

GC-MS Compositional Analysis of Essential Oil of Leaf and Fruit Rind of *Citrus maxima* (Burm.) Merr. from Coastal Karnataka, India

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ABSTRACT

Essential oils derived from plants by hydro distillation possess several medicinal properties. *Citrus maxima* (Burm.) Merr. fruit is nutritive, cardiogenic, refrigerant, in stomach troubles, puerperal sepsis and insanity; while leaves are useful in epilepsy, chorea, convulsive cough and stomach pain due to indigestion. Gas chromatography coupled with mass spectroscopy (GCMS) has been used for detection and identification of volatile components from the hydro-distillate from leaf and rind of *Citrus maxima* (Burm.) Merr. of Rutaceae. Yield of oil was more in rind (0.12%) compared to leaf (0.07%). Forty two and thirty four compounds were detected from leaf and fruit respectively, among which citronellol (28.26%) was the major component in the leaves while D-limonene (89.04%) in the rind. Volatile compounds responsible for various medicinal properties are identified from hydro-distillate of *Citrus maxima* (Burm.) Merr. from Dakshina Kannada using GC-MS.

INTRODUCTION

Due to the resistance developed by microbes to the commercially available antibiotics researchers are now concentrating on herbal remedies which have less side effects but more powerful in inhibiting the microbial colonization. The oval-shaped sacs in the flavedo or colored portion of the peel of many *Citrus* spp are the reservoirs of oils which are natural toxic barrier to many microorganisms. Terpenes from volatile oil of medicinal plants are reported to be powerful antimicrobial agents. Effect of DL-limonene in inhibition of cell growth, ethanol formation, and sugar utilization by *S. cerevisiae* has also been reported (Unal *et al.*, 2012). Essential oil *C. limonum* and *C. aurantium* were effective in inhibition of microorganism growth in multi-species biofilms in the oral cavity, which included *Candida albicans*, *Escherichia coli*, and *Enterococcus faecalis*

responsible for atrophic candidiasis, periradicular disease, endodontic infections and several other infections (Oliveira *et al.*, 2014). Lemon fruit peels, seeds and juice also possess activity against bacteria like *Streptococcus aureus* and fungus *C. albicans* (Pandey *et al.*, 2011). *Citrus maxima* (Burm.) Merr. syn. *C. grandis* (Linn.) Osbeck; *C. decumana* Linn. is spreading, round-topped almost thorn less tree. The fruit is nutritive, cardiogenic and refrigerant. Leaves are useful in epilepsy, chorea and convulsive cough (Nadakmi, 1954; Chopra *et al.*, 1956). The fruit juice is used in stomach troubles (Dagar and Dagar, 1996); puerperal sepsis and insanity (Bortahnkur, 1996). Leaves are used in stomach pain due to indigestion (Chaudhari and Pal, 1976).

With the above background information, an attempt has been made to find out chemical constituent of grapefruit leaf and fruit rind essential oil using GC-MS.

MATERIAL AND METHODS

Plant Materials

Mature grapefruits and leaves were collected from Manjanady, Mangalore, Dakshina Kannada district Karnataka.

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Instrumentation and Techniques

Volatile oil in the drug is estimated by distilling the drug with a mixture of water and glycerin in a Clavenger's apparatus. The distillate was collected in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask. Volume of the oil collected on the surface of water in the graduated tube was measured. The content of the volatile oil is expressed as a percentage v/w. GC-MS was carried out using Shimadzu gas chromatograph with a SE-30 10% Chromosorb-W packed stainless steel column (2 m x 2 mm). Oven programme: 60°C (5 min), 60°-260°C (5°C/min), 260°C (10 min); carrier gas – nitrogen, flow rate 40 ml/min; injector temperature 240°C; detector temperature 240°C. Individual components were identified by database of mass spectra matching with literature available in the libraries like NIST and WILEY by comparison of their mass spectrum values. The LRI of a compound is an expression of its retention time on a gas chromatographic column relative to an homologous series n-alkanes. The following equation is used to calculate the Linear retention index (LRI) from the retention time (Adam, 1995).

