Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 4 [1] December 2014: 171-177

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

The Effect of Geographical Location on Sour orange (*Citrus aurantium L.*) Flower Components

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ABSTRACT
Studies have shown that oxygenated compounds are important in food products. It seems that geographical location has a profound influence on this factor. The goal of the present study is to investigate on flower components of sour orange from two different locations. In the early week of May 2012, at least 50 g flower were collected. Flower components were extracted using an ultrasonic bath and eluted with n-pentane: diethyl ether (1:2) solvent. Then all analyzed using GC-FID and GC-MS. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. The amount of oxygenated compounds ranged from 61.71% to 71.67%. Between two locations examined, Jahrom showed the highest content of oxygenated compounds. As a result of our study, we can conclude that the geographical location can influence the quantity of oxygenated compounds present in the oil.
Key words: *Flavor components, Flower oil, Geographical location, Ultrasonic bath.*

Received 23.08.2014 **Revised 01.11.2014** **Accepted 03.12.2014**

INTRODUCTION

Citrus is one of the most economically important crops in Iran. In the period 2009- 2010, the total Citrus production of Iran was estimated at around 87000 tonnes [1]. The sour orange (*Citrus aurantium L.*) is a hybrid of the mandarin and pummelo that is extensively cultivated in Iran**.** It is one of the most important Citrus used in world. Although it is as important Citrus, the flower components of sour orange have been investigated very little previously.

Citrus oils occur naturally in special oil glands in flowers, leaves, peel and juice. These valuable essential oils are composed of many compounds including: terpenes, sesquiterpenes, aldehydes, alcohols, esters and sterols. They may also be described as mixtures of hydrocarbons, oxygenated compounds and nonvolatile residues. Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc [2]. In addition, recent studies have identified insecticidal, antimicrobial, antioxidative and antitumor properties for Citrus oils [2].

The quality of an essential oil can be calculated from the quantity of oxygenated compounds present in the oil. The quantity of oxygenated compounds present in the oil, is variable and depends upon a number of factors including: geographical location [3], organ [4] and etc.

Branched aldehydes and alcohols are important flavor compounds extensively used in food products [2]. Several studies have shown that oxygenated terpenoids such as linalool, Linalyl acetate and α-terpineol are important in sour orange flavor [5]. The quality of a honey can be calculated from the amount of oxygenated components present in the honey [6]. In addition, type of flowers may influence the quality of volatile flavor components present in the honey. The effect of oxygenated compounds in the attraction of the pollinators has been proven. Therefore, the presence of oxygenated compounds can encourage the agricultural yield [7].

In this paper, we compared the flower compounds isolated from sour orange with the aim of determining whether the quantity of oxygenated compounds influenced by the location.

MATERIAL AND METHODS

Sour Orange Trees

In 1989, sour orange trees were planted at 8×4 m with three replication at Ramsar research station [Latitude 36° 54' N, longitude 50° 40' E; Caspian Sea climate, average rainfall and temperature were 970 mm and 16.25°C per year respectively; soil was classified as loam-clay, pH ranged from (6.9 to 7)]. Also, trees were planted at 8 × 4 m with three replication at an orchard around the Jahrom in 1989 (latitude 28° 50′ N, longitude 53° 33′ E; dry climate, average rainfall 200 mm per year and average temperature 21.0°C; soil was classified as loam-clay with lime and gypsum in some parts; pH=8.5). Sour orange was used as plant material in this experiment (Table 1, Fig.1).

Preparation of Flower Sample

In the early week of May 2012, at least 50 g flower were collected from many parts of the same trees, located in orchards of the Ramsar and Jahrom, early in the morning (6 to 8 am) and only during dry weather. The selection method of all samples was on a random basis.

Flower Extraction Technique

The methodology used in this study, was described by Allissandrakis *et al*. [6]. In order to obtain the volatile compounds from the flowers, 50 g of fresh flowers were placed in a 2000 ml spherical flask, along with 300 ml of n-pentane :diethyl ether (1:2). The flask was covered and then placed in an ultrasonic water bath for 20 min. Extraction experiments were performed with an ultrasound cleaning bath-Fisatom Scientific-FS14H (Frequency of 40 KHz, nominal power 90 W and 24 × 14 × 10 cm internal dimensions water bath). The temperature of the ultrasonic bath was held constant at 25°C. The extract was subsequently filtered through MgSO4 monohydrate. The extract was finally concentrated under a gentle stream of nitrogen to 1 ml and placed in a vial. Vial sealed and was kept in the freezer at -4°C until the GC-MS analysis.

