Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(7): 958-962

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vitro study of biological activity essential Oil of *Origanum vulgare* L. subsp. vulgare L.

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ABSTRACT

In Ayurveda the medicinal values of plants are well documented and revealed in the literature since ancient times. The expeditions for such medicinal plants are increasing day by day on account of man's quest for finding out newer compounds to health benefit. The potential source of vascular plants is still not completely explored for the community utility. The screening of such plants for phytochemical compounds in order to evaluate pharmacological effect has become a random tool, very few vascular plants group with respect to antibacterial activity were studied. In search for alternative ways of infectious disease control; essential oil from oregano were used in the present study to check their antifungal/antibacterial properties against pathogenic bacteria,yeast and Fungi Imperfecta using standard disc diffusion method In vitro.

Keywords: antibacterial activity, antifungal activity, Oregano (*ORIGANUM VULGARE*), pathogenic microorganisms.

INTRODUCTION

Aromatic plants are considered of great interest for their flavours and for their medicinal properties, along with human consumption, animal foodstuff and ornamental uses; thus, they are especially suitable for multifunctional sustainable crop models. Currently, there is an increased interest by consumers in new sources of aromas, flavors and biological activities (e.g. natural antioxidants and biocides). Essential oils are economically important products derived from producing herbs, spices and medicinal plants (HSMP), they are complex mixtures of components and composed by hydrocarbons with or without oxy-gen (monoterpenes, sesquiterpenes, and phenylpropanoids). A large number of these aromatic species belong to the family *Lamiaceae*. Within this family, oregano (*Origanum vulgare* subsp. *vulgare*) is probably one of most widely used aromatic plant, whose essential oils are particularly rich in mono- and sesquiterpenes [6]. Oregano essential oils have been shown to possess antioxidant, antibacterial, antifungal, diaphoretic, carminative, antispasmodic and analgesic activities [5,10,11,12] and, among these, the antimicrobial potential is of special interest. In recent years, a large number of researches have reported the efficacy of essential oils from several *Origanum* species against a panel of bacterial strains [3,13], and Başer et al. [2] identified carvacrol as the main responsible for this biological activity. Owing to the new attraction for natural products like essential oils, despite their wide use and being familiar to us as fragrances, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment.

In this paper, the antifungal and antibacterial activity of oregano oil were used to check their antimicrobial properties against pathogenic yeast, fungi, and bacteria using standard disc diffusion method *In vitro*.

EXPERIMENTAL SECTION

Test organisms

Aspergillus niger, Penicillium claviforme, Saccharomyces cerevisae, Candida albicans 8673 and Candida glabrata 72 were obtained from the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria. *Escherichia coli 3398, Staphylococus aureus 745, Bacillus subtilis 6633, Salmonella Typhymurium 3591, Listeria monocytogen 863 and Enterobacter aerogenes 3691* were obtained from the Collection of the Department of General and Applied Microbiology, Sofia University. All the isolates werechecked for purity and maintained in slants of Nutrient agar (Biolife 272-20128, Milano, Italia).

Media used

Yeast and fungi were maintained on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) plates at 29^{o}C and subcultured on a monthly basis until sporulation. The spores were harvested after establishing a good growth rate of each of the fungal cultures and were filtered with sterile cotton filter, to avoid the presence of conidia and mycelia. The spore's suspensions in PBS (pH e 7.0) were adjusted to the final concentrations in the range of 10^{5}-10^{6} spores/mL. Nutrient Agar (Biolife 272-20128, Milano, Italia) was the medium used as the growth medium for the bacterias.

Essential oil

Essential oil from *Origanum vulgare* is commercial product from pharmacies

Assay for Antifungal/Antibacterial Activity

The antibacterial activity of essential oil was determined with the disc diffusion method (9). Agar medium was added to sterile Petri dishes seeded with 100 µl of each test bacterial strains. Wells of equal distance were dug on the seeded plates. Each well was filled up with 100 µl of the oregano oil at different concentrations (diluted in DMSO) and antibiotics tested. Negative controls were prepared only with DMSO. The solutions of oregano oil (50 mg/ml, 25 mg/ml, 12,5 mg/ml, 6,25 mg/ml and 3,125 mg/ml) were freshly prepared in DMSO. After staying at 4°C (2 h), all Petri dishes were incubated at 37°C for 48 hours except *L. monocytogenes* that was incubated during 48 h. Antibacterial/antifungal activity was evaluated by measuring the radius of the inhibition zones to the nearest millimetre, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited growth of bacteria. All experiments were performed in triplicate.

To control used antibiotics Fluconazole (150 mg/ml) for yeast, Chlornitromycin (250 mg/ml) for fungi, and Sefpotec (250 mg/ml) from bacteria.

