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ORIGINAL ARTICLE

Volatile components, antioxidant and antimicrobial activity of *Citrus acida* var. sour lime peel oil

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Abstract Essential oil of *Citrus acida* Roxb. var. sour lime was analyzed by GC–MS. Out of 59 components 18 were identified from their fragmentation pattern. Among the identified constituents, *o*-cymene (16.62%) was found as a major component followed by α-cedrene (10.57%), decadienal (8.043%), bisabolene (5.066%) and β-humelene (4.135%). Citronellyl acetate (2.371%), linalool acetate (2.371%), carvone (1.806%), decanone (1.474%), isopulegol acetate (1.296%), farnesol (1.254%), 4′-methoxyacetophenone (1.207%), and Δ-carene (1.070%) were found in minor quantities whereas α-terpineole (0.607%), dihydroxylinalool acetate (0.650%), *cis*-nerone (0.574%), caryophyllene oxide (0.433%), and 2,2-dimethyl-3,4-octadienal (0.375%) were found in minute amounts.

The antimicrobial activity of the essential oil of *C. acida* was determined by disc diffusion method, against different bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes, Salmonella typhymurium*) and fungi (*Aspergillus ficuum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavis*, *Fusarium saloni*, *Fusarium oxysporum*, *Pencillium digitatum, Candida utilis*). Maximum zone of inhibition was resulted against *B. subtilis* (22 mm) followed by *C. utilis* (20 mm) and *B. cereus* (19.8 mm), whereas the minimum zone of inhibition was shown by *P. digitatum* (10 mm). The inhibition zones, measured after 48 h and 96 h, showed that it is active against all tested bacteria and fungi. The results of antioxidant activity of essential oil of *C. acida* var. sour lime showed that it was able to reduce the stable radical DPPH to yellow-colored DPPH-H reaching 91.7% of DPPH scavenging effect comparative to ascorbic acid being a strong antioxidant reagent.

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1. Introduction

The genus citrus (Rutaceae) comprises of trees, shrubs and herbs of various sizes and uses. They are the most widespread arboreal plants in the world and represent one of the most important crops (Starrantino, 1994; Jacquemond et al., 2002). One of the important products of citrus fruits is the essential oil, which is obtained from citrus peels (Mondello

et al., 2005). Citrus essential oils are mixture of volatile components as terpenes and oxygenated compounds (Sato et al., 1996). These oils are used in pharmaceutical, perfumery and food industries (Huet, 1991). Genus citrus is represented by 10 species in Pakistan (Nasir and Ali, 1978). *Citrus acida* is one of them (Chopra et al., 1958; Nadkarni, 1954).

The essential oil of *C. acida* is pleasant in smell and rich in aroma. It is used as flavouring agent in beverages, liquors and other food product like baked goods, ice-creams, dessert and confectionery (Johanna et al., 1989). It is also used as an ingredient in soaps, detergents, cosmetics and perfumes (Quintero et al., 2003). A formulation for a cosmetic lotion from *C. limetta* oil for the treatment of corns, warts and other skin lesions was developed (Caccioni, 1998). Similarly, papaya (*Carica papaya*) latex was found to be bacteriostatic to different strains of food borne pathogens including *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *P. vulgaris* (Akihisa et al., 2000).

Citrus oils of different species have been studied (Vlisidis and Israilidis, 1998; Song et al., 2000; Kirbaslar and Kirbaslar, 2003; Fengxia et al., 2004). However, essential oil of *C. acida* var. sour lime (a local variety) has yet not been studied. The aim of the present studies is to determine the composition, antioxidant and antimicrobial activity of essential oil of *C. acida* cultivated in Pakistan Council of Scientific & Industrial Research Labs gardens against different food borne pathogens of public health significance.

2. Experimental

2.1. Extraction of essential oil

The fruit was washed, peeled off and cut into small pieces. The cut pieces were subjected to hydro-distillation (Sattar, 1989) by using Reverse Dean–Stark apparatus. The distillate was removed and separated from water by using a separating funnel. It dried over anhydrous sodium sulphate. The yield was 0.313%. It was stored in a refrigerator until further analysis.

