



Antibacterial Effect of Alcoholic Extract of *Juglans regia* L. stem bark and Inner Stratum of Oak Fruit (Jaft) on *Staphylococcus aureus* isolated from wound infection

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ABSTRACT

Staphylococcus aureus can cause a wide spectrum of infections ranging from wound infections to life threatening systemic illnesses. These bacteria have multi drug resistance and can be difficult to treat. This study included a total of 20 *Staphylococcus aureus* isolated from wound infection, identification of *Staphylococcus aureus* was based on growth on manitol salt agar, DNase test, coagulase test. Alcoholic extract of inner stratum of Oak (Jaft) and stem bark of *Juglans regia* L. were tested for the activity against this bacterium by determination the Minimum Inhibitory Concentration (MIC). The results showed that both Jaft and *Juglans regia* L. stem bark extract have effect against *Staphylococcus aureus*, the values of MIC of Jaft extract against isolates ranged between 0.39 -12.5mg/ml with average of 3.9 mg/ml, and the values of MIC of *Juglans regia* L. stem bark ranged between 0.39-12.5mg/ml with average of 6.3 mg/ml.

This study aimed to know the effect of alcoholic extract of inner stratum of oak (Jaft) and stem bark of *Juglans regia* L. on *Staphylococcus aureus* isolated from the wound infection by using MIC method.

Introduction

A wound infection can be resulted from microbial invasion that reaching the skin from the external source or from the bloodstream [1] the most common pathogens associated with wound infection are *Staphylococcus aureus*, *Streptococcus* species, *Pseudomonas aeruginosa* and anaerobes, some bacteria produce toxins (pyrogenic exotoxin) that cause wound infection such as *Staphylococcus aureus* [2].

Staphylococcus aureus is an important pathogens responsible for a variety of clinical disease ranging from wound infections[3] to endocarditis, osteomyelitis to pneumonia, and food poisoning to septic shock [4] *Staphylococcus aureus* strains release a number of pyrogenic toxins including enterotoxin, toxic shock syndrome toxin, exfoliative toxin A and B, hemolysin and panton valentine leukocidin [5], these toxins allow *Staphylococcus aureus* to adhere, avoid the immune system and cause harmful effect to the host [6], because the organisms rapidly develop resistance to many antibiotics drug, it is difficult to

eradicate pathogenic *staphylococci* from infected person [7]. There is a urgent need of antimicrobial substances due to rapid appearance of multiple drug – resistance pathogenic bacteria, herbal medicines are known as a protection system against pathogenic bacteria [8]. The genus *juglans* is from herbal medicines from family Juglandaceae, has been used in traditional medicines from ancient times. All parts of the plant such as shells, seeds, bark, kernel, leaves are used in the medicine and beauty industry [9]. *Juglans regia* L. stem bark have been described as anticancer activities, antimicrobial, detergent, diuretic, anthelmintic, laxative and is a good gargle for a mouth disease and for throat inflammation also used in the treatment of tuberculosis and tuberculosis of cervical glands, it is reported that bark contains ketones such as juglone, regiolone, sterol and flavonoid [10]. Phenolic compounds of *Jugland regia* L. are important for human health by reduction of oxidative stress and inhibition of macromolecular

oxidation, and they are also useful for treatment of fungal infection in skin [11].

Oak is also extracted from herbal medicines that uses in this study which exists in many mountainous regions of the world is contain many major component such as tannin, flavonoid, Alkaloid and several aromatic component. Inner stratum of oak (Jaft) is used in healing wound [12]. The fruit of oak was used in traditional medicin, people in Iran use Jaft for treatment of microbial infection from antient times such as vaginitis, researchs indicate that Jaft has antibacterial and antifungal activity by its effective content [13]. It was supposed that in the near future the Jaft extract may become a new substances to control oxidative stress pathogenesis and inflammatory disease[14].