$$\text{LRI} = 100 \left(\frac{t - t_n}{t_{n-1} - t_n} + n \right)$$

t = retention time of component

n = carbon number of preceding n-alkane

n+1 = carbon number of subsequent n-alkane

RESULT AND DISCUSSION

From 1500 g of leaves of grapefruit tree around 1 ml (0.07%) of pale white light volatile oil has been obtained by hydro-distillation using Clavenger's apparatus. Similarly, 1.5 ml of pale white light volatile oil was obtained from 1250 g (0.12%) of fruit rind. The composition of the volatile oil was analyzed by GC-MS. Fourty two constituents were detected from leaf, of which, 15 could not be identified as the mass fragmentation showed low similarity. Three major compounds eluted at RT 12.127, 14.481 and 21.014 mins were identified as β -Caryophyllene (16.89%), (-)-Spathulenol (9.32%), and Citronellol (28.26%) respectively. Other high concentration constituents were τ -Cadinol (3.16), 1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene) cyclohexane (2.66%), α -Cadinol (2.51 %), α -Caryophyllene (2.48%), 1-Bromo-4-bromomethyl decane (2.43%), 2-n-Hexylcyclopentanone (2.22%). A major compound at RT 32.034 which accounted for 11.69% could not be identified (Table 1).

Thirty four constituents were detected from fruit rind, of which, 10 could not be identified as the mass fragmentation showed low similarity index. Limonene was the major compound accounting for 89.04%. Three other major compounds were β -Pinene (2.25%), β -Myrcene (2.06%) and β -Copaene (1.76%). All other compounds were found to be present in traces (Table 2).

Surprisingly, none of the identified compounds were found occurring in both leaf and rind, though both are from same botanical origin. The two different parts found to have

distinguished chemical composition (Table 3). Antimicrobial resistance is increasing rapidly leading for the need to explore herbal remedies which does not have side effects. Thought was given to find out the components of the essential oil to explore Pomelo/Shaddock/Chakkotha, one of the fruits belonging to genus *Citrus* and commonly cultivated for consumption in Southern India, as a possible source of antimicrobial chemicals.

Composition of volatile oil of plants growing at different localities largely depends on the geographical conditions, and there are no studies in report on the composition of oil from this plant growing in Western Ghats. Leaf and fruit rind was considered for this study as volatile oil of *Citrus* species is mainly concentrated in these parts.

This result of the present study is similar to the few studies done on other species of *Citrus* in the past (Uysal *et al.*, 2011; Droby *et al.*, 2008; Kirbaşlar *et al.*, 2009; Fuselli *et al.*, 2008) where the major compounds detected were D-Limonene, β -myrcene, α -pinene, caryophyllene, octanal and β -phellandrene. Some of the minor components included δ -cadinene, copaene, methyl phthalate, linalool and 3-carene. The compounds detected from leaves of orange (*Citrus sinensis*), bigaradier (*Citrus aurantium*), mandarin (*Citrus limonum*) and lemon (*Citrus reticulata*) were limonene (7.18%), β -pinene (4.35-30%), and linalool (0.21-63.03%), and these compounds were found to be effective against fungi and their spores (Uysal *et al.*, 2011). The percentage yield of volatile oil is very less; the sample examined in the present study revealed 0.12% oil in fruit rind and 0.07% essential oil in leaves by hydro-distillation method employing Clavenger's apparatus. In a study on Algerian species of *Citrus* by Zohra *et al.*, 2015, percentage yield of volatile oil from orange, lemon, mandarin and bigaradier were in the range of 0.96, 1.02, 0.51 and 0.73 respectively. Another study by Javed *et al.*, 2014 reported a yield of 0.28-0.45%, where Grapefruit, Malta, Mandarin, Mousami, and Tangerine respectively yielded 0.45%, 0.37%, 0.33%, 0.30%, 0.28%. The variation seen in the percentage yield may be due to the differences in climatic conditions, soil quality and methods of extraction. In several studies on citrus varieties, peel oil consisted of limonene as the most predominant component followed by β -myrcene, α -pinene, β -phellandrene, 3-carene and nonane, β -pinene, γ -terpinene (Zohra *et al.*, 2015; Okunuwo *et al.*, 2013; Javed *et al.*, 2014; Vasudeva and Sharma, 2012; Sorrou *et al.*, 2013), another study (Takaya *et al.*, 2008) on oil extract of peel of *Citrus aurantium* L. found β -pinene (0.62%–19.08%), limonene (0.53%–94.67%), trans- β -ocimene (3.11%–6.06%), linalool (0.76%–58.21%), and α -terpineol (0.13%–12.89%) as major components. In earlier studies on this plant, leaf oil is reported to contain α -pinene, (2.6%), β -pinene (6.6%), linalool (42.34%) and linyl acetate (44.18%) (Nigam and Dutt, 1945; Nigam and Kumari, 1962). But this study shows entirely different chemical composition of leaves.

