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The antimicrobial activity of alcoholic and aqueous extracts of pomegranate fruit peel on *Staphylococcus aureus* isolated from hair in Najaf

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Abstract :

There has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms and many compounds of plant products have been shown to be specifically targeted against antibiotic resistant pathogens. The present study was aimed to evaluate the antimicrobial activity of the alcoholic and aqueous extract of pomegranate peel against *Staphylococcus aureus* isolated from hair. The plant extracts prepared and The antimicrobial effects of the pomegranate extracts were evaluated using method disk inhibition zone , the results shows that the extracts of pomegranate have a good antibacterial growth activity against *Staphylococcus aureus* isolated from hair with significant differences ($P<0.05$) with concentrations of the type extract in which there are more susceptible to 100 mg/ ml(the highest concentration) and the alcoholic extract.

الفعالية ضد جرثومية للمستخلص الكحولي والمائي لقشور ثمرة نبات الرمان على *Staphylococcus aureus* المعزولة من شعر الرأس في الإنسان في محافظة النجف الاشرف

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الخلاصة :

تزايد الاهتمام باستخدام المواد النباتية للسيطرة على نمو الأحياء المجهرية المرضية ، إذ وجد العديد من المركبات المستخرجة من المنتجات النباتية التي لها هذه القابلية خاصة ضد البكتريا المقاومة للمضادات. والدراسة الحالية تهدف إلى تحديد الفعالية التثبيطية للمستخلصات المائية والكحولية لقشرة ثمرة نبات الرمان ضد البكتريا المرضية *Staphylococcus aureus* المعزولة من شعر راس الإنسان. تم تحضير المستخلصات النباتية ودرست الفعالية التثبيطية بواسطة طريقة قياس منطقة تثبيط القرص وأظهرت النتائج بان المستخلصات النباتية (الماء المغلي و الكحول الايثيلي) لديها القدرة على تثبيط البكتريا مع وجود فروق معنوية ($P<0.05$) بين التراكيز المستخدمة ونوع المستخلص المستخدم ، إذ كانت البكتريا أكثر حساسية لأعلى تركيز 100 ملغ / مل وكذلك للمستخلص الكحولي أكثر من المستخلص المائي.

Introduction :

The development of drug resistance in pathogens against commonly used treatment has necessitated a search for new therapeutic

agents from other sources. Recently , there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms⁽¹⁾.The

systemic screening of antimicrobial plant extracts represents a continuous efforts to find new compounds with the potential to act against multi – resistant pathogenic bacteria and fungi⁽²⁾.

Many plants found in the world have been submitted to pharmacological tests like pomegranate (*Punica granatum L.*), which is considered one of the oldest known edible fruit that is mentioned in Koran , the Bible , the Jewish Torah and the Babylonian Talmud as “ Food of God ” that is symbolic of plenty, fertility and prosperity⁽³⁾. In ancient medicine pomegranate is considered “ a pharmacy unto itself “⁽⁴⁾ that for example its used in wound healing properties , dried fruit peel is used for diarrhea⁽⁵⁾ , also several publicity using of pomegranate in Iraq and other countries , one of them , it is used to treat skin and hair.

However , to date , very few studies have been conducted on the antimicrobial activity of pomegranate peel , therefore the present study was aimed to evaluate the antimicrobial activity of the alcoholic and aqueous extract of pomegranate peel against *Staphylococcus aureus* isolated from hair.

Material & methods:

Plant collection : Ripened and freshly harvested pomegranate fruits (season October 2012) were obtained randomly from local market in Najaf. The collected fruit peels were washed under running tap water , air dried , homogenized to a coarse powder in an electric grinder and stored in air – tight bottles at 4°C.

Preparation of plant extracts :

Coarse powdered *P. granatum* peel was extracted by ethanol (70%) using 100 gm powder in 200 ml ethanol in an electric blender at room temperature for 30 min. This suspension was filtered three times for removal of peel particles. Then ethanol was

removed in a rotary evaporator to produce a dry powder , the extract was stored in refrigerator at 4°C until use. The final material was dissolved in ethanol for obtaining concentrations⁽⁶⁾ 25(1) ,50 (2) and (3) 100 mg/ml . The aqueous extract (boiling water) obtained with the same method mention with ethanol extract.

Bacterial isolates :

Staphylococcus aureus were obtained from hair and incubated for 24 hours and colonies are isolated and then confirmed using staining and bacteriological tests .

A nutrient agar , blood agar and manitol salt agar plates are used to incubated the *Staphylococcus aureus*.

Antimicrobial activity:

The antimicrobial effects of the pomegranate extracts were evaluated using method namely, disk inhibition zone. In disk inhibition zone method, the Mueller-Hinton agar medium was inoculated with freshly prepared cells of *Staphylococcus aureus* bacteria to yield a turf of growth. After solidification of the agar, a number of sterilized disks were dipped into the solvents (negative controls) or extract solutions and placed on the plates. After incubation at 37°C for 24h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using commercial disks⁽⁷⁾ , streptomycin & chloramphenicol.