Determination of minimum inhibitory concentrations (MICs)

The estimation of MIC of the crude extracts was carried out using the broth dilution method. [8] and MICs were read in mg/ml after over night incubation at 37^{o}C. All experiments were made in replicate.

Determination of Minimum fungal/ bacteriocidal concentration (MFC/MBC)

The MFC/MBC were carried out to check whether the test microbes were killed or only their growth was inhibited. Nutrient Agar agar was prepared and sterilized at 121^{o}C for 15 minuts, the medium was poured into sterile petridishes and were allowed to cool and solidify. The contents of the MFC/MBC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37^{o}C for 24 h, after which each plate was observed for colony growth. The lowest concentration of the extracts without a colony growth was recorded as the MFC/MBC.

RESULTS AND DISCUSSION

In the present study the effects of oregano oil on five pathogenic fungi, and six pathogenic bacteria (Gram-positive and Gram-negative) were evaluated. The effects were compared with widely used antibiotic Fluconazole (150 mg/ml) for yeast, Chlornitromycin (250 mg/ml) for fungi, and Sefpotec (250 mg/ml) from bacteria. According to NCCLS, the antibiotic Chloronitromycin used is known to have broad spectrum antifungal activity, antibiotic Sefpotec used is known to have broad spectrum antibacterial activity against both gram-positive and gram-negative organisms, and antibiotic Fluconazole (150 mg/ml) used is known to have broad spectrum yeast from *Candida* sp.
The effects of oregano oil on the microorganisms were summarized in Table 1.

Table 1: Effect of oregano oil on test organisms

Microorganisms	Zone of inhibition (mm)
A. niger	22.90±0.02
P. claviforme	19.60±0.03
S. cerevisae	30.03±0.07
C. albicans 8673	22.90±0.02
C. glabrata 72	20.23±0.03
S. aureus 745 Gram- positive	18.10±0.02
E. aerogenes 3691 Gram-negative	22.20±0.03
E. coli 3398 Gram-negative	23.03±0.07
B. subtilis 6633 Gram- positive	24.87±0.02
L. monocytogen 863 Gram- positive	19.40±0.03
S. Typhymurium 745 Gram-negative	21.07±0.05
Sefpotec 250 µg/ml	22.50±0.19
Chlornitromycin 250 mg /ml	19.93±0.19
Fluconazole 150 mg /ml	20.60±0.02

Data are presented as average values ± standard deviation in mm.

The sensitivities of the test organisms to infusions were indicated by clear zone around the wells (Figure 1).

A solution of oregano oil at concentration 50 mg/ml for 24 hours notably inhibited growth of *B. subtilis 6633* (24.87 mm mean zone of inhibition), *E. coli 3398* (23.03 mm mean zone of inhibition) and *C. albicans 8673* (22.90 mm mean zone of inhibition). On the contrary, solutions of oregano oil at concentration 50 mg/ml had no activity against *S. aureus 745* (18.10 mm mean zone of inhibition).

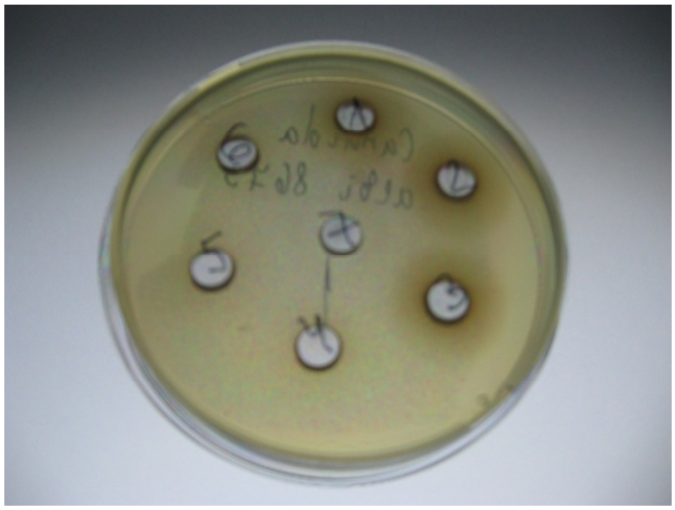

Figure 1. Showing Zone of inhibition with solutions of oregano along with tested antibiotic Fluconazole of 24 hous *C. albicans 8673*
Position 1,2 and 3) solutions of oregano oil in a concentration 50 mg/ml ; 4, 5 and 6) negative control; 7) positive control Fluconazole

Our assay for antifunal/antibacterial activity of oregano oil was conducted by testing different concentrations of the oregano oil on various pathogens to determine the MIC/MFCs. We used four concentrations – 50 mg/ml; 25 mg/ml; 12.5 mg/ml; 6.25 mg/ml and 3.125 mg/ml. The results are shown in table 2.

