American Journal of Essential Oils and Natural Products
Available online at www.essencejournal.com
AkiNik
AJEONP American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2018; 6(3): 35-39

Received: 20-05-2018
Accepted: 22-06-2018

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Chemical composition of peel, leaf and flower essential oils from 'Newhall' and 'Gannanzao' navel oranges

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Abstract
The essential oils of peel, leaf and flower of 'Newhall' and 'Gannanzao' navel orange were extracted by simultaneous distillation extraction method and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of seventy-two constituents were identified. Twenty-seven, Forty-four and thirty-nine compounds were identified in peel, leaf and flower essential oil of 'Newhall' navel orange, respectively; thirty-eight, thirty-nine and Forty-two compounds were identified in peel, leaf and flower essential oil of 'Gannanzao' navel orange, respectively. Limonene was the prominent component of the peel oil of both oranges, accounting for 81.62% and 88.07% of the total composition, respectively. There were no dominant components in the leaf and flower essential oils. Sabinene, limonene, linalool, and citronellal were abundant components of leaf essential oils. Sabinene, linalool, and nerolidol were main components in flower essential oils. The components of the same organs of these two navel oranges showed certain similarities, but some differences in content with different organs.

Keywords: 'Newhall' navel orange, 'Gannanzao' navel orange, essential oil, GC-MS

1. Introduction

Citrus is an important fruit which is not only rich in vitamins, organic acids, polysaccharides and other nutrients, but also contains a variety of important bioactive substances, such as essential oil, pectin, flavonoid, and carotenoid [1, 2]. Citrus essential oil (EO) is mainly found in peels, leaves and flowers of the plant. It is a kind of natural product with important economic value and a large market demand, widely used in flavor, fragrance, medicine and other fields.

EOs and their individual volatile constituents have potential pharmaceutical and therapeutic applications [3]. Dosoky *et al*. summarized the biological activities and safety considerations of citrus EOs [4]. Their review has indicated that the peel, leaf and flower EOs from different citrus species presented important bioactivities such as anti-oxidation, anti-bacteria, anti-inflammatory, and anti-cancer activities. Citrus EO has a good inhibitory effect on the growth of microorganisms and is becoming a new type of bacteriostatic agent [5-7]. Yang *et al*. found that navel orange EO could induce apoptosis of cancer cells [8]. Most citrus EOs are obtained from peel, and a small portion is derived from leaf and flower. Citrus species have been characterized by specific EO patterns depending on different organs. Terpenes are the predominant components in citrus peel EO, while citrus leaf EO and flower EO contain more oxygenated terpenes, such as linalool, citronellol, nerolidol, citronellal, citral and other important aroma compounds [9, 10].

China is one of the world's leading producers of citrus and Gannan is China's largest navel orange planting area with an annual output of more than one million tons [5]. 'Newhall' navel orange (*Citrus sinensis* Osbeck cv. Newhall) is the main cultivar in Gannan. 'Gannanzao' is a new variety selected from the bud mutation of 'Newhall' navel orange, which has the characteristics of early maturity (the harvest time is one month earlier than the latter), fine flavor, and easy peeling [11]. At present, there is no research report on the chemical constituents of EOs from different organs of 'Gannanzao' navel orange. Our research studied the composition characteristics of EOs in different organs of 'Gannanzao' and 'Newhall' navel oranges and compared the similarities and differences of EOs. It provides a reference to further study the composition characteristics and identification of varieties of related citrus EOs, which may benefit for the comprehensive utilization of citrus resources.

2. Materials and Methods

2.1 Materials

Orange flowers were collected in March 2017, leaves were collected in April 2017, and mature fruits were collected in October (for ‘Gannanzao’) and November (for ‘Newhall’) 2017, from the germplasm resources of Gannan Normal University, Ganzhou City of Jiangxi Province, China.

