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EVALUATION OF ANTIBACTERIAL ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF POMEGRANATE PEELS (*PUNICA GRANATUM* LIN.) AGAINST SOME BACTERIA

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ABSTRACT

Currently, uncontrolled and repeated use of antibiotic may be cause evolution of microbial resistance among pathogenic agents. Consequently, the use of new artificial and natural antimicrobial compounds is inevitable. In this study was designed to evaluated the antibacterial effectiveness of aqueous and methanol extract of pomegranate peels against six types of bacteria (*Staphylococcus aureus, Escherichia coli and Pseudomonas aeroginosa, Klebsiella pneumonia, Streptococcus pyogenes, Proteus mirabilis*). The antibacterial effectiveness of pomegranate peel extract was studied and compared with Tetracycline which used as Positive Control.The various Concentrations (25, 50,75, 100mg/ml) of the methanol and aqueous peel extracts were tested and Evaluations were done by using agar well diffusion technique for the inhibition zone determination. The results obtained were encouraging as the methanol extract showed greater zones of inhibition against the various bacteria tested in comparison with the aqueous extract. The greatest zone of inhibition induced by the action of pomegranate peel extracts was obtained from *Staphylococcus aureus* 22.33 mm at (100) mg/ml in methanol extract while the smallest zone of inhibition was obtained from *pseudomonas aerognosa* 5.5 mm at (25) mg/ ml in aqueous extract. The antibacterial effect of pomegranate peels extracts was more effectiveness against Gram-positive bacteria than Gram-negative bacteria.

KEYWORDS: Pomegranate peel, Tetracycline, methanol and aqueous peel extracts, Antibacterial activity.

INTRODUCTION

In recent years Interest has increased with medicinal plants and herbs for uses as sources to production of medical drugs or as effective materials that interference in the composition of the drug, different studies have been conducted around the influence of plant extracts on microbial growth and hence could be used in the treatment of some of the diseases resulting from microbes.[1]

The medical plants are important source to getting a diversity of therapeutic factors in depend of World Health Organization (WHO) and Many medicinal plants have been used as a medical source to a treatment of many diseases in everyday life of worldwide.[2]

The increase of antibiotic-resistant bacteria has led for the evolution of new antimicrobial drugs that are effective against these bacteria. Also, it has been large interest in the utilize of medicinal plant substances as an alternate medicine for treat some of diseases and several compounds of plant products have been exactly targeted against resistant bacteria.[3]

In addition to its olden historical uses for Pomegranate, also it has been generally used as a traditional drug to the therapy of different types of infections.[4]

Punica granatum L. (Pomegranate) back to family Punicaceae, is a fruit deciduous shrub, native of Iran Southeast Asia, tropical Africa.[5]

Different portions of pomegranate similar bark, leave, immature fruits, and fruit peel have various medical importance.[6] In addition, Pomegranate is reported to have good antibacterial and antifungal,[7] and antioxidant activities due to an excellent source of phytochemical compounds such as polyphenolic compounds[8] include falvonoids, anthocyanins, condensed and hydrolysable tannin.[9] Therefore the extractions prepared from different part of *Punica granatum* was informed to have antimicrobial activity against *E.coli* O157:H7, *B. cereus*, *S. typhi*, *Ps. aeruginosa*, *S.sonnie*, *S. aureus* and many other species.[10,11]

Several phytochemical compounds have been described to be found in various portions of the pomegranate fruit making it pharmacologically invaluable.[12]

The polyphenols are main group of pomegranate phytochemicals that prevail in the fruit. These polyphenols comprising flavonoids, condensed tannins and hydrolysable tannins and Hydrolysable tannins (HTs) are present in the peels membranes and piths of the fruit.[13]

Ellagic acid found mainly in pomegranate peel and arils extracts is responsible for its antibacterial effectiveness.[14,15]

MATERIALS AND METHODS

Plant samples

Collection of Pomegranate fruits samples were purchased from the local market,the peels were separated from fruits and dried for a week at room temperature. The peels were grinded into powdered and stored in refrigerator until used.

Extraction Methods

Aqueous peel extract was prepared from 10gm of fruits peel powder with 100ml of boiling water and heated on stirrer hot plate for 30min. The mixture was filtered by out filter paper (Whatman No.1) to remove of peel remains. The filtrate is concentrated and used to determination of antibacterial effectiveness.[16]

Methanolic peel extract was prepared from 10gm of pomegranate fruit peel powder was dissolved in 100ml of methanol (80%), the mixtures were placed at room temperature for 24h on shaker with 150 rpm. The solution was filtered by a Whatman No. 2 filter paper and concentrated by vacuum evaporator and stored in refrigerator until further use.[17]

Microorganisms and Culture

Different bacterial strains were used in this study involved (*E.coli, S. aureus, p. mirabilis, Ps. aeruginosa, K. pneumonia,*and *St. pyogenes*) were provided from Microbiology Lab. in Faculty of Medicine \University of Kufa. The bacterial growth were maintained at 4°C on brain heart infusion broth media until used for limitation of antibacterial effectiveness.

Assessment of Antibacterial Activity

An agar-well diffusion method was used to limitation of antibacterial activities.[18] The isolates were suspended in sterile water and diluted to 10^6 CFU/mL. The suspension was spread onto the surface of Mueller Hinton agar by sterile cotton swab and wait to 30 min for dry. Wells (4.6 mm in diameter) were cut from the agar by using a sterilized cork borer. Each well was filled with 50µl of the crude extracts with the help of micropipette. Negative controls were using sterile water and positive control were using Tetracycline. All plates were incubated at 37°C for 24 h. The antibacterial activity was estimated by measuring the

diameter of inhibition zone around wells of tested bacteria also all tests were done in triplicates.

