ANTIMICROBIAL EFFICACY OF OREGANO EXTRACTS

Nawras N.Jaber ، Alaa Tariq Abdul wahid ، Alya´a Sebti Jasim

College of Veterinary Medicine, University of Basrah.Basrah.Iraq
(Received 28November 2011, Accepted 4 March 2012)

Keywords: Oregano (*Origanum vulgare*), Antibacterial activity, Gram-positive bacteria,

ABSTRACT

Invegstigation of antibacterial activity of water,ethanolic and methanolic extracts of Oregano**(***Origanum vulgare*)and compared with Vancomycin, Erythromycin, cloxacillin ciprofloxacin and Streptomycin antibiotics was carried out on gram positive and Gram microorganisms for different extracts. The zone of inhibition varies depending on bacterial
ranges from 12 to 26, 14 to 20 and 18 to 22 for water,ethanolic and methanolic With anMICs of(0.167 0.1033) mg/ml against *Bacillus subtilis* and against *Staphylococcus aureus* was(0.21±0.102) µg/ml. when comparing the extracts MICs with those of antibiotics. All extracts showed pronounced antibacterial effects against both Gram positive
and Gram negative bacteria with a significant differences between the effect of extracts & antibiotics ($p < 0.05$)

INTRODUCTION

Traditional medicine is an important source of products for developing countries in treating common infectious bacteria. The emergence of multiple drug resistant infectious bacteria, high cost of synthetic compounds as well as undesirable side effects of certain drugs insist on pharmaceutical companies to look for new therapeutic agents from other alternative sources including medicinal plants.In recent years,different reports,from different countries were published showing the antimicrobial activities of medicinal plants [1].

,. It is an important culinary herb. It is mostly used for flavoring meat, especially for mutton and lamb. In barbecue and kebab restaurants, it can be usually found on table, together with paprika, salt and pepper [2]. In addition, it has been used in the folk medicine to treat several illnesses as spasmodic,antimicrobial,digestive,rheumatoid arthritis,expectorant and aromatic for the whooping and convulsive coughs [3]. In recent years,the biological activities of Oregano has received the increased attention of researchers and industry, as well as consumers [4]. There have been a number of indication that the phytotherapeutic use of this plant might be a viable option in controlling different microbial species[5]. The

purpose of this study was to evaluate the antibacterial activity of Oregano extract on bacterial growth.

MATERIALS AND METHODS

Plant material and Extraction

Origanum vulgare L flowers were purchased from the local market of Basrah and grounded to a powder then kept in dry container. Three types of extract were prepared in the present study: ethanolic, methanolic and water based extracts. The ethanolic extract was prepared by mixing 75 gm of Oregano powder with 200 ml of 70% ethanol for 12 hours. This mixture was cooled and filtered by filter paper (Wattman No.1). The filtrate was dried and concentrated using rotary evaporator at 55c. The methanolic extract was prepared by the same way except that methanolic was used instead of ethanolic. Water based Oregano extract was prepared in the same way except that distilled water was used instead of alcohol [6].

Preliminary qualitative Chemical tests

Some chemical tests were done on ethanolic, methanolic and water based extracts of *Origanum vulgare* L to determine it's active groups, as follows:

Flavonides :

Five ml of each extract was treated with 1ml of potassium hydroxide alcohol, the development of white or brown precipitate is an indication of the presence of flavondies[7].

Tanine:

Five ml of each extract was treated with few drops of 1% lead acetate; the development of white, gelatinous precipitate is an indication of the presence of tanine [8]. ..

Alkalodis:

About 1ml of each extract treated with 1ml of dragnadroff reagents, the development of orange precipitation is indicating the present of alkaloids[9].

Saponine:

Five ml of each extract was shaken vigorously in test tube, if foam froth appeared and stayed for long time indicate the presence of saponine.

Glycosides :

Five ml of ammonical silver nitrate was added to 5ml of each extract in test tube. Left in boiling water bath for 10 minutes, then cooled. The appearance of silver mirror on wall of test tube indicates the presence of saponine[10].