2.2. GC–MS analysis

GC–MS of Varian, Saturn model 2000, equipped with ion trap detector (ITD) was used for the identification of different components of essential oil of peels of *C. acida*. Sample was injected on a DB-5MS (30 m × 0.25 mm id, 0.25 u film thickness) column. Helium was used as a carrier gas with a flow rate of 7.0–9.5 psi and split ratio 1:5. The column temperature was maintained at 75 °C for 5 min with a 2.5 °C rise per min to 250 °C.

Various components were identified by their retention time and peak enhancement with standard samples in gas chromatographic mode and NIST library search from the derived mass fragmentation pattern of various components of the essential oil.

2.3. Antibacterial and antifungal activity of C. acida peel oil

The agar disc diffusion method was employed for the determination of antibacterial and antifungal activity of *C. acida* peel oil following the procedure (Baydar et al., 2004) against different food borne pathogens including bacteria (*B. subtilis*, *Bacillus cereus*, *S. aureus*, *E. coli*, *Enterobacter aerogenes, S. typhymurium*) and fungi (*Aspergillus ficuum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavis*, *Fusarium saloni*, *Fusarium oxysporum*, *Pencillium digitatum, Candida utilis*). Standard culture media (CM139, CM271, CM145, CM69, CM7 and CM201) from Oxoid were employed through out the present investigation for the purpose of culture maintenance at their respective temperatures that is 25 °C for fungi and 37 °C for bacteria.

Sterile and dried 4 mm paper discs (Difco) were impregnated with filtered sterilized (0.45 mm Millipore filter) newly extracted ginger oil and placed on freshly seeded microbial lawns (4 discs in each plate) with a control. All experiments were conducted in triplicate. The petri plates were incubated at their respective temperatures and zones of inhibition thus developed against tested microorganisms were measured in millimeters after a period of 48 and 96 h. The results of antimicrobial

Table 1 GC–MS analysis of essential oil of *Citrus acida* peels.

Sr.	Component	% Age	*m/z* value
1	Δ-Carene	1.07	M^+ (136,30) (9,100) (121,25) (105,18) (79,50) (41,35)
2	α-Cedrene	10.57	M^+ (204,3) (189,5) (161,16) (119,100) (105,45) (93,57) (79,15) (41,35)
3	*o*-Cymene	16.62	M^+ (134,30) (119,100) (104,6) (91,45) (77,22) (51,13) (41,5)
4	α-Terpinolene	0.61	M^+ (136,75) (121,70) (105,25) (93,100) (79,33) (67,15) (41,20)
5	Decadienal	8.04	M^+ (152,6) (137,10) (81,100) (67,12) (55,2) (41,7)
6	*cis*-Nerone	0.57	M^+ (194,3) (179,9) (165,11) (137,55) (111,80) (81,100) (47,45)
7	Bisabolene	5.07	M^+ (220,7) (205,3) (137,100) (111,40) (81,98) (43,20)
8	4′-Methoxyacetophenones	2.07	M^+ (150,30) (135,100) (107,15) (63,9)
9	Citronellyl acetate	2.83	M^+ (198,5) (152,75) (109,80) (81,70) (67,55) (43,100)
10	Linalool acetate	2.37	M^+ (196,3) (181,10) (152,75) (121,50) (105,20) (93,55) (67,70) (43,100)
11	β-Humulene	4.14	M^+ (204,10) (189,5) (161,2) (121,50) (93,45) (81,100) (67,33)
12	Decanone	1.48	M^+ (156,3) (141,50) (137,66) (96,30) (67,70) (43,100)
13	Isopulegol acetate	1.29	M^+ (196,4) (181,9) (136,25) (109,80) (81,50) (43,100)
14	Carvone	1.81	M^+ (152,25) (137,33) (110,75) (95,100) (81,79) (67,55) (41,60)
15	Caryophyllene oxide	0.43	M^+ (20,10) (205,30) (167,35) (91,75) (79,90) (41,100)
16	Dihydrolinalool acetate	0.65	M^+ (198,6) (138,18) (123,85) (109,100) (81,95) (67,75) (55,80) (43,85)
17	Farnesol	1.25	M^+ (222,1) (204,9) (189,15) (151,85) (123,100) (107,50) (69,50) (41,55)
18	2,2-Dimethyl-3,4-octadienal	0.38	M^+ (152,55) (137,30) (123,100) (107,55) (95,40) (67,30) (43,95)

activity of peel oil against different microorganisms were expressed as resistant, intermediate and sensitive.