2.2 Extraction of essential oils
Sample preparation: The fresh orange peel was cut to small pieces and crushed. Fresh orange leaves were washed, dried by air in a ventilated place, and cut into filaments. Fresh orange flowers were collected and used directly.
Extraction method: 200 g of the sample was weighed, placed in a 2000 mL distillation flask, and 1000 mL of distilled water and 10 g of sodium chloride were added. 300 mL of petroleum ether was added to another 500 mL distillation flask. The flasks containing the material and the extraction solvent were connected to both ends of the extractor. The solvents were heated to reflux for 3 h. The mixture was collected and partitioned in a separation funnel. The organic phase was separated and dried over anhydrous sodium sulfate. After filtration, the solvent was removed by a rotary evaporator to obtain the corresponding EO. The extraction yield of EO (Y) is calculated defined as follows: Y (%) = (EO mass/sample mass) × 100

2.3 GC-MS Analysis
Orange EOs were analyzed by gas chromatography-mass spectrometry of SHIMADZU GCMS-QP2010SE equipped with a SH-Rxi-5SilMS capillary column (30.00 m × 0.25 mm × 0.25 μm). Mass spectra were obtained by electron ionization (EI) at 70 eV with a spectra range of 50 to 500 m/z. The injector and detector temperatures were operated at 150 °C and 250 °C, respectively. The oven temperature was maintained at 80 °C for 4 min, and subsequently raised to 250 °C (5 °C/min) and kept at 250 °C for 10 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The split ratio was 50:1. The components were identified by comparing Kovats retention indices and their mass spectra with those of the computer mass libraries of The National Institute of Standards and Technology (2010).

3. Results & Discussion
Orange EOs were obtained by simultaneous distillation extraction method. The extraction yield of peel, leaf and flower EO was 0.89%, 0.14% and 0.19% (‘Newhall’) and 0.97%, 0.16% and 0.24% (‘Gannanzao’), respectively. Six EO samples were detected by GC-MS. The EO components were identified by NIST library and retention indices. A total of 72 compounds were identified, including monoterpenes, sesquiterpenes, oxygenated monoterpenes, oxygenated sesquiterpenes and other non-terpene substances. The components and contents of EOs were shown in Table 1 and Figure 1. For convenience, ‘N’ was used to present ‘Newhall’ and ‘G’ to present ‘Gannanzao’.

Table 1: Components of essential oils from peel, leaf and flower of navel oranges

Compounds	RI[a]	Content/% (Newhall)			Content/% (Gannanzao)		
		peel	leaf	flower	peel	leaf	flower
(*Z*)-3-Hexen-l-ol	849	-	0.10	-	-	0.43	-
1-Hexanol	862	-	0.09	-	-	0.17	-
α-Thujene	927	-	0.23	0.21	-	0.12	0.17
α-Pinene	936	1.13	0.84	1.20	1.14	1.02	1.01
Benzaldehyde	966	-	-	0.20	-	-	0.56
Sabinene	979	1.22	10.13	13.32	0.39	11.12	11.27
β-Pinene	983	4.07	1.08	1.47	0.05	1.46	1.13
β-Myrcene	992	-	2.98	3.32	4.93	3.07	3.13
Octanal	1002	0.51	-	-	0.06	-	-
α-Phellandrene	1006	-	-	0.18	0.08	-	0.15
3-Carene	1015	1.42	6.12	1.34	0.97	6.37	2.33
α-Terpinene	1020	-	0.76	1.21	-	0.37	0.45
p-Cymene	1027	-	0.47	-	-	1.31	0.49
Limonene	1035	81.62	8.41	12.54	88.07	7.24	11.97
cis-β-Ocimene	1050	0.11	7.55	7.95	0.02	4.48	5.19
γ-Terpinene	1061	-	1.37	2.55	0.08	0.53	1.79
1-Octanol	1070	0.28	-	-	-	-	-
cis-Sabinene hydrate	1072	-	0.34	-	-	0.67	-
trans-Linalool oxide	1075	-	-	0.09	-	-	0.09
Terpinolene	1087	0.30	1.61	1.00	0.21	1.47	0.76
Linalool	1104	2.03	3.39	15.64	0.26	9.46	20.03
Nonanal	1120	-	-	-	0.04	-	-
trans-*p*-Mentha-2,8-dien-1-ol	1123	0.05	-	-	0.02	-	-
cis-*p*-Mentha-2,8-dien-1-ol	1127	-	0.24	-	-	0.39	-
trans-Limonene oxide	1139	-	0.07	-	0.05	-	-
Benzyl nitrile	1151	-	-	1.97	-	-	3.14
Citronellal	1157	0.20	8.65	0.07	0.11	9.01	0.04
Lilac aldehyde	1158	-	-	0.08	-	-	0.03
Terpinen-4-ol	1186	0.30	3.18	6.59	0.16	2.73	5.47
α-Terpineol	1198	0.81	0.33	1.42	0.29	0.94	1.10
Decanal	1206	0.36	0.08	-	0.19	0.13	-
Piperitol	1211	-	0.08	-	-	-	-
Nerol	1227	0.89	-	-	0.18	-	-
Citronellol	1232	-	4.39	-	-	7.05	-
Neral	1242	1.08	2.40	-	0.27	3.95	-

