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Short communication

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AN INVESTIGATION ON ANTIMICROBIAL ACTIVITY OF ENDEMIC *ORIGANUM SOLYMICUM* AND *ORIGANUM BILGERI* FROM TURKEY

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Abstract

The methanol extracts obtained from endemic *Origanum solymicum* P. H. Davis and *Origanum bilgeri* P. H. Davis have been investigated for their antimicrobial activity. Antimicrobial activity was determined *Escherichia coli* ATCC 11230, *Stapylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403 and *Kluyveromyces fragilis* ATCC 8608 by disc diffusion method. The plant extracts demonstrated antimicrobial effect against bacteria and the yeast cultures used in this study at different levels.

Key words: Antimicrobial activity, *Origanum solymicum, Origanum bilgeri*

Introduction

Traditional cultures, without the benefits of modern research, somehow understood that culinary spices and herbs added more to food than flavour. They knew certain spices and herbs were important for health and longevity. Today, science has identified the unique compounds responsible for these benefits.

The name *Oreganum* is the contraction of two Greek words, *oros* meaning mountain and *ganos* meaning joy (Grieve, 1982). The common spice oregano (*Origanum vulgare*) was used extensively by the Greeks for conditions ranging from convulsions to heart failure. Nineteenth-century American Eclectic physicians (doctors who recommended herbal medicines) employed oregano as both a general tonic and to promote menstruation (Castleman, 1991). The genus *Origanum* (Labiatae) is represented in Turkey by 22 species or 32 taxa, 21 being endemic to Turkey. Out of 52 known taxa of *Origanum*, 60 % are recorded to grow in Turkey. This high rate is suggestive that the gene centre of *Origanum* is in Turkey (Baser, 2002).

Origanum solymicum P.H.Davis and *Origanum bilgeri* P.H. Davis are endemic to Turkey (Davis, 1978). Although there are many investigation on *Origanum* species (Stiles et al., 1995; Hammer et al., 1999; Salgueiro et al., 2003, Kokkini et al., 2004; Novak et al., 2004; Tepe et al., 2004), these plants have not been previously investigated. Therefore, our aim was

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to determine the antimicrobial effects of plant extracts obtained from these endemic species against microorganisms.

Materials and Methods

Plant Materials

Aerial parts of *Origanum solymicum* P.H.Davis and *Origanum bilgeri* P.H.Davis were collected from different localities in Turkey during the months of September-October of 2004. Voucher specimen (voucher number BD212 and BD213, respectively) of the plants was deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale-Turkey and identified by Dr. Emin Ugurlu.

Preparation of Extracts

The plant parts were air-dried. Each dry powdered plant material (20 g) was extracted with 150 ml 80% methanol (Merck, Darmstadt) for 24 h by using Soxhlet equipment (Khan et al., 1988). The extract was filtered using Whatman filter paper no.1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55 °C. Dried extracts were stored in labeled sterile screw capped bottles at – 20 °C.

Microorganisms

Escherichia coli ATCC 11230, *Stapylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403 and *Kluyveromyces fragilis* ATCC 8608 were used as test microorganisms. They were obtained from culture collection of Ege University, Faculty of Science, Basic and Industrial Microbiology Department.

Screening for antimicrobial activities

The dried plant extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 200 mg/ml and sterilized by filtration through a 0.45 µm membrane filter. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Schull No. 2668, Dassel, Germany) were each impregnated with 50 µL of extract (10 mg/disc) at a concentration of 200 mg/ml. All the bacteria mentioned above were incubated at 35±0.1 °C for 24 h by inoculation into Nutrient Broth (Difco Laboratories, Michigan, USA), and the yeast cultures studied were incubated in Malt Extract Broth (Difco Laboratories, Michigan, USA) at 25±0.1 °C for 48 h. An inoculum containing 10^6 bacterial cells or 10^8 yeast cells/ml was spread on Mueller-Hinton Agar (Oxoid Ltd., Hampshire, England) plates (1 ml inoculum/plate). The discs injected with extracts were placed on the inoculated agar by pressing slightly. Petri dishes were placed at 4 °C for 2 h, plaques injected with the yeast cultures were incubated at 25±0.1 °C and bacteria were incubated at 35±0.1 °C for 24 h (Collins et al., 1989; Ali-Stayeh et al., 1998). At the end of the period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in triplicate. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganisms for comparing.

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Result and Discussion

Table 1 shows antimicrobial activities of the plant extracts. Besides, the inhibition zones formed by standard antibiotic discs are indicated in table 2.