GC and GC-MS

An Agilent 6890N gas chromatograph (USA) equipped with a DB-5 (30 m $\times$0.25 mm i.d ; film thickness = 0.25 μ m) fused silica capillary column (J&W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 60 ^{o} C (3min) to 250 ^{o} C (20 min) at a rate of 3 ^{o} C/ min. The injector and detector temperatures were 260 ^{o} C and helium was used as the carrier gas at a flow rate of 1.00 ml/min and a linear velocity of 22 cm/s. The linear retention indices (LRIs) were calculated for all volatile components using a homologous series of n-alkanes (C9-C22) under the same GC conditions. The weight percent of each peak was calculated according to the response factor to the FID. Gas chromatography-mass spectrometry was used to identify the volatile components. The analysis was carried out with a Varian Saturn 2000R. 3800 GC linked with a Varian Saturn 2000R MS.

The oven condition, injector and detector temperatures, and column (DB-5) were the same as those given above for the Agilent 6890 N GC. Helium was the carrier gas at a flow rate of 1.1 mL/min and a linear velocity of 38.7 cm/s. Injection volume was 1 μL.

Identification of Components

Components were identified by comparison of their Kovats retention indices (RI), retention times (RT) and mass spectra with those of reference compounds [8].

Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 8 flower components. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at $P <$ 0.01. The correlation between pairs of components was evaluated using Pearson's correlation coefficient.

RESULTS

Flower Compounds of the Sour Orange

GC-MS analysis of the compounds extracted from sour orange flower using ultrasonic bath allowed identification of 39 volatile components (Table 2, Fig. 2): 18 oxygenated terpenes [2 aldehydes, 12 alcohols, 3 esters, 1 ketone], 18 non oxygenated terpenes [13 monoterpens, 5 sesqiterpens] and 3 other components.

Aldehydes

Two aldehyde components that identified in this analysis were neral and geranial (Table 3). In addition they were quantified from 0.19% to 0.28%. The concentration of geranial was higher in our samples. Geranial has a citrus-like aroma and is considered as one of the contributors to sour orange flavor [9]. Between two locations examined, Jahrom showed the highest content of aldehydes (Table 3). Since the aldehyde content of citrus oil is considered as one of the most important indicators of high quality, location apparently has a profound influence on this factor (Table 3).

Alcohols

Twelve alcoholic components identified in this analysis were linalool, phenyl ethyl alcohol, terpinene-4-ol, α -terpineol, lilace alcohol B, lilace alcohol D, nerol, geraniol, indol, (Z)-2,6-dimethyl-2,7-octadien-1,6-

diol, (E)-nerolidol and E,E-cis-farnesol (Table 3). The total amount of alcohols ranged from 48.37% to 55.68%. Linalool was identified as the major component in this study and was the most abundant. Linalool has been recognized as one of the most important components for sour orange flavor [5]. Linalool has a floral aroma [9] and its level is important to the characteristic favor of Citrus [2]. Between two locations examined, Jahrom showed the highest content of alcohols (Table 3).

Esters

Three ester components identified in this analysis were linalyl acetate, neryl acetate and geranyl acetate. The total amount of esters ranged from 13.15% to 15.70%. Linalyl acetate was identified as the major component in this study and was the most abundant. Linalyl acetate has been recognized as one of the most important components for sour orange flavor [5]. Linalyl acetate has a floral like smell [9] and its level is important to the characteristic favor of Citrus. Between two locations examined, Jahrom showed the highest content of esters (Table 3).

Ketones

One component identified in this analysis was cis-jasmone. The total amount of ketones ranged from 0.00% to 0.01%. Between two locations examined, Jahrom showed the highest content of ketones (Table 3).

