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Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations

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Abstract

Chemical composition, antioxidant and antimicrobial activities of the essential oils from aerial parts of basil (*Ocimum basilicum* L.) as affected by four seasonal, namely summer, autumn, winter and spring growing variation were investigated. The hydro-distilled essential oils content ranged from 0.5% to 0.8%, the maximum amounts were observed in winter while minimum in summer. The essential oils consisted of linalool as the most abundant component (56.7–60.6%), followed by *epi*-α-cadinol (8.6–11.4%), α-bergamotene (7.4–9.2%) and γ-cadinene (3.2–5.4%). Samples collected in winter were found to be richer in oxygenated monoterpenes (68.9%), while those of summer were higher in sesquiterpene hydrocarbons (24.3%). The contents of most of the chemical constituents varied significantly ($p < 0.05$) with different seasons. The essential oils investigated, exhibited good antioxidant activity as measurements by DPPH free radical-scavenging ability, bleaching β-carotene in linoleic acid system and inhibition of linoleic acid oxidation. Evaluation of antimicrobial activity of the essential oils and linalool, the most abundant component, against bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pasteurella multocida* and pathogenic fungi *Aspergillus niger*, *Mucor mucedo*, *Fusarium solani*, *Botryodiplodia theobromae*, *Rhizopus solani* was assessed by disc diffusion method and measurement of determination of minimum inhibitory concentration. The results of antimicrobial assays indicated that all the tested microorganisms were affected. Both the antioxidant and antimicrobial activities of the oils varied significantly ($p < 0.05$), as seasons changed.

Keywords: Seasonal variation; GC–MS; Linalool; IC_{50}; Antimicrobial; *Staphylococcus aureus*; *Aspergillus niger*

1. Introduction

Diets rich in selected natural antioxidants such as polyphenols, flavonoids, vitamin C and vitamin E are related to reduced risk of incidence of cardiovascular, other chronic diseases and certain types of cancer has lead to the revival of interest in plants-based foods (Choi, Jeong, & Lee, 2007; Dorman & Hiltunen, 2004; Majhenic, Skerget, & Knez, 2007; Mata et al., 2007). A large number of plant species have already been tested for their potential biological, therapeutic and pharmaceutical activities (Majhenic et al., 2007; Mata et al., 2007; Sokovic & Van Griensven, 2006; Wannissorn, Jarikasem, Siriwangchai, & Thubthimthed, 2005).

On the other hand, food borne diseases are major dilemma in the third world and developing countries, and even in developed nations (Sokmen et al., 2004). The consumption of foods contaminated with some microorganisms represents a serious health risk to humans. The subsistence and growth of microorganisms in foods may lead to spoilage, formation of toxins and quality deterioration of food products (Celiktas et al., 2007).

In recent years, the essential oils and herbal extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically

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active compounds (Bozin, Mimica-Dukic, Simin, & Anackov, 2006; Tepe, Daferera, Tepe, Polissiou, & Sokmen, 2007; Wannissorn et al., 2005). The antimicrobial and antioxidant activities of essential oils have formed the basis of many applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Bozin et al., 2006; Celiktas et al., 2007). Efforts have also been made to explore the potential of some essential oils for the treatment of infectious diseases in order to substitute standard pharmaceutical remedies (Celiktas et al., 2007; Kelen & Tepe, 2007; Politeo, Jukic, & Milos, 2007; Sokovic & Van Griensven, 2006).

Common basil (*Ocimum basilicum* L.; *O. basilicum*), a member of the Lamiaceae family is an annual herb which grows in several regions around the world. Among more than 150 species of the genus *Ocimum*, basil is the major essential oil crop which is cultivated commercially in many countries (Sajjadi, 2006). Traditionally, basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries (Telci, Bayram, Yilmaz, & Avci, 2006). The leaves and flowering tops of the plant are perceived as carminative, galactogogue, stomachic and antispasmodic in folk medicine (Sajjadi, 2006). However, recently the potential uses of *O. basilicum* essential oil, particularly as antimicrobial and antioxidant agents have also been investigated (Lee et al., 2005; Politeo et al., 2007; Sartoratotto et al., 2004; Suppakul et al., 2003; Wannissorn et al., 2005). The *O. basilicum* essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (Da-Silva et al., 2003; Sajjadi, 2006).

To the best of our knowledge, antioxidant and antimicrobial activities of the essential oils from *O. basilicum* indigenous to sub-continental regions or elsewhere with regards to the seasonal variation have not yet been reported. The present work was undertaken with the main objective to investigate the physico-chemical composition of the essential oil isolated from the aerial parts of *O. basilicum* indigenous to Pakistan as affected by different growing seasons along with their antioxidant and antimicrobial activities.

2. Materials and methods

2.1. Collection and pretreatment of plant materials

Aerial parts of cultivated *O. basilicum* L. at full flowering stage were collected during summer (June 2005), autumn (September 2005), winter (December 2005), and spring (March 2006) from the botanical garden at the University of Agriculture, Faisalabad, Pakistan. The mean values for maximum and minimum temperature (°C) for the months of June, September, December 2005 and March 2006 were 43.3 ± 3.2, 28.7 ± 2.9 (average 36.0); 37.4 ± 3.1, 26.0 ± 2.2 (average 31.7); 22.1 ± 2.3, 8.9 ± 2.3 (average 15.5); 28.1 ± 3.1, 14.6 ± 1.8 (average 21.4), respectively. The average relative humidity and total rainfall of the months of June, September, December 2005 and March 2006 were $26.2 \pm 9.0\%$ and 55.6 mm; $41.0 \pm 14.3\%$ and 76.2 mm; $57.4 \pm 10.2\%$ and 46.2 mm and $40.7 \pm 9.3\%$ and 37.0 mm, respectively. The specimens were identified and authenticated by Dr Muhammad Ashraf, Department of Botany, University of Agriculture, Faisalabad, Pakistan. The specimens were dried at 30 °C in a hot air oven (IM-30 m Irmeco, Germany) to constant weight.