The results revealed variability in the inhibitory concentrations of solutions of oregano oil for given bacteria. MIC of solutions of oregano oil at concentration 50 mg/ml for 24 hours notably inhibited growth of *S. aureus 745 L. monocytogen 863.* MIC of solutions of oregano oil at concentration 25 mg/ml for 24 hours notably inhibited growth of *C. albicans 8673, E. aerogenes 3691, B. subtilis 6633, S. Typhymurium 745* and *C. glabrata 72.* MIC of solutions of oregano oil at concentration 12.5 mg/ml for 24 hours notably inhibited growth of *A. niger, P. claviforme, S. cerevisae and E. coli 3398.*

Our next task was to determine the Minimum fungicidal/bacteriocidal concentration (MFC/MBC) in regards with determining the bactericidal or bacteriostatic activity of the examined solutions of oregano oil.

Table 2. The MIC of solutions of oregano

MO	MIC, mg/ml				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
A. niger			+		
P. claviforme			+		
S. cerevisae			+		
C. albicans 8673		+			
C. glabrata 72		+			
S. aureus 745 Gram- positive	+				
E. aerogenes 3691 Gram-negative		+			
E. coli 3398 Gram-negative			+		
B. subtilis 6633 Gram- positive		+			
L. monocytogen 863 Gram- positive	+				
S. Typhymurium 745 Gram-negative		+			

Results are mean ±SEM of three separate trails.

We used five concentrations – 50 mg/ml; 25 mg/ml; 12.5 mg/ml; 6.25 mg/ml and 3.125 mg/ml. The results are shown in table 3.

MFC/MBC of solutions of oregano oil at concentration 50 mg/ml for 24 hours notably inhibited growth only of *S. aureus 745*. For eucariotes microorganisms, MFC is 12.5 mg/ml. For *E. aerogenes 3691, B. subtilis 6633, E. coli 3398, L. monocytogen 863 and S. Typhymurium 745* MBC is 25 mg/ml.

Table 3. The MFC/MBC of solutions of oregano

MO	MFC/MBC mg/ml				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
A. niger			+		
P. claviforme			+		
S. cerevisae			+		
C. albicans 8673			+		
C. glabrata 72			+		
S. aureus 745 Gram- positive	+				
E. aerogenes 3691 Gram-negative		+			
E. coli 3398 Gram-negative		+			
B. subtilis 6633 Gram- positive		+			
L. monocytogen 863 Gram- positive		+			
S. Typhymurium 745 Gram-negative		+			

Results are mean ±SEM of three separate trails.

Regarding their biological properties, it has to be kept in mind that essential oils are complex mixtures of numerous molecules, and one might wonder if their biological effects are the result of a synergism of all molecules or reflect only those of the main molecules present at the highest levels according to gas chromatographical analysis. In the literature in most cases, only the main constituents of certain essential oils like terpineol, eugenol, thymol, carvacrol, carvone, geraniol, linalool, citronellol, nerol, safrole, eucalyptol, limonene, cinnamaldehyde, were analyzed [1]. Synergistic functions of the various molecules contained in an essential oil, in comparison to the action of one or two main components of the oil, seems questionable. However, it is possible that the activity of the main components is modulated by other minor molecules [7]. Moreover, it is likely that several components of the essential oils play a role in defining the fragrance, the density, the texture, the colour and above all, cell penetration [4], lipophilic or hydrophilic attraction and fixation on cell walls and membranes, and cellular distribution. This last feature is very important because the distribution of the oil in the cell determines the different types of radical reactions produced, depending on their compartmentation in the cell [1].

In that sense, for biological purposes, it is more informative to study an entire oil rather than some of its components because the concept of synergism appears to be more meaningful.

Based on the results obtained we can conclude that the examined solutions of oregano дсв has bactericidal activity towards pathogenic bacteria, yeast and Fungi Imperfecta, but in different concentrations.

The results obtained show the existence of antifungal/antibacterial activity of solutions of oregano oil towards various pathogenic eukaryotic and procatiotic microorganisms.

CONCLUSION

The study demonstrated that oregano oil represents an economic source of natural mixtures of antifungal compounds that can be as effective as modern medicine to combat pathogenic microorganisms and safe alternative to treat infectious diseases.

Acknowledgement
Support from the Research Fund of the Konstantin Preslavsky University of Shumen (Project No. RD-08-66/02. 02. 2016, Department of Biology).

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