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Short Communication

Comparison between Two Groups of Pathogenic Bacteria under Different Essential Oil Extract of *Ocimum basilicum* L.

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Abstract

This study was conducted to assessment the antibacterial activities of different part of basil essential oil on the standard gram-negative bacteria include *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and gram-positive ones including *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogen*. The basil essential oil was provided from two part of the plant (leaf and herb) at the two different developmental stages. The antibacterial properties of basil essential oil was studied on the standard bacteria by agar disk diffusion, then minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were detected. The results of agar disk diffusion tests showed the inhibition zones as follow: *Listeria monocytogen* 17.11-17.42 mm, *St. aureus* 29.20-30.56 mm, *B. cereus* 14.73-16.06 mm, *E. coli* 21.60-23.58 mm, *Salmonella typhi* 21.63-24.80 mm and for *P. aeruginosa* the maximum inhibition zones were observed on leaf essential oil. From the herb part of basil almost similar results were obtained: *Listeria monocytogen 17.02-17.67 mm*, *St. aureus* 29.60-30.41 mm, *B. cereus* 10.66-16.11 mm, *E. coli* 17.48-23.54 mm, *Salmonella typhi* 21.58-21.64 mm and for *P. aeruginosa* the maximum inhibition zones were observed. The MICs for gram-positive bacteria were as: *B. cereus* ranging 36-18 µg/mL, *S. aureus* 18 µg/mL, *Listeria monocytogen* 18-36 µg/mL and for gram-negative bacteria of *E. coli*, *Salmonella typhi* and *P. aeruginosa* were 18-9 µg/mL. Some of basil oil component increased by water stress. Amount of the main constituents of the oil such as linalool, methyl chavicol, 1,8–cineole and trans α-bergamotene significantly affected by water stress.

Keywords: Basil (*Ocimum basilicum*) essential oil, Gram-positive and gram negative bacteria, Antibacterial activity, MIC, MBC

Introduction

Basil called sweet basil in India (*Ocimum basilicum* L.) belonging to the plant family Lamiaceae. It is an annual plant usually producing white-purple flowers [1,2]. The main constituents include chavicol methyl ether, linalool and eugenol [3-5]. The antimicrobial activity of basil and they found to be high for *Staphylococcus aureus*, moderate for *Escherichia coli*, low for Bacillus, and *Pseudomonas aeruginosa* was highly tolerant investigated by Pandu Sastry, *et al.* [6]. The studies in the literature suggest linalool as the main active agent responsible for antibacterial activity [7] and other studies [8] suggest this plant can be suitable for using as an antibacterial against corrupting and poisoning microbes of food products [9]. Another researches showed that, the essential oils of *Ocimum basilicum* L., *Ocimum kilimandscharicum* Gürke, and *Ocimum gratissimum* L. strongly inhibit the growth of all the studied microorganisms, especially the gram-negative strains [10]. The leaves and flowering tops of the plant are perceived as carminative, galactogogue, stomachic and

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antispasmodic in folk medicine [11]. Traditionally, basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries [12]. However, the potential uses of *O. basilicum* essential oil, particularly as antimicrobial and antioxidant agents have also been investigated [5-17].

Material and Methods

For pretreatment of the plant materials, aerial parts (leaf and herb separately) of cultivated *O. basilicum* at the beginning of flowering stage were collected twice during summer. The plant materials were placed in a Clevenger type apparatus. The essential oil was isolated by hydro distillation for 3 h [18].

Test microorganisms

The susceptibilities of the isolates to 16 antimicrobial agents were on standard the gram-positive and negative bacteria. In vitro antimicrobial studies were carried out on four bacteria strains, the gram-negative ones include Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa, and for gram-positive ones, and Listeria monocytogen, Bacillus cereus and Staphylococcus aureus. All strains were grown on nutrient broth (Merck).

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 [5-9,14,15].