2.2. Chemicals

Butylated hydroxytoluene (BHT), linoleic acid, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, Tween 40, Tween 80 and homologous series of C_9–C_{24} *n*-alkanes and various reference chemicals used for identification were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals, analytical grade, i.e. anhydrous sodium carbonate, ferrous chloride, ammonium thiocyanate, chloroform and methanol used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise. All culture media and standard antibiotic discs were purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK).

2.3. Isolation of the essential oils

One hundred grams of dried and ground (80 mesh) aerial parts (both stems and leaves), wooden parts were separated and hydro-distilled for 4 h, using a Clevenger-type apparatus (Anonymous, 1996). The distilled essential oils were dried over anhydrous sodium sulphate, filtered and stored in sealed vials at 4 °C, prior to further analyses.

2.4. Essential oils analyses

2.4.1. Physical analysis

The refractive index and density of *O. basilicum* essential oils were determined following standard methods (Guenther, 1960). A digital refractometer RX-7000α (Atago Co. Ltd., Tokyo, Japan) was used for the determination of refractive index of oils.

2.4.2. Chromatographic analysis

2.4.2.1. Gas chromatography (GC) analysis. The essential oil was analyzed on a Perkin–Elmer gas chromatograph model 8700 equipped with flame ionization detector (FID) and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm; Agilent-Technologies, Little Falls, CA, USA). Injector and detector temperatures were set at 220 and 290 °C, respectively. Column temperature was programmed from 80 °C to 220 °C at a rate of 4 °C/min, lower and upper temperatures were held for 3 and 10 min, respectively. Helium was used as carrier gas at a flow rate of 1.5 mL/min. A sample of 1.0 μL was injected, using split mode (split ratio, 1:100). All quantifications were carried out using a built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin–Elmer, Norwalk, CT, USA). The composition

was reported as a relative percentage of the total peak area.

2.4.2.2. Gas chromatography/mass spectrometry (GC/MS) analysis. GC–MS analysis of the essential oil was performed using Agilent-Technologies 6890N Network gas chromatographic (GC) system, equipped with Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto injector (Agilent-Technologies, Little Falls, CA, USA). Chromatographic conditions were the same as described above. For MS detection, an electron ionization mode with ionization energy of 70 eV was used. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively.

2.4.2.3. Compounds identification. The identification of the essential oil constituents was based on a comparison of their retention indices relative to (C_9–C_{24}) *n*-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their MS data compared to the NIST mass spectral library and published mass spectra (Adam, 2001).

2.5. Antioxidant activity

2.5.1. DPPH radical-scavenging assay

The antioxidant activity of the *O. basilicum* essential oil and the major component, linalool, were assessed by measuring their scavenging abilities to 2,2′-diphenyl-1-picrylhydrazyl stable radicals. The DPPH assay was performed as described by Bozin et al. (2006). The samples (from 0.5 to 15.5 μg mL^{-1}) were mixed with 1 mL of 90 μM DPPH solution and filled up with 95% MeOH, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. Butylated hydroxytoluene (BHT) was used as a positive control. For each sample, three replicates were recorded. The disappearance of DPPH was read spectrophotometrically at 515 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Inhibition of free radical by DPPH in percent (%) was calculated in the following way:

$$I\ (\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. IC_{50} values, which represented the concentration of essential oils that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

2.5.2. Percent inhibition in linoleic acid system

The antioxidant activity of *O. basilicum* essential oil and of its major component linalool was determined using inhibition of linoleic acid oxidation, following the method described by Singh and Marimuthu (2006) with modification. The test samples (50 μL) were dissolved to a 1 mL of ethanol, mixed with linoleic acid (2.5%, v/v), 99.5% ethanol (4 mL) and 4 mL of 0.05 M sodium phosphate buffer (pH 7). The solution was incubated at 40 °C for 175 h. The extent of oxidation was measured by peroxide value using the colorimetric method described by Yen, Duh, and Chuang (2000). To 0.2 mL sample solution, 10 mL of ethanol (75%), 0.2 mL of an aqueous solution of ammonium thiocyanate (30%) and 0.2 mL of ferrous chloride solution (20 mM in 3.5% HCl) were added sequentially. After 3 min of stirring, the absorbance was measured at 500 nm, using a spectrophotometer (U-2001, Hitachi Instruments, Inc., Tokyo, Japan). A control was performed with linoleic acid without essential oils. Butylated hydroxytoluene (BHT) was used as positive control. Inhibition of linoleic acid oxidation expressed as percent was calculated as follows:

$$\%\ \text{inhibition of linoleic acid oxidation} = 100 - [(\text{Abs. increase of sample at 175 h}/\text{Abs. increase of control at 175 h}) \times 100]$$

2.5.3. Bleachability of β-carotene in linoleic acid system

Antioxidant activity of the *O. basilicum* essential oil and of it main component linalool, was assessed by bleaching of β-carotene/linoleic acid emulsion system as described by Kulisic, Radonic, Katalinic, and Milos (2004) with modification. A stock solution of β-carotene–linoleic acid mixture was prepared by dissolving 0.1 mg β-carotene, 20 mg linoleic acid and 100 mg Tween 40 in 1.0 mL of chloroform (HPLC grade). The chloroform was removed under vacuum in rotary evaporator at 50 °C. Then, 50 mL of distilled water saturated with oxygen (30 min, 100 mL/min) was added and mixture shakes. A 5.0 mL of this reaction mixture was dispensed to test tubes containing 200 μL of the essential oil or linalool solution, prepared at 4.0 g/L concentrations and the absorbance as $t = 0$ measured at 490 nm against a blank, consisting of an emulsion without β-carotene. Then emulsion was incubated for 50 h at room temperature and the absorbance was recorded at different time intervals. The same procedure was applied for BHT and blank.