Microorganisms Test:

Six types of pathogenic bacteria were previously isolated and identified by other workers were used. The antimicrobial activity of Oregano extract was determined by the well diffusion method[11]. Muller-Hinton agar medium was used for bacterial growth, Wells of(6mm diameter) were made in Mueller Hinton agar. Plates were seeded with a 24h old culture of the pathogenic bacteria. Plant extracts were added to the wells. The inoculated plates were incubated at 37°C for 24-48hrs. The diameter of the inhibition zones were measured for each plate by scale and compared with the control. (Ethanol,Methanol,D.W). Vancomycin, Erythromycin, cloxacillin , Ciprofloxacin and Streptomycin antibiotics were used in this study to evaluate the antibacterial efficacy of *Origanum vulgare* L (Oregano)extracts. Muller Hinton Agar was used with different antibiotic disc to measure MICs. The Minimum inhibitory concentration (MIC) was determined by the micro dilution method [12].

RESULTS

The chemical tests were done on Origanum vulgare L seeds to determine its active groups are shown in Table 1. The antibacterial activity of *Origanum vulgare* L seeds was evaluated against both Gram positive and negative bacteria are summarized in Table 2. The table shows the means of diameter of inhibition zone induced *Origanum vulgare* L seed extracts on the growth of microorganisms. The inhibition zones induced by extract also illustrated by photographs which are listed in Figure1. The present study reveals different influences of extractions on microorganisms due to different type of these extracts . The average diameter of inhibition zones ranges from 12 to 26mm,14 to 20mm and 18 to 22mm for *Origanum vulgare* L seeds water, ethanolic and methanolic ex-

tract,respectively. The Methanol extract of Oregano revealed the highest antibacterial activity. It was followed by Ethanol extract while water extract of oregano did not show inhibitory potential against Gram negative bacteria. The largest diameter of inhibition zone was observed from water,ethanolic and methanolic extracts on the growth of *Streptococccus* sp.The highest bacterial activity with an MICsof 0.16±0.1033 against *Bacillus subtilis* and against *Staphylococcus aureus* was 0.21±0.1021 µg/ml(table 3).

When comparing the extracts MICS with those of antibiotics. All extracts showed pronounced antibacterial effects against both Gram positive and Gram negative bacteria with a significant difference between the effect of extracts and antibiotic $p<0.05$ (Table 4)

Table 1 : Preliminary qualitative Chemical tests for *Origanum vulgare* L seeds

Type of extract	Flavonides	Tanine	Alkaloids	Saponine	Glycosides
Ethanol	++	++	-	-	+
Methanol	+	+	-	-	+
Water	+	+	-	-	+

Table 2 : Mean of Diameter of the Inhibition zones Induced by Oregano Extract on

Microorganisms .Microorganism	Water DIZ	Ethanol DIZ	Methanol DIZ
Gram Positive			
Bacillus subtilis	**12**	**14**	**18**
Staphylococcus aureus	**18**	**20**	**20**
Streptococccus sp.	**26**	**25**	**25**
Gram Negative			
Escherichia coli	-	**20**	**20**
Klebsiella pneumoniae	-	-	**22**
Pseudomonas aeruginosa	-	-	-

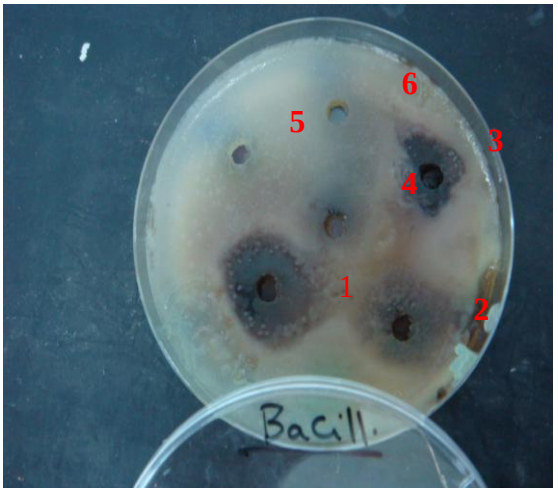
DIZ=Diameter of Inhibition Zone Measured in Millimeter.