Linalyl acetate	1250	-	-	-	-	-	0.08
Geraniol	1254	0.32	1.36	-	-	2.57	-
Geranial	1272	1.26	3.20	-	0.36	5.38	-
Undecanal	1305	-	-	-	0.02	-	-
Indole	1308	-	-	3.64	-	-	2.42
2-Methoxy-4-vinylphenol	1313	-	0.96	0.67	-	-	0.51
Methyl geranate	1321	-	0.52	-	-	0.47	-
Methyl anthranilate	1349	-	-	2.44	-	-	6.65
Citronellyl acetate	1352	-	4.05	-	0.04	2.03	-
Neryl acetate	1371	-	3.37	-	0.15	1.93	0.09
α-Copaene	1375	0.04	-	-	-	-	-
Geranyl acetate	1382	-	2.97	-	0.07	1.58	-
β-Elemene	1387	-	4.83	1.26	0.08	3.27	1.14
cis-Jasmone	1393	-	-	0.14	-	-	0.12
Dodecanal	1398	-	-	-	0.04	-	-
β-Caryophyllene	1429	0.06	1.72	0.81	0.05	1.12	0.78
Geranylacetone	1448	-	-	0.06	-	-	0.09
β-Farnesene	1453	-	0.36	0.56	0.06	0.19	0.61
α-Humulene	1459	-	0.9	0.31	-	0.6	0.29
Germacrene D	1477	-	-	-	0.02	-	-
β-Selinene	1491	-	0.16	0.05	-	0.15	0.06
Valencene	1494	0.66	-	0.16	0.60	-	0.12
α-Selinene	1499	0.01	-	-	0.05	-	-
α-Farnesene	1504	-	0.23	0.25	0.05	0.14	0.29
δ-Cadinene	1529	0.04	-	-	-	-	-
β-Sesquiphellandrene	1533	-	-	0.06	-	-	0.06
Elemol	1549	-	0.11	-	-	-	-
trans-Nerolidol	1554	-	-	9.32	-	-	10.73
Caryophyllene oxide	1583	0.03	0.19	-	0.03	0.19	-
β-Sinensal	1696	-	2.56	1.92	0.21	1.02	1.35
Farnesol	1722	0.11	-	0.09	-	-	0.05
Farnesal	1730	-	-	0.15	-	-	0.1
α-Sinensal	1750	-	0.80	0.97	0.07	0.32	0.65
Nootkatone	1823	0.03	-	-	0.06	-	-
Phytone	1834	-	-	0.09	-	-	0.05
n-Hexadecanoic acid	1964	-	0.18	-	-	-	-
Phytol	2113	-	2.98	-	-	1.65	-
Monoterpenes		89.87	41.55	46.29	95.94	38.56	39.84
Sesquiterpenes		0.81	8.20	3.46	0.91	5.47	3.35
Oxygenated monoterpenes		6.94	38.54	23.81	1.96	48.16	26.90
Oxygenated sesquiterpenes		0.17	3.66	12.45	0.37	1.53	12.88
Others		1.15	4.39	9.29	0.35	2.38	13.57
Total		98.94	96.34	95.30	99.53	96.10	96.54

'-' Not detected. [a]RI = Retention indices determined with reference to a homologous series of *n*-alkanes (C_7-C_{30}) on SH-Rxi-5SilMS column.