2.6. Antimicrobial activity

2.6.1. Microbial strains

The essential oils were individually tested against a set of microorganisms, including two Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Bacillus subtilis* (*B. subtilis*) ATCC 10707, two Gram-negative bacteria: *Escherichia coli* (*E. coli*) ATCC 25922 and *Pasteurella multocida* (*P. multocida*), and five pathogenic fungi: *Aspergillus niger* ATCC 10575 (*A. niger*), *Mucor mucedo* (*M. mucedo*), *Fusarium solani* (*F. solani*), *Botryodiplodia theobromae* (*B. theobromae*) and *Rhizopus solani* (*R. solani*). The pure bacterial and fungal strains were obtained from Biological Division of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Purity and identity were verified by the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial

strains were cultured overnight at 37 °C in Nutrient agar (NA, Oxoid) while fungal strains were cultured overnight at 30 °C using Potato dextrose agar (PDA, Oxoid).

2.6.2. Disc diffusion method

The antimicrobial activity of *O. basilicum* essential oils and linalool were determined by agar disc diffusion method (NCCLS (National Committee for Clinical Laboratory Standards)., 1997). Briefly, 100 μL of suspension of tested microorganisms, containing 10^8 colony-forming units (cfu)/mL of bacteria cells and 10^4 cfu/mL spores of fungal strains spread on NA and PDA medium, respectively. The filter discs (6 mm in diameter) were individually soaked with 15 μL of essential oils or linalool and placed on the agar plates which had previously been inoculated with the tested microorganisms. Disc without samples were used as a negative control. Amoxycillin (30 μg/disc) and Flumequine (30 μg/disc) were used as positive references for bacteria and fungi, respectively, to compare sensitivity of strain/isolate in analyzed microbial species. The Petri dishes were kept at 4 °C for 2 h. The plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungal strains. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones in millimeters (including disc diameter of 6 mm) for the test organisms and comparing to the controls. The measurements of inhibition zones were carried for three sample replications, and values are the average of three replicates.

2.6.3. Determination of minimum inhibitory concentration (MIC)

For the determination of MIC, which represents the concentration that completely inhibit the growth of microorganisms, a micro-dilution broth susceptibility assay was used, as recommended by National Committee for Clinical Laboratory Standards (NCCLS (National Committee for Clinical Laboratory Standards)., 1999). All tests were performed in Nutrient broth (NB) and Sabouraud dextrose broth (SDB) supplemented with Tween 80 detergent to a final concentration of 0.5% (v/v) for bacteria and fungi, respectively. Bacterial strains were cultured overnight at 37 °C in NB and the fungi were cultured overnight at 30 °C in SDB. Dilutions series were prepared from 0.07 to 72.0 mg/mL of the essential oils or linalool in a 96-well microtitre plate, 160 μL of NB and SDB for bacteria and fungi, respectively, were added onto microplates and 20 μL of tested solution. Then, 20 μL of 5×10^5 cfu/mL of standard microorganism suspension were inoculated onto microplates. Plates were incubated at 37 °C for 24 h for bacteria, and at 30 °C for 48 h for fungi. The same test was performed simultaneously for the growth control (NB + Tween 80) and sterility control (NB + Tween 80 + test oil). Amoxycillin was used as a reference compound for antibacterial and Flumequine for antifungal activities. The growth was indicated by the presence of a white "pellet" on the well bottom.

2.7. Statistical analysis

All the experiments were conducted in triplicate and statistical analysis of the data were performed by analysis of variance (ANOVA) using STATISTICA 5.5 (Stat Soft Inc., Tulsa, OK, USA) software. A probability value at $p \leqslant 0.05$ was considered statistically significant. Data are presented as mean values ± standard deviation calculated from triplicate determinations.

3. Results and discussion

3.1. Physical analysis

Table 1 shows the yield and physical properties of the *O. basilicum* essential oils as affected by seasonal changes. The content of the essential oils was distributed unevenly among seasons. The highest amount of the oil in the *O. basilicum* was found during winter (0.8%) which decreased significantly ($p < 0.05$) in summer to 0.5%. In Pakistan, summer is quite hot with an average temperature 35–40 °C. Low essential oil yield in summer might be attributed to the high temperature and partial evaporation of some constituents of oil can be expected. Our results were in agreement to those of Trivino and Johnson (2000) who investigated that growing season has a major effect on the essential oil yield in *Origanum majorana*. Yield was generally positively correlated with nutrient level at the lower temperature, however, negatively at higher tempera-

Table 1
Seasonal variation in yield, physical properties and antioxidant activity of *Ocimum basilicum* essential oils

Parameter	Essential oils				Linalool	BHT
	Summer	Autumn	Winter	Spring		
Physical analysis						
% Yield	0.5 ± 0.0^{d}	0.6 ± 0.0^{c}	0.8 ± 0.1^{a}	0.7 ± 0.1^{b}	–	–
Refractive index (25 °C)	1.5045 ± 0.003^{a}	1.4995 ± 0.002^{a}	1.5015 ± 0.004^{a}	1.5017 ± 0.003^{a}	–	–
Density (g cm^{-3}), 25 °C	0.95 ± 0.02^{a}	0.97 ± 0.02^{a}	0.96 ± 0.01^{a}	0.97 ± 0.01^{a}	–	–
Antioxidant activity						
DPPH, IC_{50} (μg/ml)	6.7 ± 0.1^{b}	6.0 ± 0.2^{c}	4.8 ± 0.1^{e}	5.3 ± 0.2^{d}	16.4 ± 0.5^{a}	5.8 ± 0.2^{c}
Inhibition in linoleic acid system (%)	80.3 ± 3.0^{b}	84.3 ± 3.1^{ab}	91.2 ± 3.7^{a}	90.3 ± 2.9^{a}	75.2 ± 4.5^{c}	91.1 ± 2.9^{a}

Values are means ± standard deviation of three separate experiments.
Different letters in superscript indicate significant differences within seasons.

ture. Da-Silva et al. (2003), who recorded the highest percentage of basil essential oil from Brazil in January (winter) and the lowest in August (summer). According to Vekiari et al. (2002), the leaves of lemon exhibited maximum essential oil yield during November (winter). Kofidis, Bosabalidis, and Kokkini (2006) and Muller-Riebau, Berger, Yegen, and Cakir (1997) examined considerable difference in the essential oil contents of *Mentha spicata* and *Mentha pulegium* leaves with respect to seasonal variations, respectively. A substantial variation in the yield of essential oil from the aerial parts of *Artemisia verlotiorum* has also been reported through out the year (Chericoni, Flamini, Campeol, Cioni, & Morelli, 2004). Some other studies in the literature revealed that plants exhibited remarkable fluctuation in essential oil contents with the progress of seasons (Chericoni et al., 2004; Van Vuuren, Viljoen, Ozek, Demirici, & Baser, 2007).

