

## **Antibacterial activity of *Quercus infectoria* extracts against bacterial isolated from wound infection**

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### **Abstract**

This study determines the nature of microbial wound colonization in 191 patients with wound infection attending Internal Lab of Teaching Hospital and Emergency Hospital in Erbil city during the period 1-January-2007 to 31-July-2007. A total of 241 bacterial isolates were identified after culturing the swabs on different culture media. The results indicated that the most frequent isolates were *Staphylococcus aureus* (32.78%), *Pseudomonas aeruginosa* (24.90%), *Escherichia coli* (14.94%), *Enterobacter* spp. (9.96%), *Proteus mirabilis* (8.71%), *Klebsiella pneumoniae* (6.64%), *Klebsiella oxytoca* (1.24%) and *Citrobacter freundii* (0.83%). Most of the isolates showed high level resistance to commonly available antibiotic. The present study also undertaken to assess the antimicrobial effect of aqueous, methanol and ethanol extracts of *Quercus infectoria* on the isolated bacterial species. The minimum inhibitory concentration (MIC) of ethanolic extract on *Staphylococcus aureus* was 3.125 mg/ml and that effect on *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* were 6.25 mg/ml while the aqueous, methanolic and ethanolic extracts had effect at 25.0, 12.5 and 6.25 mg/ml respectively on *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumoniae* respectively.

### **Introduction**

A wound is a breach in the skin or mucosa membrane, which may allow the entry of microorganisms, possibly leading to infection (Bowler *et al.*, 2001). Wound tissue provides the rich environment necessary for the proliferation of microbes. It is characterized by hypoxia, necrosis and often an accompanying impaired immune response owing to suboptimal delivery of immune effectors molecules through damaged blood vessels (Bowler *et al.*, 1999). This compromised, necrotic, slough tissue provides a warm, moist and nutritive environment, perfect for replication of colonizing bacteria. Bacteria species which were previously harmless commensally of the human body, most commonly on the skin, may become pathogenic in a wound environment (Robson, 1997). Wound healing is the process of repair that follows injury to the skin and other soft tissues. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis

of collagen and other extracellular matrix which are later remodeled to form scar (Shittu *et al.*, 2002). 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 (Odimegwu *et al.*, 2008) . Some medicinal plants have been employed in folk medicine since time immemorial for wound care (Muhammad & Muhammad, 2005; Samy *et al.*, 2006 and Kudi & Ngbede, 2006). 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. *Quercus infectoria* is one of such plants employed by herbalists in the treatment of sores and boils. In this research, *Quercus infectoria* was studied in order to investigate its antibacterial properties. *Quercus infectoria* is a small tree or a shrub belonging to the Fagaceae (Quercaceae) family. They are found in the Mediterranean area, mainly in Greece, Asia Minor, Syria and Iran. The galls arise on young branches of this tree as a result of attack by the gall-wasp *Adleria gallae-tinctoria* (Dor, 1976). The galls of *Q. infectoria* have been shown to have many medicinal properties such as astringent, antidiabetic, antifungal, antiviral, antibacterial, larvicidal and anti-inflammatory activities (Digrak *et al.*, 1999; Hwang *et al.*, 2000; Kaur *et al.*, 2004 & Rahman *et al.*, 2006). The chemical constituents of the galls have been reported to comprise a large amount of tannins and small amounts of free gallic acids, ellagic acid and synergic acid (Dor, 1976, Ikram & Nowshad 1977). The purpose of this study was to elucidate the pattern of bacteriological isolates, which are responsible for wound infections and to evaluate the *in vitro* antibacterial activity of aqueous, ethanol and methanol extracts against bacterial species isolated from surface wounds.

## **Material and methods**

### **Collection of wound swabs and identification:**

Surface wound swabs were collected from 190 patients attending Hawler Ferkari Lab and Emergency Hospitals in Erbil 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 swab

samples were inoculated on Blood agar, MacConky agar and Chocolate 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 (Norrell & Messley, 1997).

#### **Disc diffusion method:**

Antimicrobial susceptibility was performed on Mueller Hinton agar by the standard disc diffusion method recommended by the National committee for clinical laboratory standards (NCCLS) using the following antibiotics: Gentamicin (10µg) , -Ceftazidime (30µg),-Amoxillin(25µg), - Rifampin (5µg), - Ofloxacin (5µg), - Vancomycin (30µg), - Doxycycline (30µg), - Ciprofloxacin (5µg), - Clindamycin (2µg), - Amikacin (15µg),- Imipenem(10µg),-Cefotaxime (30µg),- Trimethoprim / Sulphamethoxazole 20µg),- Polymyxin(30µg), - Ampicillin (50µg), -Erythromycin(15µg) . The surface of the Mueller-Hinton agar was inoculated with the isolated species. High potency discs were placed on the agar. After 18 hours of incubation, the plates were examined and the sensitivity result was interpreted according to NCCLS (NCCLS, 2006).