Monoterpene Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 26.37% to 29.59 %. Limonene was identified as the major component in this study and was the most abundant. Limonene has a weak citrus-like aroma [9] and is considered as one of the major contributors to sour orange flavor. Between two locations examined, Ramsar showed the highest content of monoterpenes (Table 3).

Sesquiterpene Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.85% to 0.87%. (Z)-β-caryophyllene was identified as the major component in this study and was the most abundant. Between two locations examined, Ramsar showed the highest content of sesquiterpenes (Table 3).

Results of Statistical Analyses

Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in linalool, α-terpineol, linalyl acetate, β-Pinene, limonene and (E)-β-ocimene. These differences on the 5% level occurred in geraniol and geranyl acetate. (Table 3).

Results of Correlation

Simple intercorrellations between 8 components are presented in a correlation matrix (Table 4). The highest positive values or r (correlation coefficient) were observed between linalyl acetate and linalool (100%); α-terpineol and linalool (99%); linalyl acetate and α-terpineol (99%); geranyl acetate and geraniol (99%); (E)-β-ocimene and β-pinene (99%). The highest significant negative correlations were observed between (E)-β-ocimene and α-terpineol (97%); β-Pinene and α-terpineol (96%); (E)-β-ocimene and linalool (96%); (E)-β-ocimene and linalyl acetate (96%) (Table 4).

DISCUSSION

Our observation that geographical locations had an effect on some of the components of sour orange oil was in accordance with previous findings [3]. The compositions of the flower oils obtained by ultrasonic bath from different locations were very similar. However, the relative concentration of compounds was different according to the type of location.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies [3, 4]. It may be related to environmental factors such as altitude, insolation (solar radiation), average temperature, humidity and chemical composition of soil that can influence the oil compositions [10]. However, it should be kept in mind that the extraction methods also may influence the results. Fertilizer and irrigation affects the content of oil present in Citrus [11]. Fertilization, irrigation and other operations were carried out uniform in this study so we did not believe that this variability was a result of these factors.

The discovery of geranyl pyrophosphate (GPP), as an intermediate between mevalonic acid and oxygenated compounds (Alcohols and aldehyds), led to a rapid description of the biosynthetic pathway of oxygenated compounds. The biosynthetic pathway of oxygenated compounds in higher plants is as below:

Mevalonic acid → Isopentenyl Pyrophosphate → 3.3-dimethylallylpyrophosphate→ geranyl pyrophosphate→ Alcohols and Aldehyds

This reaction pathway catalyzed by isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively [12]. The pronounced enhancement in the amount of oxygenated compounds, when Jahrom used as the location, showed that either the synthesis of geranyl pyrophosphate was enhanced or activities of both enzymes increased.

Also, the higher proportion of the detected oxygenated compounds in Jahrom was probably due to seasonal temperature [13], which is the most important environmental factor in the control of

endogenous enzymes. Solar radiations can also be involved in activation or inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway [14].

High positive correlations between pairs of terpenes suggest a genetic control [15] and such dependence between pairs of terpenes was due to derivation of one from another that was not known. Similarly, high negative correlations between pairs of terpenes indicated that one of the two compounds had been synthesized at the expense of the other or of its precursor. Non-significant negative and positive correlations can imply genetic and/or biosynthetic independence. However, without an extended insight into the biosynthetic pathway of each terpenoid compound, the true significance of these observed correlations is not clear.

Considering that acetate is necessary for the synthesis of terpenes, it can be assumed that there is a specialized function for this molecule and it may be better served by Jahrom.

Table 1. Common and botanical names for citrus taxa used as plant material.

Common name	Botanical name	Parents	Category
Sour orange	*Citrus aurantium (L.) var. amara*	Mandarin ×Pummelo	Sour orange

Table 2. Flower components of sour orange from two geographical locations.