The essential oil yield (0.5–0.8%) in the present study was comparable to reports by Politeo et al. (2007), who found the yield of the essential oils from aerial parts of *O. basilicum* by hydro-distillation to be 0.62%. Bozin et al. (2006) reported the yield of the basil essential oils from plants grown in Serbia and Montenegro to be 0.37%. While in *O. basilicum* cv. purple and *O. basilicum* cv. green from Iran, the essential oil yields were found to be 0.20% and 0.50%, respectively (Sajjadi, 2006). Such variations in the essential oil content of *O. basilicum* across countries might be attributed to the varied agroclimatic conditions of the regions.

The values of refractive index and density for the *O. basilicum* essential oils investigated were not significantly differed ($p > 0.05$) among seasons analyzed (Table 1). Data are not available in the literature regarding the variation of these two parameters of basil essential oils as affected by seasonal changes to compare our results.

3.2. Antioxidant activity

As the chemical composition of an essential oil may potentially affect its biological activities, the antioxidant properties of the isolated essential oils were recorded. The antioxidant activity of the *O. basilicum* essential oils was assessed by different in vitro tests. Free radical-scavenging capacities of the oils were measured by the DPPH assay and the results are given in Table 1. In the DPPH assay, the ability of the examined essential oils to act as donor of hydrogen atoms or electrons in transformation of DPPH$^{\cdot}$ into its reduced form DPPH-H was investigated. The examined *O. basilicum* essential oils were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H. *O. basilicum* essential oils obtained from winter and spring crops showed greater radical-scavenging activity than those collected during autumn and summer, exhibiting IC_{50} values for 4.8, 5.3 and 6.0 and 6.7 μg/mL, respectively. Linalool, the major component of *O. basilicum* essential oil, tested under the same conditions exhibited lower antioxidant activity (IC_{50} 16.4 μg/mL) than the entire oil. When DPPH scavenging activity of essential oils was compared with synthetic antioxidant BHT, the winter and spring basil essential oil samples offered comparable activity (Table 1). Our findings are in agreement with Tepe et al. (2007) and Vardar-Unlu et al. (2003), who reported that the entire essential oil of *Nepta flavida* and *Thymus pectinatus*, respectively, showed greater antioxidant activity than individual components, indicating the possible synergistic interaction of the essential oil constituents. Politeo et al. (2007) reported that the *O. basilicum* essential oil and known synthetic antioxidant BHT showed similar radical-scavenging capacities with EC_{50} values of 1.4 and 0.9 g/L, respectively. Bozin et al. (2006) found that *O. basilicum* essential oil with IC_{50} 0.4 μg/mL was more efficient than BHT with IC_{50} 5.4 μg/mL.

Table 1 shows the level of % inhibition of linoleic acid oxidation as exhibited by the essential oils of *O. basilicum* from different seasons of the year. The higher the absorbance for iron based peroxides assessment, the higher will be the concentration of peroxides formed during reaction, consequently lower will be the antioxidant activity. The assessed *O. basilicum* essential oils inhibited the oxidation of linoleic acid by 80.3–91.2%. The effectiveness of the essential oils towards inhibition of peroxidation from winter (91.2%) and spring (90.3%) crops was comparable to BHT (91.1%). Whereas, the values observed for summer (80.3%) and autumn (84.3%) samples were significantly ($p < 0.05$) lower than BHT. Linalool, the main component of essential oil showed 75.2% inhibition of peroxidation thus showing comparatively less activity than entire oil. Singh and Marimuthu (2006) found that essential oils effectively suppress the peroxide formation in linoleic acid system during incubation. The antioxidant activity of *O. basilicum* essential oils might be attributed to the presence of other phenolic compounds (Lu & Foo, 2001). No data are available in the literature regarding the antioxidant

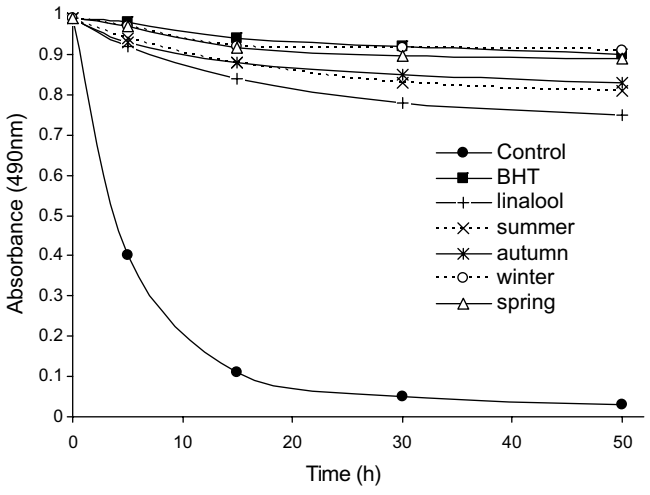

Fig. 1. Seasonal variation in bleaching of β-carotene with *Ocimum basilicum* essential oils.

activity of *O. basilicum* essential oil with respect to seasonal variations with which to compare the results of present analysis.