Table 3: The minimum inhibitory concentrations(MICs) Measured in mg/ml of Oregano Extracts ($p < 0.05$)

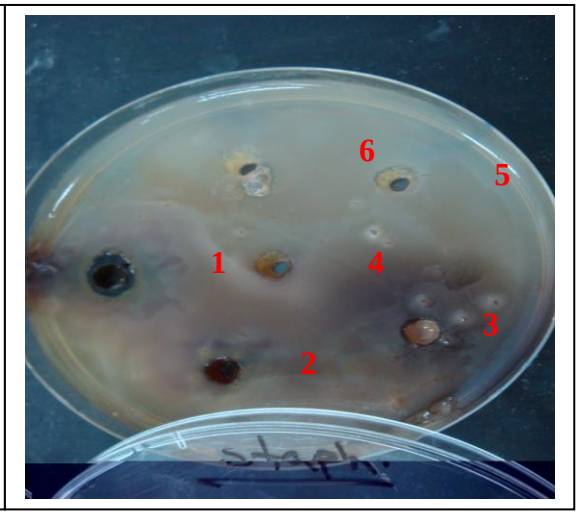
Microorganism	Mean of Minimum Inhibitory Concentrations ± standard deviation		
Gram Positive	**Water**	**Ethanol**	**Methanol**
Bacillus subtilis	*0.67 ± 8.165*	*0.167 ± 0.1033*	*0.27 ± 0.165*
Staphylococcus aureus	*0.40 ± 0.1095*	*0.21 ± 0.1021*	*0.20 ± 0.109*
Streptococccus sp.	*0.23 ± 0.1033*	*0.2 ± 0.1095*	*0.23 ± 0.1033*
Gram Negative			
Escherichia coli	*0.60 ± 0.185*	*0.6 ± 0.1095*	*0.50 ± 0.00*
Klebsiella pneumoniae	*0.43 ± 0.1053*	*0.63 ± 0.103*	*0.57 ± 0.1033*
Pseudomonas aeruginosa	*0.57 ± 0.1033*	*0.60 ± 0.1095*	*0.63 ± 0.1033*

Table 4: The minimum inhibitory concentrations(MICs)of the antibiotics on the bacteria used in the study

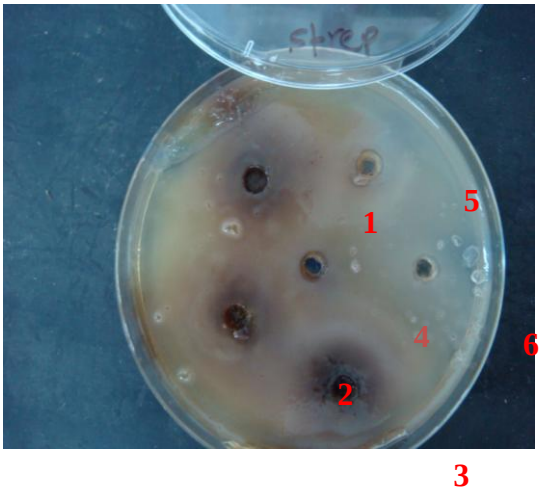
Microorganism	Antibiotic (30 mcg) (Mean±standard deviation)				
Gram Positive	Vancomycin	Erythromycin	Cloxacillin	Ciprofloxacin	Streptomycin
Bacillus subtilis	7.75±0.50	8.25±1.25	11.75±0.50	27±2.44	-
Staphylococcus aureus	12.25±0.50	23±2.45	23±2.45	12.25±0.50	4.25±1.50
Streptococccus sp.	9.5±1.91	21.5±1.91	18.75±0.95	12.75±0.50	4.5±1.91
Gram Negative					
Klebsiella pneumoniae	5.5±2.08	16.75±1.50	20.5±1.91	23.5±3.31	-
Pseudomonas aeruginosa	3.5±1	23±1.41	19.5±1	24.25±7.22	-
Escherichia coli	-	-	4.25±1.50	29±1.54	-



