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Antimicrobial and antioxidant activity of the extracts from *Origanum vulgare* L. wild growing in Bosnia and Herzegovina

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Abstract
Antifungal, antimicrobial, insecticidal and antioxidant activities of *Origanum vulgare* L. provide the basis for suggesting that oregano plant extracts may be useful for prevention and treatment of infections. The main goal of this study was to determine antimicrobial and antioxidant activities of methanolic and aqueous extracts of the leaves and flowers of *O. vulgare.* Antimicrobial testing of plant extracts was done using well diffusion method. Activity of extracts was tested against Gram positive bacteria: *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212 and five Gram-negative bacteria: *Salmonella abony* ATCC 6017, *Salmonella enterica* serovar Enteritidis ATCC 31194, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922, extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* ATCC 35218 and fungus *Candida albicans* ATCC 1023. Antibiotic ampicillin and antimycotic nystatin were used as positive control. The antioxidant activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The highest values for inhibition zone for methanolic and aqueous extracts were recorded for Gram positive *B. subtilis, S. aureus* and MRSA. Methanolic extracts exhibited antibacterial activity against tested Gram negative bacteria in variable degree while the growth of these bacteria was not inhibited by aqueous extracts. Tested fungus *C. albicans* was not sensitive to investigated oregano extracts. All the extracts showed moderate to potent antioxidant activity with methanolic flower extract demonstrating the strongest antioxidative activity with the IC_{50} value of 0.205 mg/mL. Therefore it can be concluded that flower and leaf oregano extracts have significant antibacterial and antioxidant potential.

Introduction

Lately, bacterial infections are again in the focus of research, primarily due to emergence of the resistant strains of bacteria to synthetic antibiotics. Therefore, it is necessary to improve safe way of controlling and prevention of infections caused by microbial pathogens (Bešta-Gajević et al., 2017). Over the last decade has been a rising interest in medicinal value of traditional plants in different parts of the world. Recent reports have also described investigation of therapeutic properties of plants due to their potent pharmacological, antibacterial, antifungal and antiviral activities, low toxicity and health benefits (Prashant et al., 2008).

Plants are rich in a wide variety of secondary metabolites such as phenolics, and these phytochemicals have been found to possess antioxidant and antimicrobial properties (Kaneria et al., 2012). Natural antioxidants are very important for alleviation of oxidative damage caused by reactive oxygen species and reactive nitrogen species, which are produced in biological systems and foods. Antioxidant phytochemicals play an important role in the prevention and treatment of many infections and non-communicable human diseases such as cardiovascular, cancer, diabetes and some degenerative disorders (Kumar et al., 2012).

In recent years, bacterial resistance to antibiotics has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases. This development of multidrug resistance in bacteria has compelled scientists to search for new antibacterial agents and substances from various sources such as medicinal plants and fungi which are good sources of novel antimicrobial agents (Chew et al., 2012).

Different parts of plants (root, leaf, flower, fruit, stems, bark) have been used successfully to treat many diseases caused by wide array of human pathogenic microorganisms. Their antioxidant and antimicrobial activity affect many physiological processes in the body, thus protecting against free radicals and undesirable microorganisms (Dahija et al., 2014). Aromatic plants such as herbs and spices are especially rich in phenolic content, and have been widely used to extend the shelf life of foods (Adam et al., 1998) and in traditional medicine as treatment for many health problems (Shetty, 1997). Nowadays, synthetic food preservatives do not have a good reputation but food that combines nutritional and medicinal benefits is especially popular (Ćavar et al., 2008).

Origanum vulgare L. is perennial Mediterranean herbaceous medicinal plant belonging to the family Lamiaceae that has long history of use as a food additive and in cosmetic preparations. Oregano have widely been used in the prevention and treatment of respiratory, urinary and cutaneous infections (Cervato et al., 2000; Bendini et al., 2002). Therefore, the aim of this study was to evaluate antioxidant as well as antimicrobial properties of methanolic and aqueous extracts of oregano against a wide range of pathogenic microorganisms.

Materials and methods

Plant material

Plant material was collected on the Mountain Ozren in Bosnia and Herzegovina and authenticated at The Department of Biology, Faculty of Science. The plant parts were thoroughly washed with tap water, air dried, homogenized to fine powder and deposited at 4 °C until use.

