), caryophyllene oxide (trace-3.04 %), (E)-caryophyllene (1.65-11.06 %), geranyl acetate (trace-16.41 %) and neryl acetate (0.42-8.61 %) were the other major components of *C. maxima* leaf oils. Spathulenol and caryophyllene oxide were only in trace amounts in leaf oil of *C. maxima* accession from Pathanmathitta (Table-2).

Limonene contents in *C. maxima* peel oils were high compared to its leaf oils. Oxygenated sesquiterpenes were not found in the peel oils, but they were 3.10-30.00 % in *C. maxima* leaf oils. Nootkatone, a flavour constituent in grapes[12], was found in trace amounts to 2.79 % in *C. maxima* leaf oils. α-Cadinene (trace-0.76 %), ledol (0-0.71 %) and (E, E)-α-farnesene (0-0.48 %) were also found in trace amounts in *C. maxima* leaf oils. Isoledene (trace-4.00 %) was found in *C. maxima* peel oils.

Conclusion

Chemical profiles of *C. maxima* peel and leaf oils are comparable with previous reports from various geographical locations[2,6-10]. Limonene was the major constituent in *C. maxima* peel (66.85-84.08 %) and leaf (5.29-78.45 %) oils.

Oxygenated sesquiterpenes were not found in *C. maxima* peel oils. Nootkatone was found as a minor constituent in *C. maxima* leaf oils (0-2.79 %). Among leaf oils, Pathanamthitta accession showed highest monoterpene content with 78.45 % limonene. Monoterpenes (mainly limonene) along with sesquiterpenes and their oxygenated derivatives in relatively minor quantities result in the flavour and aroma of *Citrus* volatile oils[13].

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