2.4. Antioxidant activity of C. acida peels oil

Antiradical activity was evaluated by measuring the scavenging activity of the examined *C. acida* peel oil on the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical. The DPPH assay was performed as described by Epsin et al. (2000). The samples (100 µl each) were mixed with 3 ml of DPPH solution. The absorbance of the resulting solutions and the blank (with only DPPH and no sample) were recorded after an incubation time of 30 min at room temperature against ascorbic acid as a positive control. For each sample, three replicates were recorded. The disappearance of DPPH was measured spectrophotometrically at 517 nm. The percentage of radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect } (\%) = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control at 30 min and A_1 is the absorbance of the sample at 30 min.

3. Results and discussion

The essential oil was extracted from peels of *C. acida* by hydrodistillation. The yield of oil was (0.313%). The yield is comparable as reported by Kefford and Chandler (1970).

GC–MS analysis of the essential oil revealed the presence of 59 components. Out of which 18 have been identified from their fragmentation pattern by mass spectroscopy using NIST library (Table 1). Essential oil of *C. acida* was found to be mixture of monoterpenes (22.432%), sesqui-terpenes (15.636%) and oxygenated hydrocarbons (21.854%). Among the monoterpenes *o*-cymene (16.62%) was identified as a major component followed by β-humelene (4.135%), Δ-carene (1.070%), α-terpineole (0.607%). α-cedrene (10.57%) and bisabolene (5.066%) were identified as sesquiterpenes. The oxygenated components decadienal (8.043%), citronellyl acetate (2.371%), linalool acetate (2.371%), carvenone (1.806%), decanone (1.474%), isopulegol acetate (1.296%), farnesol (1.254%), 4′-methoxyacetophenone (1.207%), dihydroxylinalool acetate (0.650%), *cis*-nerone (0.574%), caryophyllene oxide (0.433%), and 2,2-dimethyl-3,4-octadienal (0.375%) which contribute to the flavour were also present in appreciable amounts (Song et al., 2000).

Among the constituents the α-terpinolene was also reported by Baigrie et al. in the essential oil of *C. acida* (Baigrie et al., 1996). Linalool acetate a special flavouring component is also reported as one of the principle components in the essential oil of *C. acida* (Vlisidis and Israilidis, 1998). The limonene, a major component of all the citrus oils was not found in the essential oil of *C. acida* var. sour lime cultivated in Pakistan Council of Scientific & Industrial Research Labs Complex, Lahore gardens (Kefford and Chandler, 1970; Minh et al., 2002; Mahmud et al., 2006; Khan et al., 2007). The absence of limonene in the oil has increased its importance among citrus oils. Decanone and carvenone enhance the flavouring quality of oil. Presence of citronellyl acetate and linalool acetate recommends its use in high class perfumery and as a flavouring agent in food, beverages and pharmaceutical products.

The results of antifungal and antibacterial activity of *C. acida* var. sour lime peel oil, investigated against different food borne pathogens by disc diffusion method, are presented in Table 2. It was found in the present study that *C. acida* oil exhibited maximum zone of inhibition against *B. subtilis* (22 mm) as well as *C. utilis* (20 mm) and minimum zone of inhibition was shown by *P. digitatum* (10 mm) after 48 h of incubation at 37 °C. However, *A. ficuum*, *A. niger*, *A. fumigatus*, *Aspergillus falvis*, *F. saloni*, *F. oxysporium*, *B. cereus*, *S. aureus*, *E. coli*, *E. aerogenes* and *Salmonella typhimurium* gave 18.3, 16.5, 12, 14, 13, 11, 19.8, 18, 16, 10.5 and 17 mm of zone of inhibition respectively after 48 h of incubation. As the essential oil is rich in a wide variety of secondary metabolites, such as

Table 2 Antimicrobial activity of *Citrus acida* var. sour lime peel oil against tested microorganisms.