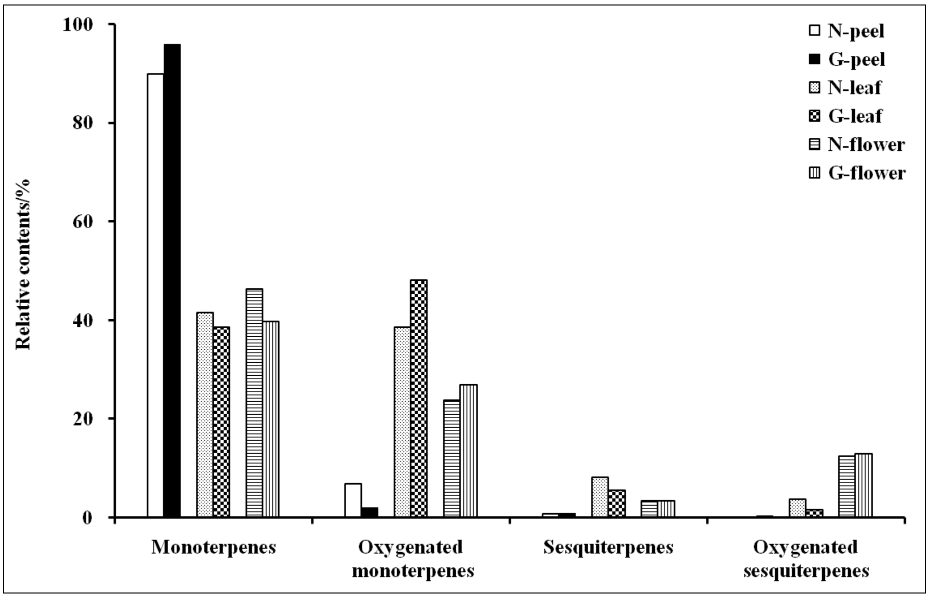

Fig 1: Relative contents of essential oils of orange peel, leaf and flower

3.1 Comparison of peel essential oils of 'Newhall' and 'Gannanzao' oranges

Twenty-seven and thirty-eight components were identified in N-peel and G-peel EOs, accounting for 98.94% and 99.53% of the compositions, respectively. There were twenty-two common components in both EOs. Five components, such as 1-octanol, geraniol and farnesol etc. were only present in N-peel EO; sixteen components, such as β-myrcene, α-phellandrene and neryl acetate etc. were only present in G-peel EO. The total amount of monoterpenes in G-peel EO (95.94%) was higher than in N-peel EO (89.87%). Limonene was the predominant component in G-peel EO (88.07%) and N-peel EO (81.62%). Percentage of oxygenated monoterpenes in G-peel EO (1.96%) was much lower than N-peel EO (6.94%). Sesquiterpenes and oxygenated sesquiterpenes presented very low content in both peel EOs (0.81% and 0.17% for N-peel EO, 0.91% and 0.37% for G-peel EO, respectively). Components of cold-pressed orange peel EO from Uganda and Rwanda [12] such as limonene (89.7%; 92.5%), myrcene (2.4%; 2.2%), α-pinene (0.5%; 2.4%), sabinene (1.6%; 0.2%), octanal (1.3%; 0.6%), and linalool (1.2%; 0.9%) had some similarities and differences in contents with N-peel EO and G-peel EO due to different varieties, extraction methods and regions. Dosoky's review [4] has indicated that there was a great variation in the chemical composition of citrus EOs due to differences in origin, genetic background, season, climate, age, ripening stage, method of extraction, etc. Our results were consistent with their conclusion.

