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Full Length Research Paper

Antimicrobial activity of essential oil extract of *Ocimum basilicum* L. leaves on a variety of pathogenic bacteria

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As in the recent years the usage of the herbal materials has been increased, it seems necessary to study the antibacterial effects of them. The basil herb, which is easily cultured worldwide, may be a potentially good candidate to be used as a plant with antibacterial activity. The essential oil was distilled using a Clevenger-type apparatus and extracted from plant leaves. The antibacterial properties of basil essential oil was studied on the standard gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive ones including *Bacillus cereus*, *Staphylococcus aureus*, then agar disk diffusion, minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were detected. The results of agar disk diffusion tests showed the inhibition zones as follow: *S. aureus* 29.20-30.56 mm, *B. cereus* 10.66-16.11 mm, *E. coli* 17.48-23.58 mm and for *P. aeruginosa* the maximum inhibition zones were seen. The results of this study showed the presence of bacteriostatic effects of basil essential oil on all the test bacteria. The MICs for gram-positive bacteria were as: *B. cereus* ranging 36-18 µg/mL, *S. aureus* 18 µg/mL, and for Gram-negative bacteria of *E. coli* and *P. aeruginosa* were 18-9 µg/mL.

Key words: Basil (*Ocimum basilicum*) essential oil, gram-positive and gram-negative bacteria, antibacterial, minimal inhibition concentration (MIC), minimum bactericidal concentration (MBC).

INTRODUCTION

Basil (*Ocimum basilicum* L.), a member of the *Lamiaceae* family is an annual herb which grows in several regions around the world. The white, rose and sometimes violet labiate flowers are in 6-blossomed, pedicled, almost sessile axillary false whorls. The calyx is bilabiate, and the corolla is 4-lobed. The lower lip is simple; the 4 stamens lie on it. Among more than 65 species of the genus *Ocimum*, basil is the major essential oil crop which is cultivated commercially in many countries (Sajjadi, 2006). Basil has a characteristic odor and sharp taste. The plant probably originated in India, Afghanistan, Pakistan, Northern India and Iran, and now is cultivated worldwide. Traditionally, basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries (Telci et al., 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; 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).The chief constituents include chavicol methyl ether or estragole, linalool and eugenol (Hussain et al., 2008; Omidbaigi et al., 2003).

The studies in the literature suggest linalool as the main active agent responsible for antibacterial activity (Ravid et al., 1997) and other studies suggest this plant

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Table 1. Comparison of the inhibition zones (mm) of different essential oils (E.O.) in different collection stages (F.C.: First cutting; S.C.: Second cutting) on the microorganisms.

Microorganisms	Cutting stage of plant (essential oil of leaf)		
	Tetracycline	F. C.	S. C.
Staphylococcus aurous	23.93	29.20	30.56
Bacillus cereus	20.53	16.06	14.73
Escherichia coli	R	21.60	23.58
Pseudomonas euriginosa	12.64	Max	Max

suitable for using as an antibacterial against corrupting and poisoning microbes of food products (Mazzanti et al.,1998; Politeo et al., 2007).

MATERIALS AND METHODS

Collection and pretreatment of plant materials

Aerial parts of cultivated *O. basilicum* at the beginning of flowering stage were collected twice during summer (23 July and 1 August, 2007), in the distance of 10 cm of the stems from the earth. The plants cultured in no-manure earth. The specimens were dried in cool shadow and in the temperature of the lab room.

Isolation of essential oil

The 50 g of plant material and 250 ml of water have been placed in a Clevenger type apparatus. The essential oil was isolated by hydro distillation for 3 h (Bernath, 1990). The obtained essential oil was stored under argon in a sealed vial, at –20°C before usage.

Test microorganisms

In this study, we investigated the antibacterial properties of basil leaves-only essential oil, on the Gram-positive and negative bacteria, purchased from Iranian Industrial Researches and Standards. *In vitro* antimicrobial studies were carried out on four bacteria strains, the Gram-negative ones include *Escherichia coli* (PTCC 1535), Pseudomonas *aeruginosa* (PTCC 1310), and for Gram-positive ones we used *Bacillus cereus* (PTCC 1015), *Staphylococcus aureus* (PTCC 1112). All strains were grown on nutrient broth (Merck).

Determination of antimicrobial activity

Determination of antimicrobial activity was done according to the standards recommended by CLSI (Clinical and Laboratory Standards Institute), namely, at first the disc diffusion method was done for all isolates, and then the MIC sensitivity method was performed for the cultures with positive results to the essential oils (Ravid et al., 1997; Mazzanti et al., 1998).

Disc diffusion method

The disc diffusion method was used to determine the antimicrobial activities by the disc diffusion method. Fresh cultures of microorganisms that were grown for 24 h were used and diluted 10-1 with sterile physiological saline solution (0.85% NaCl). 100 µl of test microorganism suspension containing 1.5×10^8 CFU/ml of bacteria were inoculated on the surface of Muller Hinton Agar (Merck) plates. The three sterile discs with a diameter of 6 mm were placed onto each agar plate containing microorganisms. Then 30 µl of extracts were dropped onto discs under sterile conditions and were incubated at +37±0.1ºC for 24 h. After incubation, the diameters of inhibition zones were measured in millimetres on all plates. All experiments were repeated three times. Tetracycline (30 µg/disc) (SIGMA) disc was used as positive control. A disc of pure dimethylsulfoxide (DMSO) was used as negative control.

