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ANTIMICROBIAL ACTIVITY OF TURKISH *CITRUS* PEEL OILS

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Abstract

The samples of the *Citrus* fruits viz., lemon (*Citrus limon* (L.) Burm. f.), grapefruit (*Citrus paradisi* Macfayden), bergamot (*Citrus bergamia* Risso et Poit.), bitter orange (*Citrus aurantium* L.), sweet orange (*Citrus sinensis* (L.) Osbeck), mandarin (*Citrus reticulata* Blanco) were collected from southern Turkey (Antalya) in November 2006 and their peel oils were obtained by cold-pressing process. The antimicrobial activities of Turkish *Citrus* peel oils were evaluated using the disk diffusion method toward 9 bacteria and the results compared with those for penicillin-g, ampicillin, cefotaxime, vancomycine, oflaxacin and tetracycline. Antifungal activities were reported for *Kluyveromyces fragilis*, *Rhodotorula rubra*, *Candida albicans*, *Hanseniaspora guilliermondii* and *Debaryomyces hansenii* yeasts, and the results were referenced against nystatin, ketaconazole and clotrimazole antifungal agents. The C*itrus* peel oils showed strong antimicrobial activity against the test organisms. Lemon and bergamot peel oils have a little higher activity than the other *Citrus* peel oils.

Introduction

Herbs and spices with antimicrobial activity have been widely used both traditionally and commercially to increase the shelf life and safety of foods (Brul & Coote, 1999; Dupont *et al*., 2006). Many natural substances may play a fundamental role in the host plant/pathogen relationship: the essential oils produced by different plant genera are in many cases biologically active, endowed with antimicrobic, allelopathic, antioxidant and bio-regulatory properties. The antimicrobial abilities of essential oils, among which citrus oils, are also shown to be a particularly interesting field for applications within the food and cosmetic industries (Caccioni *et al*., 1998). Preparation from peel, flowers and leaves of bitter orange (*citrus aurantium* L.) are popularly used in order to minimize central nervous system disorders (Pultrini *et al*., 2006). Some essential oils were used in skincare products and for acne control (Lertsatitthanakorn *et al*., 2006). It is known that oil of bergamot is receiving renewed popularity in aromatherapy.

The peel of *Citrus* fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants (Ahmad *et al*. 2006). These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries. Naringin and hesperidin have many biological activities such as antioxidant, antimutagenic effect, analgesic, anti-inflammatory etc. Principal flavanones and

polymethoxyflavones of Star Ruby grapefruits (*C. paradisi*) and Sanguinelli orange (*C. sinensis*) (Spain) acted as antifungal against *Penicillim digitatum* (Ortuno *et al*., 2006).

Citrus cultivation is probably one of the most important commercial and industrial agricultural activities of the world (Ahmed *et al.* 2006). Turkey is one of the most important countries amongst citrus fruits producer. In this study, the main components of Turkish mandarin and orange were determined and Turkish *Citrus* peel oils (orange, bitter orange, mandarin, lemon, bergamot and grapefruit, from Antalya-Turkey) were obtained by cold-pressing process and then antibacterial and antifungal activities of them were investigated.

Materials and Methods

Materials: The samples of the orange and mandarin were collected from a commercial *Citrus* plantation of Antalya-Turkey in November 2006. After the *Citrus* fruits had been washed, they were cut into six equal portions and the flesh was removed. The fruit albedo layers were peeled off carefully and discarded. Peel oils were extracted by hand pressing of the flavedo layer with exposed oil sacs and were collected in brine solution kept on ice. The extract was centrifuged (20 min at 6000 rpm) and dried in anhydrous Sodium sulphate. The oils were stored at -21°C until gas chromatography (GC) and gas chromatograph-mass spectrometer (GC-MS) analyses.

GC analysis: In GC analysis of the orange peel oils, DB-5 (60×0.25 mm, 0.25 μm film thickness) and Innowax (30 m×0.25 mm i.d., 0.25 μm film thickness) capillary columns were used. The columns were installed in a HP 6890 GC equipped with an injection port with a split ratio of 50:1 and attached to a flame ionization detector (FID). Column temperature was held at 40°C for 4 min., and then raised up at 5°C/min., to 200°C and held at 200°C for 1 min then raised up at 5°C/min 220°C where it was held for 10 min. The temperatures of the injector port and detector were set at 230°C. Ultra pure helium (99.999 %) was used as carrier gas with a flow rate of 1 mL/min. The injected volume of the samples was 1 μL of neat oil. The quantitative composition was obtained by peak area normalization, and the response factor for each component was taken to be equal to one.