Extract perparation

Herbal powder was extracted twice using 80% methanol for methanolic extracts and distilled water for aqueous extract. The extraction was performed in ultrasonic bath for 30 minutes. The extracts were weighted and dissolved to achieve concentration for 1 mg/mL for antioxidant and 5 mg/mL for antimicrobial analysis. The extracts obtained were kept in the dark at 4 °C prior to use.

Antimicrobial assay

The antimicrobial activity screening of oregano methanolic and aqueous extracts was evaluated using four different strains of Gram positive bacteria: *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, five Gram-negative bacteria: *Salmonella abony* ATCC 6017, *Salmonella enterica* serovar Enteritidis ATCC 31194, *Pseudomonas aeruginosa* ATCC 9027,

Escherichia coli ATCC 25922, extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* ATCC 35218 and fungi *Candida albicans* ATCC 1023. For *in vitro* screening well diffusion method was used to evaluate the antimicrobial activity. The bacterial cultures were maintained on Mueller Hinton Agar (Fluka Biochemica; Buchs, Switzerland) while Sabouraud Dextrose Agar (Fluka Biochemica; Buchs, Switzerland) was used for fungus *Candida albicans*. Inoculums were adjusted in sterile saline solution to the final density of 0.5 McFarland standard (~1.5 x 10^8 CFU/ml) as suggested by National Committee for Clinical Laboratory Standards (2015). Antibiotics ampicillin and antimycotic nystatin in concentration of 10 µg served as positive controls. After incubation at 35-37 ºC for 18-24 hours antimicrobial effect was evaluated based on the diameter (mm) of inhibition zones. The experiment was done in triplicate and the mean values (±SD) were presented.

Antioxidant assay

Antioxidant potential of aqueous and methanolic oregano extracts derived from leaves and flowers was determined according to the scavenging rate of DPPH radical (Aquino et al., 2002). The extracts were prepared in concentration of 1 mg/mL for antioxidant capacity evaluation. Free radical scavenging activity was calculated according to the equation:

$$AA(\%) = \frac{(A0 - A30)}{A0} * 100$$

AA – value, free radical scavenging activity; A_0 – initial absorbance; A_{30} – absorbance after 30 sec.

EC50 value represents the concentration of analyzed extract necessary for neutralization of 50% of the initial DPPH radical concentration. The calculation of IC50 value was performed by interpolation from linear regression analysis for the concentration range between 0.038 – 0.09 mg/mL for methanolic and aqueous flower and leaf extracts separately. Gallic acid was used as reference substance.

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as mean±SD. Differences were evaluated by One-way analysis of variance (ANOVA) test completed by Newman-Keuls post-hoc test. Differences were considered significant at $P<0.05$.

Results and Discussion

Antimicrobial activity

Antibacterial and antifungal activities of leaf and flower methanolic and aqueous extracts of wild growing *Origanum vulgare* L. obtained from Bosnia and Herzegovina were assessed by measuring inhibition zone diameters surrounding each well. The results presented in Table 1 demonstrate that oregano methanolic and aqueous extracts exhibit variable degree of antibacterial activity. Our results showed that Gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* were the most sensitive to investigated extracts. This antimicrobial potential supports the results of other authors who also suggest oregano as a strong antibacterial agent (Nostro et al., 2007; Liu et al., 2017). The most interesting result obtained in this study is remarkable activity against methicillin-resistant *Staphylococcus aureus* of the extracts. Finding new drugs or alternative therapies that are effective against this antibiotic-resistant bacteria is very important. The results of the antimicrobial susceptibility testing indicate that Gram negative bacteria were sensitive to methanolic extracts to variable degree while they were invulnerable to the aqueous extract. According to Arcila-Lozano et al. (2004) the most important components of oregano are limonene, gamma-cariofilene, rho-cymenene, canfor, linalol, alpha-pinene, carvacol and thymol. The antibacterial, antifungal and antioxidant properties of oregano have been attributed mainly to carvacrol and thymol, which are the major components of its essential oil (Proestos et al., 2005, Tian & Lai, 2006). Tested fungus *C. albicans* was resistant to investigated leaf and flower oregano extracts. The results of antimicrobial assay revealed that tested Gram positive bacteria were more susceptible to the investigated extracts than Gram negative strains. However, it is known that Gram negative bacteria are more resistant to antibiotics than the Gram positive bacteria which could be explained by the cell wall structure. Therefore, considering the current urgent need for new antimicrobials additional research is necessary to assess in more detail the practical application of the tested oregano extracts in therapeutic purpose.

