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Original Article

Essential oils Composition of *Ocimum basilicum var. purpurascens* from Different Ecological Zone in Iran and Antimicrobial Activity against Different Bacterial Species

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Abstract

There are distinct varieties of basil types in the genus *Ocimum* L. which makes them very special. Genus *Ocimum* L. is widespread over Asia, Africa, Central and Southern America. All basils are member of the *Lamiaceae* family. The colors of the leaves vary from bright green to purple-green and sometimes almost black. Fresh basil leaves have a strong and characteristic aroma, plant which is used in several traditional medicine systems to cure various diseases. The *Ocimum basilicum var. purpurascens* where green cultivated and collected from three different locations (Alborze, Shahr-e-Rey and Golestan provience) in Iran. The essential oils were extracted by water distillation, and then injected to Gas Chromatography (GC) and Gas Chromatography coupled with mass spectrometry (GC/MS). Main chemical composition of essential oils of leaf sample of Alborz were chavicol 81.41%, methyl acetate 2.89%, and sample of Shahr-e-Rey were chavicol 57.69%, linalool 16.43%, and sample of Gorgan city were chavicol 63.51%, and linalool 19.80% yield. In this study, extracted essential oil was investigated on bacterial with definition of MIC and MBC. essential oil of Alborz sample was examined on Gram-positive bacteria: *Staphylococcus aureus*, *Basilus subtilis* and Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, which it has growth inhibitory effect (MIC) and bactericidal (MBC), with a concentration of 5% and it has a stronger effect on *Basilus subtilis* (MBC = 5%, MIC = 2.5 %) too. Essential oil of Shahr-e-Rey was examined on the same bacterias which it has growth inhibitory effect (MIC) and bactericidal (MBC), with a concentration of 5% and it has a Stronger effect on *Basilus subtilis* (MBC = 5%, MIC = 2.5 %) too. Essential oil of Gorgan city was examined on the same bacterias which it has growth inhibitory effect (MIC) and bactericidal (MBC) with a concentration of 5% and it has a Stronger effect on *Basilus subtilis* (MBC = 5%, MIC = 2.5 %) too.

Keywords: Hydro distillation, *Ocimum*, Gram- bacteria, Chavicol, Linalool

Introduction

The genus *Ocimum* L. is ranked high among some of the astonishing herbs for having enormous medicinal potentialities. Previous studies show that there is a large number of species and varieties falls in this genus [1-5]. Several authors recognized more than 60-150 species in this genus. Characterizations of each species in this genus

