Antibacterial activity of alcohol extract of *Quercusinfectoria*Olivier

Received: 9/6/2014 accepted:30/10/2014

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Abstract:

Eighteen clinical ear and wound swaps were collected from patients attending AlfuratAl-awsatHospital in Al-KufaCity , bacterial isolates were identified after culturing the swaps , the results indicated that the most frequent isolates were *Pseudomonas aeuroginosa* and *Acinobacter baumanii.*

Extract from *Quercusinfectoria* was prepared using ethyl alcohol as a high polarity solvent then screened for antibacterial activity at the concentrations(125,250,375, and 500mg/ml) using agar well diffusion method. Crud extract exhibited a strong inhibition effect against *P. aeruginosa* ranging between (13.5-22.5mm) and moderate antibacterial effect for *A. baumanii* (13-18mm).

Key words: Antibacterial activity , pathogenic bacteria, *Quercusinfectoria*, ethyl alcohol extract

Microbiology classification : QR75-99.5

Introduction

The use of plant based medicines (local medicine) date back to 4000-5000 B.C. Nowadays huge number of allopathic medicines also contain plant based ingredients that are used for their preparation by different companies. Furthermore according to WHO about 80% of world population depend on medicinal plant for their health care needs, and more than 30% of pharmaceutical preparations are based on plants[1,2].

Oaks are one of the important trees, distributed in many regions of temperate zone in the world. They are source of raw materials, for some useful products to human race [3]. The species of oak, the (Quercus genus), are classified under the family Fagaceae. Four species of oak (*Q. aegilop, Q. infectoria, Q. libani and Q. Marcantherea*) are grown in the Iraqi Kurdistan Forest [4]. .

Quercusinfectoria oliv. or 'Manjakani' (in the Malay language) is well acknowledged in traditional medicine as a natural astringent that contains antiseptic materials and anti-oxidants, a truly generous gift from Mother nature of women[5]. Studies

indicated that *Q. infectoria* is thought to have a variety of pharmacological properties including being an astringent [6], anti-diabetic [7], anti-tremorine[8], local anesthetic, anti-viral potential [9], antibacterial [10,11,12], anti-fungal [13], larvicidal[14] and anti-inflammation [15,16].

Topical antimicrobial therapy is one of the most important methods of wound care. The goal of topical antimicrobial therapy in wound care is to control microbial colonization and subsequent proliferation thus promoting the healing of the wounds [17] . Some medicinal plants have been employed in folk medicine since time immemorial for wound care [18,19,20].

Some of these plants either promote direct wound repair or exhibit antimicrobial and other related properties which are beneficial in overall wound care. Antimicrobial principles have been isolated from some of the medicinal plants used in folk medicine for wound care. *Quercusinfectoria*is one of such plants employed by herbalists in the treatment of sores and boils[21,22].

Aim of the study:

The purpose of this study was to evaluate the in vitro activity of *Q. infectoria* extract as antimicrobial agent toward the growth inhibition of pathological bacteria which are responsible for wound infections.

Materials and methods

A-Collection and identification of wound and ear swabs :

Surface wound and ear swabs were collected from 18 patients attending Al-Furat Al-Awsat Hospital in Al-Najaf city. The wounds were first cleaned using sterile cotton swabs soaked in sterile normal saline. The specimens were collected by gently rotating sterile swab in the wound and then transported to the laboratory immediately. The study conducted in Microbiology laboratory-college of pharmacy/kufa university.

The swab samples were inoculated on nutrient agar plates and incubated overnight at 37 C° for 24 hours aerobically. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques [23].

B-Bacterial isolates:

Tested pathogenic bacteria were stored in nutrient broth containing 20% glycerol at 4°Cuntil further analysis.Testbacteria were grown overnight at 37 °C, 120 rpm in 10 mlnutrient broth. This broth was used for seeding the agarplates.The inoculum sizeof each test strain was 10^8 bacteria/ml for disc diffusion assaywhich was standardized by adjusting the optical density of thebacterial suspension to a turbidity corresponding to spectrophotometric (absorbance = 0.08 (OD_{620} = 0.08) at 620 nm).

C- Culture media

The nutrient agar(Oxoid, Germany,UK) was prepared (0.3gm/1000ml) and sterilized by autoclaving at15 psi pressure (121 °C) for 20 min. Sterilized petriplateswere prepared with an equal thickness of nutrient agar.

D- ***Plant materials***

Quercusinfectoria Oliv. (Fagaceae) barks sample was obtained commerciallyfrom the local markets and identified based on the physical characteristics.The plant samples were air dried in shadow, crushed to small pieces using a mechanical mortar intofine powder before the extraction .