As can clearly seen from Table 1, the extracts obtained from *Origanum* L. were found to be effective against all tested microorganisms used in this study in different level, showing inhibition zones of 11.2-25.6 mm. Besides, the extracts of *Origanum* L. species have an antiyeast activity against all tested yeast cultures. Notably, both *Origanum* extract have strong antibacterial effect against *Bacillus cereus*. *Staphylococcus aureus* is more susceptible to the extracts of all of the plant extracts, as compared to standard antibiotics except for OFX5 and TE 30. Similarly, the extract of the plants have strong antimicrobial effects against *Escherichia coli* and *Klebsiella pneumoniae*, as compared to the standard antibacterial antibiotics SAM20 and CTX30. While the extracts obtained from *O. bilgeri* have

Table 1:. Survey of antimicrobial activity of studied plants

	Microorganisms / inhibition zone (mm)*											
Plant species	1	2	3	4	5	6	7	8	9	10	11	12
Origanum solymicum	16.8	16.2	16.4	11.2	15.8	25.6	-	13.8	16.4	19.6	18.8	20.6
Origanum bilgeri	17.2	20.4	15.6	11.8	17.2	22.8	13.6	12.4	13.8	20.2	22.4	21.8
Methanol (control)	-	-	-	-	-	-	-	-	-	-	-	-

1 : *Escherichia coli*, 2 : *Staphylococcus aureus*, 3 : *Klebsiella pneumoniae*, 4 : *Pseudomonas aeruginosa*, 5 : *Proteus vulgaris*, 6 : *Bacillus cereus*, 7 : *Mycobacterium smegmatis*, 8 : *Listeria monocytogenes*, 9 : *Micrococcus luteus*, 10 : *Candida albicans*, 11 : *Kluyveromyces fragilis*, 12 : *Rhodotorula rubra*

* includes diameter of disc (6 mm)

Table 2: Antimicrobial activities of some standard antibiotics

	Inhibition zone (mm)						
Microorganisms	P10	SAM0	CTX30	VA30	OFX5	TE30	NY100
Escherichia coli	18	12	10	22	30	28	-
Staphylococcus aureus	13	16	12	13	24	26	-
Klebsiella pneumoniae	18	14	13	22	28	30	-
Pseudomonas aeruginosa	8	10	54	10	44	34	-
Proteus vulgaris	10	16	18	20	28	26	-
Bacillus cereus	14	12	14	18	30	25	-
Mycobacterium smegmatis	15	21	11	20	32	24	-
Listeria monocytogenes	10	12	16	26	30	28	-
Micrococcus luteus	36	32	32	34	28	22	-
Candida albicans	-	-	-	-	-	-	20
Kluyveromyces fragilis	-	-	-	-	-	-	18
Rhodotorula rubra	-	-	-	-	-	-	18

P10 : Penicillin G (10 Units), SAM20 : Ampicillin 10 µg, CTX30 : Cefotaxime 30 µg, V30 : Vancomycin 30 µg, OFX 5 : Oflaxacin 5 µg, TE30 : Tetracyclin 30 µg, NY100 : Nystatin 100 µg

antimicrobial activity against the acid-fast bacterium *Mycobacterium smegmatis*, the other plant has no antimicrobial affect against this bacterium. When the results were compared to those standard antibiotics, it was determined that *Micrococcus luteus* is more resistant. On the

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other hand, in comparison to some standard antibiotics, *Listeria monocytogenes* and *Proteus vulgaris* is more susceptible to the extracts. It was found that extracts of *Origanum* L. species have higher antiyeast effects than those of the standard antifungal antibiotics against the yeast cultures. The extracts obtained from *O. bilgeri* have more antifungal effects than the other plant.

Several studies have been conducted on the antimicrobial properties of herbs, spices and their derivates such as essential oils, extracts and decoctions (Kivanc & Akgül, 1986; Dorman & Deans, 2000; Hsieh et al., 2001; Ozcan & Erkmen, 2001). Some researches reported that there is a relationship between the chemical structures of the most abundant compounds in the tested extracts or essential oils and the antimicrobial activity (Farag et al., 1989; Deans & Svoboda, 1990). According to literature, antifungal properties of *Origanum* oil were examined both in vitro and in vivo. Using *Candida albicans* in broth cultures and a micro dilution method, comparative efficacy of origanum oil, carvacrol, nystatin and amphotericin B were examined in vitro. *Origanum* oil at 0.25 mg/ml was found to completely inhibit the growth of *C. albicans* in culture. Growth inhibitions of 75% and >50% were observed at 0.125 mg/ml and 0.0625 mg/ml level, respectively. In addition, both the germination and the mycelial growth of *C. albicans* were found to be inhibited by *Origanum* oil and carvacrol in a dose-dependent manner (Manohar et al., 2001). It was reported that *Origanum bilgeri* contains carvacrol and *O. solymicum* has p-Cymene (Baser, 2002). The antimicrobial activities of these plant extracts may possibly be due to the presence of carvacrol and p-Cymene. It was found that extracts of *Origanum* L. species have higher antiyeast effects than those of the standard antifungal antibiotics against the yeast cultures especially *Candida albicans*. Our results are similar to those reported in the mentioned study.

Our result suggest that the use of these plants as antimicrobial agents may be exploitable to prevent the deterioration of stored foods by bacteria and yeasts, as long as the taste impact is acceptable in the targeted foods.

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