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(family Lamiaceae) are based on the leaves and habitat [1]. The shape of *Ocimum tenuiflorum* L. leaves and its close relatives varies in size of leaves, vein and petioles. The color of leaves varies from bright green to dark green and sometime almost black. Though the color of plants varies, but the reason behind it, especially in basils, are not being studied yet. Medicinal and aromatic plants (MAPs) have been used for centuries as remedies for human diseases because they contain components of therapeutic value. It has been estimated by WHO that 80% of the population, the majority of this in the developing countries, still rely on plant-based medicine for primary health care needs [6]. For this purpose, various strategies have been developed, e.g. biological screening, isolation, as well as clinical trials for a variety of plants. Following the advent of modern medicine, herbal medicine suffered a setback, but during the two or three decades the advances in photochemistry and in the identification of the plant compounds, providing effective against certain chronic diseases and emergence of multidrug resistant bacteria. The genus *Ocimum* economically involves the most important medicinal and aromatic herbs, under shrubs or shrubs in the world. It belongs to *Lamiaceae*, *Ocimoideae*, and comprises more than 30 species distributed in tropical and subtropical regions of Asia, Africa, and Central and South America [7]. Essential oils are volatile material derived from physical process of odorous plant material a single botanical form and species which it agrees in name and odour. The essential oils are mixtures of up to 200 organic compounds, many of them are either terpenes (with 10 carbon atoms) or sesquiterpenes (with 15 carbon atoms) [8]. *Ocimum tenuiflorum* L. from Lamiaceae is an erect, softy hairy and aromatic herb. Two types of *Ocimum tenuiflorum* L. are met within cultivation: (i) with green leaves known as Sri or Lakshmi Tulsi and (ii) with purple leaves known as Krishna Tulsi [9]. Essential oils are fragrant, highly concentrated essences of plants which are considered to exemplify the soul or life-sourceof the plant. Essential oils are approximately 75-100 times more concentrated than dried herbs [4]. Nowadays, there are many efficient and economical extraction methods of essential oils being developed. These methods Include-Cold pressing, Hydro distillation, Steam distillation, Solvent extraction, Supercritical CO2 extraction and enfleurage. Essential oils find wide applications in a number of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The essential oil of *Ocimum* extracted via steam distillation from the leaves and flavoring tops are used to flavor foods, dental and oral products, in fragrances, and in traditional rituals and medicines. The active compounds present volatile oil from the leaves consist mainly of eugenol, thymol, citrol, geraniol, camphor, linalooll, and methyl cinematic [10-16]. The seeds contain oil composed of fatty acids and sitosterol. The roots contain sitosterol and three triterpenes A, B, and C. additionally, they also contain rosmarinic acid, thymol, methyl chavicol and, citral etc. [17], and vitamins C, A, and minerals like calcium, zinc, and iron [18], as well as chlorophyll and many other phytonutrients. Recent interest in *Ocimum* has resulted from its inhibitory activity against HIV-I reverse transcriptase and platelets aggregation induced by collagen and ADP (adenosine 5'-disphosphate) [19-20]. However, the antimicrobial activity of *Ocimum* essential oil against microorganisms has been investigated by some researchers [16, 21-25], using different techniques and their investigations mostly covered one individual or two species. Unfortunately, the published data on the former subject are difficult to compare, because the chemical composition of essential oil is known to vary with the local climate, harvest period, and environmental conditions [26], and is also dependent on type of solvent used in the extraction procedure [27]. However, with our experience in this study we reported result of essential oil of *Ocimum tenuiflorum* L. were collected from three different locations, Alborze karaj, Shahr-e-Rey near Tehran, and another one Gorgan city in Golestan provience, cultivated in Iran, therefore the current study was under taken to elucidate the chemical composition. The antimicrobial activity of some chemical compounds have been investigated and the possibility of their use for the development of antimicrobial drugs has also been registered [28]. Use of medicinal plants is economic and effective and on the other hand is easily accessible and safe to use [29] Some chemical constituents of medicinal importance of *Ocimum tenuiflorum* L. are known that many of which have shown a high biological activity [30,31]. The herb of basil has essential oil. It is Variable due to climate of

growing place from 0.5 to 1.5%. To obtain high quality and quantity of essential oil, it is recommended that the herb basil is harvested in full bloom. The essential oil of Basil is extracted from (leaves, Branches, fresh and dry flowers) [32]. The antimicrobial activity of essential oil is known for years, in addition to antimicrobial properties, Oil has antifungal, antiviral, anti-parasitic and … properties. Despite of recognize of these effects in previous years, the green consumers tend to lead to greater scientific understanding of these materials . In this study, essential oils were extracted using steam distillation and Clevenger and the structure was determined using mass spectrometry. The antimicrobial effect of essential oils was determined by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Antimicrobial activity can be measured by diffusion method, dilution method and …. [33]. MIC is mentioned as a criterion for determining the antimicrobial activity of essential oils by the majority of researchers [34]. According to studies carried out in different seasonal conditions on Basil essential oils, the oil extracted by hydro distillation is different from 0.5 to 0.8 which the highest value observed in winter, the lowest was in summer and most observed composition was Linalool and after that epi-α-cadinol. Some samples showed more oxygenated compounds. The antimicrobial activity of this seasonal essential oil on Bacillus subtilis, Staphylococcus aureus, Escherichia coli showed that this essential oil has a positive effect on all strains studied which varied according to seasonal conditions [35] The chemical composition of *Ocimum tenuiflorum* L. essential oil aerial parts of flowering which is growing on India north west showed that essential oil of this herb is rich in phenolic compounds and derivatives which most of the oil composition is Eugenol with the concentration of 82.9% among of 26 detected compounds and 98.9% of the whole essential oil [36].

Material and Methods

Plant Name

Ocimum basilicum var. purpurascens (Source)

The Leaves of *Ocimum basilicum var. purpurascens*, was collected from different location during the month of June 2015, samples belonge to Alborze, Karaj, Shahr-e-Rey in Tehran, and Gorgon city in Golestan province. Samples were collected and identification of the plants was determined by botanist in Iranian Botanical Garden (IBG).