E. Extraction conditons

Extract of dried plant materials was prepared by usinga solvent of different polarity. The dried plant materials of 100 geach were extracted by with 500ml ethyl alcohol(100%) for 24hrs using magnetic stirrer at room temperature in a dark place. The solvents used were methanol, ethanol, hexane, chloroform,and distilled water,then theextract was filtered through Whatman filter paper No.1. Thesolvent was then distilled under reduced pressure in a rotaryevaporator until it became completely dry. The weight of thesolid residue was recorded and taken as yield of crudeextracts.

Extract was stored at 4 ^{o}Cand were freshly dissolved in distilled water to prepare the following concentrations(125,250,375 and 500). The corresponding concentration was expressed in termof mg of extract per ml of solvent (mg ml^{-1}).

F- Evaluation of Antibacterial Activity

The *in-vitro* antibacterial activity of the extractwas determined by agar well diffusion assay [24,25]. Bothisolates were first grown in nutrient broth under shaking condition for 4 h 37^{0}C and afterthe incubation period 0.1ml of the test inoculums wasspread evenly with a sterile glass spreader on nutrient agar plates. The seeded plates wereallowed to dry in the incubator at 37^{0}C. Wells were madeusing sterile 6 mm cork borer in the inoculated agarplates. The wells were filled with 100µl of the extracts(125,250,375 and500mg/ml). The inoculated plates wereincubated at 37^{o}C for 24 h. The plates were observed forthe presence of inhibition of bacterial growth that wasindicated by a clear zone around the well.

The size ofzone of inhibition was measured and the bacterialactivity was expressed in term of average diameter ofthe zone of inhibition in millimeters. The results werecompared with the standard antibiotics,(Sigma company, Germany);Gentamycin (10µg /ml), Ceftazidime(30µg /ml), Cefepime(30µg /ml) and Amikacin (30µg /ml)were alsoexperimented separately which would serve as positiveantibacterial controls. Sterile distilled water served as negative control.Each experiment was carried out intriplicate and the mean diameter of the inhibition zones measured to the nearest millimeter (mm) wasrecorded.

Result And Discussion

A-bacterial isolates:

Various bacterial isolates were recovered from various infected wounds and ear swaps (Table 1). Positive growth was observed in 83.33% of wound cultures. The most frequently predominant bacterial isolate was *Pseudomonas aeruginosa* followed by *Acinobacter baumanii* .

Table (1): Bacterial species recovered from patients wound and

ear swapswith their frequency

Bacterial species	Wounds		Ear swaps	
	Positive growth	Negative growth	Positive growth	Negative growth
Acinobacter baumanii	13	2	2	1
Pseudomonaaeroginosa				
Escherichia coli				
Staphylococcus aureus				
Proteus spp.				
Total= (18 swaps)	**15 swaps**		**3 swaps**	
Percentage %	**(positive)=83.33%**		**(Negative)=16.66%**	

B. Antimicrobial assay

The antimicrobial activity of alcohol extract of *Q.infectoria* was studied by the agar diffusion method and the results are shown (Table 2, Fig.1). In the present study, the microbiological analysis reveals that *P. aeruginosa* is the leading etiologic agent of wound infection which was supported with previous reports [26,27,28]. Wounds are known to be easy portals for infection and provides suitable medium for the proliferation of microbial organisms, so both of Gram positive and Gram-negative bacteria are known to cause wound sepsis[29].

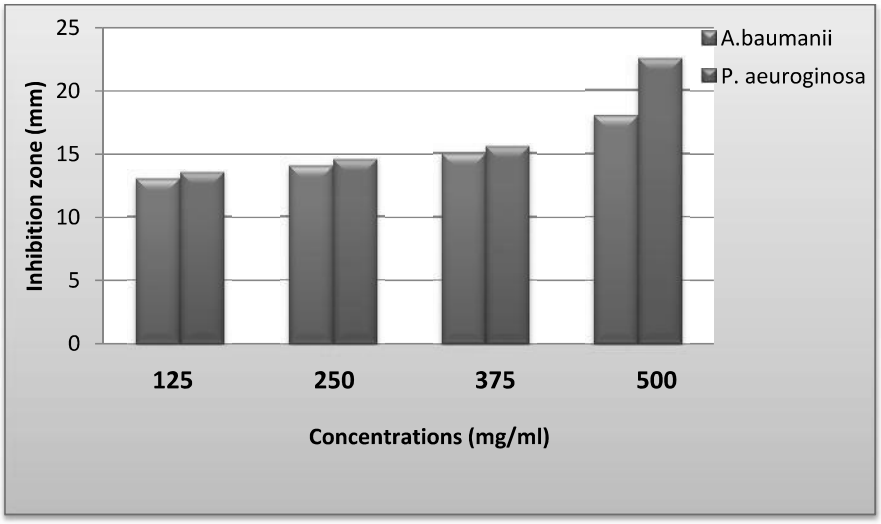
In this study the results of the investigations show that the extract from *Q. infectoria* possess antimicrobial activities against both tested microorganisms that are involved in causing wound infections at a concentration varied between (125 -500) mg/ml.Oakethanolic extract displayed an excellent activity against both gram-negative pathogenic bacterial species, it exhibited a higher antibacterial activity against *P.aeruginosa* and produced inhibition zones ranging from 13.5 to 22.5 mm in diameter while the extract exhibited a high to moderate activity against the other tested species *A. baumanii* (13-18mm). The results were compared with those of Gentamycin (10mg/ml), Ceftazidime(30mg/ml), Cefepime(30mg/ml) and Amikacin (30mg/ml)as standard antibiotics.

