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Research Article

VARIATION OF THE VOLATILE CONSTITUENTS OF FRUIT PEELS OF *CITRUS RETICULATA* BLANCO BY PHYSICAL EFFECTS

Husain Shahnaz Sultana, Ali Mohammed*, Ansari Shahid Husain
Phytochemistry Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India

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*Prof. Mohammed Ali, Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi – 110 062, India E-mail: maliphyto@gmail.com

ABSTRACT
The chemical composition of volatile oil of the fruit peels of *Citrus reticulata* Blanco (Rutaceae) of Delhi Region has been studied under different physical factors. The oil was composed mainly monoterpenes (99.1 %) constituting l-limonene (92.4 %), γ-terpene (2.6 %) and β-phellandrene (1.8 %). The volatile oil after heating at 110°C for 24 hours, exposing to sunlight for 48 hours and UV light for 24 hours and after treatment with silica gel and alumina neutral for 24 hours contained the above three major components in varied amounts. α-Pinene, β-phellandrene, l-limonene, γ- terpinene and l-linalool were detected in all oil samples. α-Thujene and trans-β-caryophyllene were produced when the oil was treated with silica gel for 24 hours. Trans-Verbenol was determined when the oil was heated at 110°C for 24 hours and exposed to sunlight for 48 hours. Terpen-4-ol disappeared in all the samples except exposition to the sunlight. Cis-β-farnesene, present in the fruit peels in trace amount, was not detected in other oil samples after impact of physical factors.
KEYWORDS: *Citrus reticulate*, Rutaceae, Fruit peel, Volatile oil, Chemical composition variation

INTRODUCTION

Citrus reticulata Blanco (Rutaceae) is commonly known as narangi or santra (orange). It is a small spiny tree with dense top of slender branches, widely grown in India[1]. Mandarin is a group name for this class of orange with thin, loose peel, which has been dubbed 'kid-glove' oranges. These are treated as members of a distinct species, *C. reticulata* Blanco. The name 'tangerine could be applied as an alternate name to the whole group, but in trade, it is usually confined to the types with red-orange skin. In the Philippines all mandarin oranges are called naranjita. The fruit possesses laxative, aphrodisiac, astringent and tonic properties[1]. It is used to relieve vomiting[2]. The fruit peel regulates the skin moisture, softens hard and rough skin and has a cleaning effect on oily skin[3]. It also helps skin tone and removes skin blemishes[2]. Chemical composition of the volatile oil of the fruit peels of this species has been reported[3-8]. The present paper describes the effect of different physical factors on the chemical composition of the essential oil of the fruit peel of *C. reticulata*.

MATERIALS AND METHODS

Plant material

The fresh fruit peels of *C. reticulata* Blanco were purchased from the Alakananda market, New Delhi –110 019 in October 2009. The plant material was identified by Dr. M. P. Sharma, Professor, Department of Botany, Jamia Hamdard. A voucher specimen No. PRL/JH/09/08 is preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard.

Isolation of the volatile oil

The fresh fruit peels (1 kg) of *C. reticulata* were hydrodistilled in all glass Clevenger apparatus according to the method recommended in the British Pharmacopoeia[9]. The colourless volatile oil was dried over anhydrous sodium sulphate and stored at 4°C in the dark. The yield was 2.95 % based on the weight of the fresh fruit peels.

Thermal effect on the volatile oil

The volatile oil (3 ml) of *C. reticulata* fruit peels was heated in a sealed vial at 110°C in an electric hot oven for 24 hours. After cooling the oil was stored in dark at 4°C.

Sunlight effect on the volatile oil

The volatile oil (3 ml) of *C. reticulata* fruit peels was exposed to sunlight for 48 hours at 15°C in a glass vial. After exposure, it was stored in the dark at 4°C.

Ultraviolet light effect on the volatile oil

The volatile oil (3 ml) of *C. reticulata* fruit peels was exposed to ultraviolet light for 24 hours at 12°C in a glass vial. After exposure, the oil was stored in the dark at 4°C.

Treatment of the volatile oil with silica gel-G

The volatile oil (3 ml) of *C. reticulata* fruit peels was treated with silica gel-G in a sealed vial for 24 hours at 12°C. It was dissolved in solvent ether, filtered and the solvent evaporated under reduced pressure on a hot water bath. The treated volatile oil was stored in the dark at 4°C.

Treatment of the volatile oil with alumina neutral

The volatile oil (3 ml) of *C. reticulata* fruit peels was treated with alumina neutral in a sealed glass vial for 24 hours at 12°C. It was dissolved in solvent ether, filtered and the solvent evaporated under reduced pressure on a hot water bath. The treated volatile oil was stored in the dark at 4°C.

