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Assessing the Antibacterial Activity of Pomegranate against *Staphylococcus aureus* obtained from wound infections.

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ABSTRACT

Punica granatum, commonly known as pomegranate, has emerged as a medicinal plant with potential antimicrobial activity. The present study was planned to evaluate this activity against Gram positive *Staphylococcus aureus* (*S. aureus*). The extracts of pomegranate fruit skin were prepared using a solvent (**95%** ethanol). Antimicrobial effect of the extracts was studied and compared with commercial antibiotics using disk inhibition zone techniques. Pomegranate extracts showed high antibacterial activity against *S. aureus.* The antimicrobial activity against *S. aureus* was comparable with those of Clindamycin, Chloramphenicol, Gentamycin, and Vancomycin**.** The extracts from pomegranate fruit skin possess strong antimicrobial activity against the tested microorganism. Therefore this plant could be an important source of new antimicrobial compounds to treat bacterial and fungal infections.

Keywords: Antibacterial activity, Ethanol, Pomegranate, *Staphylococcus aureus*, wounds

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INTRODUCTION

Infectious diseases are still one of the leading causes of death in the world Sadeghian A et al., 2000. Although conventional drugs provide effective treatment for some infections, antibiotic resistance continues to grow among key microbial pathogens such as *Staphylococcus aureus (S. aureus*), *Pseudomonas aeruginosa, (P. aeruginosa*), *Streptococcus* spp, and *Enterobacteriaceae* Bax et al., 2000; Bhavnani and Ballow, 2000. Therefore, the search for new antimicrobial agents is imperative Sadeghian A et al., 2000.

Nowadays, infections with *S. aureus* strains, especially those caused bymethicillin resistant (MRSA) strains, have become a major problem, not only in hospitals, but also in the wider community Otter JA and French GL, 2010.

The pomegranate, *[Punica granatum* L.], is one of the oldest known edible fruits Lamar AS et al., 2008. This fruit is mentioned in the Bible and Koran and is often associated with fertility Duman AD et al., 2008. It is native to Persia and from there it spread into Asia, North Africa and Mediterranean Europe Duman AD et al., 2008. *Punica granatum* L. has been widely used by traditional medicine in America, Asia, Africa and Europe for the treatment of different types of diseases Duman AD et al., 2008.

The fruits of *Punica granatum* (pomegranate) have been used to treat acidosis, dysentery, microbial infections, diarrhoea, helminthiasis, haemorrhage, and respiratory pathologies Duman AD et al., 2008.

Most pomegranate fruit parts are known to possess substantial antioxidant activity Kaur G et al., 2006. The flower Kaur G et al., 2006, seed oil Nuamsetti T et al., 2012 , seed extract, and peel extract of pomegranate also have a potent antioxidant activity Nuamsetti T et al., 2012 .

The antimicrobial activity of some of the common pomegranate cultivars has also been studied Reddy MK et al., 2007 andJurenka J.2008.

Braga et al., 2005 showed that pomegranate extracts inhibit and delay *S. aureus* growth and subsequent enterotoxin production at 0.01, 0.05 and 1% v/v concentrations.

Melendez PA and Capriles VA, 2006 have also reported that extracts from *Punica granatum* fruits possess strong in vitro antibacterial activity against many bacterial strains tested. Therefore, the aim of this study was to determine antibacterial effect of pomegranate on *S. aureus* isolates obtained from wound infections.

METHODOLOGY

I- Pomegranate fruit and preparation of the extracts:

Pomegranate fruits were purchased from local markets in Duhok. After opening the fruit, the seeds were manually separated from the peels. Collected peels were then rinsed with tap water. These peels were ground in a blender. Fifty grams of blended peels were placed in 250ml flask, followed by adding 100 ml of solvent (95% ethanol). The flask was then shaken at room temperature for 18 h prior to filtration. The filtrate was concentrated under reduced pressure with an evaporator at 40 °C. These crude extract was kept at 4 °C until use, this extract of Pomegranate was considered as the (100%) concentration. Then the concentrations (75%, 50%, and 25 %) were made by diluting the concentrated extract of Pomegranate with suitable volumes of sterile distilled water.

II- Bacterial isolates:

Twenty isolates of *S. aureus* were subjected to Pomegranate extracts, the isolates of *S. aureus* have been obtained from wound infections in Azadi teaching hospital in Duhok city, and all samples were identified by routine conventional methods.