	Component	Ramsar	Jahrom	KI		Component	Ramsar	Jahrom	KI
1	α -thujene	*	*	925	21	Neral	*	*	1240
2	α - Pinene	*	*	933	22	Geraniol	*	*	1255
3	Sabinene	*	*	974	23	Linalyl acetate	*	*	1257
4	β - Pinene	*	*	978	24	Geranial	*	*	1267
5	β -myrcene	*	*	989	25	Indol	*	*	1296
6	α - phellandrene	*	*	1003	26	δ- elemene	*		1343
7	δ - 3-carene	*	*	1029	27	Neryl acetate	*	*	1363
8	Limonene	*	*	1031	28	(Z)-2,6-dimethyl-2,7-octadien-1,6-diol	*	*	1366
9	(Z)-β-ocimene	*	*	1035	29	Geranyl acetate	*	*	1384
10	(E)-β-ocimene	*	*	1052	30	Cis-jasmone		*	1396
11	Cis-sabinene hydrate	*		1068	31	(Z)-β-caryophyllene	*	*	1417
12	(Z)- Linalool oxide	*	*	1073	32	(Z)- β -farnesene	*	*	1451
13	α- terpinolene	*	*	1088	33	α - humulene	*	*	1461
14	Linalool	*	*	1100	34	E,E-α-farnesene	*	*	1505
15	Phenyl ethyl alcohol	*	*	1110	35	(E)-nerolidol	*	*	1562
16	Terpinen-4-ol	*	*	1179	36	E,E-cis-farnesol	*	*	1731
17	α - terpineol	*	*	1192	37	Octadecane	*		1795
18	Lilace alcohol B		*	1217	38	Caffeine	*		1846
19	Lilace alcohol D		*	1233	39	Nonadecane		*	1892
20	Nerol	*	*	1235					

*There is in oil

Table 3- Statistical analysis of variation in flower Components from different locations.

Compounds	Ramsar		Jahrom		
	Mean	St.err	Mean	St.err	F value
a) Aldehyds					
1) Neral	0.06	0.006	0.09	0.006	
2) Geranial	0.13	0.02	0.19	0.02	
total	0.19	0.03	0.28	0.03	
b) Alcohols					
1) Linalool	33.93	0.26	36.55	0.19	**F****
2) Phenyl ethyl alcohol	1.03	0.11	1.24	0.13	
3) Terpinen-4-ol	0.78	0.07	0.95	0.09	
4) α-terpineol	5.04	0.15	7.73	0.17	**F****
5) Lilace alcohol B			0.05	0.006	
6) Lilace alcohol D			0.11	0.01	
7) Nerol	0.97	0.07	1.11	0.07	
8) Geraniol	2.22	0.14	2.52	0.11	**F***
9) Indol	0.51	0.04	0.72	0.07	
10) (Z)-2,6-dimethyl-2,7-	0.78	0.07	1.03	0.10	

octadien-1,6-diol					
11) (E)-nerolidol	1.22	0.10	1.49	0.08	
12) E,E-cis-farnesol	1.89	0.14	2.18	0.16	
total	48.37	1.15	55.68	1.19	
d) Esteres					
1) Linalyl acetate	9.43	0.23	11.41	0.18	**F****
2) Neryl acetate	1.34	0.12	1.54	0.12	
3) Geranyl acetate	2.38	0.19	2.75	0.12	**F***
total	13.15	0.54	15.70	0.42	
e) Ketones					
1) Cis-jasmone			0.01	0	
total					
Monoterpenes					
1) α-thujene	0.03	0.006	0.01	0	
2) α-pinene	0.77	0.09	0.59	0.05	
3) Sabinene	1.26	0.09	1.17	0.07	
4) β-Pinene	7.66	0.19	5.85	0.16	**F****
5) β-myrcene	1.71	0.10	1.66	0.15	
6) α - phellandrene	0.30	0.03	0.10	0.01	
7) δ - 3-carene	0.81	0.08	0.74	0.06	
8) Limonene	10.03	0.32	11.10	0.20	**F****
9) (Z)-β-ocimene	0.47	0.04	0.41	0.04	
10) (E)-β-ocimene	5.78	0.12	4.22	0.09	**F****
11) Cis-sabinene hydrate	0.07	0.01	0.02	0.00	
12) (Z)-Linalool oxide	0.16	0.02	0.07	0.01	
13) α-terpinolene	0.54	0.04	0.43	0.03	
total	29.59	1.14	26.37	0.87	
Sesquiterpenes					
1) δ- elemene	0.12	0.02			
2) (Z)-β-caryophyllene	0.52	0.05	0.59	0.04	
3) (Z)-β-farnesene	0.06	0.006	0.08	0.006	
4) α - humulene	0.10	0.01	0.08	0.01	
5) E,E-α-farnesene	0.07	0.006	0.10	0.01	
total	0.87	0.09	0.85	0.07	
Other compounds					
1) Octadecane	0.05	0.006			
2) Caffeine	0.07	0.006			
3) Nonadecane			0.37	0.02	
total	0.12	0.01	0.37	0.02	
Total oxygenated compounds	61.71	1.72	71.67	1.64	
Total	**92.29**	**2.96**	**99.26**	**2.60**	

