



Research Article

**Volatile Constituents , Antimicrobial and Antifungal activities
of fresh peels oil of *Citrus reticulata* Blanco**

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Steam distilled volatile oil obtained from fresh peels of fruits of *Citrus reticulata* Blanco. belonging to family-Rutaceae, was analysed by capillary GC and GC-MS. The oil is composed of nine monoterpenes which includes, five terpenic alcohols(45.5%), two terpenic esters(18.2%) and two were terpenic hydrocarbons(18.2%).The predominant monoterpenes were α -pinene (30.5%), terpinen-4-ol(17.4%), Benzyl acetate hydrocarbon(12.8%), *p*-cymene(8.8%).

Key words: *Citrus reticulata* Blanco., Rutaceae, essential oil, α -pinene, terpinen-4-ol.

Introduction:

Citrus reticulata Blanco.(Rutaceae), commonly known as Madder is widely grown in India¹.Orange peel oil is used as an antiseptic,antifungal, antimicrobial while the dried peels are used as antidyspeptic, antiscorbutic, astringent & Carminative. Flowers are stimulating¹⁻³. The main chemical constituents present in essential oil are α -pinene, Nerol, terpinen-4-ol, β -phellandrene, β - sitosterol, *p*-cymene, Myrcene, Sabinene, Tangeretin,

Syringin, Citral, Methyl-anthranilate, α -tocopherol, germacrene-B, Stigmesterol, α -terpineol, Benzyl acetate, 3-glucoside, 3-acetyl glucoside, 3-coumaryl-6,8-di-C-glucosyl Apigenins⁴⁻⁸.

Experimental

Plant Material: Fresh peels of fruit Mandarin were obtained from the herbal garden of Ram-eesh Institute, Greater Noida and then identified by Dr. M. P.Sharma Reader, Department of Botany, Faculty of Science, Jamia Hamdard, Hamdard University, New-Delhi. A

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voucher specimen is preserved in the herbarium of Ram- Eesh Institute.

Isolation: The fresh peels were hydro-distilled for 3 hrs. according to the method recommended in the British Pharmacopoeia 1998. The light yellowish green coloured oil obtained was dried and stored in the dark. The yield was 3.5% w/w based on fresh weight of the sample.

GC-Analysis: Analytical GC was carried out on a Varian 3300 GC fitted with a silicone DB-1 capillary column (30m × 0.25 mm.), film thickness 0.25 µm, carrier gas Nitrogen, flow rate 1.5 ml/min., split mode, temperature programmed 80-250°C

at 4°C/min. Injector temperature and detector temperature were 250°C and 300°C respectively. Detector used was FID. Injector volume for all samples was 0.1µl.

GC-MS Analysis: GC-MS analysis was carried out on a QP-2000 instrument at 70 eV and 250°C. GC column Ulbon HR-1 fused silica capillary 0.25mm×50m with film thickness 0.25µm. The initial temperature was 100°C for 6 min. and then heated at a rate of 10°C / min. to 250°C. Carrier gas Helium, flow rate 2ml/min. detector used was FID.

Identification: The volatile components were identified by comparing their retention indices of GC chromatograph

with those of literature. Further identification was done by GC-MS. The fragmentation patterns of Mass spectra were compared with those of the spectrometer data base using NBS 54 AL and Wiley L-built libraries and also with those reported in the literature⁹⁻¹⁵. Many constituents were identified by comparing their retention indices with those of authentic standards available in author's laboratory.

Anti-microbial Activity

Preparation of sample: The volatile oil (0.1% v/v, 0.5% v/v, 1.0% v/v) was dissolved in dimethyl sulfoxide (DMSO) for anti-microbial activity.

Preparation of Standard Drugs

Solution: Chloramphenicol and ketoconazole were used as standard solution of comparison of anti-microbial study. Both the standard drugs were taken in DMSO. The concentration of both standard drug solutions was 0.10 mg/ml.

Anti-microbial Activity: The antibacterial and antifungal activities of volatile oil were performed by the Department of Micro-biology, Ram-Eesh Institute. The identification of microbial strains were based on morphological, cultural and biochemical tests. The in-vitro antimicrobial activity of the fresh peels oil of *Citrus reticulata* fruits was studied by the cup plate method¹⁶

Table 1. Chemical composition of volatile oil of fresh peels of *Citrus reticulata* Blanco. fruits

S.N.	Component	R.I.	Percentage
1.	α -Pinene	928	30.5
2.	<i>p</i> -Cymene	1022	8.8
3.	<i>p</i> -Cymene-8-ol	1185	6.5
4.	α - Terpineol	1181	6.3
5.	Benzyl acetate	1182	12.8
6.	α - Terpineol	1185	6.4
7.	Terpinen-4-ol	1187	17.4
8.	Nerol	1218	5.7
9.	Linalyl acetate	1240	0.2
10.	7-Hydroxy-3,7-dimethyl octanal	1269	3.8
11.	α – Terpenyl acetate	1328	1.5

against various microorganisms listed in Table-2. Chloramphenicol and ketoconazole were used as standard and the activity of volatile oil was compared with corresponding concentration of standard drugs. The plates were incubated

at $37 \pm 2^\circ\text{C}$, after 48 hrs. of incubation. The Petri-dishes were taken out from the incubator and the anti-microbial activities of the fresh peels oil of *Citrus reticulata* fruits were compared by measuring the diameter of the zone of inhibition (Table-2).

Table 2: Anti-microbial activity of fresh peels oil of *Citrus reticulata* Blanco. fruits

S.No.	Test organism	Zone of Inhibition in mm ^a					
		Conc. of volatile oil			Alcoholic extract	Standard Chloramphe.	Standard Ketocona.
		0.1 % v/v	0.5 % v/v	1.0 % v/v	5.0% w/v	(0.1mg/ml)	(0.1mg/ml)
1.	<i>S.aureus</i>	7.2	10.5	21.5	9.8	17.4	-
2.	<i>E.coli</i>	6.4	9.2	11.5	8.9	16.6	-
3.	<i>A.nidulans</i>	6.8	11.4	12.4	9.8	16.3	17.5
4.	<i>A.niger</i>	6.7	10.5	12.2	9.4	15.6	17.0
5.	<i>F.oxysporum</i>	6.9	10.8	12.0	9.5	15.8	17.2

^aan average of triplicate

Chloramphenicol- Against all micro-organism [gram(+ve) and gram(-ve) bacteria and fungal strains]; **Ketoconazole-** Against fungal strains only.



Results and Discussion: The volatile component of fresh peels oil of *Citrus reticulata* fruits are listed in Table-1. Components are arranged in order of GC elution on QP-2000 column. The oil was characterized by a large amount of monoterpene constituents (100%) with α -pinene, *p*-cymene, *p*-Cymene-8-ol, α -Terpineol, Terpinen-4-ol, Nerol, α -Terpenyl acetate. α -pinene(30.5%) and terpinen-4-ol(17.4%) were the major constituents identified in the oil sample. The essential oil of fresh peels of *Citrus reticulata* fruits (Table-2) displayed significant inhibitory activity against gram(+ve)(*S.aureus*) and gram(-ve)bacteria(*E.coli*), in addition, it was also active against the fungus, *Aspergillus nidulan*, *Aspergillus niger* and *Fusarium oxysporum* 1% v/v concentration of volatile oil shows maximum inhibitory activity against bacteria and fungi. However, the maximum inhibitory activities of essential oil were on *S.aureus* and *Aspergillus niger*.

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