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Antimicrobial activity of Origanum magorana against bacteria isolated from the patient with pneumonia

***Thamer Mutlag Jasim, *Baydaa Hameed Abdullah, **Suhad Faisel Al Mugdadi and *Mayssam Camel**

Mustansiriya University, College of Pharmact, Department of Pharmaceutical Microbiology
****medical city, laboratory department***

الخلاصة

تم أخذ 150 عينة من البصاق للمرضى المراجعين إلى مستشفى مدينة الطب ومستشفى ابن البلدي والمصابين بالتهاب رئوي للفترة من شهر شباط 2009 ولغاية شهر أيار 2009 وكانت أكثر العزلات هي:

Klebsiella pneumonia 18 (22.5%), *Pseudomonas eruginosa* 16 (20%), *Acinetobacter spp*, 4 (5%), *Eschereshia coli*, 5 (6.25%), *Candida albicans* 5 (6.25), (5%), *Mycobacterium tuberculosis* 4 (5%), *Streptococcus pneumonia* 3 (3.75%), Proteus 3 (3.75%), Citrobacter (1.25%), *Enterobacter* 1 (1.25%), *Nocardia* 3(3.75%).

اختبرت حساسية البكتريا للمضادات الحياتية التوبرامايسين والجنتامايسين واليسفوثاكسيم ووالترايميثوبريم والبنسلين وا لايريثرومايسين والفانكومايسين والتتراسايكلين والايوكمنتين من بقية العزلات وكانت المكورات الرئوية أكثر مقاومة اختبرت الخلاصة المائية للمزرنجوش الباردة بتركيز 5ملكرام لكل مل ضد البكتريا المعزولة قيد الدراسة ووجدت إنها لم توثر على البكتريا المعزولة بينما الخلاصة المائية الحارة كان لها تأثير قليل على البكتريا المعزولة واختبرت الخلاصة الكحولية للمزرنجوش وكان تأثيرها على الاشريشية القولونية والمكورات العنقودية الذهبية فقط و اختبرت خلاصة الزيت وكان لها تاثيرعلى البكتريا المعوية والمكورات العنقودية.

Abstract:

A total of 150 sputum sample from the patient attendants to medical city and Ibn Al-Balady hospital during the period from February 2009 to May 2009 with signs and symptoms of pneumonia only 57 sputum samples 53. 5 gave bacterial growth positive, the result indicated the most frequent microorganisms were 18 isolate (22.5%) *Klebsiella pneumonia*, 16 isolate (20%) *Pseudomonas aeruginosa*, 5 isolate (6.25%) *Candida albicans*, 5 isolate (6.25%) *Acinetobacter spp*, 5 isolate (6.25%) *Eschereshia coli*, 4 isolate (5%) *Mycobacterium tuberculosis*, 3 isolates (3.75%) *proteus spp*, 1 isolate (1.25%) *Enterobacter*, (1.25%) *Citrobacter Acinetobacter* spp. 5 (6.25%), 3 isolate

(3.75%) *Streptococcus Pneumonia*, *Enterobacter* 1 (1.25%), *Mycobacterium tuberculosis* 4 isolates (5%). Aqueous of cold extract were investigated for their antibacterial activities against bacteria not effect. Hot water extract can inhibit the growth of microorganisms. Oil extract of *Origanum magorana* effect on *E.coli* and *Staphylococcus aurous*.
Key words: *Origanum margorana*, antibacterial.

Introduction:

Origanum majorana , a member of the Labiate family. Majorana extract of its leaves and flowers has been prescribed in folkloric medicine for relieving the symptoms of hay fever, sinus congestion, asthma, stomach pain, headach, dizziness, colds, cough, and nervous disorders. The plant extract contains mainlyterpinenes, aroma- active compounds, carvacrol and thymol, alkaloids, flavonoids and essential oils [1, 2].

Plant contains numerous biologically active compounds many of which have been shown to have antimicrobial properties [3]. Plant derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in plants as source of agents to fight microbial diseases. Nowadays multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [4]. In addition to the problem, antibiotic are sometimes associated with adverse effects. On the host including hypersensitivity, immune suppression allergic reactions [5]. Yet no previous studies on the Iraq *Origanum majorana* report species show its antimicrobial activity. Thus the present paper report the antibacterial activate of *Origanum majorana* aqueous (hot, cold, oil) extract against bacterial isolate from sputum of patient attended to medical city of medicine Ibn Al-Balady hospital.

Materials and methods:

Bacterial isolates

Isolation and detection

The following bacteria were isolate from the patient attendant's medical city and Ibn Al-Balady hospital during the period from February to may 2009. *Klebsiella pneumonia*, *pseudomonas aeaurginosa*, *Eschereshia coli*, *Proteus spp, Citrobacter spp*, *Enterobacter Nocardia*, *Mycobacterium tuberculosis*. The specimen were transport to lab for diagnosis, Gram stain, Diehl nelson stain for acid fast bacilli, culture on blood, chocolate, MacConkey agar and Seaboards agar for fungal, biochemical test, indole, TSI urea citrate, motility for gram negative, suspected colony (Api 20, biomeriex for gram negative), Mannitol salt agar for *Staphylococcus aureus*, coagulase positive, optician for *Streptococcus pneumonia*.

Antibiotic susceptibility

All bacterial pathogens isolated were subsequently evaluated for susceptibility to the following antibiotic Amikacin (AK), ciproflacine (CTX), Cotrimaxazol Trimethoprim (STX), pipracillin (pip), Augmentin (Ame), Tetracycline (TE), Penicillin (p), Erythromycin (E), Vancomycin (VA), susceptibility test were performed by the agar diffusion disk method advocated by the national commit for clinical laboratory standard [6].

