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# *In Vitro* Studu of Antibacterial Activity of Oregano (*Origanum Vulgare*)

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**Abstract:** ***Background and Purpose****: Screening of natural medicinal plants is common because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. 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 antibacterial properties against Gram-negative and Gram-positive using standard disc diffusion method In vitro.*

***Experimental approach:*** *Antimicrobial 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 solutions 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 antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Minimum bacteriocidal concentration (MBC):The 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 MIC 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 MBC; Minimum inhibitory concentrations(MICs): The estimation of MIC of the crude extracts was carried out using the broth dilution method [11] and MICs were read in mg/ml after over night incubation at 37$^{o}$C. All experiments were made in replicate.*

***Key Results:*** *This study has demonstrated that solution of oregano at concentration 50 mg/ml for 24 hours notably inhibited growth of S. aureus (29.10 mm mean zone of inhibition), E. aerogenes (26.20 mm mean zone of inhibition) and E. coli (24.03 mm mean zone of inhibition). On the contrary, solutions of oregano at concentration 50 mg/ml had no activity against L. monocytogen (20.40 mm mean zone of inhibition) and S. Typhymurium(20.37 mm mean zone of inhibition). MIC of solutions of oregano at concentration 50 mg/ml for 24 hours notably inhibited growth of S. aureus 745, L. monocytogen 863 and S. Typhynurium 745. MIC of solutions of oregano at concentration 6.25 mg/ml for 24 hours notably inhibited growth only of E. coli 3398. In contrast, MIC of solutions of oregano at concentration 3.125 mg/ml for 24 hours notably inhibited growth of E. aerogenes 3691 and B. subtilis 6633. The probable reason for the higher MIC reported for some bacteria is the complex structure of their cell wall. MBC of solutions of oregano at concentration 50 mg/ml for 24 hours notably inhibited growth only of Gram-positive bacteria L. monocytogen 863. For Gram-positive bacteria S. aureus 745, MBC is 25 mg/ml. For E. aerogenes 3691, E. coli 3398 and B. subtilis 6633 MBC is 3.125 mg/ml.*

***Conclusions and Implications:*** *Based on the results obtained we can conclude that the examined solutions of oregano has bactericidal activity towards both Gram-positive bacteria and Gram-negative bacteria, but in different concentrations.*

*The results obtained show the existence of antibacterial activity of solutions of oregano towards various pathogenic bacteria.*

*The study demonstrated that oregano represents an economic source of natural mixtures of antibacterial 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 significantanti bacterial activity on selected pathogens inclinical isolates.*

**Keywords:** *antimicrobial activity,* ***Oregano (ORIGANUM VULGARE)****, pathogenic bacteria.*

## 1. INTRODUCTION

Microorganisms occur nearly everywhere in nature and affect the well being of people in a great many ways. Many different microbial species normally inhabit various parts of our bodies, such as the oral cavity, skin and intestinal tract. New antibiotics were produced by pharmacological industries in the last three decades [2]. However, these antibiotics have failed to discourage the growth of many bacteria that have genetic ability to transmit and acquire resistance to drugs[6]. Thus, infections with these bacteria are associated with high morbidity and mortality especially with immunocompromised patients [7,8].

Medicinal Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones [1,10], which have been used worldwide in traditional medicine to treat several diseases and infection [9]. Many studies all over the world have been showed that these plants and their extract have multi-antimicrobial properties [2,4].

Biological effects of these plants on prokaryotic and eukaryotic organisms have been discussed by few studies [1,4].

Screening of natural medicinal plants is common because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [12]. In search for alternative ways of infectious disease control aqueous decoction from oregano were used in the present study to check their antibacterial properties against Gram-negative and Gram-positive using standard disc diffusion method *In vitro*.

## 2. MATERIALS AND METHODS

### 2.1. Test Organisms

*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.

### 2.2. Media Used

Nutrient Agar (Biolife 272-20128, Milano, Italia) was the medium used as the growth medium for the microbes.

### 2.3. Plant Material

*Origanum vulgare* ssp. *Vulgare* growing wildin thevicinity 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 floweringstage. The plant specimens were identified and authenticated by Zh. Nanova (Taxonomist), Faculty of Natural Sciences, Shumen University, Bulgaria.Collected plant materialswere dried at a room temperature [3].

*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), Sefpotec (250 mg/ml) were freshly prepared in distilled water.

*Assay for Antimicrobial Activity:* Antimicrobial assay was performed by the well diffusion method [5] 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 solutions 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 antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. All experiments were performed in triplicate.

### 2.4. Determination of Minimum Inhibitory Concentrations(Mics)

The estimation of MIC of the crude extracts was carried out using the broth dilution method. [**11**] and MICs were read in mg/ml after over night incubation at 37$^{o}$C. All experiments were made in replicate.

### 2.5. Determination of Minimum Bacteriocidal Concentration (MBC)

The 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 MIC 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 MBC.

## 3. RESULTS AND DISCUSSION

In the present study the effects of solutions of oregano on six pathogenic bacteria (Gram-positive and Gram-negative) and were evaluated. The effects were compared with widely used antibiotic Sefpotec. According to NCCLS the antibiotic Sefpotec used is known to have broad spectrum antibacterial activity against both gram-positive and gram-negative organisms.