Microdilution assay

The minimal inhibition concentration (MIC) values were also studied for the microorganisms which were determined as sensitive to the extracts in the disk diffusion assay. The inocula of the microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity (Dastouri et al., 2008). The *O. basilicum* essential oil of leaves dissolved in 10% dimethylsulfoxide (DMSO) were first diluted to the highest concentration (600 µg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 9.37 to 600 µg/ml in 10 ml sterile test tubes containing Mueller-Hinton broth. MIC values of *O. basilicum* essential oils against bacterial strains were determined based on a micro-well dilution method with some modifications. The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and microorganism suspension containing 1.5×10^8 CFU/ml of bacteria. 100 µl from *O. basilicum* essential oils initially prepared at the concentration of 600 µg/ml was added into the first wells. Then 100 µl from their serial dilutions was transferred into four consecutive wells. The last well containing 195 µl of Mueller-Hinton broth without compound and 5 µl of the inoculum on each strip was used as negative control. The final volume in each well was 200 µl. The contents of every well were mixed on plate-shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance measurement at 600 nm using the ELx 800 universal micro plate reader (Biotech Instrument Inc., USA) and the results were compared and confirmed by plating 5 µl samples from clear wells on nutrient agar medium. The extract tested in this study was screened two times against each organism and for two essential oils and also for two stages of collections. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms (Adiguzel et al., 2005).

RESULTS

The antibacterial properties of the several essential oils were studied in 24 h. The results suggest that the essential oils of the two collection stages (first and second cutting) have antibacterial activities (Tables 1). The comparison of the inhibition zone due to the essential oils and the control disc (Tetracycline), the

Table 2. Results of MIC of different essential oils (E.O.) in different collection stages (F.C.: first cutting; S.C.: second cutting) on the microorganisms (ND: non-determinate).

Cutting stage	*Staphylococcus aureus*		*Bacillus cereus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
E. O. of Leaf F.C.	18	*	18	*	9	ND	9	ND
E. O. of Leaf S.C.	18	*	36	*	18	ND	18	ND

essential oil of both collection stages showed better results for Gram-negative strains of *Escherichia coli* and *P. aeruginosa*. Among Gram-positive germs, the study revealed better results against *Staphylococcus aureus.*

The MIC results of different essential oils have been tabulated in Table 2. The best MIC effect was related to the Gram negative bacteria (in concentration of 9 µg/ml) for *E. coli* and *P. aeruginosa*, of essential oil extracted from the leaves of the first harvest. The MBC concentration for the latter bacteria was not countable. It was due to the too lower concentration of the extract active against the bacteria than our test settings in comparison to the rest dilutions.

DISCUSSION

Many differences in the papers of the literature including differences of methodologies of evaluating the antimicrobial properties, and also differences in herbal contents and compositions from different geographical regions, makes it difficult and even impossible comparing the studies on the antimicrobial activities of *O. basilicum*. The results of our study showed the better activity of essential oil of *O. basilicum* on Gram-negative germs than Gram-positive ones.

This disagrees with the results of the study done by Prasad et al. (1986) in which the oil extract of *O. basilicum* collected from different geographical regions, had high effectiveness on the Gram-positives in comparison to the Gram-negative ones. Although in the same study, the oil extract of *O. basilicum* had high efficacy on *Salmonella* strains (Prasad et al., 1986; Opalchenovaa and Obreshkova, 2003). In another study, however, Sinha and Gulati (1990) have reported the effectiveness of the *O. basilicum* on *E. coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella boydi*, *Proteus vulgaris*, and *S. aureus*. The results of our study revealed that the differences in the plant compounds and the extracting methods may affect the antimicrobial activities. Some studies also confirm our suggestion. As the essential oils were different (from two cutting stages), it is predictable that their antimicrobial properties will be different. It is due to the physiological differences of the different stages of its growth; hence this affects the composition and extraction content of the final essential oil.

Additionally, the environmental factors, including day length, light intensity and ambient temperature influence on the quantity and quality of the essential oil content and eventually will affect the medicinal properties of the plants. It has been shown that in the family *Lamiaceae,* the day length increases the content of their extractions, and the duration of day light augments the quality of their active materials.

The essential oil used in this study is related to the plants, which were cut in two stages, including 23 July for the first cutting and 1 August for the second cutting. As the day length and light intensity and also the mean monthly temperature of the first cutting stage is more than the second cutting period, the extract percentage and the quality of active materials were better than the second stage. The result revealed that, the antimicrobial activity of the essential oil from the first cutting is more significant than the second one. On the other hand, the quantity of the active materials of only-leaves extraction was more that the whole-plant part, which was account for the higher efficiency of the extract in inhibiting the growth of the bacteria.

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