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The antibacterial activity of essential oil of oregano (*Origanum vulgare* L.)

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Abstract

Antibacterial activities of the essential oil of oregano (*Origanum vulgare* L.) and ampicillin on *Escherichia coli* RSKK 340, *Klebsiella pneumoniae* RSKK 06017, *Pseudomonas aeruginosa* RSKK 06021, *Salmonella enteritidis* RSKK 96046, *Streptococcus pyogones* RSKK 413/214, *Bacillus cereus* RSKK 1122, *Staphylococcus aureus* RSKK 96090 and methicilline-resistant *Staphylococcus aureus* (MRSA) were determined. The minimum inhibitory concentration (MIC) for oregano oil against various bacteria varied: while *M.luteus* (16 µg/ml) and *B.cereus* (32 µg/ml) were susceptible to oregano oil, the others exhibited partly resistance. Except MRSA *S. aureus* (250 µg/ml), ampicillin had more effect on *K. pneumoniae* (128 µg/ml) and *C. albicans* (128 µg/ml) than oregano oil, but very low concentrations of the essential oil were sufficient to prevent microbial growth. Of the bacteria tested, *Micrococcus luteus* and *Bacillus cereus* proved to be most susceptible to oregano oil. Oregano oil possessed strong antimicrobial activity compared with the antibiotic. Gram-positive bacteria were more sensitive to the antimicrobial agent in spice than Gram-negative ones.

Key words: Antibacterial effect, oregano, *Origanum vulgare* L., essential oil, antibiotic.

Introduction

The antibacterial activities of spices and their essential oils have been known for a long time, and a number of researches on the antibacterial effect of spices, essential oils and their derivatives have been reported. Plant essential oils are the potentially useful source of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microorganisms, including foodborne pathogens [1-6]. Essential oils are natural products extracted from vegetal materials. They are used as natural additives in many foods due to their antibacterial, antifungal, antioxidant and anti-carcinogenic properties [7, 8]. Screening of natural medicinal plants is now being popular because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind [9].

The use of natural antimicrobial compounds is important not only for the preservation of food but also for the control of human and plant diseases of microbial origin [10]. Recently, there has been considerable emphasis on studies involving essential oils and extracts of spices and their constituents for inhibiting the growth of pathogen microorganisms. It has also been known for some time that certain crude drugs and spices contain substances with antifungal effect in their derivatives [11-13]. There are a few reports on the antimicrobial activity of essential oils or their major constituents [8, 9, 14-18]. Recent studies have shown that essential oils of oregano (*Origanum vulgare*), and clove (*Eugenia caryophyllata*) are among the most active compounds on the strains of *E.coli* [14, 19, 20].

In this study, the antibacterial effect of *Satureja hortensis* L., *Thymus vulgaris* L. essential oils, and their major constituents were determined using the disc diffusion method. These essential oils were reported to prevent *Erwinia amylovora* growth [18]. These alternative preservatives possess antimicrobial activity and have no any adverse effect on health. In this study, various spice essential oils were tested for their inhibitory activity against the growth of some microorganisms [12]. This paper reports the results of antibacterial effect of oil obtained from aerial parts of *Origanum vulgare* L. by hydrodistillation.

Materials and Methods

Materials: The aerial parts of oregano (*Origanum vulgare* L.) were collected from Mersin (Büyükeceli-Gülnar) in Turkey in the summer of 2009.

Recovery of the essential oils: Dried aerial parts of the plant (200 g) were ground and submitted to hydrodistillation for 4 h using a Clevenger-type apparatus and the oils obtained were dried over anhydrous sodium sulfate. The essential oils were light dark-yellow.

Determination of antibacterial activity: *In vitro* antimicrobial activity was tested using broth micro-dilution technique [21] and using pathogenic strains of *Escherichia coli* RSKK 340, *Klebsiella pneumoniae* RSKK 06017, *Pseudomonas aeruginosa* RSKK 06021, *Salmonella enteritidis* RSKK 96046, *Streptococcus pyogones* RSKK 413/214, *Bacillus cereus* RSKK 1122, *Staphylococcus aureus* RSKK 96090 and methicilline-resistant *Staphylococcus aureus* (MRSA). Ampicillin was used as control of assays.

The minimal inhibitory concentration (MIC) was determined using broth micro-dilution methods. For MIC determination, the inoculum was prepared using 4-6 h of broth culture of each bacterial strain, adjusted to a turbidity equivalent to a 0.5 McFarland standard, diluted in Nutrient broth media to give concentration of ≈10^6 cfu/mL for bacteria. Two-fold serial dilutions of oregano were prepared in Nutrient broth in 96-well plates starting from a stock solution of compounds (1,024 mg/ml DMSO). An equal volume of bacterial inoculums was added to each 11 well of first row on the microtitre plates. In this manner, final concentration of compounds ranged from 256.128 to 0.5 µg/ml and 5x10^5 cfu/ml bacteria in each well (last wells were broth, only negative control well and penultimate wells were without drug positive control). The same process was repeated for 15 different microorganisms for each row of microtitre plates. The inoculated microtiter plates were then incubated at 37°C for 24 h, and the growth was recorded spectrophotometrically at 620 nm using a microplate reader (µQuant, BioTek). These procedures were used as control standards for Ampicillin (IE Ulagay, Turkey). The MIC values were defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells (broth only, without inoculum). Ampicillin was used for comparative purposes and quality control of the method.

