COMPARISON STUDIES ON LEAF OILS OF EGYPTIAN CITRUS VARIETIES

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SUMMARY: A comparison was made between the essential leaf oils separated from seven citrus varieties growing in Egypt. The volatile components were analyzed by using Gas Liquid Chromatography on polar and non polar phase. Most of the identified components are common to all varieties. The difference in the characteristic aroma for each variety might be attributed to the differences in the concentration of the specific components in each case.

Key words: Leaf oils, citrus varieties.

INTRODUCTION

Volatile oils play an important role in the medical and food aspects as well as in the cosmetics. In Egypt, most of the essential oils used in different industries are important, so it is deamed of interest to evaluate the leaves of citrus varieties as possible potential local source of volatile oils.

Although the essential oils obtained from citrus peels play a major role in food and beverage flavouring, the quit different oils obtained from citrus leaves might have an equal importance as fragrances while extensive studies have been done on the peel oils (1-5), the compositional analysis of volatile oil of local orange peel had been studied in our laboratory, Fadel and Soliman (6). Increasing interest for the commercially available citrus volatile oils for use in citrus hold and cosmetic products revealed the need for more information on the constitution of citrus leaf oils. However, data on leaf oil composition had been reported in literature (7-15).

On the other hand, the literature is almost devoid of information regarding the chemical composition of leaf oils of typical local Egyptian citrus species.

In the present study, the chemical composition of leaf oils of seven different Egyptian citrus species will be reported.

MATERIAL AND METHODS

Leaves of seven Egyptian citrus varieties were used, namely, *Citrus reticulate* (cv. Balady mandarine, *Citrus aurantifolia* (cv. Balady lime) and *Citrus siensis* which includes 5 varieties; cv. Valinchia orange, cv. Navel orange, cv. Sweet orange, cv. Balady orange and cv. Jaffa orange. The leaves of all citrus varieties were collected after harvest from mature trees during the season, 1990.

Preparation of leaf oil from citrus leaves

The leaf oil was prepared by steam distillation under reduced pressure (30 m Hg) for about 2 hours. The aroma was collected in traps of ether cooled to -10°C (6). The percentage of the collected concentrate of the individual seven citrus varieties was 1% for lime, 3% for mandarine and 2-4% for the five orange varieties. The authentic samples and solvents used were extra pure or analytical grade.

Aroma panel test

Paired comparison test were run on volatile oil samples obtained from the different citrus varieties vs. control samples. For each test, equal volume of individual sample were placed in identical 5 ml screw capped vial and presented at room temperature. The panel consisted of 12 experienced number each of whom was given two samples and asked to indicate whether the samples were the same or different, Mosnones (16).

Gas liquid chromatographic analysis

The seven samples were subjected to GLC analysis using Varian 3700 instrument equipped with dual flame ionization detector and computing integrator polar and non polar phases were used for the analysis. The first column was packed with 15% diethylene glycol succinate (DEGS) on chromosorb W (60-80 mesh) carrier gas flow rate 30 ml/min. (N2), column temperature 70-195°C with programming rate 4°C/min. The second column was packed with 5% OV_{101} on chromosorb G_{11} (80-100 mesh), column temperature was 100-295°C with programming rate 6°C/min.

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Table 1: Volatile components of Egyptian citrus leaf oils.

