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Antimicrobial Effects of *Ocimum basilicum* (Labiatae) Extract

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Abstract: Ethanol, methanol, and hexane extracts from *Ocimum basilicum* Labiatae (sweet basil) were investigated for their invitro antimicrobial properties. A total of 146 microbial organisms belonging to 55 bacteria, and four fungi, and a yeast species were studied using a disk-diffusion and minimal inhibition concentration (MIC) method. The result showed that none of the three extracts tested have antifungal activities, but anticandidal and antibacterial effects. Both the hexane and methanol extracts, but not the ethanol extracts, inhibited three isolates out of 23 strains of *Candida albicans* studied. All three extract of *O. basilicum* were different in terms of their antibacterial activities. The hexane extract showed a stronger and broader spectrum of antibacterial activity, followed by the methanol and ethanol extracts, which inhibited 10, 9 and 6% of the 146 bacterial strains tested, respectively. The minimal inhibition zones (MIC) of the hexane, methanol, and ethanol extracts ranged from 125 to 250 µl/ml, 62.50 to 500 µl/ml, and 125 to 250 µl/ml, respectively.

Key Words: *Ocimum basilicum* Labiatae., antimicrobial activity, medicinal plant

Ocimum basilicum Labiatae Ekstraktının Antimikrobiyal Etkisi

Özet: *Ocimum basilicum* Labiatae (sweet basil)'un etanol, metanol ve hegzan ekstraktlarının antimikrobiyal özelliği invitro koşullarda araştırıldı. 55 bakteri, 4 fungus ve bir mayadan oluşan toplam 146 mikroorganizma, disk difüzyon ve minimal inhibisyon konsantrasyonu (MIC) yöntemleri kullanılarak çalışıldı. Sonuçlar, test edilen 3 ekstrakttan hiçbirinin antifungal aktivite göstermediğini fakat antikandidal ve antibakteriyal etkiye sahip olduğunu ortaya koydu. Hem hegzan hem de metanol ekstraktları 23 *Candida albicans* türünden 3 tanesine karşı inhibisyon etkisi gösterirken, etanol ekstraktı göstermedi. *O. basilicum*'un 3 ekstraktı da farklı oranlarda antibakteriyal etki gösterdi. Hegzan ekstraktı, metanol ve etanole ekstraktlarına göre daha güçlü ve geniş oranda antimikrobial etkiye sahip olup, test edilen 146 bakteri strainine karşı sırasıyla %10, 9, 6 oranlarında inhibisyon etkisi gösterdi. Hegzan, metanol ve etanol ekstraktlarının minimal inhibisyon konsantrasyon (MIC) oranları sırasıyla, 125-250 µl/ml, 62.50-500 µl/ml ve 125-250 µl/ml'dir

Anahtar Sözcükler: *Ocimum basilicum* Labiatae, antimikrobial aktivite, tıbbi bitki

Introduction

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (1,2). The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80 % of the worlds population (3). Turkish people have a tradition of using a number of plant species for the treatment of infectious diseases and various ailments (4).

Sweet basil (*O. basilicum* L.) is known locally as "fesleğen or reyhan", is native to Asia and is widely grown as an ornamental or field crops from seed in the Mediterranean countries, including Turkey (5,6). Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic, and tonic agents (7-9). They have been also used as a folk remedy to treat various ailments such as; feverish illnesses, poor digestion, nausea, abdominal cramps, gastro-enteritis, migraine, insomnia, depression, gonorrhea, dysentery, and chronic diarrhea exhaustion (10). Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites, and skin infections (11). However, *O . basilicum*, like many other *Ocimum* species, has not been investigated very well in terms of antimicrobial activities. In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (12-14). In addition to this problem, antibiotics are sometimes associated with adverse effects

on the host which includes hypersensitivity, immunosuppression, and allergic reactions (15). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources, including medicinal plants (16,17).

The purpose of this study was to evaluate the potential antimicrobial activities of the ethanol, methanol, and hexane extracts of *O . basilicum* plants.

