



## Original Research Article

### *In vitro* Study of Antifungal Activity of Oregano (*Origanum vulgare*)

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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 and aqueous decoction from oregano were used in the present study to check their antifungal properties against pathogenic yeast and Fungi Imperfecta using standard disc diffusion method *In vitro*. *In vitro* antifungal test: *Aspergillus niger*, *Penicillium claviforme*, *Saccharomyces cerevisiae*, *Candida albicans* 8673 and *Candida glabrata* 72 were treated for 24 hours with solution of oregano (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml), Chloronitromycin (250 mg/ml). The antifungal activity was assayed by the well diffusion method. *Determination of minimum inhibitory concentrations (MICs)*: The MIC of solution of oregano, that shows antifungal activity, were determined by 2-fold dilution methods as described by [8] and MICs were read in µg/ml after overnight incubation at 37°C. All experiments were made in replicate. *Determination of Minimum fungal concentration (MFC)*: The MFC was carried out to check whether the test microbes were killed or only their growth was inhibited. Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) was prepared and sterilized at 121°C for 15 minutes, the medium was poured into sterile petridishes and were allowed to cool and solidify. The contents of the MIC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37°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. The solutions of oregano had higher antifungal activity than tested antibiotic even from this fourth generation – Chloronitromycin. This study has demonstrated that solution of oregano at concentration 12.5 mg/ml for 24 hours notably inhibited growth of *S. cerevisiae* and *C. glabrata* 72. MIC of solutions of oregano at concentration 6.25 mg/ml for 24 hours notably inhibited growth only of *C. albicans* 8673. In contrast, MIC of solutions of oregano at concentration 3.125 mg/ml for 24 hours notably inhibited growth of Fungi Imperfecta *A. niger* and *P. claviforme*. The probable reason for the higher MIC reported for eukaryotic microorganisms is the complex structure of their cell. MFC of solutions of oregano at concentration 12.5 mg/ml for 24 hours notably inhibited growth only of *S. cerevisiae*. For Fungi Imperfecta *A. niger* and *P. claviforme*, MFC is 3.125 mg/ml. For *C. glabrata* 72 MFC is 6.25 mg/ml. Based on the results obtained we can conclude that the examined solutions of oregano has bactericidal activity towards both towards both pathogenic yeast and Fungi Imperfecta, but in different concentrations. The results obtained show the existence of antifungal activity of solutions of oregano towards various pathogenic eukaryotic microorganisms. The study demonstrated that oregano 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. The solutions of oregano at 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml concentrations showed significant antifungal activity on selected pathogens in clinical isolates.

## Introduction

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 (Abascal and Yarnell, 2002).

The compounds obtained from plants were rich in phytochemicals such as phenolic acids, flavonoids, tannins, lignin and other small compounds (Cowan, 1999). Such plants signify rich source of active principle exhibits numerous health related effects such as antimicrobial, antimutagenic, anticarcinogenic and vasodilatory activity (Bidlack *et al.*, 2000).

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 (Kroschwitz and Howe-Grant, 1992; Srivatsava *et al.*, 1996). Apart from medical uses, some of the plants are used as ingredients in composting in the manufacture of organic manure. These plants contain phyto-chemicals which inhibits the growth of pathogenic microbes causing disease in plants.

In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997; Mary Shobha Rani *et al.*, 2013; Reynolds, 1996).

In this paper, the antifungal activity of aqueous decoction from oregano were used

to check their antibacterial properties against pathogenic yeast and fungi using standard disc diffusion method *In vitro*.

## Materials and Methods

### Test organisms

*Aspergillus niger*, *Penicillium claviforme*, *Saccharomyces cerevisiae*, *Candida albicans* 8673 and *Candida glabrata* 72 were obtained from the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria. All the isolates were checked for purity and maintained in slants of nutrient agar.

### Media used

They were maintained on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) plates at 29°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 7.0) were adjusted to the final concentrations in the range of  $10^5$ – $10^6$  spores/mL.

### Plant material

*Origanum vulgare* ssp. *Vulgare* growing wild in the vicinity of Shumen (Velino, Bulgaria) (latitude 43°18' N; longitude 27°01' E, altitude 227 m) was used in this study. The aerial parts of oregano plants were collected at the flowering stage. The plant specimens were identified and authenticated by Zh. Nanova (Taxonomist), Faculty of Natural Sciences, Shumen University, Bulgaria. Collected plant materials were dried at a room temperature (Asya Pencheva Dragoeva *et al.*, 2014).

**Preparation of aqueous decoction:** Aerial parts of oregano plants, collected in June-July, cut about 20 cm from the top, were used in laboratory tests. The dried stems, leaves and flowers were covered with boiling distilled water, left for 60 min and then allowed to cool to room temperature. After cooling the contents of flask were filtered. The solutions of oregano (50 mg/ml, 25 mg/ml, 12,5 mg/ml, 6,25 mg/ml and 3,125 mg/ml), Chloronitromycin (250 mg/ml) were freshly prepared in distilled water.

### **Assay for antifungal activity**

Antifungal assay was performed by the well diffusion method using soft 0,8% agar. 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 BA-1 and antibiotics tested. After adjusting the pH at 6.5 by NaOH, the activity of the plant extracts was checked. The plates were incubated at 37°C for 48 hours. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well (Bertrand –Herb *et al.*, 2003). All experiments were performed in triplicate.