The aim of this study was to evaluate antibacterial effect of alcoholic extract of Jaft and *Juglans regia* L. stem bark against *Staphylococcus aureus* isolated from a wound infection by using Minimum Inhibitory Concentration (MIC) method.

Metrials and method

1- Collection of bacteria

20 strains of *S. aureus* were isolated from patient with a wound infection (from Tikrit Military Hospital) of both genders and at different age groups using cotton swabs. samples were streaked immediately on blood agar and identification of *S. aureus* strains were based on growth in manitol salt agar and also by measuring production of catalase, DNase test ,slide and tube coagulas test (7).

2-Preparation of ethanolic extract of Jaft and *Juglans regia* L. stem bark

The stem bark of *Juglans regia* L. and Jaft was obtained from a local market and were ground to powder . Fourty gram of each powder were solved in 160 ml of ethanol 95% and were placed in room temperature for 24 hours, the solvents were filtered by Whatman filter paper and then the extract was put in petri dish at room temperature for drying. The residues obtained were stored in a freezer until future tests (15).

3-Determination of Minimum Inhibitory Concentration (MIC).

Broth dilution methods were used to determine MIC .Cultures of *S. aureus* were prepared in Brain-heart infusion broth and incubated in 37 C for 24h and then adjusted to Mcfarland turbidity standard (0.5). One gram of alcoholic extract was mixed with 10 ml of D. W. sterile for making stock of 100mg/ml. Aserial dilution of extract was prepared in steril distilled water (50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.195 mg /ml) then inoculated with *S.aureus* and incubated at 37 C for 24 h. The concentration of the lowest dilution with no detectable bacterial growth was considered as MIC. Absence of growth confirmed by absence of turbidity (16).

Result

In this study, the MIC method was evaluated for the antimicrobial activity of ethanolic extracts of Jaft and

Juglans regia L. stem bark against *S. aureus* isolates and the results were shown in table (1) and (2), according to the obtained MIC values, ethanol extract of Jaft indicate activity against all the *S. aureus* isolates and the values of MIC ranged between 0.39 to 12.5 mg/ml with average of 3.9 mg/ml, also ethanol extract of *Juglans regia* L. showed activity against all *S. aureus* isolates and the values of MIC ranged between 0.39 to 12.5 mg/ml with average of 6.3 mg/ml.

Table (1) : MIC values of ethanolic extracts of Jaft against *S.aureus*

| Isolates of <i>S.aureus</i> | Values of MIC mg/ml |
|-----------------------------|---------------------|
| 1 | 0.78 |
| 2 | 1.56 |
| 3 | 1.56 |
| 4 | 1.56 |
| 5 | 0.78 |
| 6 | 0.39 |
| 7 | 3.12 |
| 8 | 3.12 |
| 9 | 6.25 |
| 10 | 3.12 |
| 11 | 3.12 |
| 12 | 1.56 |
| 13 | 6.25 |
| 14 | 0.78 |
| 15 | 6.25 |
| 16 | 6.25 |
| 17 | 12.5 |
| 18 | 6.25 |
| 19 | 1.56 |
| 20 | 6.25 |

Table (2) : MIC values of ethanolic extracts of *Juglans regia* L. stem bark against *S.aureus*

| Isolates of <i>S.aureus</i> | Values of MIC mg/ml |
|-----------------------------|---------------------|
| 1 | 12.5 |
| 2 | 3.12 |
| 3 | 12.5 |
| 4 | 6.25 |
| 5 | 6.25 |
| 6 | 12.5 |
| 7 | 0.39 |
| 8 | 6.25 |
| 9 | 3.12 |
| 10 | 6.25 |
| 11 | 12.5 |
| 12 | 6.25 |
| 13 | 6.25 |
| 14 | 6.25 |
| 15 | 0.78 |
| 16 | 6.25 |
| 17 | 3.12 |
| 18 | 3.12 |
| 19 | 1.56 |
| 20 | 12.5 |