GC Analysis

Analytical GC was carried on a Varian 3300 gas chromatography fitted with a silicon DB-1 capillary column (30 m x 0.25 mm), film thickness 0.25 µm; carrier gas nitrogen, flow rate 1.5 ml/min, split mode, temperature programmed 80º-225ºC at 4ºC/min. Injector temperature 250º C, detector used FID, detector temperature 300º C. Injection volume for all samples was 0.1µl.

GC-MS Analysis: GC-MS analysis were carried out on a QP-2000 instrument fitted with a fused silica column Ulbon HR-1 (25 m x 0.22 mm), film thickness 0.22 µm and FID, carrier gas He, flow rate 1.5ml/min. The initial temperature was 100º C for six minutes and then heated at a rate of 10º C per minute to 250º C. The chromatograph was coupled to a HP 5971 A mass selective detector (70 eV).

Identification

The most constituents were identified by GC comparing their retention indices with those of authentic standard available in the laboratory or with the retention indices in close agreement with reference (Adams, 1995). α- and β-Pinenes, α-thujene, β-phellandrene, l-limonene, linalool, α-terpineol and linalyl acetate, available in the laboratory were procured from Sigma, Mumbai. Further identification was achieved by GC/MS. The fragmentation patterns of mass spectra was compared with those stored in the spectrometer data base using the NBS 54 KL and Wiley L-built libraries and with those reported in the literature[10-15].

RESULT AND DISCUSSION

A comparative variation of the volatile components of the fruit peels of *C. reticulata* are tabulated in Table-1. The components are arranged in order of GC elution on DB-1 column. The oil of the fresh fruit peels was characterized mainly by monoterpenes (99.1 %). All the components of the volatile oil were positively identified. There were six monoterpene hydrocarbons (97.7 %), three monoterpene alcohols (1.0 %) and one monoterpene ester occurring in trace amount. The predominant monoterpene was *l*-limonene (92.4 %) followed by *γ*-terpinene (2.6 %) and *β*- phellandrene (1.8 %). The component, detected in trace amounts included *α*- and *β*-pinenes, *n*-heptane, *α*-terpinolene, linalool, terpen-4-ol, *α*-terpeineol, linalyl acetate, longipinene and *cis*- *β*-farnesene. Except *n*-heptane, no other aliphatic constituent was detected in the volatile oil. Longipinene and *cis*- *β*-farnesene were the only sesquiterpenes detected in the oil. *α*-Pinene, *β*-phellandrene , γ-terpinene and linalool were detected in all the oil samples.

The percentage composition of *α*-pinene varied in trace amounts from 0.1 to 0.6 %; whereas the variation in the amounts of *β*- phellandrene differed from 1.2 to 2.2 %. *l*-Limonene was the predominant component in all the oil samples and except the UV light exposed sample, its percentage varied from 92.4 % in normal oil to 94.7 % in the alumina treated sample. The percentage yield of γ-terpinene varied widely from 2.4 % in alumina treated sample to 19.7 % in the UV exposed sample. The percentage of linalool differed from 0.2 % in the silica get G and alumina treated samples to 0.7 % in the normal oil. *α*-Thujene was only detected in trace amounts in the silica gel G treated sample. Except heated oil and UV light exposed sample, *β*-pinene was detected in all oil samples in 0.2 – 0.4 % yield. *n*-Heptane was only detected in normal and silica gel G treated sample. Except the alumina treated sample *α*-terpinolene was present in all the oil samples and its percentage yield markedly differed from 0.1% in sunlight exposed sample to 2.6 % in the UV light exposed sample. *trans*-Verbenol in the heated oil and sunlight exposed sample and terpen-4-ol in the normal and sunlight exposed samples were detected in 0.1% yield in each case .The percentage yield of *α*-terpineol remained constant as 0.2 % in the normal, heated and sunlight exposed samples and in other samples it was not characterized. Except alumina treated sample, linalyl acetate was present in all other samples and its amounts varied from 0.2 to 0.4 %.The concentration of longipinene , absent in the UV exposed and silica gel treated samples, differed from 0.1% in the sunlight exposed sample to o.4 % in the normal oil . *trans*- *β* –Caryophyllene and *cis*- *β* –farnesene were only detected in 0.1 % yield each in the silica gel-G treated and normal oil samples, respectively. From this study it has been concluded that *α*-pinene, *β* –phellandrene, *l*-limonene, γ-terpinene and linalool were less susceptible to the physical changes whereas *β*-pinene, *n*-heptane, terpen-4-ol, *α*-terpineol, longipinene and *cis*- *β*-farnesene were transformed to other components on subjection to physical changes.

In conclusion, the essential oil obtained after exposure to different physical factors varied in chemical constituents and their relative amounts. These qualitative and quantitative differences of oil might be due to interference of the components of the oil with light, heat and chromatographic adsorbents. Therefore, by using these trials, it is possible to increase the quality and quantity of specified contents and composition of the essential oil for industrial, nutritional and biological purposes.

