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Vijender Singh
BBS Institute of Pharmaceutical & Allied Sciences, Knowledge Park-III, Greater Noida, Gautam Budh Nagar, Uttar Pradesh-201310, India.

Deepti Katiyar
Department of Pharmacognosy, KIET School of Pharmacy, Ghaziabad- 201206, India

Mohammed Ali
Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India

Correspondence:
Vijender Singh
BBS Institute of Pharmaceutical & Allied Sciences, Knowledge Park-III, Greater Noida, Gautam Budh Nagar, Uttar Pradesh-201310, India.

Comparative study of volatile constituents and antimicrobial activities of leaves and fruit peels of *Citrus sinensis* Linn.

Vijender Singh, Deepti Katiyar, Mohammed Ali*

Abstract

A steam distilled volatile oil from the fresh leaves of *Citrus sinensis* Linn. analysed by capillary-GC and GC-MS yielded eight components, of which seven were monoterpenic (99.6%) and one ester (0.4%). *Cis*-sabinene hydrate (35.1%) was the predominant monoterpene, followed by *l* - limonene (30.1%), citral (27.9%), lavendulol (2.5%), perillaldehyde (2.0%), *α*-pinene (1.4%) and perillyl alcohol (0.6%). The ester obtained was tert.-butyl benzoate (0.4%). Whereas volatile oil from its fresh fruit peels yielded five components of which three were monoterpenes (76.5%) and remaining two were non-terpenic compounds. *α*-pinene (60.80%) was the predominant monoterpene, followed by verbenone (15.40%) and *α*-thujene (0.30%).The non-terpenic compounds obtained was *N*-pentylcyclopentane (11.80%) and cyclopentenyl ethyne (11.70%). The maximum antibacterial activity was shown with 1%v/v of volatile oil collected from Greater Noida, U.P on *Staphylococcus aureus* (16.4 mm) followed by *Escherichia coli* (14.2 mm), and maximum anti-fungal activity was shown on *Candida albicans* (13.1 mm) followed by *Aspergillus niger* (12.5 mm).

Keywords: *Citrus sinensis*, *cis*-sabinene hydrate, l-limonene, citral, anti-microbial activity.

Introduction

Fruits are an essential part of our diet and are known to reduce the risk of several chronic diseases, including cancer.[1] Citrus fruits belonging to the Rutaceae family contain various bioactive phytoconstituents e.g. polyphenols which are responsible for their protective nature. *Citrus sinensis* commonly known as sweet orange (Mosambi),[2] cultivated both in the subtropical regions of North India and the tropical, humid regions of South India.[3,4] The volatile phytoconstituents include citral, limonene, γ-terpine, α- and β- pinene, camphene, linalool, α- and β-carotene, methyl anthranilate, cryptoxanthin, limocitrin, nerol and sabinene.[5,6] It also contains flavone glycosides (neohesperidin, naringin, hesperidin, narirutin), pigments (anthocyanin, beta-cryptoxanthin, zeaxanthin), vitamins (B1, B2, B3, B5, B6 and vitamin C) and minerals (calcium, iron, magnesium, zinc, phosphorus, potassium).[7] It is an excellent anti-oxidant agent due to its flavonoid content.[8,9] It possesses some cardioprotective constituents-vitamin C, flavonoids & carotenoids. Limonene is responsible for its cholesterol lowering property[10] and anti-carcinogenic activity of sweet orange.[11,12] It also posseses anti-ulcer[13], anti-anxiety,[14,15] anti-typhoid,[16] anti-bacterial,[17] larvicidal,[18] anti-diabetic,[19,20] anti-fungal[21] and anti-inflammatory activities.[22] As a part of our investigation on aromatic and medicinal plants of India, we describe in this communication, the comparative study on the chemical composition and anti-microbial activity of oil isolated from the fresh leaves and fruit peels of Sweet orange by modern sophisticated techniques.[23]

Materials and Methods

Plant material: Fresh leaves of *Citrus sinensis* were collected from Neha Nursery, Greater Noida, U.P. and fruit peels were obtained from its fresh fruits purchased from local market, Greater Noida, U.P. and both were authenticated by Dr.M.P.Sharma, Reader, Deptt. of Botany, Jamia Hamdard, New Delhi and a voucher specimen is preserved in the herbarium of Phytochemistry laboratory, Faculty of Pharmacy, BBS Institute of Pharmaceutical & Allied Sciences, Greater Noida, U.P.