Mean is average composition in % over the different locations used with three replicates. St. err = standard error. F value is accompanied by its significance, indicated by: NS = not significant, * = significant at P = 0.05, ** = significant at P = 0.01.

Table 4. Correlation matrix (numbers in this table correspond with main components mentioned in Table 3).

	Linalool	**α-terpineol**	**Geraniol**	**Linalyl acetate**	**Geranyl acetate**	**β-Pinene**	**Limonene**
α-terpineol	0.99**						
Geraniol	0.89*	0.87*					
Linalyl acetate	1.00**	0.99**	0.90*				
Geranyl acetate	0.88*	0.86*	0.99**	0.89*			
β-Pinene	-0.95**	-0.96**	-0.71	-0.94**	-0.71		
Limonene	0.96**	0.95**	0.96**	0.97**	0.95**	-0.85*	
(E)-β-ocimene	-0.96**	-0.97**	-0.75	-0.96**	-0.75	0.99**	-0.87*

*=significant at 0.05
**=significant at 0.01

Fig. 1. Flowers were collected from two geographical locations in Iran

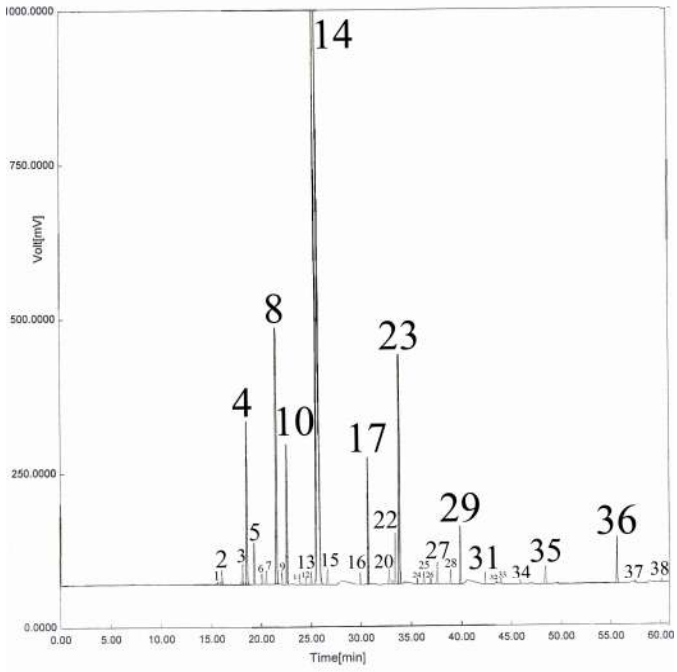

Fig. 2. HRGC chromatogram of flower oil of sour orange from Ramsar

CONCLUSION

In the present study we found that the amount of flower compositions was significantly affected by locations and there was a great variation in most of the measured characters between two locations. The present study demonstrated that volatile compounds in flower can vary when different locations are utilized. Between two locations examined, Jahrom showed the highest content of oxygenated compounds. The lowest of oxygenated compounds content were produced by Ramsar. Studies like this are very important to determine the amount of chemical compositions existing in different locations. Further research on the relationship between geographical locations and oxygenated compounds is necessary.

ACKNOWLEDGEMENTS

The author would like to express his gratitude to Z.Kadkhoda from Institute of Medicinal Plants located at Supa blvd-Km 55 of Tehran – Qazvin (Iran) for her help in GC-MS and GC analysis.

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CITATION OF THIS ARTICLE

Behzad B D. The Effect of Geographical Location on Sour orange (*Citrus aurantium L.*) Flower Components. Bull. Env. Pharmacol. Life Sci., Vol 4[1] December 2014: 171-177