Table 1. Antimicrobial activity of flower and leaf extracts of *Origanum vulgare* L.

		Zone of inhibition (mm)								
		E. coli	ESBL	*P. aerugi nosa*	*S. enterica*	*S. aureus*	MRSA	*B. subtilis*	*E. faecalis*	*C. albicans*
Methanolic extracts	Flower	11.00± 1.00a	-	-	11.33± 0.57b	16.66± 0.57b	15.33± 0.57a	22.33± 2.08c	11.33± 0.57c	-
	Leaf	11.33± 0.57a	10.66± 0.57	10.66± 0.57b	11.66± 0.57b	13.66± 2.08c	12.66± 1.15b	36.33± 1.52b	12.00± 0.00b	-
Aqueous extracts	Flower	-	-	-	-	14.00± 1.00c	14.66± 0.57a	13.33± 0.57d	-	-
	Leaf	-	-	-	-	11.66± 0.57d	-	14.66± 0.57d	-	-
Ampicillin		8.93± 0.31b	NI	13.10± 0.21a	17.01± 0.22a	32.97± 0.25a	NI	48.13± 0.32a	16.91± 0.37a	-
Nystatin		-	-	-	-	-	-	-	-	21.32± 0.68

Inhibition zone diameters are expressed as Mean±SD. The Mean±SD within column followed by the same letter are not significantly different (Newman-Keuls test critical ranges: P= 0.05). NI = no zone of inhibition.

Antioxidant activity

Evaluation of antioxidant potential of methanolic and aqueous extracts of leaves and flowers of oregano was performed using DPPH method. Comparing obtained results of antioxidant activity, the rate of DPPH radical scavenging was higher for methanolic extracts irrelevant of the used plant part (87.86 and 91.40 for flower and leaf extracts respectively).

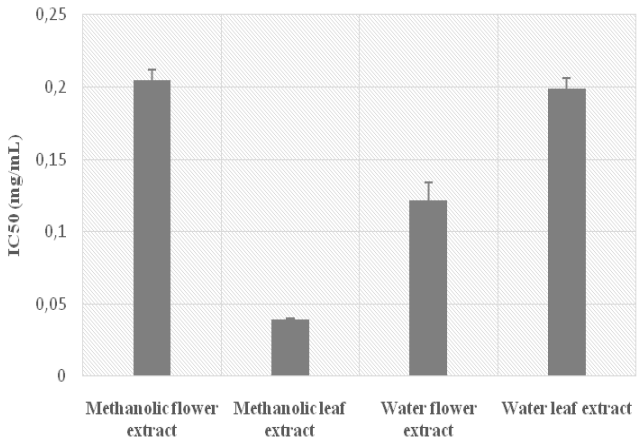


Figure 1. IC50 (μg/mL) values of plant extracts for free radical scavenging activity by DPPH test.

Aqueous extracts showed low antioxidant potential, 36.63% for flower and 29.54% for leaf extracts. IC_{50} values indicated that the highest antioxidant potential, of the tested extracts, can be attributed to methanolic leaf extract (IC_{50} = 0.03 mg/mL; Figure 1). Due to its high antioxidant potential oregano extracts can be considered as potentially safer alternative to synthetic antioxidants in pharmaceutical and food industries (Stanojević et al., 2018). Thymol and carvacrol are found to be main antioxidant components in *Origanum* species (Barrata et al., 1998; Puerters-Mejia et al., 2002) and they are probably mostly responsible for such high DPPH radical scavenging activity of methanolic extracts as previously recorded for oregano essential oil (Kulišić et al., 2004; Sahin et al., 2004; Stanojević et al., 2018).

Conclusions

From this study it can be concluded that wild growing *Origanum vulgare* L. presents potential source in the search for novel antibacterial agents especially against multidrug resistant strains such as methicillin-resistant *S. aureus*. The results of our research justified the use of oregano in traditional medicine as treatments for bacterial infections and as a food additive. In this research oregano extracts showed high antioxidant capacity and potentially could substitute synthetic antioxidants in food, pharmaceutical and cosmetic industries. Our results extend the supporting evidence of antimicrobial and antioxidant activity of oregano extracts and ensures way for further studies and research.

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