Isolation: The fresh leaves and peels (1.0 kg each) were hydro distilled for three hours, according to the method recommended in the British Pharmacopoeia 2009 [24]. The light yellowish green colored oil (0.33%v/w) and colorless oil (0.55% v/w) were obtained from leaves and peels respectively. The collected volatile oil was dried over anhydrous sodium sulphate and stored at 4 ^{0}C in the dark.

GC Analysis: Analytical GC was carried out on a Varian 3300 GC fitted with a silicone DB-1 capillary column (30m × 0.25mm), film thickness 0.25µm, carrier gas Nitrogen, flow rate 1.5 ml/min., split mode, temperature programmed 80 - 250 ^{0}C at 4 ^{0}C/min. Injector temperature and detector temperature were 250 ^{0}C and 300 ^{0}C respectively. Detector used to be FID. Injection volume of all samples was 0.1µ.

GC-MS Analysis: GC-MS Analysis was carried out on a QP-2000 instrument at 70eV and 250 ^{0}C. GC column Ulbon HR-1 fused silica capillary 0.25 mm × 50 m with film thickness 0.25 µm. The initial temperature was 100 ^{0}C for six minutes and then heated at a rate of 10 ^{0}C per min. to 250 ^{0}C. Carrier gas Helium, flow rate 2ml/min., detector used was FID.

Identification of volatile constituents: 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 spectrometer data base using NBS 54AL and Wiley L-built libraries and also with those reported in the literature[25-28].

Anti-microbial Activity

Preparation of sample:

The volatile oil (0.1%v/v, 0.5 %v/v, 1%v/v) and dried alcoholic extract (5.0%w/w) were dissolved in dimethyl sulfoxide (DMSO) for anti-microbial activity.

Preparation of Standard Drugs Solution: Chloramphenicol and Ketoconazole were used as standard solutions for comparison of anti-bacterial and anti-fungal studies. Both the standard drugs were taken in DMSO. The concentration of both standard drug solutions was 10 mg /ml.

Anti-microbial Activity: The antimicrobial activities of volatile oil and dried alcoholic extract of fresh leaves and fresh fruit peels of *Citrus sinensis* were performed in the Deptt. of Micro-biology, BBS Institute of Pharmaceutical & Allied Sciences, Greater Noida. The identification of microbial strains was based on morphological, cultural and biochemical tests. The in-vitro antimicrobial activity of various oil concentrations were studied by the cup plate method. [29-30]

Chloramphenicol and Ketoconazole were used as standard and the activity of each concentration was compared with the corresponding concentration of standard drugs. The plates were incubated at 37 $\pm$ 2 ºC for antibacterial activity and 25 $\pm$ 2 ºC for anti fungal activity, after 48 hrs of incubation. The Petri dishes were taken out from the incubator and the anti-microbial activity of different concentrations of oil and dried alcoholic extract of fresh leaves and fresh fruit peels of *Citrus sinensis* were compared by measuring the diameter of the zone of inhibition.

The control DMSO showed no inhibition of growth, while all the concentrations of oil were effective against *Escherichia coli* & *Staphylococcus aureus* when compared to chloramphenicol and Ketoconazole .

Result and Discussion

Analysis of the volatile oil of sweet orange leaves by GLC and GC-MS resulted in the identification of eight components of which seven were monoterpenoids (99.6%) and one ester (0.4%). *cis*-sabinene hydrate (35.1%) was the predominant monoterpene, followed by *l*-limonene (30.1%), citral (27.9%), avendulol (2.5%), perillaldehyde (2.0%), *α*-pinene (1.4%) and perillyl alcohol (0.6%). The ester obtained was *tert*.-butyl benzoate (0.4%). Out of the monoterpenes, lavendulol and citral are acyclic monoterpenes. *l*-limonene, perillaldehyde and perillyl alcohol are monocyclic monoterpenes of the *p*-menthane structural types. *cis*-Sabinene hydrate and *α*-pinene are bicyclic monoterpenes of the *α*-thujene and *α*-pinene structural types respectively. **(Table-1)**

Where as analysis of the oil of sweet orange peel by GLC and GC-MS resulted in identification of the five compounds of which three were monoterpenoids (76.5%). Quantitatively the oil was characterized by high amounts of monoterpenes (76.5%) namely *α*-thujene, *α*-pinene & verbenone and *α*-thujene and α-pinene were hydrocarbons where as verbenone was a ketone, remaining two were non terpenic compounds namely cyclopentenylethyne and *n*-pentylcyclopentane. (**Table 2**)

Table 1: Chemical composition of volatile oil of the fresh leaves of *Citrus sinensis* Linn.

