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Composition and Antibacterial Activity of the Essential Oil of a Green Type and a Purple Type of *Ocimum basilicum* L. from Iran

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Composition and Antibacterial Activity of the Essential Oil of a Green Type and a Purple Type of *Ocimum basilicum L.* from Iran

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Abstract

A comparison of the chemical composition and antimicrobial activity of the essential oils obtained from the aerial parts of two types of *Ocimum basilicum* L.—*O. basilicum* L. (green type) and *O. basilicum* (purple type)—were carried out. The oils were obtained by hydrodistillation and were analyzed by GC and GC/MS. The main components of the oil of the *O.basilicum* (green type) were methyl chavicol (62.5%), geranial (12.5%) and neral (9.9%) while in the oil of *O.basilicum* (purple type), *trans*-a-bergamotene (17.5%), linalool (17.0%) and 1,8-cineole (9.0%) were the prominent components. The antimicrobial activity of each oil was determined by measurement of the growth inhibitory zone, against three Gram positive, one Gram negative and one fungus using the well diffusion assay.

Key Word Index

Ocimum basilicum, Lamiaceae, essential oil composition, methyl chavicol, geraniol, neral, *trans*-α-bergamotene, linalool, 1,8-cineole.

Introduction

The genus *Ocimum* (family Lamiaceae), collectively called basil, consists of about 160 species, and is spread over the tropical, subtropical and warmer parts of the temperate regions of both hemispheres raging from sea level to 1800 ft altitude (1).

Basil has traditionally been used to treat head colds and as a cure for warts and worms, as well as an appetite stimulant, carminative, and diuretic. In addition, it has been used as a mouthwash and astringent to cure inflammations of the mouth and throat. Alcoholic extracts of basil have been used in creams to treat slowly healing wounds (2). Basil is more widely used as a medicinal herb in the Far East, especially in China and India. It was first described in a major Chinese herbal around AD 1060 and has since been used in China for spasms of the stomach and kidney ailments, among other applications. It is especially recommended for use before and after parturition to promote blood circulation. The whole herb is also used to treat snakebite and insect bites (3).

It contains both cultivated herbs and shrubs and ruderal species. Some species are used in traditional medicine for different applications, especially in many Asian and African countries (4,5). Anti-inflammatory, anti-arthritic, anti-stress and anti-pyretic pharmacological activities of *O. tenuiflorum* have been reported (6,7). The oil of *O. gratissimum* is used to flavor foods, dental and oral products, in fragrances and aromatherapy, and in traditional rituals and medicines (8).

Sweet basil, *Ocimum basilicum*, is the major culinary and essential oil source of this genus (9). As a result of its medicinal properties, the plant is used as a carminative, diuretic and stimulant (10). The oil, mainly used in food industries and perfumery, has also been reported to possess antimicrobial properties (11). The recurring polymorphism determines a large number of subspecies, different varieties and forms producing essential oils with varying chemical compositions (12,13). So, *O. basilicum* comprises many chemotypes. The oil of *O. basilicum* varies in chemical constituents and there is extensive diversity in the constituents of oils from various parts of the world (14,15). There are usually considerable variations

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in the major components within this species (16). In most cases, four compounds—methyl chavicol, linalool, methyl cinnamate and eugenol—occur in the oil in varying degrees of abundance (14). As a consequence, there are several chemotypes of basil oils identified according to their major compounds, including methyl chavicol, methyl chavicol-methyl eugenol, methyl eugenol, eugenol, methyl cinnamate, linalool, linalool-geraniol, methyl chavicol-linalool, methyl chavicol-linalool-methyl cinnamate, linalool-geraniol-eugenol (17), etc.

The present study deals with the analysis and antimicrobial activity of the oils obtained from the aerial parts of two types of *Ocimum basilicum* L. (type green and type purple) growing wild in Iran.