Leaves were separated from the plants making sure no foreign matter or any other part of the plant was mixed with them. The drying leaves was done on air flow in shadow for a few days. Extraction of Essential Oils, done bay about 300g leaves of *Ocimum basilicum var. purpurascens*, subjected to hydro-distillation (Clevenger type apparatus) for 2 hours. The essential oil was separated from aqueous layer using a 100 mL capacity separator funnel, and was dried by filtration over anhydrous sodium sulfate. Oil yield from leaves of Alborze Karaj was 99.91 %, Shahr-e-Rey 99.9 % and Gorgon was 99.34%, respectively. The oils were stored in sealed vials at 2 °C before analysis. The composition of essential oils was analyzed by gas chromatography (GC) and gas chromatography, coupled to mass spectrometry (GC-MS).

Antimicrobial activities of the plant extracts against the planktonic form of the bacteria were determined using the disc diffusion method. MIC and MBC values were evaluated using macro broth dilution technique. Anti-biofilm effects were assessed by microliter plate method.

Gas Chromatography

GC analyses were performed using a gas chromatography, Ultra-Fast Module–GC, made in Italia. Profile column machine brand Ph-5 capillary column, manufactured by Shimadzu with Length of 30 mm and an inner diameter of1/0 mm thick 25/0 mm, the inner surface of the stationary phase material is covered Phenyl Dimethyl Siloxane 5%. Column temperature program: initial temperature 60 °C to start the final temperature of 210° C. The initial 3 °C per minute to be added and then injected into the chamber to a temperature of 280 °C. The carrier gas inlet pressure to the column: helium with a purity of 99/99% of the inlet pressure to the column equal to 5/1 kilogram per square centimeters set.

Gas Chromatography-Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used. The column was same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V. The identity of the oil components was established from

their GC retention indices, relative to C7- C25 n-alkanes, by comparison of their MS spectra with those reported in the literature [37-39], and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results

Result from dry Leaves essential oils is shown in table - 1. Main component from Alborz sample were chavicol 81.41%, methyl acetate 2.89 and sample from Shahr-e-Rey were chavicol 57.69%, linalool 16.43%, and sample from Gorgan city were chavicol 63.51%, and linalool 19.80%.

Preparation of essential oils

Extracted essential oils of *Ocimum basilicum var. purpurascens* was from Shahre-Rey Tehran, Alborz and Gorgan was tested for microbial.

Microbial Tests

To determine the MIC, Micro dilution technique was used [40]. In short, 24-hour culture of micro-organisms on Mueller Hinton agar medium in sterile saline was prepared McFarland bacterial suspension with a turbidity equivalent to half [41]. This suspension has an absorbtion about 0.08-0.1 on wavelength of 625 nm. then, concentration of 0.3125, 0.625, 1.25,2.5 and 10.5 were prepared on 96-well plate using Hilton muller brath (MHB) and Dilution 1 to 150 of the bacterial suspension were added to it. After 24 hour Incubation on 37 °C, concentration of last wells that did not make it turbidity on it, was determined as MIC by definition [41]. To evaluate the MIC (Minimum Bactericidal Concentration), study on solid MHA medium (agar) were performed. 100 µl of three wells before the MIC House were cultured on Muller Hilton for 24 hours and were inoculated on 35 °C. The lowest concentration of essential oils that the bacteria did not grow on it, was reported as MBC. All the above tests were repeated three times and the results were reported.

Table 1 Percentage composition of volatile oils of *Ocimum basilicum var. purpurascens*.