These findings support the use of this plant in the management of wound infection. Ethanolic extracts showed the strongest activity an indication that ethanol is a better extractant than other solvents and this may be due to the ability of the ethanol to extract a wide range of chemical constituent of the plant. Our finding was supported by other researches who reported that the crude powder of the galls of *Q. infectoria* was found to be active against Gram-positive and Gram-negative bacteria [30,31,32].

Table (2):- Antibacterial activities of alcohol extract of *Q.infectoria*

Concentrations mg/ml	Diameter of inhibition zone (mm)	
	Acinobacter baumanii	*Pseudomonas aeruginosa*
125 mg/ml	13	13.5
250 mg/ml	14	14.5
375 mg/ml	15	18.5
500mg/ml	18	22.5
Gentamycin(10µg/ml)	13-14	13-14
Ceftazidime(30µg/ml)	15-17	15-17
Cefepime (30µg/ml)	15-17	15-17
Amikacin (30µg/ml)	15-16	15-16

Mean value of threedeterminations, each from a different plate.



Figure(1):- Antibacterial activities of alcohol extract of *Q.infectoria*

The inhibitory effects of gall nut may be due to the presence of some phytochemical components, and based on previous studies. High amounts of tannin (50-70%) present in the galls of *Q. infectoria* implied that tannin is the active compound for the antibacterial activity in this study. Tannins are a group of polymeric phenolic substances characterized by antibacterial activity owing to inactivation of bacterial adhesions, cell envelope and transport proteins [33]. In addition, tannin is potent inhibitors of microbial enzymes like protease[34]. Other studies showed that tannin inhibits the growth of both *E. coli* and *S. aureus* and has been attributed to a similar inhibitory action of the mechanism of tannin binding with the protein of the bacterial cell walls[30]. *Quercus* species have been reported to contain high levels of tannins in both hydrolysable and condensed form which form irreversible complexes with proline-rich protein resulting in the inhibition of the cell protein synthesis. It can be concluded that the *Q. infectoria* extracts has beneficial effect as antiseptic and can use for the

treatment of wound infection caused by pathogenic bacteria[2,5].

On the other hand, the *Q. infectoria* have quercetin which has activity against microbial. The quercetin markedly enhanced antibacterial activitywhich was at least partially attributed to the inhibition of DNA gyrase[11,35].

CONCLUSIONS

In conclusion, the extracts of the galls of *Q. infectoria* have high potential as antibacterial agent. This finding provides an insight into the usage of the galls of Q. *infectoria* in traditional treatment of wounds or burns associated with bacterial infections. The results of present study supports the traditional usage of plant and *Q. infectoria* plant extracts which posses compounds with antibacterial properties.

Acknowledgement

The authors would like to thank Mr. Faáz Al-Salami/ Microbiology Laboratory in Al-Furat Al-Awsat Hospital for his support and technical assistance during the course of this research especially in bacterial identification.

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الفعالية المضادة للجراثيم للمستخلص الكحولي لنبات العفص

Quercusinfectoria Olivier

تاريخ الاستلام: 2014/6/9 تاريخ القبول:2014/10/30

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لخلاصة:

شملت الدراسة الحالية جمع (18) عينة (مسحات من الأذن والجروح) من مرضى مراجعين في مستشفى الفرات الأوسط- مدينة الكوفة , وبعد زراعة هذه المسحات وتشخيصها وجد إن البكتريا *Pseudomonas aeuroginosa* and *Acinobacter baumanii* هما الأكثر تكرارا وشيوعا .

حضر مستخلص نبات العفص *Quercusinfectoria* باستخدام الكحول الاثيلي عالي القطبية واختبرت فعاليته المضادة للجراثيم عند التركيز (125,250,375 و500 ملغ/مل) باستخدام طريقة الانتشار عبر الاكارالمغذي . أظهرت النتائج الحالية أن المستخلص الخام له فعالية قوية تجاه الجنس *P. aeuroginosa* إذ بلغت (13.5-22.5-ملم) في حين كانت المستخلص اقل تجاه الجنس *A. baumanii* (13-18- ملم) .

الكلمات المفتاحية: الفعالية المضادة للجراثيم , البكتريا المرضية , نبات العفص ,مستخلص كحولي

Microbiology classification : QR75-99.5