Table I. Percentage composition of the oils of two types of *Ocimum basilicum*

Compound	RI	*O. basilicum* [a] (%)	*O. basilicum* [b] (%)
α-pinene	939	0.1	1.2
sabinene	979	-	0.5
β-pinene	980	-	1.0
6-methyl-5-heptene-2-one	985	1.6	-
myrcene	991	-	1.0
p-cymene	1026	0.6	-
limonene	1031	0.5	-
1,8-cineole	1033	-	10.0
(E)-β-ocimene	1050	-	0.7
fenchone	1087	1.8	7.0
linalool	1098	-	17.0
camphor	1143	-	2.5
trans-verbenol	1143	0.8	-
methyl chavicol	1195	62.5	-
neral	1240	10.0	-
geraniol	1255	12.5	-
(E)-anethole	1283	1.5	-
α-copaene	1376	-	1.5
β-bourbonene	1384	-	0.6
β-elemene	1391	-	3.5
methyl eugenol	1401	1.3	-
β-caryophyllene	1409	1.6	7.5
trans-α-bergamotene	1415	0.3	17.5
α-humulene	1454	0.8	3.0
(E)-β-farnesene	1458	-	4.1
germacrene D	1480	0.2	6.0
β-selinene	1485	0.6	-
bicyclogermacrene	1494	-	4.0
trans-β-guaiene	1500	-	2.5
(Z)-α-bisabolene	1504	0.7	-
δ-cadinene	1524	-	0.5
trans-calamenene	1532	0.5	-
α-cadinene	1538	0.3	-
Caryophyllene oxide	1581	-	0.5
Total		98.6	92.1

RI= relative retention indices
a: green type
b: purple type

Experimental

***Plant material**:* The aerial parts of two types of *O. basilicum;* 200 g of *O.basilicum* (green) and 150 g of *O. basilicum* (purple) were collected during the flowering stage from Karaj, just north of Tehran, Iran, in June 2006. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

***Oil isolation**:* The air-dried aerial parts of both types [*O. basilicum* (purple) and *O. basilicum* (green)] were separately subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. After decanting and drying of the oils over anhydrous sodium sulfate, the corresponding oils were isolated in yield of 0.2% and 0.4% v/w, respectively.

Analysis: The GC analysis was carried out on Shimadzu 15A gas chromatograph equipped with a spilt/splitless injector (250°C) and a flame ionization detector (250°C). Nitrogen was used as carrier gas with a flow rate of 1.5 mL/min and the capillary used was DB-5 (50 m × 0.2 mm, film thickness: 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C- R4A chromatopac without the use of correction factors.

***GC/MS*:** Analyses were performed on a Hewlett-Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness: 0.25 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. MS were taken at 70 eV. The retention indices for all the components were determined according to the Van Den Dool method, using n-alkanes as standards (17). The compounds were identified by (RRI, DB5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or with published mass spectra (19,20).

***Antimicrobial activity**:* The in vitro antibacterial activity of the two types of *O. basilicum* were determined by measuring the growth inhibitory zones (well diffusion assay) against three Gram-positive bacteria: *Staphylococcus aureus* PTCC 1399, *Listeria monocytogenes* PTCC 1431, *Bacillus cereus* PTCC 1301; one Gram-negative bacteria: *Escherichia coli*

Table II. Antibacterial activities of two types of *Ocimum basilicum*

Microorganisms	Gram +/-	Green type	Purple type	Gentamicine
Staphylococcus aureus PTCC 1113	+	28	35	31
Listeria monocytogenes PTCC 1431	+	8	10	9
Bacillus cereus PTCC 1301	+	27	45	32
Escherichia coli PTCC 1399	-	8	15	15
Candida albicans PTCC 5027		-	22	15

Values are the mean diameter of inhibitory zones (mm)

PTCC 1399; and one fungus: *Candida albicans* PTCC 5027. The microorganisms were obtained from Iranian Research Organization for Science & Technology (IROST). Microorganisms (obtained from enrichment culture of the microorganisms in 1 mL of Muller-Hinton broth, incubated at 3°C for 12 h) were cultured on Muller-Hinton agar medium. The inhibitory activity was compared with that of standard antibiotics, such as gentamicine (10 µg), which were obtained from the Iran Daru Company. After drilling wells on medium using a 6 mm cork borer, 100 µL of oils obtained from two types of *O. basilicum* were poured into each well. The plates were incubated at 37°C overnight, the diameter of inhibition zone was measured to the nearest millimeter.