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REFERENCES

1. Anonymous. The Wealth of India. A Dictionary of Indian Raw Materials a Industrial Products, National Institute of Science Communication (CSIR), New Delhi.3: 65 – 69.
2. Chopra RN, Nayar SL, Chopra IC**.** Glossary of Indian Medicinal Plants Council of Scientific and Industrial Research.1956; 69.
3. Khan MA, Ali M, Alam P. Phytochemical investigation of the fruit peels Of the fruit peels of *Citrus reticulata* Blanco. Natural Prod. Res. 2010; 24: 610-620.
4. Mahalwal V, Ali M. Volatile constituents of the fruit peels of *Citrus reticulata* Blanco, Journal of Essential oil Bearing Plants. 2001; 4: 45 – 49.
5. Anne-Laure F, Felix T, Francois L, Jean MD, Joseph C**.** Chemical variability of peel and leaf oils of mandarins. Flavour and Fragrance Journal. 2005; 21 (2): 359 – 367.
6. Rincon AM, Vasquez AM, Padilla, FC**.** Chemical composition and bioactive compounds of flavour of orange (*Citrus sinensis*), tangerine (*Citrus reticulata*) and grapefruit (*Citrus paradise*) peels cultivated in Venezuela, Arch. Latinoam Nutr. 2005; 55 (3): 305 – 310.
7. Qian S, Chen L**.** Studies on the chemical constituents of *Citrus reticulata*, Zhong Yao Cai. 1998; 21(6): 301 -302.
8. Simon MN, Hiroshi K, Mercy M , Minh Tu NT, Masayoshi S.. Essential oils of Kenyan Citrus fruits: volatile components of two varieties of mandarins (*Citrus reticulata*) and a tangelo (*C. paradise x C. tangerina*). Flavour and Fragrance Journal. 2004; 20 (1): 74 – 79.
9. British Pharmacopoeia**.** British Pharmacopoeia Commission, London. 2007; 3: 2343.
10. Adams, R.P**.** Identification of essential oil components by gas Chromatography/Qudrupol mass spectrometry. Allured publication Corp. Carol Stream, Illinois. 2001.
11**.** Ali M. Techniques in terpenoid identification. Birla Publications (Regd.) ,Delhi. 2001.
12. Kondjoyana K, Berdague JLA**.** Compilation of relative retention indices for the analysis of aromatic compounds, ed. Laboratorie Flaveur, INR de Theix, France. 1996.
13. Joulain D, Konig W**.** The Atlas of spectral data of sesquiterpenes hydrocarbons, E.B. Verlag, Hamburg, Germany. 1998.
14. Libey LM. A paradox of GC-MS data on components of essential oils and Other volatiles, J. Essential Oil. Res. 1991; 3: 193 -194.
15. McLafferty FW**.** The Wiley Registry of Mass Spectral Data, 6th edition, New York, John Wiley and Sons. 1994 .

Table 1. Comparative variation of the volatile oil components of the fruit peel of *Citrus reticulata* after different treatments

S.No.	Component	R.I.	A %	B%	C %	D %	E %	F %
1	*Alpha-Thujene*	922	---	---	---	---	0.1	--
2	*α*- Pinene	925	0.6	0.3	0.1	0.6	0.6	0.6
3	*β*-Pinene	964	0.2	---	0.4	---	0.3	0.2
4	*β*-Phellandrene	1003	1.8	1.2	1.5	1.6	2.2	1.6
5	*n*-Heptane	1013	0.4	---	---	---	0.5	---
6	*l*-Limonene	1022	92.4	94.0	93.6	74.9	92.3	94.7
7	γ-Terpinene	1043	2.6	2.9	2.9	19.7	3.0	2.4
8	*α*-Terpinolene	1070	0.1	0.2	0.1	2.6	0.2	---
9	Linalool	1086	0.7	0.5	0.6	0.3	0.2	0.2
10	*trans*-Verbenol	1140	---	0.1	0.1	---	---	---
11	Terpen-4-ol	1149	0.1	---	0.1	----	---	---
12	*α*-Terpineol	1177	0.2	0.2	0.2	---	---	---
13	Linalyl acetate	1240	0.4	0.3	0.3	0.2	0.3	---
14	Longipinene	1258	0.4	0.2	0.1	---	---	0.2
15	*trans- β*-Caryophyllene	1403	---	---	---	---	0.1	---
16	*cis-β*-Farnesene	1446	0.1	---	---	---	---	---

R.I. = Retention Indices, A = Volatile oil of the fruit peels without any treatment (Normal), B = Volatile oil heated at 110°C for 24 hours, C = Volatile oil exposed to sunlight for 48 hours, D = Volatile oil exposed to UV light for 24 hours, E = Volatile oil treated with silica gel-G for 24 hours, F = Volatile oil treated with alumina neutral for 24 hours.

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