Thirty five compounds were identified accounting to 98.9% of essential oil from peel. Limonene was found to be the major constituent (93.02%).

Table 1: The composition of the volatile oil from leaf.

Peak	RT	Name	%
1.	8.152	4-Terpinenol	0.19
2.	10.779	3-Methyl-6-(1-methylethylidene) cyclohexene	0.44
3.	11.649	β -Elemene	0.74
4.	12.127	β -Caryophyllene	16.89
5.	12.639	α -Caryophyllene	2.48
6.	12.963	--	0.19
7.	13.287	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene) cyclohexane	2.66
8.	13.560	--	0.52
9.	13.645	--	0.91
10.	14.105	(Z)-1,3-Bis-(2-cyclopropyl,2-methyl cyclopropyl)-but-2-en-1-one	0.38
11.	14.174	--	1.27
12.	14.361	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	0.28
13.	14.481	(-)-Spathulenol	9.32
14.	14.566	Caryophyllene oxide	1.03
15.	14.685	Viridiflorol	0.75
16.	14.839	--	0.40
17.	14.924	Humulene 1,2-epoxide	1.02
18.	15.163	α -Bergamotene	0.74
19.	15.316	τ -Cadinol	3.16
20.	15.521	α - Cadinol	2.51
21.	15.760	--	0.18
22.	16.340	Cyclopentaneundecanoic acid, methyl ester	0.17
23.	16.408	1,3-Bis-(2-cyclopropyl,2-methyl cyclopropyl)-but-2-en-1-one	0.33
24.	16.681	--	0.59
25.	17.364	--	0.17
26.	17.858	6,10-Dimethyl-2-undecanone	1.35
27.	18.848	15-Methyl- hexadecanoic acid, methyl ester	0.24
28.	19.103	3,5,11,15-Tetramethyl-1-hexadecen-3-ol	0.26
29.	20.093	3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	0.29
30.	20.878	Oxalic acid, allyl pentadecyl ester	0.22
31.	21.014	Citronellol	28.26
32.	21.321	2-n-Hexylcyclopentanone	2.22
33.	21.441	3,7-Dimethyl-6-octen-1-ol, formate	0.71
34.	21.662	--	0.28
35.	27.872	2,6-Dimethyl-1,5-heptadiene	0.29
36.	32.034	--	11.69
37.	32.904	1-Bromo-4-bromomethyl decane	2.43
38.	33.041	--	1.93
39.	33.382	--	0.31
40.	33.587	--	0.79
41.	33.740	--	0.27
42.	33.945	--	1.14

-- Unidentified

Table 2: The composition of the volatile oil from rind.

Peak	RT	Name	%
1.	4.490	α -Pinene	0.41
2.	4.605	--	0.24
3.	4.783	--	0.25
4.	5.184	β -Phellandrene	0.43
5.	5.298	β -Pinene	2.25
6.	5.438	β Myrcene	2.06
7.	6.265	D-Limonene	89.04
8.	6.494	β -Ocimene	0.33
9.	6.946	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	0.09
10.	7.226	--	0.08
11.	7.430	Linalool	0.49
12.	7.856	--	0.11
13.	8.098	cis-p-Mentha-2,8-dien-1-ol	0.11
14.	8.842	Terpinen-4-ol	0.11
15.	9.058	L- α -Terpineol	0.39
16.	9.141	--	0.24
17.	9.733	Neral	0.18
18.	10.140	Geranial	0.13
19.	11.100	--	0.06
20.	11.139	2-Carene	0.06
21.	11.896	--	0.06
22.	11.972	--	0.05