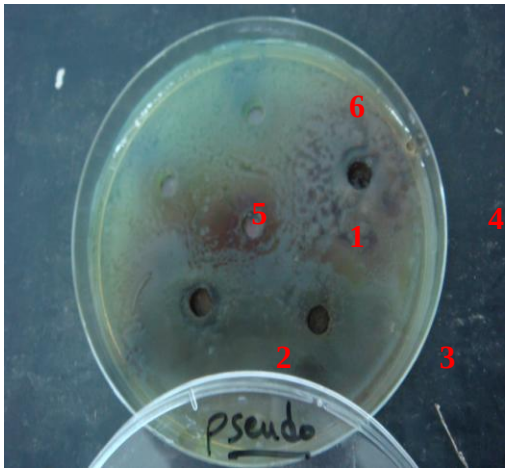
Bacillus subtilis



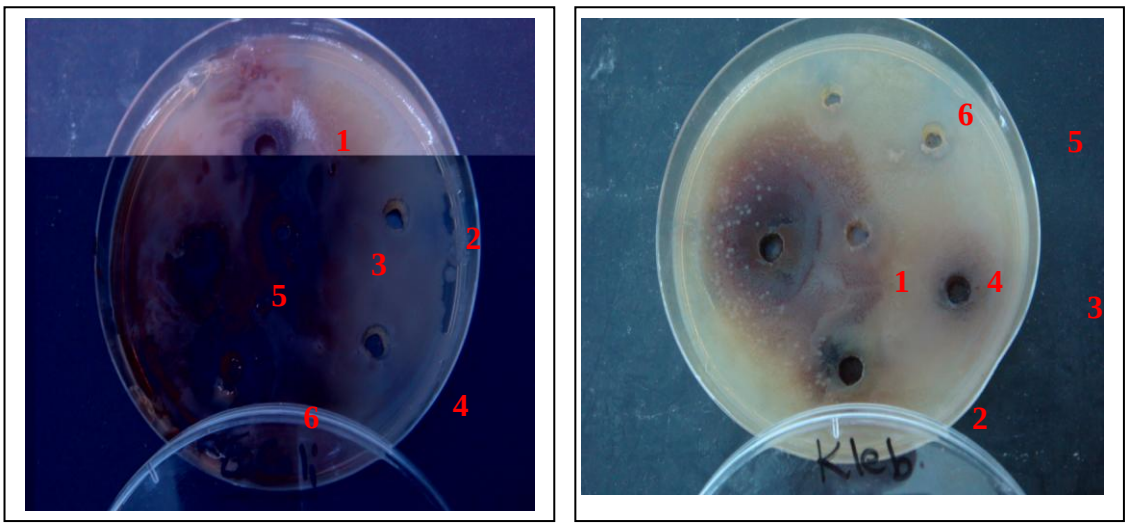
Staphylococcus aureus



Streptococcus sp .



Pseudomonas aeruginosa



Escherichia coli ***Klebsiella pneumoniae***
Escherichia coli ***Klebsiella pneumonia***

Figure 1: Inhibition Zones Induced by Oregano Extract on Microorganisms Used in This Study.1. Methanol Extract ; 2. Ethanol Extract ; 3.Water Extract ; 4. Ethanol Solvent ; 5. Methanol Solvent 6. D.W

DISCUSSION

Present study exhibited the medical importance of plants through the existence of antimicrobial activity in the crude extracts of *Origanum vulgare* L seeds. Microorganisms showed a variable susceptible to the action of oregano extracts. It was observed that Gram positive bacteria were high susceptible more than Gram negative bacteria. Generally verified greatest resistance of Gram-negative bacteria to oregano extract has been attributed in part to the great complexity of the double membrane-containing cell envelope of these microorganisms in contrast with the single membrane structures of Gram-positive bacteria and the cell membrane of Gram negative bacteria contain 90-95% lipids. These contains were not provided suitable medium to reaction with extracts. The results agreed with [13].

The antimicrobial activity seemed to depend on the antimicrobial compound in the oregano extract including the limonene ,gamma-cariofilene ,rho-cymenene,canfor,linalool,Alpha-pinene,carvacol and thymol, Among them thymol and carvacol are the main components of the oregano essential oil which are responsible for its antioxidative,antimicrobialand antifungal effects [14].

The ethano l extract showed the lowest MICs compared to other types of extracts and this may be due to the large quantity of active substances that were precipitated during the extraction process due to the effect of solvent it self [15]**.** When compared with antibiotics, all extracts showed more antibacterial activity Compared to those of antibiotic. We concluded Traditional using of this spice may help in protecting from several bacterial diseases spontaneously and may aid in control of bacterial growth in foods.