Material and Methods

Plant materials and extraction procedure

Aerial parts (leaves, flowers and stems) of wild sweet basil (*Ocimum basilicum* L., Labiatae) plants were collected from Balıkesir in the Aeagean region of Turkey. The taxonomic identification of plant materials was confirmed by Meryem Şengül in the Department of Biology, Atatürk University, Erzurum, Turkey. Collected plant material was dried in the shade and ground in a grinder with a 2 mm diameter mesh. The dried and powdered plant materials (500 g) were extracted successively with 1 L of ethanol, methanol and hexane by using Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent (18). The aqueous extracts were filtered using Whatman filter paper (No. 1) and then concentrated *in vacuo* at 40 °C using a Rotary evaporator. The residues obtained were stored in a freezer at -80 °C until further tests.

Microorganisms

A total 146 microbial cultures belonging to 55 bacteria, one yeast, and four fungi species, were used in this study. The list of microorganisms used is given in Tables 1 and 2. Microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine; and the Plant Diagnostic Laboratory, Faculty of Agriculture at Ataturk University, Erzurum, Turkey. Identity of the microorganisms used in this study was confirmed by Microbial Identification System in the Biotechnology Application and Research Center at Ataturk University.

Antimicrobial activity

The dried plant extracts were dissolved in the same solvent (hexane, ethanol and methanol) to a final concentration of 30 mg/ml and sterilized by filtration by 0.45 µm milipore filters. Antimicrobial tests were then carried out by disk diffusion method (19) using 100 µl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast and 10^4 spore/ml of fungi spread on nutrient agar (NA), sabourand dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The disks (6 mm in diameter) were impregnated with 10µl of the extracts (300 µg/disk) at the concentration of 30 mg/ml and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Penicillin-G (10 µg/disk) was used as a positive reference standard to determine the sensitivity of one strain from each bacterial species. The inoculated plates were incubated at 30 ºC for 24 h for bacterial strains, 48 h for yeast and 72h for fungi isolates. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

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. The *O. basilicum* extracts 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 nutrient broth. MIC values of *O. basilicum* extracts against bacterial strains and *C. albicans* isolates were determined based on a micro-well dilution method (20-23) with some modifications. The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and 5 µl of the inoculum. A 100 µl from *O. basilicum* extracts 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 six consecutive wells. The last well containing 195 µl of nutrient 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. Maxipime (Bristol-Myers Squibb) at the concentration range of 500–7.8 µg/ml was prepared in nutrient broth and used as standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each 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 at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, Vermont, USA) 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. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

Results and Discussion

The antimicrobial activities of *O. basilicum* (ethanol, methanol, and hexane) extracts against the microorganisms examined in the present study, and their potency, were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter, and MIC values. The results are given in Tables 1-3.

Table 1. Antimicrobial activity of *Ocimum basilicum* extracts (300 µg/disk) against the bacterial strains tested based on disk diffusion method.

Microorganisms	Number of Strains/ origins	Inhibition zone in diameter (mm) around test disk			
		Ocimum basilicum extracts (300 µg/disk)			Positive Control (Disk) (10 µg/disk)
		Hexane	Ethanol	Methanol	
Bacteria					
Acinetobacter baumanii	3/Clinic	-	-	-	-
Acinetobacter calcoaceticus	2/Clinic	11 mm/1	16 mm/1	17 mm/1	16 mm ([a]OFX)
Acinetobacter lwoffii	1/Clinic	-	-	-	27 mm (OFX)
Acinetobacter johnsonii	1/Clinic	-	-	-	25 mm (OFX)
	1/Plant	-	-	-	32 mm (OFX)
Alcaligenes pacificus	1/Plant	-	-	-	
Alcaligenes xylosoxydans	2/Clinic	-	-	-	12 mm ([b]SCF)
Agrobacterium radiobacter	1/Plant	-	-	-	28 mm (SCF)
Bacillus amyloliquefaciens	1/Plant	7 mm/1	-	-	27 mm (SCF)
Bacillus atrophaeus	2/Plant	-	-	-	21 mm (SCF)
Bacillus cereus	2/Clinic	-	-	-	30 mm (OFX)
Bacillus licheniformis	1/Plant	-	-	-	[d]NT mm (SCF)
Bacillus macerans	1/Plant	11 mm/1	8 mm/1	7 mm/1	26 mm (SCF)
Bacillus megaterium	4/Plant	8 mm/1	-	7 mm/1	9 mm (SCF)
Bacillus pumilus	2/Plant	-	-	-	23 mm (OFX)
Bacillus sphaericus	1/Plant	-	-	-	18 mm (OFX)
Bacillus substilis	4/Plant	-	-	-	28 mm (OFX)
Brevundimonas diminuta	1/Clinic	-	-	-	34 mm (SCF)
Brucella abortus	15/Clinic	10 mm/1	-	-	12 mm (SCF)
Brucella melitensis	11/Clinic	-	-	-	18 mm (OFX)
Burkholdria cepacia	1/Clinic	-	-	-	--- mm (SCF)
Burkholdria gladioli	1/Plant	-	-	-	22 mm ([c]NET)
Citrobacter freundii	1Clinic	-	-	-	23 mm (SCF)
Clavibacter michiganense	1/Plant	-	-	-	25 mm (SCF)
Curtobacterium flaccumfaciens	2/Plant	-	-	-	37 mm (OFX)
Enterobacter aerogenes	1/Clinic	-	-	-	12 mm (OFX)
Enterobacter cloacae	2/Clinic	-	-	-	10 mm (OFX)
Enterobacter intermedius	2/Plant	-	-	-	27 mm (SCF)
Enterobacter pyrinus	1/Clinic	-	-	-	NT mm (-)
Erwinia amylovora	1/Plant	-	-	-	12 mm (OFX)
Erwinia carotovora	1/Plant	-	-	-	30 mm (OFX)