S. No.	Volatile constituents	R I	%age
1	*α* – Pinene	928	1.4
2	*l* – Limonene	1014	30.1
3	*cis*- Sabinene hydrate	1045	35.1
4	Citral	1136	27.9
5	Lavandulol	1153	2.5
6	Perillaldehyde	1253	2.0
7	Perillyl alcohol	1281	0.6
8	*tert*.- Butyl benzoate	1287	0.4

Identified as monoterpenes 7 and Other components (non terpenic compound) 1

Table 2: Chemical composition of volatile oil of the fresh fruit peels of *Citrus sinensis* Linn.

S. No.	Volatile constituents	R.I.	%age
1.	*α*-Thujene	923	0.30
2.	*α*-Pinene	928	60.80
3.	Cyclopentenylethyne	935	11.70
4.	*N*-pentylcyclopentane	-	11.80
5.	Verbenone	1171	15.40

RI: Retention index; %age: Percentage; Identified as monoterpenes 3 and Other components (non terpenic compound) 2

Antimicrobial activities of dried alcoholic extract and different concentration of volatile oil of fresh leaves and fresh fruit peels of *Citrus sinensis* from Greater Noida, U.P. were summarized in **Table 3 & 4**. The maximum antibacterial activity was shown with 1%v/v of volatile oil of *Citrus sinensis* fresh leaves from Greater Noida, U.P on *Staphylococcus aureus* (16.4 mm) followed by *Escherichia coli* (14.2 mm), and maximum anti-fungal activity was shown by 1% v/v of volatile oil on *Candida albicans* (13.1 mm) followed by *Aspergillus niger* (12.5 mm).

Table 3: Anti-microbial activity of volatile constituents of the fresh leaves of *Citrus sinensis* Linn.

S. No.	Test Organism	Zone of Inhibition in mm[a]					
		Conc. of Volatile Oil			Dried Alcoholic Extract 5.0 %w/v	Standard Chloramphenicol (0.1 mg/ml)	Standard Ketoconazole (0.1 mg/ml)
		0.1 %v/v	0.5 %v/v	1.0 %v/v			
1	*Staphylococcus aureus*	7.4	9.6	16.4	9.2	20.9	-
2	*Escherichia coli*	6.7	8.8	14.2	8.2	18.8	-
3	*Candida albicans*	6.4	7.9	13.1	7.2	16.8	17.9
4	*Aspergillus niger*	5.9	7.3	12.5	6.7	15.9	17.6

Table 4: Anti-microbial activity of volatile constituents of the fresh fruit peels of *Citrus sinensis* Linn.

S. No.	Test Organism	Zone of Inhibition in mm[a]					
		Conc. of Volatile Oil			Dried Alcoholic Extract 5.0 %w/v	Standard Chloramphenicol (0.1 mg/ml)	Standard Ketoconazole (0.1 mg/ml)
		0.1 %v/v	0.5 %v/v	1.0 %v/v			
1	*Staphylococcus aureus*	7.0	9.2	15.9	8.9	20.8	-
2	*Escherichia coli*	6.6	8.7	14.0	8.4	18.9	-
3	*Candida albicans*	6.6	7.9	12.9	7.6	16.6	17.8
4	*Aspergillus niger*	6.1	7.4	12.3	7.2	15.7	17.3

a an average of triplicate; **Chloramphenicol**- Against all micro-organism [gram + ve, gram - ve bacteria and fungal strains]; **Ketoconazole**- Against fungal strains only.

Whereas in case of fruit peels maximum antibacterial activity was shown on *Staphylococcus aureus* (15.9 mm) followed by *Escherichia coli* (14.0 mm), and maximum anti-fungal activity was shown on *Candida albicans* (12.9 mm) followed by *Aspergillus niger* (12.3 mm).

Conclusion

It was concluded from the above studies that, in case of *Citrus sinensis*, the leaves are more effective as anti-microbial than fruit peels, the reason might be that the leaves have shown to possess more number of volatile constituents than the fruit peels.

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