Results and Discussion

The essential oil from oregano was inhibitory to the growth of all the bacteria under test (Table 1). *Micrococcus luteus* and *Bacillus cereus* were the most susceptible to oregano oil. The minimum inhibitory concentration (MIC) for oregano oil against various bacteria varied: while *M.luteus* (16 µg/ml) and *B.cereus* (32 µg/ml) were susceptible to oregano oil, the others exhibited partly resistance. Except MRSA *S. aureus* (250 µg/ml), ampicillin had more effect on *K. pneumoniae* (128 µg/ml) and *C. albicans* (128 µg/ml) than oregano oil, but very low concentrations of the essential oil were sufficient to prevent microbial growth. The obtained data showed that Gram-positive bacteria were more sensitive to the antimicrobial agent in spice than did Gram-negative ones. Oregano oil possessed stronger antimicrobial activity compared with the antibiotic. There was a relationship between the chemical structures of the most abundant compounds such as thymol and carvacrol in the essential oil and their antimicrobial activity. The essential oil extracted from *Origanum vulgare* was found to have antibacterial properties as determined *in vitro* medium.

Table 1. Minimum inhibitory concentration (MIC) in µg/ml of oregano oil against tested bacterial strains by microdilution method.

Microorganisms	Oregano oil	Ampicillin
Escherichia coli	250	16
Klebsiella pneumoniae	250	128
Listeria monocytogenes	250	<0.5
Proteus mirabilis	64	<0.5
Proteus vulgaris	64	4
Pseudomonas aeruginosa	64	64
Candida albicans	64	128
Streptococcus pyogenes	64	<0.5
Streptococcus mutans	64	<0.5
Salmonella enteritidis	128	<0.5
Bacillus cereus	32	<0.5
Staphylococcus aureus	64	<0.5
Streptococcus pneumoniae	128	<0.5
Micrococcus luteus	16	<0.5
MRSA Staphylococcus aureus	250	250

Steam distilled volatile oil of marjoram (*Origanum majorana* L.) was evaluated with respect to its antibacterial and antifungal activities. The oil had considerable higher inhibitory effects on several bacteria species, including the food-poisoning bacterium *Staphylococcus aureus* [13]. Bagamboula *et al.* [8] reported the antimicrobial effect of basil and thyme essential oils and its major constituents. Thyme essential oil, thymol and carvacrol showed an inhibition effect on *Shigella* sp. in the agar well diffusion assay. The oregano oil exhibited significant inhibitory activity against *Citrobacter* spp., *Salmonella typhii* and *Escherichia coli* [22]. Sağdıç *et al.* [15] determined the antimicrobial activities of the extracts of seven spices (cumin, *Helichrysum compactum* Boiss (HC), laurel, myrtle, oregano, sage and thyme) against the growth of *Escherichia coli* O157:H7. Of the spices tested, thyme and oregano showed promising results because of inhibiting the microbial growth both in paper disc and agitated liquid culture assays. Two thyme (*Thymus vulgaris* L. and *Thymus serpyllum* L.) and three oregano (*Origanum vulgare* L., *Origanum onites* L. and *Origanum majorana* L.) hydrosols were tested for their inhibitory effects against four pathogenic bacteria (*Escherichia coli* ATCC 25922, *E. coli* O157:H7 ATCC 33150, *Staphylococcus aureus* ATCC 2392 and *Yersinia enterocolitica* ATCC 1501) and the most inhibitive of the spice hydrosols on the four pathogenic bacteria were *O. onites* L. and *O. majorana* L. [23]. The antimicrobial effect of thyme essential oil (EO) at the concentrations of 0.3, 0.6, or 0.9%, that of nisin at 500 or 1000 IU/g, and their combination effect against *Listeria monocytogenes* were examined in both tryptic soy broth (TSB) and minced beef meat. Thyme EO at 0.3% level possessed a weak antibacterial activity against the pathogen in TSB, whereas the 0.9% level of thyme EO showed unacceptable organoleptic properties in minced meat. Thus, only the level of 0.6% of EO was further examined against the pathogen in minced meat [17]. Inhibitory effects of essential oils of Turkish oregano (*Origanum minutiflorum* O. Schwarz & P. H. Davis), bay laurel (*Laurus nobilis* L.), Spanish lavender (*Lavandula stoechas* subsp. *stoechas* L.), and fennel (*Foeniculum vulgare* Mill.) on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* were determined. After the essential oils were applied on the foodborne pathogens at doses of 0 (control), 5, 10, 20, 30, 40, 50, and 80 µl/ml, the resultant numbers of cells surviving were counted. Results revealed that all essential oils exhibited a very strong antibacterial activity against the tested bacteria [24]. The antibacterial effect of different concentrations (0.01 to 15%) of thyme (*Thymus vulgaris*), peppermint (*Mentha piperita* L.) caraway seed (*Carum carvi*), fennel (*Foeniculum vulgare*), tarragon (*Artemisia dracunculus*) and pennyroyal (*Mentha pullegium*) essential oils on *Staphylococcus aureus* and *Escherichia coli* was studied in nutrient broth medium. The MIC values of peppermint, fennel, thyme, pennyroyal and caraway essential oils against *Escherichia coli* were determined to be 0.5±0.03, 1±0.03, 0.3±0.01, 0.7±0.03 and 0.6±0.02% and in contrast, these values obtained for *Staphylococcus aureus* were determined to be 0.4±0.01, 2±0.13, 0.1±0.01, 0.5±0.02 and 0.5±0.02%, respectively [25]. Most of the antimicrobial activity in essential oils from spices and culinary herbs appeared to be associated with phenolic compounds [26]. In general, levels of essential oils and their compounds necessary to inhibit microbial growth were higher in foods than in culture media. This was due to interactions between phenolic compounds and the food matrix [27].

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