Tested microorganisms	Incubation temperature (°C) and culture media	Colony morphology	Incubation zones (mm)		Percentage decrease in zone inhibition after 96 h	Efficiency Y
			After 48 h	After 96 h		
Aspergillus ficuum	25 °C CM139	White, later green/black	18.3	16.6	9.29	S
Aspergillus niger	25 °C CM139	White, later green/black	16.5	15.2	7.88	S
Aspergillus fumigatus	25 °C CM139	White, later green	12	10	16.67	I
Aspergillus falvis	25 °C CM139	White, later green	14	12.5	10.71	I
Fusarium saloni	25 °C CM139	White, cottony	13	10.8	16.92	I
Fusarium oxysporum	25 °C CM139	White, later pink, rinkled	11	8.3	24.55	R
Penicillum digitatum	25 °C CM139	White, later green	10	8.1	19	I
Candida utilus	25 °C CM139	Creamy white	20	19.1	4.50	S
Bacillus subtilis	37 °C CM271	Gram +ve rods	22	21.8	5.46	S
Bacillus cereus	37 °C CM271	Gram +ve rods	19.8	18.5	6.57	S
Staphylococcus aureus	37 °C CM145	Gram +ve cocci	18	16.6	7.78	S
Escherichia coli	37 °C CM69	Gram −ve rods	16	14.2	11.25	I
Enterobacter aerogenes	37 °C CM7	Gram −ve rods	10.5	8.8	16.19	I
Salmonella typhimurium	37 °C CM201	Gram −ve rods	17	15.2	10.59	I

S = Sensitive, I = Intermediate, R = Resistant.
CM139 = Potato dextrose agar, CM271 = Blood agar base, CM145 = Staphylococcus medium 110, CM69 = Eosin methylene blue, CM7 = Macconkey agar, CM201 = Bismithsulphite agar.
pH of he assay medium ranges from 6.4 to 7.3 depending upon the tested microorganisms.

tannins, terpenoids, alkaloids and flavonoids, that are known to possess antimicrobial properties (Porter, 1999).

The present investigation for the assessment of antimicrobial activity of *C. acida* peel oil against different microbes of public health significance indicated a percent decrease in clear zone of inhibition after 96 h ranging between 5% and 17% for fungi while for bacteria it ranged between 6% and 16%. This decrease in clear zone of inhibition hours by sterilized *C. acida* peel oil preparation may be either due to inactivation or low concentrations of diffusible water soluble active constituents. However, in a similar study it is reported that the sweet lime peel oil was found to be very effective against *S. aureus* and *P. vulgaris* (Wan et al., 1998). Thus low or absence of activity in sterile *C. acida* peel oil might be due to a number of factors such as collection time of plant material, its storage, climate, which might, in turn, affect the amount of the active principal constituents (tannins, terpenoids, alkaloids, flavonoids etc.) in the plant material (Murphy, 1999; Suresh et al., 1997). Our findings are in agreement with earlier reports (Saddique et al., 1996), where a decrease in inhibitory zone from 15 to 26 mm after a period of 96 h of incubation was observed to cause decrease from 9.52% to 34.8% against the tested strains (Saddique et al., 1996).

Several natural compounds are known to quench free radicals (Youdim et al., 1999). In the current study (Table 2) essential peel oil was able to reduce the stable radical DPPH to yellow-colored DPPH-H reaching 91.7% of DPPH scavenging effect. The comparison of DPPH scavenging activity of *C. acida* peel oil with well known antioxidant ascorbic acid showed that peel oil has equally strong antioxidant potential.

4. Conclusion

The results of our present study showed that the essential oil of *C. acida* var. sour lime peels possessed antimicrobial and antioxidative potential which might be utilized in food and chemical industry and further studies are warranted.

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