#### **Preparation of extracts:**

The galls of *Q. infectoria* used in this study were collected from different area of Erbil city. The galls were washed with distilled water, and dried in air. The galls were crushed in mechanical mortar. Aqueous, methanol and ethanol extractions were performed by the following method. 50 gm of gall powders were used with 300 ml of solvents with an extraction period 24-72 hours. The extracts were filtered using filter paper and the solvents were evaporated using rotary distillation apparatus. In order to obtain a completely dry extract, the resultant extracts were transferred to glass dishes, and were left in 50 C° ovens for 24 hours. Then, they were left at 4 C° until assessment of their antimicrobial activities (Mashhadian & Rakhshandeh, 2004).

#### **Minimum inhibitory concentration (MIC):**

A quantity of 0.2 g of each extract was dissolved in 4 ml sterile nutrient broth which yields an initial concentration of 50 mg/ml. Subsequently, two folds serial dilution were made from the stock of 4 ml containing 50 mg/ml. Nutrient broth was used to obtain the following concentrations 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390 and 0.195mg/ml. One milliliter of standardized inoculums ( $1 \times 10^8$  cells/ml) of each isolated species was

introduced into each extract nutrient broth mixture and then incubated at 37C° for 24 h. The MIC was considered the lowest concentration of the extract that prevents visible growth in the liquid media (NCCLS, 2006).

## **Result**

Two hundred and forty one bacterial isolates were recovered from various infected wounds averaging 1.35 bacteria per specimen. Positive growth was observed in 93.68% of wound cultures, 119 (62.63%) solitary isolates were cultured from as many wounds whereas twin and triple isolates were cultured from 55 (28.94%) and 4 (2.11%) wounds respectively and only twelve (6.32%) wound swabs were sterile Table(1), Table (2) showed the types of organisms cultured from the surface wounds. The most frequently predominant bacterial isolate was *Staphylococcus aureus* 79 (32.78%) followed by *Pseudomonas aeruginosa* 60 (24.90%), *Escherichia coli* 36 (14.94%), *Enterobacter* spp. 24 (9.96%), *Proteus mirabilis* 21 (8.71%), *Klebsiella pneumoniae* 16 (6.64%), *Klebsiella oxytica* 3 (1.24%) and *Citrobacter freundii* 2 (0.83%). Antibiotic sensitivity patterns revealed that many of the isolated species were resistant to commonly used antibiotics like Gentamicin, Ampicillin, Amoxicillin/Clavulanic acid, Rifampin, Doxycycline, Trimethoprim + Sulfamethoxazole, Polymyxin and Clindamycin which are being indiscriminately used as empirical basis for prolonged duration of time Table(3). The most effective antibiotics for *Staph. aureus* were Vancomycin (98.73%). Amikacin (73.41%) and Clindamycin (69.62%), while the most effective antibiotic for the Gram-negative (*K. pneumoniae*, *E. coli*, *Enterobacter* spp, and *Pr. mirabilis*) were Ciprofloxacin, Ofloxacin, Imipenem, Amikacin and Cefotaxime. *Pseudo. aeruginosa* was most sensitive to Polymyxin (93.33%), Imipenem (70.0%) and Amikacin (63.33%). The highest degree of multidrug resistance to all the drugs was found in *Pseudo. aeruginosa*. Minimum inhibitory concentration (MIC) of Aqueous, methanol and ethanol extracts from *Q. infectoria* against all tested organisms was shown in Table (4). The results showed that The minimum inhibitory concentration (MIC) of ethanolic extract on *Staphylococcus aureus* was 3.125 mg/ml and that effect on *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* were 6.25 mg/ml while the aqueous, methanolic and ethanolic extracts had effect at 25.0, 12.5 and 6.25mg/ml respectively on *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumoniae* respectively.

Table (1): Distribution of bacterial isolates cultured

Isolates	Number (n = 190)	Percentage%
Solitary	119	62.63
Twin	55	28.94
Triple	4	2.11
Nil	12	6.32

Table (2): Bacterial species recovered from patients wound and their frequency

Bacterial species	Frequency of isolation	Percentage %
<i>Staphylococcus aureus</i>	79	32.78
<i>Pseudomonas aeruginosa</i>	60	24.90
<i>Escherichia coli</i>	36	14.94
<i>Enterobacter Spp.</i>	24	9.96
<i>Proteus mirabilis</i>	21	8.71
<i>Klebsiella pneumonie</i>	16	6.64
<i>Klebsiella oxytoca</i>	3	1.24
<i>Citrobacter freundii</i>	2	0.83
Total	241	100

Table (3): Sensitivity of bacterial species against various antibiotics:

Antibiotic (final conc.)	N=79(%) 1	N=60(%) 2	N=36(%) 3	N=24(%) 4	N=21(%) 5	N=16(%) 6	N=3(%) 7	N=2(%) 8
Gentamycin	2(2.53)	0.0	4(11.11)	2(8.33)	1(4.76)	2(12.5)	0.0	0.0
Ceftazidime	-	8(13.33)	6(16.66)	8(33.33)	10(52.38)	4(25.0)	0.0	0.0
Amoxillin	7(8.86)	0.0	2(5.55)	0.0	11(52.38)	2(12.5)	0.0	0.0
Rifampin	4(5.06)	0.0	6(16.66)	0.0	0.0	0.0	0.0	0.0
Ofloxacin	5(6.32)	6(10.0)	27(75)	18(75.0)	18(85.71)	9(56.25)	2(66.66)	1(50.0)
Vancomycin	78(98.73)	-	-	-	-	-	0.0	0.0
Doxycycline	3(3.79)	1(1.66)	0.0	5(20.83)	0.0	1(6.25)	0.0	0.0
Ciprofloxacin	-	0.0	28(77.77)	20(83.33)	19(90.47)	10(62.5)	2(66.66)	0.0
Clindamycin	55(69.62)	3(5.0)	0.0	1(4.16)	2(9.52)	-	0.0	0.0
Amikacin	58(73.41)	38(63.33)	21(58.33)	22(91.66)	18(85.71)	11(68.75)	3(100.0)	1(50.0)
Imipenem	-	42(70.0)	29(80.55)	18(75.0)	18(85.71)	14(87.5)	3(100.0)	2(100.0)
Cefotaxime	-	0.0	18(50)	14(58.33)	11(52.32)	13(81.25)	0.0	0.0
Trimethoprim/Su lphamethoxazole	-	0.0	2(5.55)	1(4.16)	0.0	0.0	0.0	0.0
Polymyxin	-	56(93.33)	3(8.33)	0.0	0.0	1(6.25)	0.0	0.0
Ampicillin	1(1.26)	-	1(2.77)	0.0	1(4.76)	2(12.5)	0.0	0.0
Erythromycin	27(34.17)	-	-	-	-	-	0.0	0.0

N= Number of isolated species, 1-*Staphylococcus aureus*, 2- *Pseudomonas aeruginosa*, 3- *Escherichia coli*, 4- *Enterobacter spp.*, 5- *Proteus mirabilis*, 6- *Klebsiella pneumonie*, 7- *Klebsiella oxytoca*, 8- *Citrobacter freundii*.

Table (4): Minimum inhibitory concentration (MIC) of the crude extracts of *Quercus infectoria* on wound bacterial species:

Microorganisms	Minimum inhibitory concentration (mg/ml)		
	Aqueous	Methanol	Ethanol
<i>Staphylococcus aureus</i>	12.5	6.25	3.125
<i>Pseudomonas aeruginosa</i>	25.0	25.0	25.5
<i>Escherichia coli</i>	12.5	6.25	6.25
<i>Enterobacter spp.</i>	12.5	12.5	6.25
<i>Proteus mirabilis</i>	12.5	12.5	12.5
<i>Klebsiella pneumoniae</i>	6.25	6.25	6.25
<i>Klebsiella oxytoca</i>	6.25	12.5	0.781
<i>Citrobacter freundii</i>	12.5	6.25	6.25

## **Discussion**

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. In the present study, the microbiological analysis reveals that *Staph. aureus* is the leading etiologic agent of wound infection with previous reports (Onche & Adedeji, 2004, Khorvash *et al.*, 2008, Isibor *et al.*, 2008) but is in contrast to other studies which report *Pseudo. aeruginosa* as predominant organisms (Agnihotri *et al.*, 2004, Anupurba *et al.*, 2008). *Staph. aureus* is the most frequently isolated microorganism from body surface and this could easily be introduced into the wound either by the patients dressing materials or through the object that causes the injury. Microbiological investigations have noted that this organism is the single causative bacterium in approximately 25% to 69% of cutaneous abscess (Meislin *et al.*, 1977, Brook & Finegold, 1981), and the same microorganism has also been recognized as the most frequent isolate in superficial infections seen in Hospital accident and Emergency Department. The second most frequent organism cultured in this study was *Pseudo. aeruginosa* (24.90%) followed by *E. coli* (14.94%) and *Enterobacter spp.* (9.96%). These results are in accordance with other studies (Agnihotri *et al.*, 2004 & Masaadeh & Jaran, 2008). This microorganism has, since the mid. Twentieth century, been held responsible for the majority of invasive wound infections in many hospitals worldwide (Olayinka *et al.*, 2004 & Rastegar *et al.*, 2005). In general the increase rate of occurrence of *Pseudo. aeruginosa* is not unrelated with indiscriminate use of antibiotics without laboratory