Bleaching β-carotene with linoleic acid system as antioxidant activity of the essential oils of *O. basilicum* in Fig. 1 are presented. The greater the effectiveness of an antioxidant, the slower will be the color depletion. In Fig. 1 smaller decrease in absorbance of β-carotene, indicates a lower rate of oxidation of linoleic acid and higher antioxidant activity in the presence of essential oils from *O. basilicum*. Control showed the highest rate of color depletion and the least antioxidant activity. Essential oils of *O. basilicum* of winter sample exhibited better antioxidant activity than BHT. Based on these results, order of antioxidant activity of *O. basilicum* essential oils was as follows: winter > BHT > spring > autumn > summer > linalool. No data are available in the literature regarding the antioxidant activity of *O. basilicum* essential oil with respect to seasonal variations with which to compare the results of present analysis.

3.3. Chemical composition of the essential oils

The chemical composition of *O. basilicum* essential oils, which differed considerably with regard to the seasons of the year in Table 2, is presented. A total of 29 compounds representing 98.0–99.7% of the oils were identified (see Fig. 3). Linalool was the main constituent of *O. basilicum* essential oil (56.7–60.6%), followed by *epi*-α-cadinol (8.6–11.4%), α-bergamotene (7.4–9.2%), γ-cadinene (3.3–5.4%), germacrene D (1.1–3.3%) and camphor (1.1–3.1%). Analyzed essential oils mainly consisted of oxy-

Table 2
Chemical composition of *Ocimum basilicum* essential oils

Components[A]	RI[B]	% Composition				Method of identification
		Summer	Autumn	Winter	Spring	
Monoterpene hydrocarbon						
Limonene	1031	t	t	0.3 ± 0.0^{a}	0.2 ± 0.0^{b}	a, b, c
cis-β-Ocimene	1041	–	–	0.8 ± 0.0^{a}	0.6 ± 0.0^{b}	a, b
Oxygenated monoterpenes						
1,8-Cineole	1034	0.2 ± 0.0^{d}	0.4 ± 0.0^{c}	1.2 ± 0.0^{a}	1.1 ± 0.0^{b}	a, b, c
Fenchone	1088	t	t	1.0 ± 0.0^{a}	0.9 ± 0.0	a, b
Linalool oxide	1090	t	t	1.1 ± 0.0	1.0 ± 0.0^{b}	a, d
Linalool	1099	56.7 ± 1.7^{b}	60.5 ± 1.8^{ab}	60.6 ± 1.2^{a}	58.6 ± 1.3^{ab}	a, b, c
Camphor	1150	1.1 ± 0.0^{b}	1.1 ± 0.0^{b}	3.0 ± 0.1^{a}	3.1 ± 0.1^{a}	a, b, c
α-Terpineol	1191	0.9 ± 0.0^{c}	1.0 ± 0.0^{b}	0.7 ± 0.0^{d}	1.0 ± 0.0^{a}	a, b
cis-Geraniol	1255	0.9 ± 0.0^{c}	0.5 ± 0.0^{d}	1.3 ± 0.0^{a}	1.0 ± 0.0^{b}	a, b, c
Linalyl acetate	1257	0.5 ± 0.0^{a}	0.3 ± 0.0^{b}	t	–	a, b
Bornyl acetate	1292	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	t	t	a, b
Sesquiterpene hydrocarbon						
α-Copaene	1379	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{c}	–	a, b
β-Cubebene	1389	0.5 ± 0.0^{a}	0.4 ± 0.0^{b}	t	t	a, b
β-Caryophyllene	1421	1.7 ± 0.0^{a}	1.9 ± 0.1^{a}	1.2 ± 0.0^{c}	1.4 ± 0.0^{b}	a, b, c
α-Bergamotene	1439	9.2 ± 0.2^{a}	7.4 ± 0.3^{b}	7.8 ± 0.2^{b}	7.6 ± 0.2^{b}	a, b
α-Humulene	1457	t	t	0.2 ± 0.0^{b}	0.4 ± 0.0^{a}	a, b, c
γ-Muurolene	1483	0.9 ± 0.0^{a}	0.8 ± 0.0^{b}	0.5 ± 0.0^{d}	0.7 ± 0.0^{c}	a, b
Germacrene D	1489	3.3 ± 0.1^{a}	2.0 ± 0.1^{b}	1.1 ± 0.0^{c}	2.0 ± 0.1^{b}	a, b
β-Selinene	1491	0.8 ± 0.0^{a}	0.4 ± 0.0^{d}	0.6 ± 0.0^{b}	0.4 ± 0.0^{c}	a, b
Bicyclogermacrene	1503	1.1 ± 0.0^{a}	1.0 ± 0.0^{a}	0.6 ± 0.0^{c}	0.8 ± 0.0^{b}	a, b
γ-Cadinene	1515	5.4 ± 0.2^{a}	5.3 ± 0.2^{a}	3.2 ± 0.1^{b}	4.9 ± 0.1^{a}	a, b
(−) Calamenene	1519	1.0 ± 0.0^{a}	0.8 ± 0.0^{b}	0.6 ± 0.0^{d}	0.7 ± 0.0^{c}	a, b
Oxygenated sesquiterpene						
Spathulenol	1580	t	t	0.4 ± 0.0^{b}	0.5 ± 0.0^{a}	a, b
Caryophyllene oxide	1584	t	t	0.7 ± 0.0^{a}	0.3 ± 0.0^{b}	a, b, c
Viridiflorol	1595	1.7 ± 0.0^{b}	1.8 ± 0.0^{a}	1.3 ± 0.0^{d}	1.6 ± 0.1^{c}	a, b
Cadinol, *epi*-α	1645	11.4 ± 0.2^{b}	12.4 ± 0.4^{a}	8.6 ± 0.2^{d}	10.0 ± 0.3^{c}	a, b
β-Eudesmol	1656	t	t	0.2 ± 0.0^{b}	0.3 ± 0.0^{a}	a, b
α-Cadinol	1658	0.5 ± 0.0^{a}	0.2 ± 0.0^{c}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	a, b
α-Bisabolol	1689	t	t	0.4 ± 0.0^{a}	0.2 ± 0.0^{b}	a, b
Total		98.6	99.0	98.0	99.7	