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

**Table1.** *Effect of solutions of oregano on test organisms.*

| **Microorganisms** | **Zone of inhibition (mm)** |
|---|---|
| ***S. aureus 745* Gram- positive** | **29.10**±0.02 |
| ***E. aerogenes 3691* Gram-negative** | **26.20**±0.03 |
| ***E. coli 3398* Gram-negative** | **24.03**±0.07 |
| ***B. subtilis 6633* Gram- positive** | **21.85**±0.02 |
| ***L. monocytogen 863* Gram- positive** | **20.40**±0.03 |
| ***S. Typhymurium 745* Gram-negative** | **20.37**±0.05 |
| **Sefpotec 250 µg/ml** | **20.50**±0.19 |

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 2).

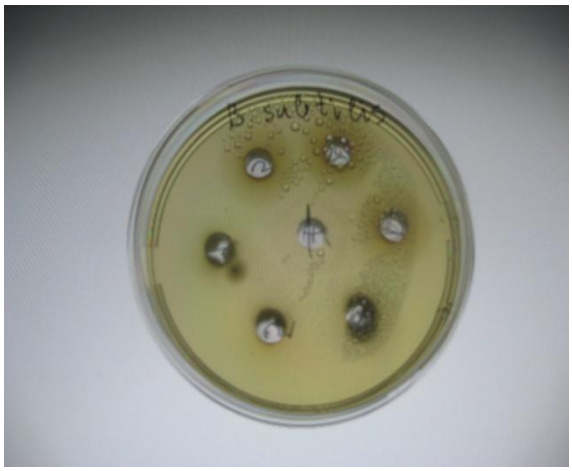

**Figure2.** *Showing Zone of inhibition with solutions of oregano along with tested antibiotic Sefpotec of 24 hous B. subtilis 6633*

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) Sefpotec

A solution of oregano at concentration 50 mg/ml for 24 hours notably inhibited growth of *S. aureus* (29.10 mm mean zone of inhibition), *E. aerogenes* (26.20 mm mean zone of inhibition) and *E. coli* (24.03 mm mean zone of inhibition). On the contrary, solutions of oregano at concentration 50 mg/ml had no activity against *L. monocytogen* (20.40 mm mean zone of inhibition) and *S. Typhymurium*(20.37 mm mean zone of inhibition).

Our assay for antibacterial activity of solutions of oregano was conducted by testing different concentrations of the compound on various pathogens to determine the MICs. 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.

**Table2.** *The MIC of solutions of oregano*

| Microorganisms | MIC (mg/ml) | | | | |
|---|---|---|---|---|---|
| | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.25mg/ml | 3.125mg/ml |
| ***S. aureus 745*** | + | | | | |
| ***E. aerogenes 3691*** | | | | | + |
| ***E. coli 3398*** | | | | + | |
| ***B. subtilis 6633*** | | | | | + |
| ***L. monocytogen 863*** | + | | | | |
| ***S. Typhynurium 745*** | + | | | | |

Results are mean ± SEM of three separate trails.

The results revealed variability in the inhibitory concentrations of solutions of oregano for given bacteria. MIC of solutions of oregano at concentration 50 mg/ml for 24 hours notably inhibited growth of *S. aureus 745, L. monocytogen 863 and S. Typhynurium 745.* MIC of solutions of oregano at concentration 6.25 mg/ml for 24 hours notably inhibited growth only of *E. coli 3398*. In contrast, MIC of solutions of oregano at concentration 3.125 mg/ml for 24 hours notably inhibited growth of *E. aerogenes 3691* and *B. subtilis 6633.* The probable reason for the higher MIC reported for some bacteria is the complex structure of their cell wall.

Our next task was to determine the Minimum bactericidal concentration (MBC) in regards with determining the bactericidal or bacteriostatic activity of the examined solutions of oregano. 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 3.

**Table3.** *The MBC of solutions of oregano*

| Microorganisms | MIC (mg/ml) | | | | |
|---|---|---|---|---|---|
| | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.25mg/ml | 3.125mg/ml |
| ***S. aureus 745*** | | + | | | |
| ***E. aerogenes 3691*** | | | | | + |
| ***E. coli 3398*** | | | | | + |
| ***B. subtilis 6633*** | | | | | + |
| ***L. monocytogen 863*** | + | | | | |
| ***S. Typhynurium 745*** | | | | + | |

Results are mean ± SEM of three separate trails.

MBC of solutions of oregano at concentration 50 mg/ml for 24 hours notably inhibited growth only of Gram-positive bacteria *L. monocytogen 863*. For Gram-positive bacteria *S. aureus 745,* MBC is 25 mg/ml. For *E. aerogenes 3691, E. coli 3398* and *B. subtilis 6633* MBC is 3.125 mg/ml.

Based on the results obtained we can conclude that the examined solutions of oregano has bactericidal activity towards both Gram-positive bacteria and Gram-negative bacteria, but in different concentrations.

The results obtained show the existence of antibacterial activity of solutions of oregano towards various pathogenic bacteria.

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

## 4. CONCLUSION

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 significantanti bacterial activity on selected pathogens inclinical isolates.

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## Author Contributions

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

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