تقييم الفعالية المضادة للميكروبات لمستخلص البردقوش

نورس نوري جابر ،ألاء طارق عبد الواحد ،علياء سبتي جاسم
كليه الطب البيطري ، جامعه البصرة ، البصرة ،العراق

الخلاصة

تم دراسة الفعالية المضادة للميكروبات لثلاثة أنواع من مستخلص نبات البردقوش (المائي ، الكحول الاثيلي والكحول المثيلي)وقورنت مع ال Streptomycin,cloxacilln, Ciprofloxacin, Erthromycin,vancomycin, باستخدام طريقه انتشار القرص وطريقه التركيز المثبط الأدنى و قد اختيرت بعض انواع البكتريا المرضية الموجبة والسالبة لصبغه غرام . بينت الدراسة ان هذه الميكروبات لها حساسية مختلفة تجاه المستخلص وحسب نوع الميكروب ونوع المستخلص المستخدم ، تراوح معدل قطر المنطقة المثبطة لجرثومة*Bacillus subtilis* / من 12- 26 ملم ، 14-20 ملم و 18-22 ملم للمستخلص المائي، الكحول الاثيلي والكحول المثيلي على التوالي ,افتراضيا فأن المستخلص المثيلي كان له التأثير الضد جرثومي الأعلى. كما أظهرت النتائج أن تركيز المثبط الادنى لمستخلص البردقوش الاثيلي كان 0.1033 ± 0.167 ملغم /مل ضد جرثومة *Bacillus subtilis* و 0.1021 ± 0.21 ملغم /مل ضد جرثومة ال *Staphylococcus aureus* مقارنة مع التراكيز المثبطة الدنيا للمضادات الحياتية. كل المستخلصات أظهرت تأثيرات ضد جرثومية عالية للجراثيم الموجبة والسالبة لصبغة كرام مع وجود فروقات معنوية بين تأثير المستخلصات وتأثير المضادات الحياتية ($p < 0.05$) .

REFERENCES

1. Nimri LF., Meqdam MM.and A. ALkofani (1999). Antibacterial Activity of Jordanian Medicinal Plants . Pharmaceutical Biology, 37: 196 -201.
2. http://en.wikipedia.org/wiki/Oregano

3. Novak, J.; Zambori-Nemeth, E.; Horvath, H.; Seregely,Z. and K.Kaffka.(2003). Study of essential oil components in different Origanum species by GC and sensory analysis. Acta Alimentaria, 32, 141-150.

4. Arcila-Lozano, C.C., G. Loarca-Pina, S. Lecona-Uribe and E.M. Gonzalez.(2004). Oregano : properties, composition and biological activity. Arch. Latinoam. Nutr., 54(1): 100-111.

5. Digrak, M., M. H. Alma, and A. I`lcim.(2001). Antibacterial and antifungal activities of Turkish medicinal plants. Pharm. Biol.39:346–350.

6. Dulger,B. and A.Gonuz,(2004). Antimicrobial activity of certain plants used in Turkish traditional medicine.Asain J.Plant Sci.,3:104-107.

7. Al-Khazraji,S.M.(1991). Biopharmacological study of *Artmisia herbaalba*. M.Sc. Thesis, College of Pharmacy. University of Baghdad.

8. Justesen U and Knuthsen P (2001). "Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes". Food Chem. 73 (2): 245–50..

9. Jawad,A.A.(1996). Ethological studies and assessing the anti-aggressive effects of some Iraq medical plants in laboratory mice(*Mus musulus*).Ph.D. Thesis, College of Education. University of Basrah.

10. Harrbone,J.B.(1984).Phytochemical methods. Chapman and Hall,London,U.K.,PP.: 566-568

11. National Committee for Clinical Laboratory Standards (NCCLS),Performance standards for antimicrobial disk susceptibility tests,NCCLS, Pennsylvania, USA, 1993, M2-A5.

12. Chung, K. T., Wong T. Y. Wei, C. I.,Huang Y. W. and Y.Lin.(1998). Tannins and human health: A review. Crit. Rev. Food Sci. Nutr. 38:421–464.

13. Sabahat S. and P. Tariq(2009). Antibacterial activity of Oregano (Origanum vulgare L) against gram positive bacteria. Pak.J.Pharm.Sci.,pp.421-424.

14. Tian,H.andD.M.Lai.(2006).Analysis on the volatile oil in origanum Vulgare,Zhong.Yaocai,29(9):920-921.

15. Proestos ,C.;N.,Choriano Poulos;G.A.Nychas andM.Komaitis.(2005).RP-HPLC analysis of the phenolic compounds of plant extracts investigation of their antioxidant capacity and antimicrobial activity.J.Agric.Food chem.,53:1190-1195.