diagnosis and antibiotic sensitivity report. This single factor could eliminate the normal flora and provide a non-competitive environment for *Pseudo. aeruginosa*. The resistant nature of this organism to antimicrobial agents, nutritional versatility and the difficulties encountered in maintaining proper hygienic standards especially among personal involved with wound dressing and general care of patients may have contributed to the high rate of *Pseudo. aeruginosa* infection (Oguntibeju & Rau, 2005). Antibiotic sensitivity patterns revealed that many of the isolates were resistant to commonly used antibiotics. The results indicated that there was a significantly high percentage resistance among Gram-negative bacilli to aminoglycosides like Gentamicin, Amikacin, Amoxicillin, Ciprofloxacin, Ceftazidime and Cefotaxime. This alarming trend was also seen for *Staph. aureus* and *Pseudo. aeruginosa*. A similar results of multidrug resistant Gram-positive and negative bacteria to various antibiotic routinely used has been reported from several studies (Howell-Jones *et al.*, 2005; Basri, & Fan, 2005 & Dhar *et al.*, 2007). Since high antimicrobial resistance is probably promoted due to selective pressure exerted bacteria due to numerous reasons like non adherence to hospital antibiotic policy, and excessive and indiscriminate use of broad-spectrum antibiotics. These multi drug resistant strains establish themselves in the hospital environment in area like sinks, taps, railing mattress, toilets and thereby spread from one patient to another. In this study the results of the investigations show that the three extracts from *Q. infectoria* possess antimicrobial activities against tested microorganisms that are involved in causing wound infections at a concentration varied between 0.781 mg/ml and 25 mg/ml. These findings therefore support the use of this plant in the management of wound infection. Ethanolic extracts showed the strongest activity followed by methanolic extracts and aqueous extracts an indication that ethanol is a better extractant than the two other solvents used in this study 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 (Vorovuthikuchai *et al.*, 2004; Makkar *et al.*, 2006 & Muskhazli *et al.*, 2008). The inhibitory effects of gall nut may be due to the presence of some phytochemical components, and based on previous studies, *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 (Hagermant & Butler, 1981). 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.

## References

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## الفعالية التثبيطية لنبات العفص *Quercus infectoria* ضد البكتريا المعزولة من اصابات الجروح

سهيلة نافع داروغه

كلية تربية العلوم – جامعة صلاح الدين / اربيل

### الخلاصة

حددت هذه الدراسة الطبيعة البكتيرية لاستعمار الجروح في ١٩١ مريضا ممن يشكون من اصابات في الجروح و المراجعين للمختبر الداخلي التابع لمستشفى اربيل التعليمي و مستشفى الأميرجيني الطواري في مدينة اربيل للفترة من ١/ كانون الثاني/ ٢٠٠٧ الى ٣١/ آب / ٢٠٠٧. تم تشخيص ٢٤١ عزلة بكتيرية بعد استنبات المسحات على اوساط زرعية مختلفة وكانت البكتريا الاكثر شيوعا وتكرارا من العزلات *Staphylococcus aureus* بنسبة ( ٣٢,٧٨%) تلتها *Pseudomonas aeruginosa* ( ٢٤,٩٠%)، *Escherichia coli* (١٤,٩٤%)، *Enterobacter spp.* (٩,٩٦%)، *Proteus mirabilis* (٨,٧١%)، *Klebsiella pneumoniae* (٦,٦٤%)، *Klebsiella oxytoca* (١,٢٤%) و *Citrobacter freundii* (٠,٨٣%). وتم التحري عن حساسية البكتريا الملوثة للجروح تجاه ١٦ مضادا حيويا ضمن مجاميع مختلفة وكانت النتائج مقاومة عالية للعزلات البكتيرية تجاه المضادات الحيوية. تضمنت هذه الدراسة ايضا معرفة التأثير الاحيائي للمستخلص المائي، الميثانولي والايثانولي لنبات العفص *Quercus infectoria* على العزلات البكتيرية من مسحات الجروح. اظهرت النتائج ان التركيز المثبط الادنى للنمو MIC لبكتريا *Staphylococcus aureus* كانت ٣,١٢٥ ملغم/مل، *Escherichia coli*، *Klebsiella pneumoniae* و *Citrobacter freundii* كانت ٦,٢٥ ملغم/مل للمستخلص الايثانولي في حين كان التركيز المثبط الادنى لنمو *Pseudomonas aeruginosa* و *Proteus mirabilis* و *Proteus mirabilis* ٢٥,٠ ملغم/مل، ١٢,٥ ملغم/مل و ٦,٢٥ ملغم/مل على التوالي للمستخلصات الثلاثة.