Peak no.	t_R	Cv-Balady Mandarine	Cv-Balady Lime	Cv-Valinchia Orange	Cv-Navel Orange	Cv-Sweet Orange	Cv-Balady Orange	Cv-Jaffa Orange	Components
1	2.31	1.30	3.22	2.60	0.83	0.75	0.97	-	Acataldehyde
2	3.27	4.22	3.40	26.36	8.66	15.59	15.81	20.37	α - Pinen
3	4.00	18.85	20.06	14.45	15.40	25.00	10.48	11.03	B - Pinen
4	6.10	1.00	T	3.00	7.91	5.00	-	0.74	Sabinen
5	7.65	0.69	2.28	T	1.86	0.47	0.20	-	B - Myrcene
6	8.00	0.10	T	0.30	T	0.51	0.49	0.10	Octanol
7	10.16	0.66	0.03	T	T	-	-	0.37	α - Telhinen
8	11.95	3.50	23.88	21.46	4.68	14.55	19.16	11.19	Limonen
9	13.09	1.80	5.04	1.50	0.28	5.63	4.28	T	Trans-2 hex-1-al
10	14.06	4.50	10.52	6.00	1.00	7.96	10.03	7.78	γ - Terpinen
11	14.97	0.20	0.11	1.21	0.83	-	-	0.18	Trans Ocimen
12	16.11	0.14	0.60	1.79	1.55	0.15	2.20	0.18	B - Cymene
13	17.00	0.21	-	0.86	1.15	T	T	1.10	Terpinolene
14	17.98	0.16	0.09	0.26	0.16	T	0.17	0.49	Cis-2-Pent-1-al
15	18.10	T	0.14	0.12	0.03	T	-	-	n-Hexyl Alcohol
16	19.07	0.25	-	0.16	0.08	1.88	0.36	0.06	Cis-3 hex-1-ol.
17	20.79	0.50	0.11	0.08	0.13	0.08	0.46	0.06	Trans-2 hex-1-ol.
18	21.51	0.08	0.01	0.17	0.12	T	-	-	α-3-Dimethyl Styr.
19	22.60	0.60	T	0.26	0.15	0.16	-	-	Citronelal
20	23.01	54.51	4.30	0.79	46.63	10.74	14.19	25.43	Linalool
21	23.91	T	0.20	0.40	0.03	0.08	0.03	1.43	Linalyl acetate
22	24.61	1.00	0.40	-	0.02	-	-	-	Thymol Methyl eth.
23	25.51	0.66	0.90	0.20	0.65	0.75	3.65	3.31	Terpinen-4 ol.
24	26.40	0.50	0.40	1.53	-	0.22	0.80	T	Caryophyllene
25	27.00	1.27	0.19	4.00	2.99	1.76	2.85	1.88	α - Terpineol
26	27.82	1.20	0.82	0.26	2.91	1.24	3.79	0.09	B - Selinene
27	28.66	T	0.31	0.06	T	0.45	0.73	0.42	Linalool oxide
28	29.70	T	0.95	0.23	-	-	T	T	B - Terpineol
29	30.90	T	-	0.14	-	-	T	T	Unidentified
30	31.50	0.90	0.20	0.30	0.12	0.37	0.73	0.14	Decanal
31	32.34	T	0.20	1.00	0.20	3.71	0.24	4.00	Unidentified
32	33.46	-	-	0.04	T	-	-	0.76	Unidentified
33	34.84	0.50	5.40	-	0.16	-	-	-	Neral
34	36.81	0.07	7.25	0.26	0.40	-	1.47	1.65	Geranial
35	38.00	-	3.41	-	-	-	-	-	Nerol
36	39.93	-	2.00	0.03	0.06	T	-	0.16	Geraniol
37	41.26	-	-	-	1.00	1.50	4.75	5.00	Unidentified
38	42.90	0.04	-	-	-	-	-	1.00	Unidentified
39	43.50	0.10	T	1.20	0.01	1.50	2.00	0.90	Unidentified
40	45.73	0.49	0.03	-	-	-	-	-	Thymol
41	50.00	0.01	-	-	T	-	T	T	Unidentified

Identification of the components was done tentatively using authentic reference Compounds under identical conditions. For confirmation, the GC-Ms analysis of some samples under suitable conditioned was done and compared with GC analysis.

RESULTS AND DISCUSSION

Each of the concentrates of the seven Egyptian leaf oils mentioned above were analyzed. The results were compared for compositional differences of their components (Table 1).