-- Unidentified

23.	12.347	Caryophyllene	0.20
24.	12.475	Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene	0.10
25.	12.659	γ -Muurolene	0.03
26.	12.761	--	0.03
27.	13.143	β -Copaene	1.76
28.	13.327	--	0.24
29.	13.384	γ -Muurolene	0.05
30.	14.479	Hexadecane	0.09
31.	16.718	Octadecane	0.08
32.	17.838	7,9,Di-tert-butyl-1-oxaspiro(4,5)eca-6,9-diene-2,8-dione	0.11
33.	18.741	Eicosane	0.05
-- Unidentified			

Table 3: Linear retention index of the compounds identified from leaf and rind.

SN	LRI	Sample Name	Leaf	Rind
1.	-	4-Terpinenol	+	
2.	97	α -Pinene		+
3.	1014	β Myrcene		+
4.	1029	Geranial		+
5.	1033	β -Ocimene		+
6.	1044	β -Pinene		+
7.	1061	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate		+
8.	1064	β -Elemene	+	
9.	1071	2-n-Hexylcyclopentanone	+	
10.	1072	L- α -Terpineol		+
11.	1075	3-Methyl-6-(1-methylethylidene) cyclohexene	+	
12.	1077	Terpinen-4-ol		+
13.	1078	D-Limonene		+
14.	1135	3,7-Dimethyl-6-octen-1-ol, formate	+	
15.	1315	15-Methyl- hexadecanoic acid, methyl ester	+	
16.	1486	1-Bromo-4-bromomethyl decane	+	
17.	1519	Caryophyllene	+	
18.	1532	Hexadecane		+
19.	1541	Caryophyllene oxide	+	
20.	1541	Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene		+
21.	1542	τ -Cadinol	+	
22.	1543	Viridiflorol	+	
23.	1546	α -Cadinol	+	
24.	1548	β -Caryophyllene	+	
25.	1558	(-)-Spathulenol	+	
26.	1561	α -Caryophyllene	+	
27.	1564	6,10-Dimethyl-2-undecanone	+	
28.	1666	Octadecane		+
29.	1694	Eicosane		+
30.	1724	1,3-Bis-(2-cyclopropyl,2-methyl cyclopropyl)-but-2-en-1-one	+	
31.	1785	Oxalic acid, allyl pentadecyl ester	+	
32.	1820	3,5,11,15-Tetramethyl-1-hexadecen-3-ol	+	
33.	1855	7,9,Di-tert-butyl-1-oxaspiro(4,5)eca-6,9-diene-2,8-dione		+
34.	2030	Citronellol	+	
35.	2055	3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	+	

The other compounds identified were myrcene, α -pinene, sabinene, linalool, octylacetate, neral, geranial, germacrene D, citropten, 4-methyl-2-pentanone, butyl acetate, β -pinene, (*E*)- β -ocimene, nonanol, *cis*-limonene oxide, citronellal, α -terpineol, geraniol, decanal, undecanal, δ -elemene, neryl acetate, geranyl acetate, β -elemene, dodecanal, β -caryophyllene, α -salimene, germacrene B, nootkatone, hexadecanal, isobergaptene, osthole and auraptene (Bordoloi *et al.*, 1999). Results of this study also showed limonene as the major constituent accounting to 89.04%. But the minor constituents were different in contrast to the earlier reports.

Citrus peels are rich sources of biologically active antimicrobial compounds like flavanones and polymethoxylated flavones, which are not found in other trees. The difference in the constitution may be due to the genetic and ecological variation of

the plants. They are mostly used in food industries and also used as flavoring agents. It is also known to possess anti-inflammatory and anti-cancer properties. Constituents, such as simple sugars, carotenoids, vitamin C, flavonoids, limonoids, folic acid, fibres and potassium are good for human health. Major portion of citrus fruits produced are processed into juices, jams and concentrates (Francis and Hoang, 2015).

CONCLUSION

Citrus fruits are the most widely available and maximum consumed fruits. It has so many medicinal properties and very less toxic effect. The detection of compounds may be helpful in the study and association of various medicinal properties in the extract of leaves and fruit rind of *Citrus maxima* (Burm.) Merr. from Dakshina Kannada.

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