GC-MS analyses of the oils were performed on the same chromatograph equipped with a Hewlett Packard 5973A model high resolution mass spectrometer (double focusing, magnetic sector) with the following settings; ionization voltage 70 eV, interface temperature 230°C, ion source temperature 230°C, scan mass range 35-700 amu, GC column and conditions were the same as above. Mass spectra and individual GC peaks were identified by a computer search of the commercial libraries (WILEY, NIST), followed by expert matching of MS data and published data (Kubeczka, 2002; Jennings & Shibamoto, 1980; Adams 1995).

Antimicrobial activity: The antimicrobial activities were evaluated against Gram positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* La 2971) and Gram negative (*Escherichia coli* ATCC 11230, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427) and the yeast cultures (*Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra* DSM 70403, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432) using disk diffusion technique.

Antimicrobial screening: Disk diffusion method: Sterilized antibiotic discs (6 mm) were used following the literature procedure (NCCLS, 1993; Collins *et al*., 1989). The discs were impregnated with 20 μL of these solutions. All the bacteria were incubated and activated at 30°C for 24 h inoculation into Nutrient Broth (OXOID) and the yeasts were incubated in Malt Extract Broth (OXOID) for 48 h. Inoculums containing 10^6 bacterial cells or 10^8 yeast cells per cm^3 were spread on Mueller-Hinton Agar (OXOID) plates (1 cm^3 inoculum for each plate). The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35°C (24 h) and at 25°C (72 h) for bacteria and yeast, respectively. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganisms. In each case triplicate tests were performed and the average was taken as the final reading.

Results and Discussions

Compositions of the Citrus peel oils: In our previous studies, compositions of the peel oils of Turkish lemon, grapefruit, bergamot and bitter orange were investigated (Kırbaşlar *et al*., 2006; Kırbaşlar & Kırbaşlar, 2003; Kırbaşlar *et al*., 2001). In total 42 components have been identified in Turkish lemon peel oil. Turkish lemon peel oil has high content of monoterpene hydrocarbons (89.9%) with limonene (61.8%), γ-terpinene (10.6%) and β-pinene (8.1%); sesquiterpene hydrocarbons (3.3%) were second major class of substances. The most prominent sesquiterpenes in the lemon oil were β-bisabolene (1.6%), trans-α-bergamotene (1.0%) and β-caryophyllene (0.7%). The major oxygenated components (5.1%) of the oils were found; aldehydes components (2.4%); geranial (1.3%), neral (0.7%), octanal (0.1%), decanal (0.1%) and alcohol components (0.9%); linalool (0.2%), nerol (0.1%), geraniol (0.1%) and ester components (1.8%); neryl acetate (1.2%) and geranyl acetate (0.6%). In total 27 components have been identified in Turkish grapefruit peel oil. Grapefruit oil has monoterpene hydrocarbons (96.4%) of which limonene (92.5%) and myrcene (2.6%) were the first two major components; the major sesquiterpene components (0.8%) were β-caryophyllene (0.4%) and δ-cadinene (0.2%) and nootkatone (0.2%). The major oxygenated components (1.2%) of the oils were found; aldehydes components (0.7%); octanal (0.2%), decanal (0.2%) geranial (0.1%) and neral (0.1%), alcohol components (0.3%); linalool (0.2%), α-terpineol (0.1%) and ester components (0.2%); neryl acetate (0.1%) and geranyl acetate (0.1%) (Kırbaşlar *et al*., 2006). In the Turkish bergamot peel oil samples 47 components have been identified. The major monoterpenes (49.0%) of the bergamot peel oils were found limonene (36.8%), γ-terpinene (5.6%), β-pinene (3.4%), myrcene (1.3%). The major sesquiterpenes (1.5%) component was β-bisabolene (0.6%), trans-α-bergamotene (0.4%). The major oxygenated components (49.1%) of the oils were found following: Aldehyde components (0.6%); neral (0.4%), alcohol components (13.9%); linalool (13.5%), ester components (34.7%); linalyl acetate (32.6%), neryl acetate (0.9%), geranyl acetate (0.6%) (Kırbaşlar *et al*., 2001). In the Turkish bitter orange peel oil samples 29 components have been identified. The major monoterpenes (97.3%) of the bitter orange peel oils were found limonene (94.1%), myrcene (1.8%), β-pinene (0.5%). The major sesquiterpenes (0.1%) component was β-caryophyllene (0.1%). The major oxygenated components (2.5%) of the oils were found following: Aldehyde components (0.5%); decanal (0.2%), geranial (0.1%), alcohol components (0.5%); linalool (0.4%), ester components (1.4%); linalyl acetate (1.2%), geranyl acetate (0.08%) (Kirbaşlar & Kirbaşlar, 2003).