Statistical analysis :

The data of our study were collected and calculated by Chi- test X^2 to determinate the significance differences among these data.

Results:

Bacterial isolates from hair that we studied passed through many tests after staining to ensure that we dealing with of *Staphylococcus aureus* , table 1. showed the result of these tests.

Table (1): The result of bacteriological tests on *Staphylococcus aureus*

Test name	Result
Catalase	+
Oxidase	-
Urease	-
Coagulase	+

Pomegranate peel ethanolic , aqueous extract showed various degrees of inhibition against the growth of investigated microorganism (*S. aureus*) as shown in figure 1 showed that there were significant differences ($P < 0.05$) among inhibition effects on the tested microorganism in contrast with the concentrations and the type of extract. Hence, the inhibitory effect of Pomegranate peel ethanolic , aqueous extract increased by increasing their concentrations. In the other hand the ethanolic extract is more effective than aqueous extract.

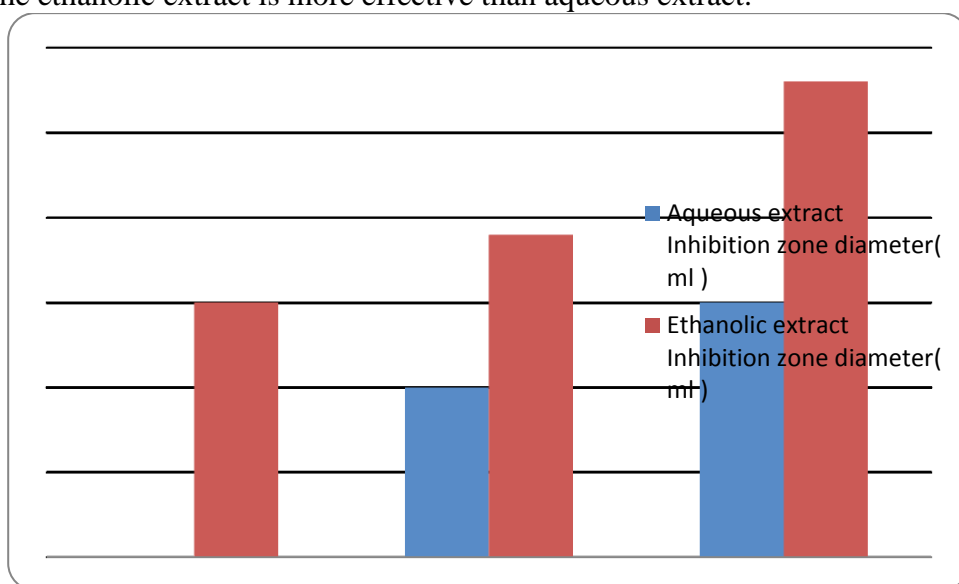


Figure 1 : The inhibitory effect of Pomegranate peel ethanolic , aqueous extract.

The ethanolic extract in our study showed that 100 mg/ ml concentration of pomegranate plant is best inhibition agent to bacteria *Staphylococcus aureus* than antibiotic (Figure 2).

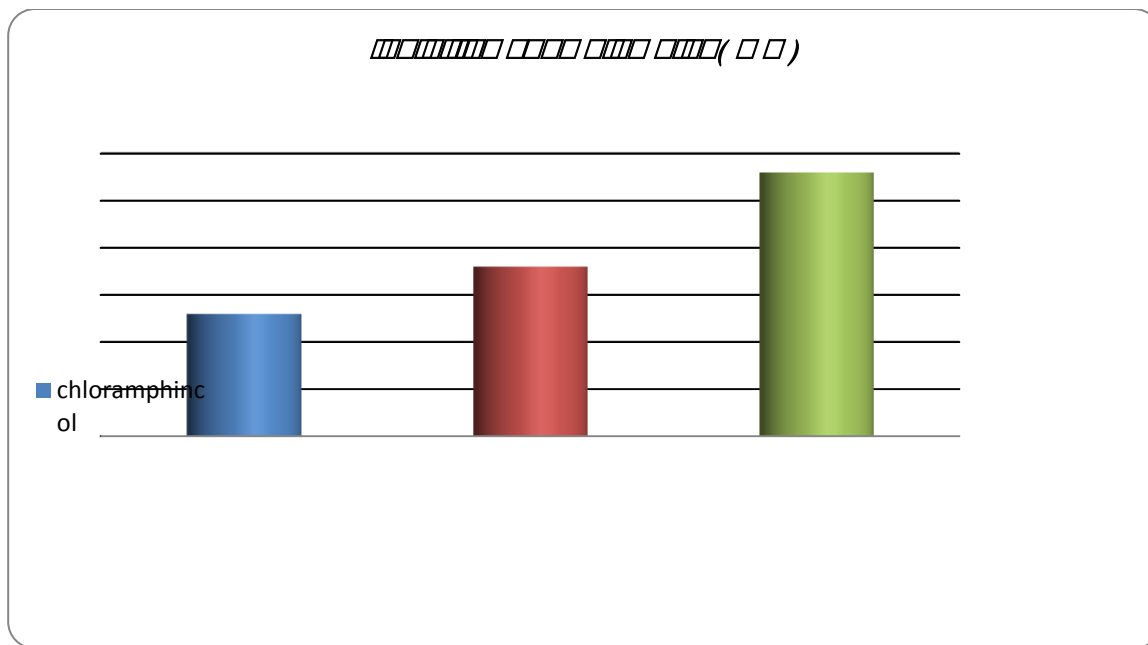


Figure 2: The difference between ethanolic peel extract and antibiotic action on bacteria *Staphylococcus aureus*.