Discussion

Traditional treatment is cheaper and have more effect than the modern one,in this study evaluating the

antibacterial activity of Jaft and stem bark of *Juglans regia* L. on *S. aureus*. The MIC values indicate that the Jaft extract is potent against *S. aureus*, in many studies antimicrobial evaluation of different extract of oak indicates activity of oak against bacteria, this extract contain the potential content such as tannin, oil, gersit ,amidand and nitosan, antimicrobial activity of Jaft may involve complex mechanisim like the inhibition of cell wall, nucleic acid, cell membrane and inhibition of metabolism. Oak extract showed activity against some microbes such as *E. coli*, *S. aureus*, *Bacillus subtilis*, *Micrococcus leutus*, *Bodetella branchiceus* and *Candida albicans*[13]. In wound infection treatment, antiseptic solutions such as betadine and acetic acid, which are poisoning for fibroblasts, lymphocytes and the cell which need to be repaired. Oak is among plants that contains tannin, and researchs have explained that tannin is a good substances in healing wound and to regeneration of skin by increase of fibroblast proliferation, fibroblast is responsible for manufacture of collagen fibers [12].

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In this study also *Jugland regia* L. stem bark showed the potential effect on *S. aureus* isolates that indicate can be used this extract in treatment of wound infection. In several studies the extract of *Juglans regia* L. showed inhibition activity of growth of many pathogenic bacteria such as *S.aureus*, *Streptococcus mutans*, *E coli* and *Pseudomonas aeruginosa*, *Juglans regia* L. bark in many studies analyzed chemically it contain essential oil, phenolic compound, flavonoid, alkaloid, terpenoid and steroid, and all these chemical composition has antimicrobial activity [9]. Moori Bakhtiari and Khalafi (2015) in Iran reported the antibacterial effects of *Juglans regia* L. stem bark hydro-alcoholic extract on *S. aureus* ,the values of MIC was 62.5 mg/ml for *S.aureus* ,and also reported that this plant was more effectively in gram positive than gram negative.

Thruhout the investigation, it is significant to use antimicrobial substances in treatment a wound infection.

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التأثير الضد البكتيري لمستخلص الكحولي للحاء ساق الجوز والغلاف الداخلي لفاكهة البلوط (الجفت)
على العنقوديات الذهبية المعزولة من أخماج الجروح

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الملخص

العنقوديات الذهبية تسبب طيف واسع من الاصابات يشمل من أخماج الجروح الى أمراض جهازية مهددة للحياة. تمتلك هذه البكتيريا مقاومة متعددة للمضادات الحيوية وتكون صعبة في المعالجة. تضمنت الدراسة جمع (20) عزلة من بكتيريا العنقوديات الذهبية من أخماج الجروح أتمتت تشخيصها على نموها في وسط ماننتول الملحي واختبار محلل الدنا واختبار أنزيم الخثرة. أختبرت فعالية المستخلص الكحولي لغشاء الداخلي لفاكهة البلوط (الجفت) ولحاء ساق الجوز تجاه بكتيريا العنقوديات الذهبية بطريقة تحديد التركيز المثبط الأدنى للبكتيريا. أظهرت النتائج بأن كلا المستخلص الكحولي للجفت ولحاء ساق الجوز لهما تأثير فعال تجاه العنقوديات الذهبية حيث تراوحت قيم (MIC) لمستخلص الجفت من 0.39 الى 12.5 ملغرام/ مل وبمعدل 3.9 ملغرام / مل أما قيم (MIC) لمستخلص لحاء ساق الجوز تراوحت من 0.39 الى 12.5 ملغرام / مل و بمعدل 6.3 ملغرام / مل.

هدفت الدراسة الى معرفة تأثير المستخلص الكحولي للغشاء الداخلي للبلوط (الجفت) ولحاء ساق الجوز على بكتيريا العنقوديات الذهبية المعزولة من أخماج الجروح باستخدام طريقة (MIC).