Compounds name	R.I.	Alborz	Shahe ray	Gorgan
α- pinene	942	-	0.19	0.27
β- pinene	975	-	0.64	0.65
α-phellandrene	1005	-	0.30	0.36
Z- β- ocimene	1053	0.17	0.24	0.25
E- β- ocimene	1055	0.26	3.45	1.90
Cis-sabinene yhdrate	1063	1.16	2.02	3.57
Terpinolene	1095	-	-	0.19
Linalool	1111	2.19	16.43	19.80
Cis-pinene hydrate	1121	-	0.31	-
Menthol	1179	0.17	0.80	1.11
Methyl chavicol	1219	-	0.32	0.88
Chavicol	1240	81.41	57.69	63.51
n-decanol	1268	2.20	-	-
Methyl acetate	1294	2.89	-	-
Trans-caryophyllene	1425	0.39	1.24	0.70
β- copaene	1431	1.91	0.57	0.49
Cis-muurola-4,5-diene	1465	1.10	4.16	1.33
Germacrene D	1476	1.64	1.43	0.60
α-bulnesene	1513	0.64	0.89	-
δ – cadinene	1522	0.24	0.33	-
α- cadinene	1534	1.27	1.88	0.84
Elemol	1546	0.32	1.26	0.51
Cis-muurol-5-en-4- α-ol	1559	-	1.40	-
E-nerolidol	1562	1.44	-	-
Trans-methyl dihydro jasmonate	1680	-	0.53	0.30
n-heptadecane	1703	0.51	3.5	2.08
Oplopanone	1731	-	0.32	-

Table 2 Used Microorganisms

#	Microorganism	NO.	Microorganism Kind
1	*Staphylococcus aureus*	PTCC 1431	Gram –Possitive
2	*Bacillus subtilis*	PTCC 1023	Gram –Possitive
3	*Escherichia coli*	PTCC 1330	Gram_ Negative
4	*Pseudomonas aeruginosa*	PTCC 1430	Gram_ Negative

Table 3 MIC & MBC value for dried leaf Essential oils extracted by Clevenger

Dried leaf oil extracted by Clevenge (Shahre-Rey)				
Microorganism	PTCC	Inhibition zone	MIC	MBC
Staphylococcus aureus	1431	20.1±0.2	5	5
Basilus subtilitis	1023	17.5±0.1	5	5
Escherichia coli	1330	10.5±0.8	5	5
Pseudomonas aeruginosa	1430	9.7 ±0.2	5>	5>

Table 4 MIC & MBC value for Essential oils extracted by Clevenger

Dried leaf oil extracted by Clevenger(Alborz Province)				
Microorganism	PTCC	Inhibition Zone	MIC	MBC
Staphylococcus aureus	1431	19.3±0.2	5	5
Basilus subtilitis	1023	25.6±0.1	2.5	5
Escherichia coli	1330	13.1 ±0.8	5	5
Pseudomonas aeruginosa	1430	10.6±0.2	5>	5>

Table 5 MIC & MBC value for dried leaf Essential oils extracted by Clevenger

Dried leaf oil extracted by Clevenger (Gorgan Province)				
Microorganism	PTCC	Inhibition Zone	MIC	MBC
Staphylococcus aureus	1431	20.5 ±0.2	5	5
Basilus subtilitis	1023	26.8 ± 0.1	2.5	5
Escherichia coli	1330	12.3 ± 0.8	5	5
Pseudomonas aeruginosa	1430	9.1±0.2	5>	5>

Method

Microbial strains were prepared from Iran Industrial Standards Institute (PTCC).

Result and Discussion

Essential oils have several chemical groups and they are able to eliminate bacteria with different mechanism. They have entered to cell membrane of mitochondria and lipids and disturb their lives which it can be due to their hydrophobic properties. Research shows oxygenated compounds in oil are able to eliminate bacteria.

Essential oils sample of *Ocimum basilicum var. purpurascens* from Shahrerey Zone, which they show the Positive result with 5% concentration on the tested bacteria and on Gram-Positive bacteria (*Bacillus subtilis*) has a strong result (2.5%) for MIC and (5%) for MBC. Result are shown on table 3. Essential oils of Alborz. Zone extracted by Clevenger. Which shows the same result (Table 4). Essential oils of Gorgan Zone, in this way that inhibited growth zone and Killing Gram-negative and Gram-Positive tested bacteria with 5% concentration shows that there is no difference in essence behavior on microbial strains (Table 5).

The results are logical due to major chemical group of Essential oils which are oxygenated phenolic and alcoholic compounds. Therefore, we can confidently determine the antibacterial activities of the basil essential oil in dry state benefit. According to the potential of *Ocimum basilicum var. purpurascens* extracts to inhibit the test bacteria, it can be suggested that extracted this verity can be applied as antimicrobial agents against the pathogenic bacteria.

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