Each test was carried out in triplicate and the average was calculated for inhibition zone diameters. The antibacterial activity was recorded as the radial extent of the area cleared of bacterial growth around the well.

Results and Discussion

***Chemical composition**:* The chemical composition of the oils of the two types of *O. basilicum* are listed in Table I, in which the percentage and retention indices of components are given. Twenty components were identified in the oil of *O. basilicum* (green type), making up 98.5% of the total composition. Methyl chavicol (62.5%), geraniol (12.5%) and neral (9.9%) were the major components in this oil. Monoterpene and sesquiterpene fractions of the oil were relatively small, representing 26.7% and 50%, respectively. According to high methyl chavicol content, this sample could be classified as methyl chavicol-chemotype.

Twenty-one components were identified in the oil of the purple type of *O. basilicum* representing 92.1% of the total oil with *trans*-α-bergamotene (17.5%), linalool (17.0%), and 1,8-cineole (10.0%) as main constituents. Other compounds that were present in appreciable amounts in this oil were b-caryophyllene (7.5%), fenchone (7.0%), germacrene D (6.0%), (E)-ß-farnesene (4.1%) and bicyclogermacrene (4.0%). The oil was characterized by large amounts of sesquiterpenes (51.2%) and monoterpenes (40.9%). As can be seen from the above information, the oils of two varieties of *O. basilicum* are dissimilar. The chemovar I oil was rich in phenyl propanoid compounds, while the chemovar II oil was rich in sesquiterpenes and monoterpenes.

The composition of the oil of some *Ocimum* species has been reported (21-28). The oil of the chemotype of *O. basilicum* from Cuba (24) and Nigeria (25) have methyl chavicol, similar to our results from var. green of the plant, whereas the oil from plants growing in India showed camphor in high content (26) and the oil of the plant from Bulgaria has linalool as the main constituent (27).

The composition of the oils of three chemotypes of the plant, *O. basilicum* L., *O. basilicum* L. var *purpurascens* Benth. and *O. basilicum* L. var *minimum* from Brazil, were reported (28). In the latter oil, methyl chavicol was the major constituent, while in the other two oils linalool predominated.

***Antibacterial activity**:* The antimicrobial activities of two types of *O. basilicum* oils were assayed against three Gram-positive and one Gram-negative bacteria and one fungus, and the results, presented in Table II, were compared with standard antibiotics, such as gentamicine. The present study revealed that both oils were active against the Gram-positive bacteria *Listeria monocytogenes* and Gram-negative bacteria *Escherichia coli*. *Candida albicans* was insensitive to the oil of chemvar I of the plant, whereas the chemvar II was active against the fungus *C. albicans*. Wan et al. (29) and Lachowicz et al. (30) reported the antimicrobial activity of basil oil against *E. coli, S. aureus* and some genera of *Candida*. Application of basil oil as an insecticidal fumigant/powder (31) and as an alternative to conventional antimicrobial additives in food (32) has been proposed. Koga et al. (33) reported a basil-resistant strain of *Vibrio parahaemolyticus,* which showed a higher resistance to heat and H_2O_2 than the parent strain and conversely a heat-adapted *V. parahaemolyticus* also showed a higher resistance to basil oil than non-adapted cells.

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In vitro Antimicrobial Activities of Essential Oils from *Origanum minutiflorum* and *Sideritis erytrantha* subsp. *erytrantha* on Phytopathogenic Bacteria

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Abstract

This study was designated to evaluate the antimicrobial activity of the essential oils obtained from the aerial parts of *Origanum minutiflorum* and *Sideritis erytrantha,* both endemic species in Turkey, against 19 plant pathogenic bacteria. Two essential oils were analyzed by GC-FID and GC/MS. The major components of *O. minutiflorum* oil were carvacrol (73.93%) and p-cymene (7.20%). The essential oil of *O. minutiflorum* exhibited an extremely strong activity

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