Table 1. Antimicrobial activity data of Turkish *Citrus* peel oils (inhibition zone, mm)

Microorganisms	Grapefruit	Bitter orange	Orange	Mandarin	Lemon	Bergamot
Escherichia coli	10	12	13	12	14	15
Staphylococcus aureus	11	12	12	14	14	16
Klebsiella pneumoniae	11	12	11	13	13	14
Bacillus cereus	10	11	12	12	13	15
Micrococcus luteus	11	12	13	13	12	14
Proteus vulgaris	17	15	15	14	16	15
Mycobacterium smegmatis	11	10	11	12	13	14
Listeria monocytogenes	11	12	12	13	16	15
Pseudomonas aeruginosa	12	11	10	12	10	11
Kluyveromyces fragilis	11	12	14	15	16	14
Rhodotorula rubra	11	12	12	14	14	15
Candida albicans	10	12	12	13	14	13
Hanseniaspora guilliermondii	11	12	14	12	11	12
Debaryomyces hansenii	11	13	13	14	13	15

Table 2. Antimicrobial activities of some standard antibiotics and antifungals (inhibition zone, mm).

Microorganisms	P10	A10	CX30	VA30	OFX5	TE30	N100	KT20	CL10
Escherichia coli	18	12	10	22	30	28	-	-	-
Staphylococcus aureus	13	16	12	13	24	26	-	-	-
Klebsiella pneumoniae	18	14	13	22	28	30	-	-	-
Bacillus cereus	14	12	14	18	30	25	-	-	-
Micrococcus luteus	36	32	32	34	28	22	-	-	-
Proteus vulgaris	10	16	18	20	28	26	-	-	-
Mycobacterium smegmatis	15	21	11	20	32	24	-	-	-
Listeria monocytogenes	10	12	16	26	30	28	-	-	-
Pseudomonas aeruginosa	8	10	54	10	44	34	-	-	-
Kluyveromyces fragilis	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	-	-	-	-	-	-	18	22	16
Candida albicans	-	-	-	-	-	-	20	21	15
Hanseniaspora guilliermondii	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	-	-	-	-	-	-	16	14	18

P10: Penicillin G (10 Units), A10: Ampicillin 10 µg, CX30: Cefotaxime 30 µg, V30: Vancomycin 30 µg, OFX5: Oflaxacin 5 µg, TE30: Tetracyclin 30 µg, N100: Nystatin 100 µg, KT20: Ketaconazole 20 µg: CL10: Clotrimazole 10 µg

In this study, in total 54 and 45 components have been identified in Turkish sweet orange and mandarin peel oils, respectively. The major monoterpenes range of the sweet orange oils was found limonene (91.6%), α-pinene (0.9%), sabinene (1.0%) and myrcene (1.3%). The major sesquiterpene components were α-copaene (0.1%) and β-caryophyllene (0.1%). The major oxygenated components of the oils were found; aldehydes components; octanal (1.4%), decanal (0.2%) and geranial (0.2%), alcohol components; linalool (0.4%), α-terpineol (0.1%) and geraniol (0.1%) and ester components; geranyl acetate (0.1%) and neryl acetate (0.1%). The major monoterpenes of the mandarin oils were found limonene (90.7%), γ-terpinene (3.9%), myrcene (2.1%), α-pinene (0.5%), sabinene (0.3%). The major sesquiterpene component was (E)-β-farnesene (0.1%). The following major oxygenated components of the oils were found: Aldehyde components; octanal (0.2%), decanal (0.1%), alcohol components; linalool (0.4%), α-terpineol (0.1%), ester components; geranyl acetate (0.2%) and neryl acetate (0.1%).

Antimicrobial assays: The results concerning *In vitro* antimicrobial activity of the Turkish *Citrus* peel oils with the inhibition zone (mm) values of compared antibiotic and antifungal references are presented in Tables 1 and 2.

Antimicrobial activity of the peel oils is directly concerning with the components that they contain. The studies showed that essential oils, protopine and corydaline alkaloids, lactons, polyacetylene, acyclic sesquiterpenes, hypericin and pseudohypericin compounds are effective toward various bacteria. Also, these effects are related to a lot of factors such as individual change of herb's chemical contains, soil composition, daily or seasonal changes at time of collecting of the herb materials, physiological development period of herbs, extraction process, kind of bacteria used etc., (Keleş *et al*., 2001; Izzo *et al*., 1995; Martinez *et al*., 1996; Tunon *et al*., 1995).

The Turkish C*itrus* peel oils showed strong antimicrobial activity against the Gram (+) and Gram (-) bacteria and the fungi cultures studied. These results may justify the traditional use of the Turkish *Citrus* peel oils.

According to the antimicrobial activity, it would suggest that all of the *Citrus* peel oils are more effective towards *Proteus vulgaris* than the other microorganisms. Also, it is observed that all of the *Citrus* peel oils are effective on all bacteria and fungi. Generally, lemon and bergamot peel oils were higher antimicrobial activity according to the other *Citrus* peel oils (Table 1).

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