



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading

doi:

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## Influence of volatile constituents of fruit peels of *Citrus reticulata* Blanco on clinically isolated pathogenic microorganisms under *In-vitro*

Husain Shahnaz Sultana<sup>1</sup>, Mohammed Ali<sup>1\*</sup>, Bibhu Prasad Panda<sup>2</sup><sup>1</sup>Phytochemistry Research Laboratory,<sup>2</sup>Microbial & Pharmaceutical Biotechnology laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India

## ARTICLE INFO

**Article history:**

Received 21 August 2012

Received in revised form 19 September 2012

Accepted 16 December 2012

Available online 28 December 2012

**Keywords:**

Citrus reticulata

Fruit peel

Volatile oil

Antimicrobial

## ABSTRACT

**Objective:** To investigate the antimicrobial activity of volatile constituents of fruit peels of *Citrus reticulata* Blanco on clinically isolated pathogenic microorganisms. **Methods:** Extraction of volatile oil was carried out by Clevenger's apparatus. Volatile chemical components were measured by GC-MS. Antimicrobial activity was carried by Agar well diffusion assay with reference to standard fluconazole and tetracycline. **Results:** The chemical composition of volatile oil of the fruit peels of *Citrus reticulata* Blanco (Rutaceae) of Delhi Region was composed mainly monoterpenes (99.1 %) constituting 1-limonene (92.4 %),  $\gamma$ -terpene (2.6 %) and  $\beta$ -phellandrene (1.8 %). The volatile oil showed antibacterial and antifungal activities against the clinically isolated pathogenic microbial strains *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans* under in vitro condition. **Conclusions:** The potential antimicrobial activity of volatile oil present in fruit peels of *C. reticulata* can be useful for treatment of skin disorder and/or in aroma. Therapy, it can be incorporated into cosmetic formulations.

### 1. 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 effects of the volatile oil of *C. reticulata* has been studied against pathogenic

fungi [9], *Saccharomyces cerevisiae* [10], *Aspergillus flavus* [11], *Schistosoma mansoni* [12], *Paenibacillus larvae* [13] and other microorganisms [14–19]. The volatile oil of *C. reticulata* also shows anti cancer activity [20]. The present paper describes the chemical composition and antimicrobial effect on bacteria, unicellular fungi and multi cellular fungi of the essential oil of the fruit peel of *C. reticulata* of Delhi region.

### 2. Materials and methods

#### 2.1. Plant material

The fresh fruit peels of *C. reticulata* 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.

#### 2.2. Isolation of the volatile oil

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

The fresh fruit peels (1 kg) of *C. reticulata* were hydrodistilled in all glass Clevenger apparatus. 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.

### 2.3. Gas chromatography of volatile oil

Analytical Gas chromatography (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  $\mu$  m; carrier gas nitrogen, flow rate 1.5 ml/min, split mode, temperature programmed 80 to 225°C at 4°C/min. Injector temperature 250°C, detector used FID, detector temperature 300°C. Injection volume for all samples was 0.1  $\mu$ l.

### 2.4. GC–MS analysis

GC–MS analysis was 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  $\mu$  m and FID, carrier gas He, flow rate 1.5ml/min. The initial temperature was 100°C for six minutes and then heated at a rate of 10°C per minute to 250 °C. The chromatograph was coupled to a HP 5971 A mass selective detector (70 eV).

### 2.5. Identification

The most of the 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 [21]. 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 [22–24].

### 2.6. Antimicrobial activity

#### 2.6.1. Microbial strains

Pure cultures of pathogenic bacterial species *Escherichia coli* and *Staphylococcus aureus* were obtained from Microbiology Department of Majeedia Hospital, Jamia Hamdard, New Delhi, India. Clinically isolated pathogenic fungi *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans* were obtained from the Institute of Genomics and Integrative Biology (CSIR), New Delhi, India. All the bacterial cultures were maintained on nutrient agar medium and fungal culture were maintained on potato dextrose agar media at 4°C.

#### 2.6.2. Standard antimicrobial substance

Anti bacterial antibiotic tetracycline and antifungal fluconazole solution (50  $\mu$  gml<sup>-1</sup>) was prepared in dimethyl

sulphoxide.