Table 1. (Continued).

Microorganisms	Number of Strains/ origins	Inhibition zone in diameter (mm) around test disk			
		Ocimum basilicum extracts (300 μg/disk)			Positive Control (Disk) (10 μg/disk)
		Hexane	Ethanol	Methanol	
Erwinia chrysanthemi	1/Plant	-	-	-	25 mm (SCF)
Escherichia coli	7/Clinic	7-9 mm/2	13-14 mm/2	11-12 mm/2	- mm (OFX)
Flavobacterium blastinum	1/Plant	-	-	-	32 mm (OFX)
Klebsiella pneumoniae	2/Clinic	-	-	-	12 mm (OFX)
Klebsiella trevisanii	1/Clinic	-	-	-	23 mm (OFX)
Kocuria varians	1/Clinic	-	-	-	18 mm (OFX)
Leclercia adecarboxylata	1/Clinic	-	-	-	NT (-)
Micrococcus luteus	1/Clinic	-	-	7 mm/1	- mm (OFX)
Neisseria spp	1/Clinic	-	-	-	- mm (OFX)
Pantoea agglomerans	3/Plant	-	-	-	24 mm (NET)
Plesiomonas shigelloides	1/Clinic	-	-	-	- mm (SCF)
Proteus vulgaris	1/Clinic	-	-	-	12 mm (OFX)
Pseudomonas aeruginosa	15/Clinic	-	-	-	22 mm (NET)
	5/Plant	-	-	-	
Pseudomonas fluorescens	4/Clinic	-	-	-	30 mm (OFX)
	1/Plant	-	-	-	
Pseudomonas huttiensis	2/Plant	-	-	-	10 mm (OFX)
Pseudomonas putida	1/Plant	8 mm/1	-	-	24 mm (SCF)
Pseudomonas syringae pvs.	5/Plant	-	-	-	24 mm (OFX)
Ralstonia pickettii	1/Plant	-	-	-	25 mm (OFX)
Salmonella typhimurium	1/Clinic	-	-	-	27 mm (SCF)
Staphylococcus aureus	5/Clinic	8 mm/3	8 mm/3	9 mm/3	22 mm (SCF)
Staphylococcus epidermis	5/Clinic	9 mm/2	10 mm/2	12 mm/2	NT mm
Serratia spp.	1/Clinic	-	-	-	27 mm (SCF)
Streptococcus pneumoniae	2/Clinic	-	-	-	- mm (OFX)
Streptococcus pyogenes	2/Clinic	-	-	-	10 mm (OFX)
Xanthomonas campestris pvs	4/Plant	-	-	-	20 mm (SCF)
Total 55 bacterial species	146	7-11 mm/13 strains	8-16 mm/9 strains	7-17 mm/11 strains	

[a]OFX=Ofloxacin (10 μg/disk); [b]SCF=sulbactam (30 μg)+cefoperazona (75μg) (105 μg/disk); [c]NET=Netilmicin (30 μg/disk) were used as positive reference standards antibiotic disks (Oxoid); [d]NT=Not tested.