Forty-one components were separated, out of which 35 were identified by conventional methods, in addition to GC-MS whenever needed. The table showed that most of the identified components are common to all varieties with few exceptions for specific components in certain varieties. Generally, linalool was found to be the major constituents

Table 2: Ratio between linalool (LL) and limonen (L) (LL/L)

Citrus variety / Ratio	Cv-Balady Mandarine	Cv-Balady Lime	Cv-Valinchia Orange	Cv-Navel Orange	Cv-Sweet Orange	Cv-Balady Orange	Cv-Jaffa Orange
LL/L	15.57	0.18	0.46	9.96	0.74	0.74	2.27

of most samples, which is in accordance with the finding of Attawy (18), where as limonen; the main component in citrus peel samples; is represented in most varieties by less values. However, in mandarine, linalool reached (54.5%). B-pinen and γ-terpinen were (18.85% and 4.5%), respectively, which in accordance with Kamiyama (10). Thymol and thymol methyl ether, the characteristic volatile components of mandarine oil were identified with concentrations of 1.0 and 0.49% respectively, Moshonas (1). However, Wilson and Show (18) claimed that terpene hydrocarbons, especially B-pinen and γ- terpinen, in addition to thymol and methyl anthranelate appear to be necessary for full mandarine aroma.

In case of lime leaf oil (Table 1) it can be seen that limonen has the highest concentration where it recorded a value of 23.88% which agree with the previous data represented by Clark *et al.* (5). The other two major hydrocarbons; B-pinen and terpinen; constitute 20.06 and 10.52%; respectively, Kamiyama (10). Citral (neral and geranial) is the main contributor to lemon and lime aroma and commercially evaluated as the most important factor in determining the value of lemon and lime oil samples, Staroscik *et al.* (19). The citrus leaf aroma of *citrus Sinensis* are represented by five orange varieties mentioned above. The results show that qualitative compositions of their volatile oils were mostly identical. Limonen is the main component of Valinchia orange sample, constituting 21.46% compared to 19.66% in Balady orange, 14.55% in sweet, 11.19% in Jaffa and 4.68% in Navel orange.

Linalool showed opposite trend where it is the main components in Navel leaf oil (46.63%), it compared to 9.79 for Valinchia. However, as linalool was found to be the common citrus leaf oil, it was suggested to be the precursor of many constituents of citrus flavour (7,10). As for α-pinen and B-pinen and γ-terpinen they showed high concentration in most orange varieties. However, α-pinen is the main volatile component in Valinchia orange (26.36%) compared to 8.66 in Navel orange.

In case of B-pinen, it was found in high concentration (25%) in sweet orange while it recorded a minimum value in Balady orange (10.48%). The concentration of γ-terpinen in Balady orange (10.03%) was ten folds its value in Navel orange 1.0% (10). Although cis-3-hex-1-ol (leaf alcohol) and trans-2-hex-1-ol were identified in most of the citrus varieties in minor quantities compared to trans-2-hex-1-al (leaf aldehyde), yet these three components are reported to give the characteristic green leaf aroma (8). Calculating the ratio of linlool (LL) to limonen (L) for all the studied samples showed significant differences as shown in Table (2). These ratio can be used to distinguish between the different citrus variety. From Table (2), the higher ratio determined was for mandarine while the smallest one was that of lime. Balady and sweet oranges gave nearly same ratios; these two orange varieties gave the most preferred orange aroma.

The aroma panel test

From the paired panel test it can be determined that the lime and mandarine leaf oils have the pleasent charactristic aroma of lime and mandarine, respectively. The panel test of the orange varieties showed that the Balady and sweet oranges were the most preferred ones for their pleasant orange aroma. Since most of the specific orange aroma constituents identified were identical in all varieties, the difference in aroma panel tests might be due to the quantitative differences of the individual components in the orange varieties.

As a general conclusion, from the above study we can claim that although most of the volatile components were identified in all the citrus leaf oils each variety has different characteristic aroma which might be attributed to the differences in the concentration of the specific compounds in each case. Since panel tests showed pleasant characteristic aroma for lime, mandarine, Balady and sweet orange, these leaves can be used as potential source of volatile oils and might be commercially available to use in different fields.

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