Values are mean ± standard deviation of two different experiments. Different letters in superscript indicate significant difference within seasons.
t: trace (<0.05%).
a = Identification based on retention index; b = identification based on comparison of mass spectra; c = identification based on co-injection with authentic compounds.
[A] Compound listed in order of elution from a HP-5MS column.
[B] Retention indices on the HP-5MS column.

genated monoterpenes (60.7–68.9%) followed by sesquiterpenes hydrocarbons (16.0–24.3%) and oxygenated sesquiterpenes (12.0–14.4%). The major oxygenated monoterpenes were: linalool, camphor, *cis*-geraniol and 1,8-cineole. While, α-bergamotene, β-caryophyllene, germacrene D, γ-cadinene and bicyclogermacrene were the main sesquiterpene hydrocarbons, whereas, *epi*-α-cadinol and viridiflorol were the important oxygenated sesquiterpene. Our results are in a good agreement to those of Telci et al. (2006), who reported the oxygenated monoterpenes were the major compounds in Turkish *O. basilicum* essential oils. In *O. basilicum* essential oils from Bangladesh, linalool was reported as the main component (Mondello et al., 2002). Jirovetz and Buchbauer (2001) found a high level of linalool (71.4%) in *O. basilicum* essential oil from Bulgaria. According to Gurbuz et al. (2006), linalool (41.2%) was the main compound, identified in the hydro-distilled *O. basilicum* essential oil from Turkey. Purkayastha and Nath (2006) reported the camphor, limonene and β-selinene were the major components in *O. basilicum* essential oils from northeast India. The observed differences in the constituents of basil essential oils across countries may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants.

3.3.1. Seasonal variability

The data for the present analysis demonstrated the contents of the essential oils to be varied significantly ($p \leqslant 0.05$) with regards to seasonal changes (Fig. 2). Components that varied mostly with seasons were linalool (highest contribution in winter: 60.6%), *epi*-α-cadinol, β-caryophyllene and viridiflorol (highest amount in autumn: 12.4%, 1.9% and 1.8%, respectively), camphor (highest contribution in spring: 3.1%) and α-bergamotene, γ-cadinene and germacrene D (highest amount in summer: 9.2%, 5.4% and 3.3%, respectively; Fig. 2). The contents of oxygenated components sum of oxygenated monoterpenes and oxygenated sesquiterpenes, present in the essential oils were highest during winter (80.9%) and lowest during summer (74.3%; Fig. 2a). An opposite trend was observed for sesquiterpene hydrocarbons, the level of those reached the maximum of 24.3% in summer and the minimum in winter (16.0%). These results are in good agreement with the findings of Cristiana, Freire, Marques, and Costa (2006), who reported that the composition of essential oil obtained from *Ocimum gratissimum* leaves at different seasons of the year varied markedly. Kofidis et al. (2006) reported the seasonal variation in the chemical composition of essential oils of *M. spicata*, grown wild in Greece. The essential oil contained the maximum amounts of linalool in October (mid-autumn) and minimum in June (summer). Celiktas et al. (2007) also found the variation in the chromatographic profile of *Rosemarinus officinalis* due to different seasons. In contrast, Da-Silva et al. (2003) reported that there were no considerable changes in the chromatographic profiles of basil essential oils with regard to two different seasons of the year reported.

3.4. Antimicrobial activity

The antimicrobial activity of the essential oils obtained from *O. basilicum* collected seasonally against nine patho-

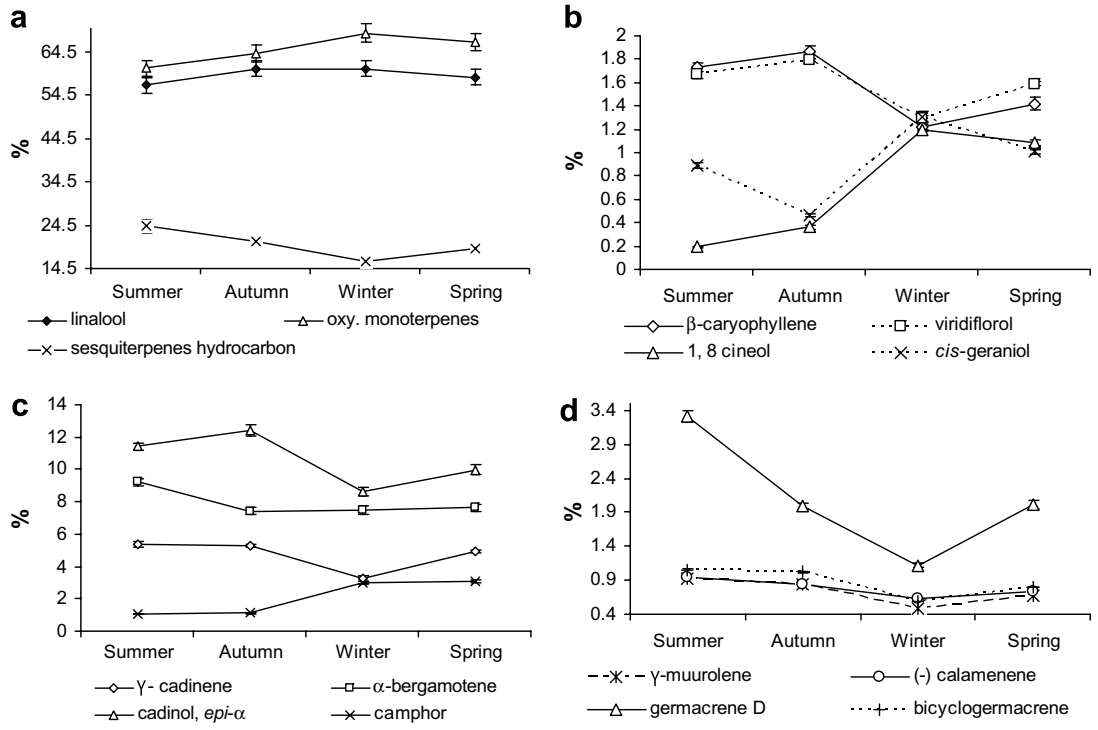

Fig. 2. Seasonal variation in the major components of *Ocimum basilicum* essential oils.