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 1.5x108 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 º ± 0.1 ºC for 24 h. After incubation, the diameters of inhibition zones were measured in millimeters on all plates. Experimental design was factorial based on completely randomized design (CRD) with triple replicate. Tetracycline (30 µg/disc) (SIGMA) disc was used as positive control. A disc of pure dimethyl sulfoxide (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 [19]. MIC values of *O. basilicum* essential oils against bacterial strains were determined based on a micro-well dilution method with some modifications. The 144-well plates were prepared by dispensing into each well 95 µL of nutrient broth and microorganism suspension containing $1.5x10^8$ CFU/mL of bacteria. A 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 [20].

Result and Discussion

The antibacterial properties of the several essential oils were studied in 24 h. Analysis of variance was presented in Table 1**.** The results showed that the essential oils of the two collection stages and two part of basil (first and second cutting, leaf and herb) have antibacterial activities (See Table 2 and Table 3). The comparison of the inhibition zone due to the essential oils and the control disc

(Tetracycline), showed better results for gram-negative strains of *E. coli* and *P. aeruginosa*. Among gram-positive germs, the study revealed better results against *S. aureus.* The MIC results of different essential oils have been tabulated in Table 3.

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. In the herb of plant and two harvest time (first and second time), MIC effect were same results (in concentration of 18 µg/ml).

Table 1 Variance analysis of various inhibition zones (mm) of essential oils in different collection stages and two parts of plant on the microorganisms

Source of Variation	Sum of Squares	df	Mean Square
Type of Bacteria (A)	4203.310	4	1050.828**
Type of Essence (B)	28874.085	5	5774.817**
Interaction (A,B)	9501.058	20	475.053**
Error	279.008	60	4.650

**: Significant at 1% probability levels

Table 2 The comparison of the inhibition zones (mm) of essential oils in different collection stages and two parts of plant on the microorganisms

Bacteria (Microorganism)		Plant Cutting Time			
		Leaf Essential Oil		Herb Essential Oil	
	Tetracycline	First Cutting	Second Cutting	First Cutting	Second Cutting
Gram + *Listeria monocytogen*	26.73±2.62	17.11	17.42	17.02	17.68
Staphylococcus aurous	23.93±0.91	29.20	30.56	29.60	30.41
Bacillus cereus	20.53±5.01	16.06	14.73	16.11	10.66
Gram - *Escherichia coli*	R	21.60	23.58	23.54	17.48
Salmonella typhi	R	24.80	21.63	21.64	21.58
Pseudomonas euriginosa	12.64±2.28	Max	Max	Max	Max

Table 3 Mic of essential oils in different collection stages and two parts of plant on the microorganisms

Harvest Stage And Part Of Plant		Gram Positive						Gram Negative					
		Listeria monocytogenes		*Staphylococcus aureus*		*Bacillus Cereus*		*Escherichia Coli*		*Salmonella Typhi*		*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	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL
Leaf Essential Oil	First Cutting	36	*	18	*	18	*	9	ND	9	ND	9	ND
Leaf Essential Oil	Second Cutting	18	*	18	*	36	*	18	ND	18	ND	18	ND
Herb Essential Oil	First Cutting	18	*	18	ND	18	*	18	*	18	ND	18	ND
Herb Essential Oil	Second Cutting	18	*	18	*	18	*	18	ND	18	ND	18	ND

ND: Non-determinate, *: can't determine

Table 4 compare the average type of Bacteria is given in the appendix but given the lack of need for it, not in the original article. (Table 4 Comparison means by Duncan test)

Bacteria type	Groups		
	1	2	3
Bacillus cereus	16.04 a	-	-
Escherichia coil	17.25 ab	17.25	-
Salmonella typhi	-	17.78 bc	17.78
Listeria monocytogen	-	-	19.16 c
Staphylococcus aurous	-	-	-
Pseudomonas euriginosa	-	-	-
Sig.	0.13	0.50	0.08

a. Uses Harmonic Mean Sample Size =15
b. Alpha=0.05

The MBC concentration for the latter bacteria was not countable. 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 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 [6,8,14,15] In another study, however, have reported the effectiveness of the *O. basilicum* on *E. coli, S. typhi, S. paratyphi, Shigella boydi*, *Proteus vulgaris*, and *Staphylococcus aureus* [16].

The results of our study revealed that the differences of 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 and two parts of plant), 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.

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