VOLATILE CONSTITUENTS OF *CITRUS AURANTIFOLIA* VARIETY "KAGHZI NIMBU"

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ABSTRACT: Hydro distillation of *Citrus aurantifolia* peels gave 0.47% of essential oil. GC-MS analysis of peel oil showed the presence of 21 different components. Out of which 14 components were identified. Limonene was found 82.84% to be the most dominating terpene followed by -thujene (0.16%), -terpinene (8.55%), -pinene (1.90%), 3-carene (0.01%), iso-terpinolene (0.55%), 4-terpineol (0.39%), -terpineol (0.39%), Trans carveol (0.33%), Geraniol (0.09%), Geranyl alcohol (0.11%), -cedrene (0.18%), -cadinene (0.18%) and -bisabolene (0.22%). The rest of the components were less than 1.0% each and 6.73% portion of the essential oil remained unidentified.

Key words: *Citrus aurantifolia*, Essential oil, Limonene.

INTRODUCTION

Citrus aurantifolia commonly known as "Kaghzi Nimbu" is one of the most important species of family *Rutaceae*. (Nasir and Ali, 1972, Njoroge *et al.,* 2006). This family has 150 genera and 1600 species distributed throughout the Mediterranean, tropical and subtropical regions of the world (Swingle, 1967, Tasneem, 1995). Out of these genra, citrus is the most important genus of this family due to its edible fruits (Mabberley, 1997).

The oil of lemon (*Citrus aurantifolia*) is one of the most important flavouring oil, used widely in all kind of beverages, soft drinks, tablets, baked goods, such as cakes, pastries, pie fillings, confectionary, soft and hard centre candies, gelatin desserts, ice creams, etc. The oil is also employed in perfumes, toiletries, colognes, and in cosmetics to which it imparts a refreshing top note (Gamara *et al*., 2006, Siharat *et al.,* 2007).

Survey of literature shows that similar investigations have also been carried on Citrus peel oils (Sattar et al, 1987,1989,1992). *Citrus aurantifolia* has been studied by John *et al.*,2005, Shivashankara *et al.,* 2002, Marie *et al.,* 2002,Hoda 1991,Marry *et al.,* 2002, and Nguyen *et al.,* 2007, for their chemical constituent in Australia, India, France, Egypt, USA and Japan respectively. The quality of essential oil of *Citrus aurantifolia* was studied by Gamara *et a.* (2006) while Siharat., *et al*., (2007) and Onyeagba *et al.* (2004), studied its toxicity and antimicrobial activity.

Numerous varieties of genus citrus have been studied. Peel and seed oil of sangtra, kinnow and tangerine were studied by Sattar *et al.,* 1987 Mahmud *et al.,* 2005 & 2006,Saleem *et al.,* 2008 respectively.

This study is the continuation of our previous work and aimed to investigate the volatile constituent of *Citrus aurantifolia* "Kaghzi Nimbu" found in the region of Punjab Pakistan.

MATERIALS AND METHODS

The fresh *Citrus aurantifolia* fruit locally known as "Kaghzi nimbu" was collected from a garden near Pattoki 100 km away from Lahore. The peels were detached from the fruits, cut into small pieces, weighed (750 gm) and subjected to steam distillation. The essential oil collected was separated from water and extracted twicely (2x100ml) with diethyl ether. The extracted oil was dried using anhydrous sodium sulphate. The pale yellow dried essential oil filtered and collected in an amber colored sample bottle.
The physico-chemical characteristics of the essential were estimated by the standard methods of Guenther (1952).

GC-MASS SPECTROSCOPY: Gas chromatographic analysis for the *Citrus aurantifolia* essential oil was conducted on a Shimadzu GC-14 gas chromatograph. This GC was equipped with flame ionization detector and 25mx 0.22mm SE-30 capillary column. Helium was used as carrier gas with a flow rate of 1ml/min.One l sample was injected with split ratio 1:100.The column oven temperature was maintained at 70°C for four minutes with 4°C rise

per minutes to 220°C respectively. Percentage composition of individual components was calculated on the basis of peak area using Shimadzu C-R4A chromatopac. Various compounds were identified by comparison of their retention time and peak enhancement with standard samples.

Jeol model JMS-AX505H mass spectrometer combined with Hewlett Packard 5890 gas chromatograph was used for analysis of essential oil. The essential oil sample was injected on a 25mx0.22mm BP5 (5% phenyl methyl siloxane) capillary column, using Helium as a carrier gas with split ratio 1100 EI (electron impact) mode was used with ionization current 300A and programmed column temperature at 70°C for 4 minutes with 4°C/minutes rise to 220°C.

Data acquisition and processing were performed by Jeol JMA-DA 5000 analysis system. The comparison of the fragmentation pattern of individual component with Nist library search helped in the identification and confirmation of the components.