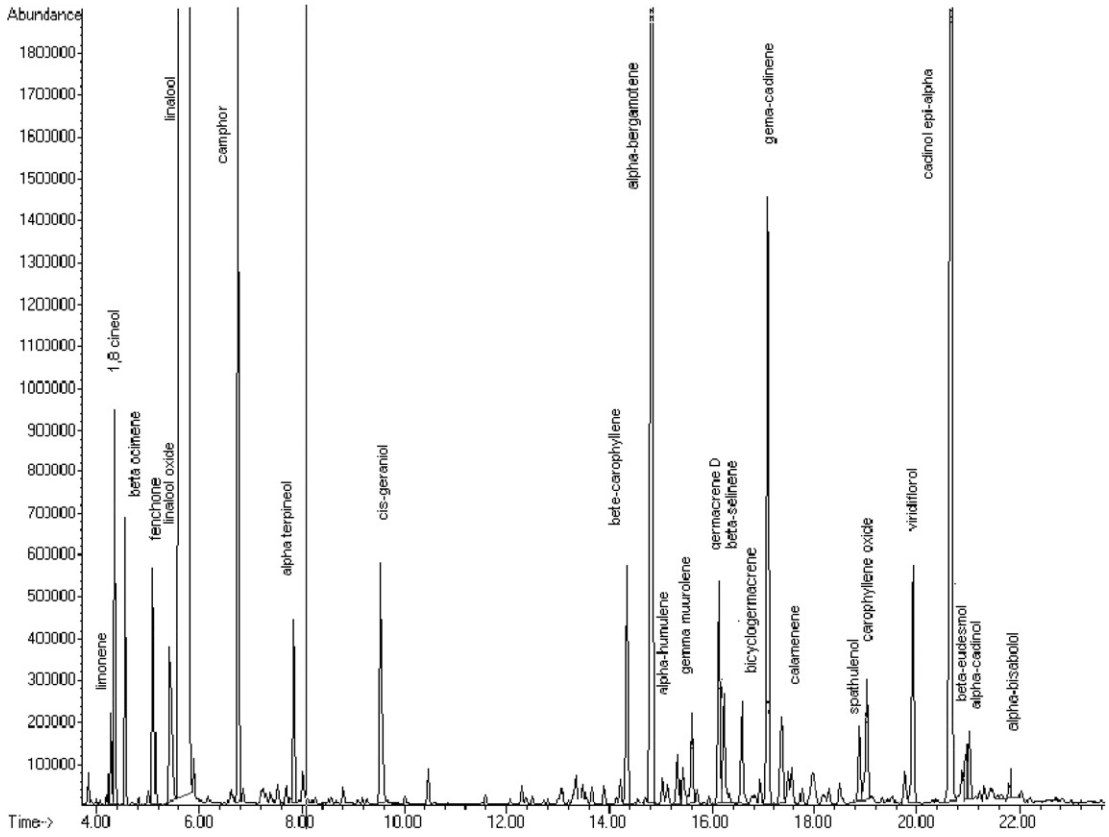

Fig. 3. Typical chromatogram of *Ocimum basilicum* essential oil components.

Table 3
Seasonal variation in antimicrobial activity of *Ocimum basilicum* essential oils