Discussion :

Plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of chemo preventive drug discovery and development⁽⁸⁾. It has been observed that many plant polyphenols, such as ellagic acid, catechins, and chlorogenic, caffeic and ferulic acids act as potent antioxidant, antimutagenic and anticarcinogenic agents

In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from the plant raw material used. Pomegranate peel is one of the important dietary sources of antioxidant phenolics. Increase of antibiotic resistance as well as undesirable side effects of synthetic drugs have triggered immense interest in the search for new antimicrobial agents of plant origin. *Staphylococcus aureus* (or *Staph. aureus*) is a type of bacteria commonly found on the skin and hair as well as in the noses and throats of people and animals, these bacteria are present in up to 25 percent of healthy people and are

even more common among those with skin, eye, nose, or throat infections⁽⁹⁾.

Responsible for *staph* on the skin, it is usually harmless, however, infants, small children, the elderly and people with compromised immune systems are at risk of becoming ill from this bacteria, *staph* can cause a wide range of infections. It causes the skin infection impetigo, staphylococcal pneumonia, sepsis and toxic shock syndrome⁽¹⁰⁾.

In the present study, extracts of pomegranate fruit skin have been tested against bacteria (*S. aureus*). Under the conditions employed here, the bacteria were found to be sensitive to both ethanolic and aqueous extracts. These findings are in accordance with the observations of McCarrell et al⁽¹⁰⁾. who found that aqueous macerated extract of pomegranate rind inhibits growth of *S. aureus*. Similarly, Al-Zoreky⁽⁸⁾ has reported that methanolic extract of pomegranate fruit peels is a potent inhibitor for *S. aureus*

Also, in the case of test bacteria, the antibacterial activity of ethanolic extract was started at lower concentration, as compared with aqueous one. So, it is reasonable to assume that the principal chemical constituents with antimicrobial activity were concentrated in the alcoholic fraction. This was in agreement with Ahmad *et al.* 1998⁽¹¹⁾ who found alcohol as a better solvent for extraction of antimicrobial active substances compared to water.

The active phytochemicals responsible for antimicrobial activity of pomegranate remained to be exactly elucidated. However, ellagitannin punicalagin is thought to be the primary constituent involved in the antimicrobial effects⁽¹²⁾. Pomegranate peel contains ellagic acid, ellagitannins and gallic acids. The presence of these polyphenols in the pomegranate peel may be responsible for antioxidant and anticarcinogenic effect of peel extracts⁽¹³⁾. Also, it can be suggested that the observed activity of pomegranate peel ethanol extract in our study due to the presence of these compounds.

Pomegranate peel extract was active and effective against the growth of tested microorganisms. Whereas, the inhibition zones ranged from < 10 to 28 mm depend on the action of plant extract on microorganism. These results provide evidence for the presence of antimicrobial phenolic compounds in pomegranate peel. These compounds can degrade the cell wall, disrupt the cytoplasmic membrane, damage membrane proteins and interfere with membrane-integrated enzymes, which may eventually lead to cell death⁽¹⁴⁾. The same observation was reported by Prashanth and colleagues⁽¹⁵⁾, they tested a number of extracts of pomegranate against a range of bacteria (*S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Bacillus subtilis* and *Salmonella typhi*), and found activity against all isolates. In a previous study, Opara *et al.* (2009)⁽¹⁶⁾ reported that

the best activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were found in fruit peel compound punicalagin, particularly from Oman, which was coincident with the highest levels of vitamin C detected in these samples. Similar findings were reported by Salgado *et al.* (2009)⁽¹⁷⁾ and Dahham *et al.* (2010)⁽¹⁸⁾ in which antibacterial and antifungal activities of pomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria and fungi were investigated. The antimicrobial effectiveness of the extracts depends on the species of bacteria evaluated, the more sensitive being the Gram-positive species *S. aureus* and *Bacillus sp.* along with *Aspergillus niger*.

In conclusion, the extracts from pomegranate fruit skin possess strong antimicrobial activity against the Gram positive bacteria *S. aureus*. Further isolation and purification of the extracts are required to determine the active components responsible for their activity. Although our results support the idea that pomegranate extracts are candidate for treatment of infectious diseases, clinical trials will be required to confirm its antimicrobial action and general safety.

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