### **Determination of minimum inhibitory concentrations (MICs)**

The estimation of MIC of the crude extracts was carried out using the broth dilution method and MICs were read in mg/ml after overnight incubation at 37°C (Madumelu Mark *et al.*, 2014). All experiments were made in replicate.

### **Determination of minimum fungal concentration (MFC)**

The MFC 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°C for 15 minutes, the medium was poured into sterile petridishes and were allowed to cool and solidify. The contents of the MFC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37°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.

### **Result and Discussion**

In the present study the effects of solutions of oregano on five pathogenic fungi and were evaluated. The effects were compared with widely used antibiotic Chloronitromycin. According to NCCLS, the antibiotic Chloronitromycin used is known to have broad spectrum antifungal activity.

The effects of solutions of oregano on the microorganisms were summarized in table 1.

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

A solution of oregano at concentration 50 mg/ml for 24 hours notably inhibited growth of *S. cerevisiae* (29.10 mm mean zone of inhibition), *A. niger* (24.91 mm mean zone of inhibition) and *C. glabrata* 72 (23.23 mm mean zone of inhibition). On the contrary, solutions of oregano at concentration 50 mg/ml had no activity against *C. albicans* 8673 (18.90 mm mean zone of inhibition).

Our assay for antifungal activity of solutions of oregano was conducted by testing different concentrations of the compound on various pathogens to determine the MICs. We used the following 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 for given bacteria. MIC of solutions of oregano at concentration 12.5 mg/ml for 24 hours notably inhibited growth of *S. cerevisiae* and *C. glabrata* 72. MIC of solutions of oregano at concentration 6.25 mg/ml for 24 hours notably inhibited growth only of *C. albicans* 8673. In contrast, MIC of solutions of oregano at concentration 3.125 mg/ml for 24 hours notably inhibited growth of Fungi Imperfecta *A. niger* and *P. claviforme*. The probable reason for the higher MIC reported for eukaryotic microorganisms is the complex structure of their cell.

Our next task was to determine the Minimum fungicidal concentration (MFC) in regards with determining the bactericidal or bacteriostatic activity of the examined solutions of oregano. We used the following 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 of solutions of oregano at concentration 12.5 mg/ml for 24 hours

notably inhibited growth only of *S. cerevisiae*. For Fungi Imperfecta *A. niger* and *P. claviforme*, MFC is 3.125 mg/ml. For *C. glabrata* 72 MFC is 6.25 mg/ml.

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

The results obtained show the existence of antifungal activity of solutions of oregano towards various pathogenic eukaryotic microorganisms.

The study demonstrated that oregano 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.

The solutions of oregano at 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml concentrations showed significant anti antifungal activity on selected pathogens inclinal isolates.

**Table.1** Effect of solutions of oregano on test organisms

Microorganisms	Zone of inhibition (mm)
<i>A. niger</i>	24.91±0.02
<i>P. claviforme</i>	20.61±0.03
<i>S. cerevisiae</i>	29.10±0.07
<i>C. albicans</i> 8673	18.90±0.02
<i>C. glabrata</i> 72	23.23±0.03
Chlornitromycin 250 µg/ml	18.93±0.19

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

**Table.2** The MIC of solutions of oregano

Microorganisms	MIC (mg/ml)				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
<i>A. niger</i>					+
<i>P. claviforme</i>					+
<i>S. cerevisiae</i>			+		
<i>C. albicans</i> 8673				+	
<i>C. glabrata</i> 72			+		

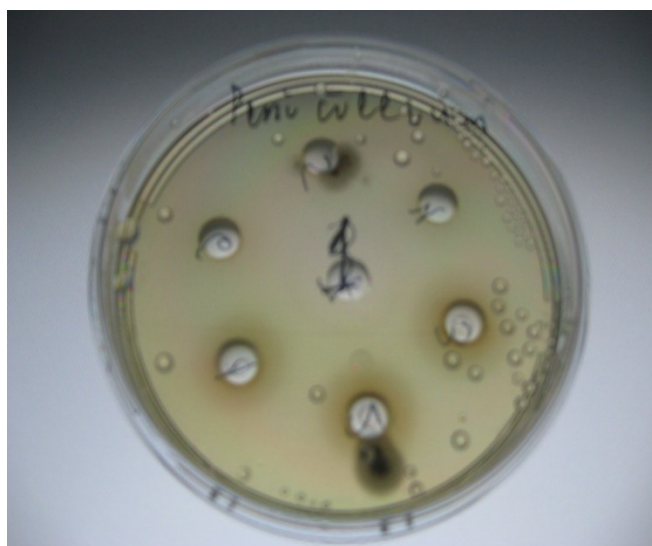
Results are mean  $\pm$  SEM of three separate trails

**Table.3** The MFC of solutions of oregano

Microorganisms	MFC (mg/ml)				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
<i>A. niger</i>					+
<i>P. claviforme</i>					+
<i>S. cerevisiae</i>			+		
<i>C. albicans</i> 8673					+
<i>C. glabrata</i> 72				+	

Results are mean  $\pm$  SEM of three separate trails

**Figure.1** Showing Zone of inhibition with solutions of oregano along with tested antibiotic Chlonitromycin of 24 hous *P. claviforme*



Position 1, 2 and 3) solutions of oregano in a concentration 50 mg/ml; 4,5 and 6) solutions of oregano in a concentration 25 mg/ml 6) Chlonitromycin

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## Author contributions

All the authors have read the final manuscript and approved for submission.

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