Tested organism	Essential oils				Linalool	Amoxicillin	Flumequine
	Summer	Autumn	Winter	Spring			
Diameter of inhibition zone (mm)[A]							
S. aureus	22.2 ± 1.3^{b}	24.4 ± 1.1^{ab}	24.0 ± 1.0^{ab}	23.2 ± 1.4^{b}	25.3 ± 1.1^{ab}	28.2 ± 1.1^{a}	–
B. subtilis	21.2 ± 1.2^{c}	20.4 ± 1.0^{c}	26.1 ± 1.1^{ab}	24.1 ± 1.2^{b}	26.9 ± 1.2^{ab}	29.3 ± 1.0^{a}	–
P. multocida	16.1 ± 1.0^{d}	13.6 ± 0.8^{e}	18.0 ± 0.9^{c}	18.4 ± 1.0^{c}	22.3 ± 1.0^{b}	31.1 ± 1.2^{a}	–
E. coli	11.4 ± 0.6^{e}	13.2 ± 0.8^{d}	16.2 ± 1.0^{c}	13.5 ± 0.8^{d}	19.5 ± 1.1^{b}	21.4 ± 0.8^{a}	–
A. niger	18.4 ± 1.2^{d}	21.6 ± 1.2^{c}	18.7 ± 0.7^{d}	20.4 ± 1.2^{cd}	23.3 ± 0.8^{b}	–	26.1 ± 0.9^{a}
M. mucedo	11.1 ± 0.9^{d}	13.6 ± 0.8^{c}	9.7 ± 0.6^{d}	11.2 ± 0.6^{d}	15.6 ± 0.8^{b}	–	18.4 ± 0.7^{a}
F. solani	15.2 ± 0.7^{c}	19.4 ± 1.1^{b}	11.7 ± 0.7^{d}	17.7 ± 1.3^{bc}	21.4 ± 1.0^{ab}	–	23.2 ± 1.1^{a}
B. theobromae	14.3 ± 1.1^{c}	17.2 ± 1.0^{b}	13.6 ± 0.8^{c}	16.6 ± 1.0^{b}	17.6 ± 1.0^{b}	–	20.2 ± 1.2^{a}
R. solani	12.3 ± 0.7^{d}	13.5 ± 0.8^{cd}	10.3 ± 0.6^{e}	14.3 ± 0.8^{c}	17.3 ± 0.8^{b}	–	19.3 ± 0.8^{a}
Minimum inhibitory concentration (mg/ml)							
S. aureus	1.3 ± 0.0^{b}	1.5 ± 0.0^{a}	0.9 ± 0.0^{d}	1.1 ± 0.0^{c}	0.4 ± 0.0^{e}	0.0 ± 0.0^{f}	–
B. subtilis	1.4 ± 0.0^{a}	1.2 ± 0.0^{b}	0.8 ± 0.0^{c}	0.8 ± 0.0^{c}	0.3 ± 0.0^{d}	0.0 ± 0.0^{e}	–
P. multocida	1.9 ± 0.1^{c}	2.0 ± 0.1^{b}	1.7 ± 0.1^{c}	2.3 ± 0.1^{a}	0.9 ± 0.0^{d}	0.3 ± 0.0^{e}	–
E. coli	2.6 ± 0.1^{a}	2.1 ± 0.1^{b}	1.6 ± 0.1^{c}	2.0 ± 0.1^{b}	0.9 ± 0.0^{d}	0.1 ± 0.0^{e}	–
A. niger	3.2 ± 0.2^{b}	2.2 ± 0.1^{d}	4.5 ± 0.2^{a}	2.7 ± 0.1^{c}	1.0 ± 0.1^{e}	–	0.4 ± 0.0^{f}
M. mucedo	4.9 ± 0.3^{a}	3.8 ± 0.2^{b}	5.1 ± 0.3^{a}	4.6 ± 0.2^{a}	1.9 ± 0.1^{c}	–	0.3 ± 0.0^{d}
F. solani	3.6 ± 0.2^{b}	2.7 ± 0.1^{c}	4.9 ± 0.2^{a}	3.2 ± 0.2^{b}	1.6 ± 0.1^{d}	–	0.2 ± 0.0^{e}
B. theobromae	4.1 ± 0.2^{a}	2.3 ± 0.1^{c}	4.6 ± 0.2^{a}	2.9 ± 0.2^{b}	1.1 ± 0.0^{d}	–	0.2 ± 0.0^{e}
R. solani	4.3 ± 0.2^{b}	3.0 ± 0.2^{c}	5.0 ± 0.3^{a}	2.9 ± 0.2^{c}	1.5 ± 0.1^{d}	–	0.2 ± 0.0^{e}

Values are means ± standard deviation of three separate experiments.
Different letters in superscript indicate significant differences within seasons.
[A] DD, diameter of inhibition zone (mm) including disc diameter of 6 mm.

genic microorganisms is shown in Table 3. The essential oils of *O. basilicum* exhibited strong antimicrobial activity against all the microorganisms tested. Results obtained from disc diffusion method, followed by measurement of

minimum inhibitory concentration (MIC), indicated that *S. aureus* and *B. subtilis* were the most sensitive microorganisms showing largest inhibition zones (22.2–24.4 mm), (20.4–26.1 mm), and lowest MIC values (0.9 mg/mL), (0.8 mg/mL), respectively. Less activity was observed against *M. mucedo* with the smallest inhibition zones (9.7–13.6 mm) and highest MIC value (3.8–5.1 mg/mL). Linalool, showed strong antimicrobial activity than the entire oil with MIC values, 0.3–1.9 mg/mL.

It was observed that *O. basilicum* essential oil and linalool showed greater activity against bacterial strains than against antifungal strains (Table 3). Overall the essential oils and linalool possessed stronger antimicrobial activity against Gram-positive bacteria than against Gram-negative bacteria. Generally, essential oils from winter and autumn crops exhibited greater antimicrobial activity which might be attributed to the high contents of linalool and other oxygenated compounds in these samples. When compared with standard drugs, Amoxicillin and Flumequine; the essential oils from winter and autumn harvests, and linalool demonstrated comparable antimicrobial activity. Analysis of variance revealed that, both, the seasonal and microorganisms variations exhibited significant ($p < 0.05$) effect on the antimicrobial activity of essential oils of *O. basilicum*. The changes in antimicrobial activity of the investigated *O. basilicum* essential oils, with regard to seasonal variations, might be attributed to the different chemical composition of the oils. Some earlier reports showed that the changes in chemical composition of an essential oil directly affected their biological activities (Celiktas et al., 2007; Van Vuuren et al., 2007).

Suppakul et al. (2003) reported that basil essential oils exhibited good antimicrobial activity against a wide range of microorganisms. Wannissorn et al. (2005) reported that *O. basilicum* essential oil showed moderate antibacterial activity. Whereas, Bozin et al. (2006) and Lopez, Sanchez, Batlle, and Nerin (2005) showed that the Gram-positive strains of bacteria showed higher sensitivity to *O. basilicum* essential oils than those of their counter part. Bozin et al. (2006) and Sokovic and Van Griensven (2006) also reported the antifungal activity of essential oils from *O. basilicum* and its main component, linalool. The antimicrobial activities of essential oils from *O. basilicum* may be in due part to the presence of high content of linalool (Koutsoudaki, Krsek, & Rodger, 2005; Sartoratotto et al., 2004; Sokovic and Van Griensven, 2006; Suppakul et al., 2003).

In general, growing season affected chemical composition, antioxidant and antimicrobial activities of the essential oils from *O. basilicum*. These differences can be attributed to the seasonal changes in temperature and humidity, also to different stage of plant metabolism. As discussed above, essential oils from *O. basilicum* showed good antioxidant and free radical-scavenging activities, and broad activity against bacteria and pathogenic fungi. The production of essential oils and their utilization as potential natural food preservants could be of economical value. However, further investigation to establish how components interact to provide the antioxidant activity is needed. Studies should also be extended to evaluating the practical effectiveness of essential oil against the growth of different food borne and spoiling microbes under the specific environmental, storage and food processing conditions and using different substrates.

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