Plant material

The plant materials for screenings were authenticated duplicate pressed specimen of reference material obtained from national Herbarium of Iraq Botany directorate at Abu-Grab.

Extraction procedure

Plant were cleaned, air dried and grounded by mortar and pestle, each 2gm of grounded substance was soaked in 10ml of distilled water and left at 37^{o}C for 24 hours with continuous shaking in Gallenkau-cooled orbital incubator then filtered through filter paper (what man No.1). The filtrate was centrifuged and condensed by rotary evaporator under vacuum at 40^{o}C until athick syrup was obtained. The syrup was left in an incubator at 37^{o}C until dryness.

Oil extraction

The oil was extracted from 250 g of herb using steam distillation method British pharmacopeias. One ml, aliquots of the oil were added to 2ml DMSO and tested to their antimicrobial activity. The dilution (1:3) was chosen since the final volume required for performing the test.

Alcohol extraction

The leaves of *Origanum majorana* were cut into pieces 25 gram of the leaves were sox let extracted using 350 ml of 95% methanol. The extraction lasted for ten hours. The volatile oil obtained was concentrated by evaporation using rotary evaporation at 100^{o}C and rotary evaporated

Result:

A total of 150 sputum samples were submitted for sputum culture 57 isolates (53.33%) were significant bacteria. Table-1 shows the number and percentage of infectious bacteria. Table- 2 shows percentage of resistance among bacterial species isolated from sputum. Evaluation of antimicrobial activities was performed by agar well diffusion method. Diameter of the zone of inhibition (ZOI) was measured for the estimation of potency of the antimicrobial substance. The zone of inhibition of Water extract (cold and hot) alcohol and oil extract of *Origanum majorana* as indicated in table-3 are shown in the following table..

Discussion:

The results of the inhibitory effects of the water extracts is less effective on the test organism than the oil extract Table no 2 *Klebsiella spp* was not inhibited in the water extract (table-1) . However, it was inhibited by the oil extracts. This may be due to the fact that this organism produce capsule which would not be readly dissolved in water [7].

These observations are likely to be the result of the difference in cell wall structure between Gram positive and Gram negative bacteria with the Gram negative outer membrane using as a barrier to many environmental substances including antibiotics [8, 9].

This antibacterial study of the plant extracts demonstrated that folk medicine can be effective as modern medicine to combat pathogenic microorganism. The millenarian use of these plants in folk medicine suggests that they represent an economic and see alternative to treat infectious disease. However, the application of any compounds to medicine will require safety and toxicity issues to be addressed. The resistance to antibiotics may be due to developing altered receptors by altering the means of entry or removal of drug or by synthesizing resistant metabolic pathway [10]. This result agreement with Enzo, et. al.[11] who report the antibacterial effectiveness of *Origanum majorana* of their use as the source of alternative antimicrobial compound. Several studies have been conducted on the antimicrobial properties of herbs, spices and their derivatives such as essential oils, extracts and decoctions [12, 13, 14, 15, 16]. *Origanum majoarana* should the best antibacterial effect on different pathogenic microorganisim this result agreement with Joshi Bishnu, et. al.[17] who report that *Origanum majorana* should the best antibacterial. Further evaluation of the antibacterial properties of the extracts and elucidation of the components responsible for the activities is warranted.

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Types of bacteria	No. of isolates	%
K.pneumonia	18	(22.5)
Pseudomonas aeruginosa	16	(20)
Acinetobacter	4	(6.25)
E.coli	5	(6.25)
Strep.pneumonia	4	(5)
Mycobacterium tuberculosis	4	(5)
Proteus	3	(3.75)
Eterobacter	1	(1.25)
Citrobacter	1	(1.25)
Enterobacter	1	(1.25)
Total	57	

Table-1: The types, numbers and percentages of bacteria isolated from 150 patients with pneumonia.

Number of Bacteria	Type of antibiotic resistance									
	AK	CIP	IMP	Tb	GM	CTX	STX	PIP	AUG	TE
E.coli				3	2	2	2	1	3	2
Kleb. pneumonia	3	2		8	9	9	8	3	8	3
Pseudomonas aeruginosa	2	2	2	3	9	1	9	3	8	3
Staph . aureus				1	2	1				
Acinetobacter. spp	2	2		5	3	3		2	2	1
Strep. pneumonia										
Citrobacte. spp										
Proteus. spp	2	2		3	3	3	2	1	4	2
Mycobacterium tuberculosis										
Enterobacter	2	2	2	2	2	2	2		2	2

Table-2: Antibiotic susceptibility bacteria from patients with pneumonia

Microorganisms	Type of extract and Inhibition Zone (mm)			
	Cold water	Hot water	oil	Alcoholic
Candida spp	-----		25mm	
Pseudomonas aeruginosa	------		12mm	
Acinetobacter spp	------		18mm	
E. coli	-------		16mm	13mm
Klebsiella pneumonia	-------	12mmzone	15mm	
Enterobacter spp	-------		20mm	
Proteus spp	-----	13	14mm	
Staphylococcus aureus	-----	15	12mm	20mm
Streptococcus pneumonia	-------	10mm	14mm	

Table-3: Diameter of inhibition zone of the water hot and cold and alcohol and oil of *Origanum majorona* on pathogenic bacteria