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III- Antimicrobial activity:

The antimicrobial effects of the pomegranate extracts were evaluated using disk inhibition zone method.

In disk inhibition zone method, the Mueller-Hinton agar medium was inoculated with freshly prepared cells of bacteria to yield a growth. After solidification of the agar, a number of sterilized disks were dipped into the extract solution and placed on the plates. After incubation at 37˚C for 24 h, 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 [Clindamycin, Chloramphenicol, Gentamycin, and Vancomycin].

RESULTS

Twenty isolates (20) of *S.aureus* were collected and transferred to the Microbiology laboratory, College of Medicine, University of Duhok, and cultured on different media. In the present investigation we used pomegranate extracts in different concentrations (100%, 75%, 50%, and 25%) by agar well diffusion method. The mean diameters of inhibition zones on *S. aureus* were measured in (mm) and the results were recorded (Table 1).

Table (1): Antimicrobial activity of different pomegranate concentrations on the growth of twenty 20) isolates of *S. aureus*.

Antibiotics	No. of isolates	Maximum inhibition zone (mm)
Clindamycin	10 (50%)	30
Chloramphenicol	4 (20%)	28
Gentamycin	4 (20%)	28
Vancomycin	2 (10%)	20
20 (100%)		

Commercial Antibiotic Sensitivity Testing: The bacterial isolates (*S. aureus* and *E. faecalis*) were also tested for their susceptibility against commonly used (commercial) antibiotics by modified Kirby-Bauer method (Table 2).

Table(2): Antibiotic sensitivity pattern with the inhibition zone for each antibiotic disk used against *S. aureus.*

Concentration of pomegranate extracts %	No. of isolates	Average diameter of inhibition zones (mm)
100	7 (35%)	36
75	6 (30%)	30
50	4 (20%)	26
25	3 (15%)	22
20 (100%)		

DISCUSSION

The emergence of drug resistance with patient's poor compliance, drugs adverse effects and the higher cost of therapy combinations, indicates a strong need for a therapy regimens with similar or higher antibiotics beneficial properties but with better adverse effects profiles Unnisa N etal.,2012.

Punica granatum, commonly known as pomegranate, has been highlighted in some studies as having this antimicrobial property Braga et al., 2005; Al- Zoreky, 2009.

The result of the present study indicates different concentrations of pomegranate extract exhibited different inhibition zone against *S. aureus*, (Table 1). The potency of pomegranate extracts on *S. aureus* ranging from (22-36) mm diameter zone of inhibition.

According to the dose response, the zone of inhibition was increased with increasing the concentration of pomegranate extracts. The Lowest concentration (50, and 25 mg/ml) were inhibited the bacteria weakly, while for the high concentrations of pomegranate extract (100, and 75mg/ml), the pomegranate extracts were recorded noticeable inhibition activity against bacteria.

The concentration (100 mg/ml) of pomegranate extract had the highest inhibitory effect about (36mm) inhibition zone for *S. aureus*. These results are in agreement with those previously published, for example, Melendez PA and Capriles VA, 2006 have reported that extracts from pomegranate fruits possess in vitro antibacterial activity against many bacteria tested showing an inhibition zones of (11-31) mm. Interestingly, they stated that in Puerto Rico, it is very common practice to use these plant extracts as remedies for colds and bacterial infections.

In India, Unnisa N et al., 2012, showed in their study that the ethanolic and aqueous extracts of pomegranate fruits showed high antibacterial activity when tested against *S. aureus* ranging from (23-26) mm diameter zone of inhibition.

A study by Al-Zoreky, 2009 showed the methanolic extract of pomegranate fruit has been induced antibacterial activity against *Listeria monocytogenes*, *S. aureus*, *Escherichia coli* and *Yersinia enterocolitica*.

The data from a South Africa study reported that methanol, ethanol, acetone, and water extracts obtained from pomegranate were active and effective against the tested microorganisms (*S. aureus, E. coli, Salmonella typhi, Vibrio cholera, S. dysenteriae, S. sonnei, S. flexneri, S. boydii*), showing an inhibition zones of 12-31 mm Mathabe et al., 2005 .

This study proved that the pomegranate extract has more effective antimicrobial agent than the antibiotics currently in use.

CONCLUSION

Besides having high antioxidant activity, pomegranate peels also have antibacterial activity and may be used as medicine for humans. This reduces the cost and the risk of antibiotic consumption. Furthermore, the peels which are the byproduct could provide health benefits to humans and may be employed in food preservation and pharmaceutical purposes.

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