#### 2.6.3. Methods of preparation of test organisms

The test organisms were maintained on slants of nutrient agar medium (for bacterial culture) potato dextrose agar medium (for fungal culture) and transferred to a fresh slant once in a month. The slants were incubated at 37°C for bacterial culture and at 25°C for *Candida albicans* for 24 hours. For multi cellular fungi *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* the incubation temperature was 25°C and incubation time is 72 hours. Using 10 ml of sterilized normal saline solution, the cells/ mycelium were washed from the slants. A dilution factor was determined which gave optical density of 1.5 at 600 nm. The amount of suspension to be added to each 100 ml agar or nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

#### 2.6.4. Anti–microbial assay

In vitro antimicrobial assay was carried out by Agar well diffusion [25]. A Previously liquefied and sterilized nutrient agar /potato dextrose agar medium (20 ml) was poured in to petri–plates of 100 mm size (to make uniform thickness) and kept for solidifying. Microbial suspensions were spread over the solidified media. Holes were made in each plate with a stainless steel borer having 6 mm ID. Different dilutions (75  $\mu$ l) of the volatile oils of fruit peels of *C. reticulata* were made having concentration of 3  $\mu$ lml<sup>-1</sup>, 5  $\mu$ lml<sup>-1</sup>, 7  $\mu$ lml<sup>-1</sup> and 9  $\mu$ lml<sup>-1</sup> of solution. Tetracycline and fluconazole solutions were used as standards. All dilutions were made in DMSO solvent. The plates were then left for standing for 3 hours at 4°C for proper diffusion of the drugs/ test solutions. After diffusion process all the Petri plate were incubated for 24 h at 37°C for bacterial culture and at 25°C for *Candida albicans*. After 24 h the plates were examined and the diameter of zones of inhibition was accurately measured. The Petri disc inoculated with multi cellular fungi *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* plate were incubated for 72 hours at 25°C and the diameter of zones of inhibition was accurately measured by zone reader.

## 3. Result and Discussion

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  $\gamma$  –terpinene (2.6 %) and  $\beta$  – phellandrene (1.8 %). The component, detected in

**Table 1**Percentage composition of volatile oil of the fresh fruit peel of *C. reticulata*

S.No.	Components	Refractive Indices	Percentage
1	$\alpha$ -Pinene	925	0.6
2	$\beta$ -Pinene	964	0.2
3	$\beta$ -Phellandrene	1003	1.8
4	n-Heptane	1013	0.4
5	l-Limonene	1022	92.4
6	$\gamma$ -Terpinene	1043	2.6
7	$\alpha$ -Terpinolene	1070	0.1
8	Linalool	1086	0.7
9	l-Terpen-4-ol	1149	0.1
10	$\alpha$ -Terpineol	1177	0.2
11	Linalyl acetate	1240	0.4
12	Longipinene	1258	0.4
13	cis- $\beta$ -Farnesene	1446	0.1

**Table 2**Antimicrobial activity of the volatile oil of fruit peels of *C. reticulata* on tested pathogenic microbes

Test drugs/standard drugs concentration	Zone of inhibition to pathogenic fungi/ bacterial strains (mm)					
	<i>S. aureus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>C. albicans</i>
3 $\mu$ lml <sup>-1</sup>	13	12	00	12	14	15
5 $\mu$ lml <sup>-1</sup>	13	13	00	13	15	15
7 $\mu$ lml <sup>-1</sup>	14	13	12	13	14	15
9 $\mu$ lml <sup>-1</sup>	16	15	13	15	15	15
Tetracycline						
50 $\mu$ gml <sup>-1</sup>	11	17	–	–	–	–
Fluconazole						
50 $\mu$ gml <sup>-1</sup>	–	–	22	12	14	30

trace amounts included  $\alpha$ - and  $\beta$ -pinenes, n-heptane,  $\alpha$ -terpinolene, linalool, terpen-4-ol,  $\alpha$ -terpineol, linalyl acetate, longipinene and cis- $\beta$ -farnesene. Except n-heptane, no other aliphatic constituent was detected in the volatile oil. Longipinene and cis- $\beta$ -farnesene were the only sesquiterpenes detected in the oil. The oil was devoid of aromatic constituents. Limonene contributes to the aromatic odour of the oil and hence the plant belongs to the limonene chemo type.

The volatile oil was examined for antibacterial activity against *E. coli* and *S. aureus* and antifungal activity against *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Candida albicans*. The Oil showed significant antimicrobial and antifungal activity against clinically isolated pathogenic microbial strains in comparison to standard, tetracycline and fluconazole. The observations were recorded in the Table 2. The antimicrobial activity is mainly due to the presence of monoterpene hydrocarbons in volatile oil [26–27].

The potential antimicrobial activity of volatile oil present fruit peels of *C. reticulata* can be useful for treatment of skin disorder and/or in aroma therapy, it can be incorporated into cosmetic formulations.

We don't have any conflict of interest.

### Acknowledgement

The authors are thankful to the Head, SAIF, Central Drug Research Institute (CDRI) for recording GLC and GC-MS data of the volatile oil samples.

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### Conflict of interest statement

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