Table-1: Physicochemical Properties Of Essential Oil Of *Citrus Aurantifolia.*

Yield	0.47%
Colour	Pale yellow
Odour	Sharp refreshing lemon like
Specific gravity at 20°C	0.8401
Refractive index at 20°C	1.0472
Acid value	1.38
Aldehyde value	1.74

Table-2: Results Of *Citrus Aurantifolia* Essential Oil Analysis By GC-MS

Sr.#	Compound	% Age	Mass fragmentation pattern M/Z value
1.	α-thujene	0.16	136 (M^+, 21), 105(5), 93(100), 77(26), 65(4), 32(8)
2.	β-terpinene	0.61	136 (M^+, 21), 121(15), 107(6), 93(100), 79(17), 69(30), 53(5), 41(22), 32(9)
3.	β-pinene	0.86	136 (M^+, 15), 121(6), 93(100), 79(12), 69(65), 53(7), 41(51), 32(6)
4.	d-limonene	82.84	136 (M^+, 56), 121(32), 107(27), 93(71), 79(25), 68(100), 53(16), 41(14)
5.	3-carene	0.01	136 (M^+, 56), 121(38), 93(100), 77(24), 65(6), 53(4), 43(15)
6.	Isoterpinolene	0.55	136 (M^+, 81), 121(100), 105(18), 93(80), 79(27), 67(9), 55(7), 41(12), 32(2)
7.	4-terpineol	0.39	154 (M^+, 35), 136(17), 111(76), 93(49), 86(32), 71(100), 55(16), 43(31), 32(4)
8.	α-terpineol	0.39	136 (M^+, 84), 121(64), 93(75), 81(40), 67(16), 59(100), 43(26), 31(6)
9.	Trans carveol	0.33	152 (M^+, 31), 137(12), 119(12), 109(100), 93(14), 84(62), 69(21), 55(24), 41(21), 32(11)
10.	Geraniol	0.39	154 (M^+, 7), 136(6), 123(10), 93(21), 84(16), 69(100), 55(12), 41(56), 31(2)
11.	Geranyl alcohol	0.11	154 (M^+, 7), 136(6), 123(13), 111(8), 93(13), 69(100), 55(7), 41(50), 32(6)
12.	α-Cedrene	0.18	204 (M^+, 11), 189(4), 161(11), 135(9), 119(100), 107(26), 93(85), 79(19), 69(30), 55(16), 41(23), 32(3)
13.	γ-Cadinene	0.18	204 (M^+, 22), 161(100), 147(5), 133(13), 119(22), 105(31), 91(21), 69(12), 55(9), 41(11)
14.	β-Bisabolene	0.22	204 (M^+, 74), 189(18), 175(3), 161(49), 147(10), 134(21), 119(36), 109(42), 93(92), 79(30), 69(100), 55(16), 41(48)

RESULTS AND DISCUSSION

The yield of the essential oil obtained was 0.47% (Table1). The yield was quite encouraging as compared to the yields of citrus oils reported by Rondeau *et al.*, 2003 (0.03% to 0.08%),Mosaddegh *et al.*, 2004, (1.1%) Jhon *et al.*, 2005 (0.41%) and Nguyen *et al.*, 2007 (0.07%). The physicochemical characteristics are given in Table-1. These are comparable to the values reported in Guenther (1952).

The gas chromatography coupled with mass spectrometric analysis revealed the presence of 21 components of the essential oil, out of which 14 components could be, identified as naturally occurring terpenic compounds.Limonene was found as major Component (82.84%), which is quite fair enough contrary to the results reported by Gamara *et al.*, 2006, Shaw, 1979, Kefford and Chandler 1970, Sattar *et al.*, 1987,1989, 1992, Mahmud et al. 2005, 2006.,Saleem *et al.*, 2008. Jin *et al.*, 2001, Zhu etal. 2003, and Mosaddegh.2004 reported the comparatively low yield of limonene (48.4%, 6.7%, 51.99%) in citrus species.

The other monoterpenes α- thujene (0.16%), β-terpinene (0.61%) β -pinene (0.86%) 3- carene (0.01%) and iso-terpinolene (0.55%) were present in minute amounts. These results are comparable to the Johan *et al.*, (2005), Marie *et al.*, (2002) but-β– pinene in Indian Kaghzi lime was quite high (22.16%) Shivashankara et al.2002. The oxygenated components 4-terpineol (0.39%) α-terpineol (0.39%) trans- carveol (0.33%), geraniol (0.09%) geranyl- alcohol (0.11%), which contributes to the flavor of oil, were also present. These oxygenated components are reported by Wolford *et al.*, (1967).Chisholm *et al.*, (2003) reported geranial and many aldehydes, alcohols ethers and many sesquiterpenes contribute to the aroma of extracted oil giving a characteristic lime tone. Selli, *et al.*, 2004, reported that the major flavor compound was terpene-4-ol. Gamarra *et al.*, 2006 reported that the aldehyde is related to the presence of the oxygenated compound especially the carbonylics have an important influence on the aroma of the essential oil. These all references supported our studies in which it is described that oxygenated components contribute to the flavor of oil.

Some sesquiterpenes were also identified including α cadinene (0.18%), γ -cadinene (0.18%) and β- bisabolene (0.22%). α cadinene and γ -cadinene are quite comparable to the

Shivashakara et al 2002 while β- bisabolene is in accordance of Marie *et al.*, 2002, John *et al.*, 2005 and Gamara *et al.*, 2006. They reported β-bisabolene 0.3, 0.2 and 0.29 respectively.

Due to distinct aroma, essential oil of *Citrus aurantifolia* is recommended for use as flavoring agent in beverages and food products. From the above studies it is suggested that after deterpination of the oil, the oxygenated fraction is suitable for flavoring purpose and the other major fraction i.e. limonene may be utilized in cheep perfumery and in leather industry.

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