The results showed that ethanol extract of *O. basilicum* has an antimicrobial effect against nine strains in the genera *Acinetobacter*, *Bacillus*, *Escherichia*, and *Staphylococcus*. On the other hand, the methanol and hexane extracts of *O. basilicum* showed antibacterial activities against 11 and 13 strains in six bacterial genera including *Acinetobacter*, *Bacillus*, *Brucella*, *Escherichia*, *Micrococcus*, and *Staphylococcus*, and anticandidal effects against three isolates of *C. albicans,* respectively (Tables 1 and 2). The maximal inhibition zones and MIC values for the bacterial strains and *C. albicans* isolates to *O. basilicum* hexane and methanol extracts were in the range of 7-11 mm and 125-250 μl/ml; and 7-17 mm and 62.50-500 μl/ml, respectively (Tables 1-3). In the case of the ethanol extract, the maximal inhibition zones and MIC values of the bacterial strains sensitive to the hexane extract were 8-16 mm and 125-250 μl/ml, respectively (Tables 1 and 3).

Based on these results, the hexane extract has a stronger and broader spectrum of antimicrobial activities compared with the ethanol and methanol extracts. This result did not confirm the previous studies which reported that hexane was a better solvent for the more consistent extraction of antimicrobial substances from

Table 2. Antimicrobial activity of *Ocimum basilicum* extracts (300 µg/disk) against yeast and fungi isolates tested based on disk diffusion method.

Microorganisms	Number of Strains/ origins	Inhibition zone in diameter (mm) around test disk			
		Ocimum basilicum extracts (300 µg/disk)			Positive Control (Disk) (10 µg/disk)
		Hexane	Ethanol	Methanol	
Yeast					
Candida albicans	23/Clinic	8-9 mm/3	-	9-11mm/3	- mm (NET)
Fungi					
Alternaria alternata	2/Plant	-	-	-	- mm (NET)
Aspergillus flavus	2/Clinic	-	-	-	- mm (NET)
Fusarium oxysporum	2/Plant	-	-	-	- mm (NET)
Penicillium spp.	2/Clinic	-	-	-	- mm (NET)
Total	31 isolates	8-9 mm/3 isolates		9-11 mm/ 3 isolates	

[a]OFX=Ofloxacin (10 µg/disk); [b]SCF=sulbactam (30 µg)+cefoperazona (75µg) (105 µg/disk); [c]NET=Netilmicin (30 µg/disk) were used as positive reference standards antibiotic disks (Oxoid); [d]NT=Not tested.

Table 3. The MIC values of *Ocimum basilicum* extracts against the microorganisms tested in microdilution assay (MIC in µg /ml).

Microorganisms	Number of Strains/ Origins	*Ocimum basilicum* extracts			
		Hexane	Ethanol	Methanol	Standard Drug (Maxipime)
Acinetobacter calcoaceticus	2/Clinic	250	125	62.50	250
Bacillus amyloliquefaciens	1/Plant	125	-	-	62.50
Bacillus macerans	1/Plant	250	125	125	250
Bacillus megaterium	4/Plant	250	-	62.50	125
Brucella abortus	15/Clinic	250	-	-	250
Escherichia coli	7/Clinic	250	125	250	125
Micrococcus luteus	1/Clinic	-	-	500	250
Pseudomonas putida	1/Plant	250	-	-	250
Staphylococcus aureus	5/Clinic	250	250	125	15.625
Staphylococcus epidermis	5/Clinic	250	125	125	62.50
Candida albicans	23/Clinic	250	-	250	500

medicinal plants compared to other solvents such as water, methanol, and ethanol (15, 17, 24). This conflict can be explained that the better extraction of antimicrobial compounds from various medicinal plants may require different solvents.

Conclusions

The results of the present study may suggest that *O. basilicum* extracts possesses compounds with antimicrobial properties against *C. albicans* and some bacterial pathogens.

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