3.2 Comparison of leaf essential oils of 'Newhall' and 'Gannanzao' oranges

Orange leaf EO is an important natural product, for example, petitgrain from Paraguay was a famous product in aroma industry [13]. Forty-four and thirty-nine components were identified in N-leaf and G-leaf EOs, with relative contents of 96.34% and 96.10%, respectively. Leaf EOs of both varieties were rich in monoterpenes and oxygenated monoterpenes. The total amount of monoterpenes in G-leaf EO was 38.56%, lower than N-leaf EO (41.55%); while the content of oxygenated monoterpenes in G-leaf EO (48.16%) was higher than N-leaf EO (38.54%). The content of sesquiterpenes (5.47%) and oxygenated sesquiterpenes (1.53%) was lower than N-leaf EO (8.20%; 3.66%). Sabinene was the most abundant component in N-leaf and G-leaf EOs, accounting for 10.13% and 11.12%, respectively. In addition, components with the high percentages in N-leaf EO were limonene (8.41%), *cis*-β-ocimene (7.55%), 3-carene (6.12%), and citronellal (8.65%) etc., which was consistent with G-leaf EO: limonene (7.24%), *cis*-β-ocimene (4.48%), 3-carene (6.37%), and citronellal (9.01%). The content of linalool, citronellol, geraniol, geranyl aldehyde in G-leaf EO was higher than in N-leaf EO; however, the content of citronellyl acetate, neryl acetate, and geranyl acetate was lower than in N-leaf EO. The contents of sabinene in EOs of orange leaves from Dubrovnik (21.0%-34.4%) [14] and Nigeria (20.9%-49.1%) [15] were much higher than N-leaf EO (10.13%) and G-leaf EO (11.12%), which may due to different planting areas, varieties and other factors [4].

3.3 Comparison of flower essential oils of 'Newhall' and 'Gannanzao' oranges

Thirty-nine and forty-two compounds were identified in N-flower and G-flower EOs, with relative content of 95.30% and 96.54%, respectively. Monoterpene content (39.84%) in G-flower EO was lower than in N-flower EO (46.29%); while oxygenated monoterpene content (26.90%) was higher than N-flower EO (23.81%). The relative contents of sesquiterpenes and oxygenated sesquiterpenes in two EOs were similar. In addition, flower EOs contained some other non-terpene compounds such as benzaldehyde, benzyl nitrile, indole, and methyl anthranilate. Linalool was the most abundant component of both flower EOs, and its content in G-flower EO (20.03%) was higher than N-flower EO (15.64%). Other abundant components in N-flower and G-flower EOs were sabinene (13.32% and 11.27%), limonene (12.54% and 11.97%), *trans*-nerolidol (9.32% and 10.73%), and *cis*-β-ocimene (7.95% and 5.19%).

4. Conclusions

In this study, we found that the percentage of EO components of 'Newhall' and 'Gannanzao' was affected by different varieties and organs. EO constituents of the same organs showed certain similarities, but some differences in content with different organs. Monoterpenes were the most abundant components in both peel EOs, in which limonene was the predominent compound. Monoterpenes and oxygenated monoterpenes were abundant in leaf and flower EOs. Other than peel EOs, leaf and flower EOs had more common chemical constituents. The composition of EOs, especially the differences in oxygenates such as alcohols, aldehydes and esters, could lead to differences of their aroma characters. The important biological activities might also be affected by chemical composition of EOs. Because orange EOs have wide applications, it is crucial to identify their chemical constituents from different species and organs. Our research might provide reference to improve comprehensive utilization of navel oranges.

5. Declaration of interest

No potential conflict of interest was reported by the authors.

6. Acknowledgments

This work was supported by Foundation of Jiangxi Province Educational Committee (KJLD14079), Natural Science Foundation of Jiangxi Province (20141BBG70002), and Innovation Team Plan of Jiangxi Province (20142BCB24007).

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