Statistical analysis

Data are brought as mean ± SD and inhibition zone for pomegranate peel extract and tetracycline were compared by using analysis of variance (One way ANOVA), followed by LSD. Microsoft Excel 2010 and SPSS (version 21) was used in Statistical analysis. Differences were considered significant when p values was $P < 0.05$.

RESULTS

The antimicrobial activity of four different concentration(25,50, 75and 100%) of methanol and aqueous Pomegranate extracts were evaluated against six of bacteria isolates (Table 1). The result showed that methanol peel extract was more effectiveness in compare with aqueous peel extract against bacterial isolates. the effect methanol extract show inhibition zone diameter ranging between (6.33±0.57 mm) to (22.33±0.57mm).

On the other hand the concentration (25% mg/ml) of methanol extracts, revealed highest inhibition zone with *S. aureus* (15.5±0.57mm) while the lowest inhibition zone was recorded with *Ps. aeroginosa* (6.33±0.57 mm) and the same inhibition zone of both *St. pyogenes* and *P. mirabilis* (13.33±0.57mm) were recorded of Figure 1. Also at a concentration (50% mg/ml), the lowest inhibition zone was noted with *Ps. aeroginosa* (8.33±0.57mm) while the highest inhibition zone with *S. aureus* (19±1mm) followed by *St. pyogenes* with inhibition zone about (17.6±0.57 mm). The highest inhibition zone was showed for *S. aureus* (21±1mm) when used concentration (75% mg/ml) followed by *St. pyogenes* (20.3±0.57 mm) but the lowest inhibition zone was observed for *K. pneumonia* (15±0.5 mm) followed by *Ps. aeroginosa* (9.66±0.57 mm).Finally The results of (100% mg/ml) concentration exhibit the highest inhibition zone with *S. aureus* (21±1mm) and show the same inhibition zone for *E. coli* and *K. pneumonia* (17.5±0.5 mm) but the lowest inhibition zone was noted for *Ps. aeroginosa* (12.16±0.57) mm. In the other hand the aqueous extract results were listed in Table 2, the highest inhibition zone was recorded for *S. aureus* (20.66±0.57mm) in concentration (100% mg/ml) while the lowest inhibition zone was noted with *Ps. aeroginosa* (10.13±0.57 mm) of concentration (25% mg/ml).However, the aqueous extract of concentration (25% mg/ml) was not give significantly ($p< 0.05$)of *E. coli* and *S. aureus* bacteria. At a concentration (50% mg/ml) show the same inhibition zone of both *E. coli* and *P. mirabilis* (12.33±0.57mm) (Figure 2). At a concentration (75% mg/ml) the highest

inhibition zone was showed for *S. aureus* (18±1mm) followed by *St. pyogenes* (17.66±0.57 mm) but the lowest inhibition zone was observed for *Ps. aeroginosa* (8.5±0.5 mm) (Table2) (Figure2).

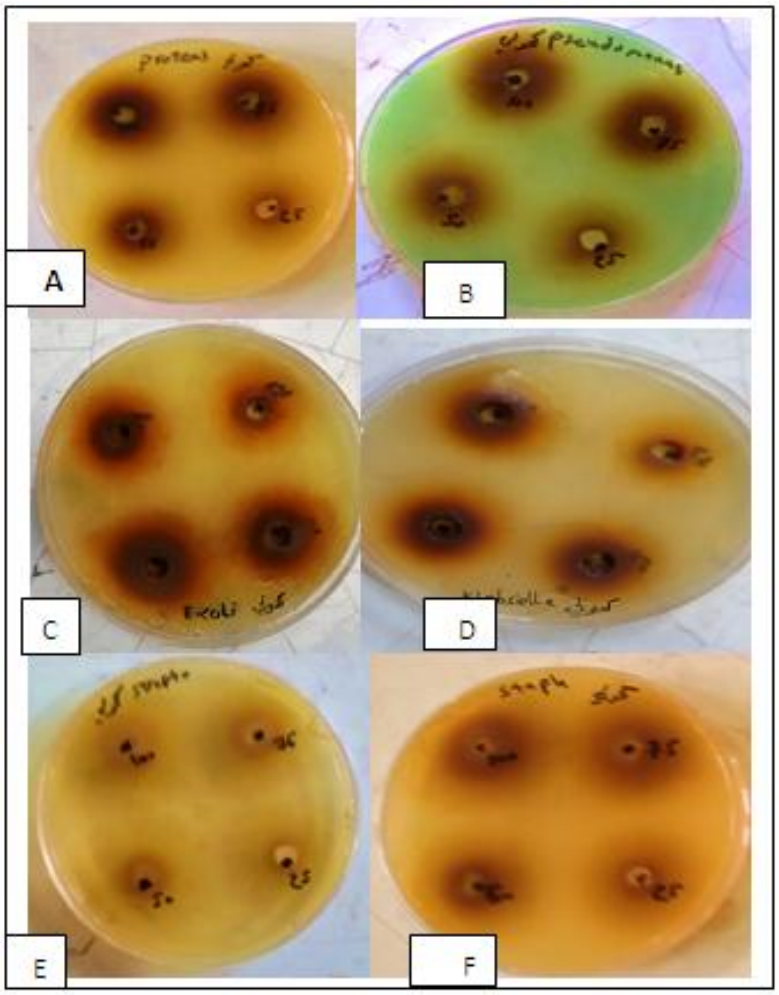

Fig. 1: Inhibition zone of bacterial isolates against various concentrations (25,50,75 and 100 mg/ml) from methanol extracts, A: *Ps. aeruginosa*,B:*P. mirabilis*., C: *K. pneumonia*, D: *E.coli*, E:*S. aureus*, F: *St. pneumonia*.

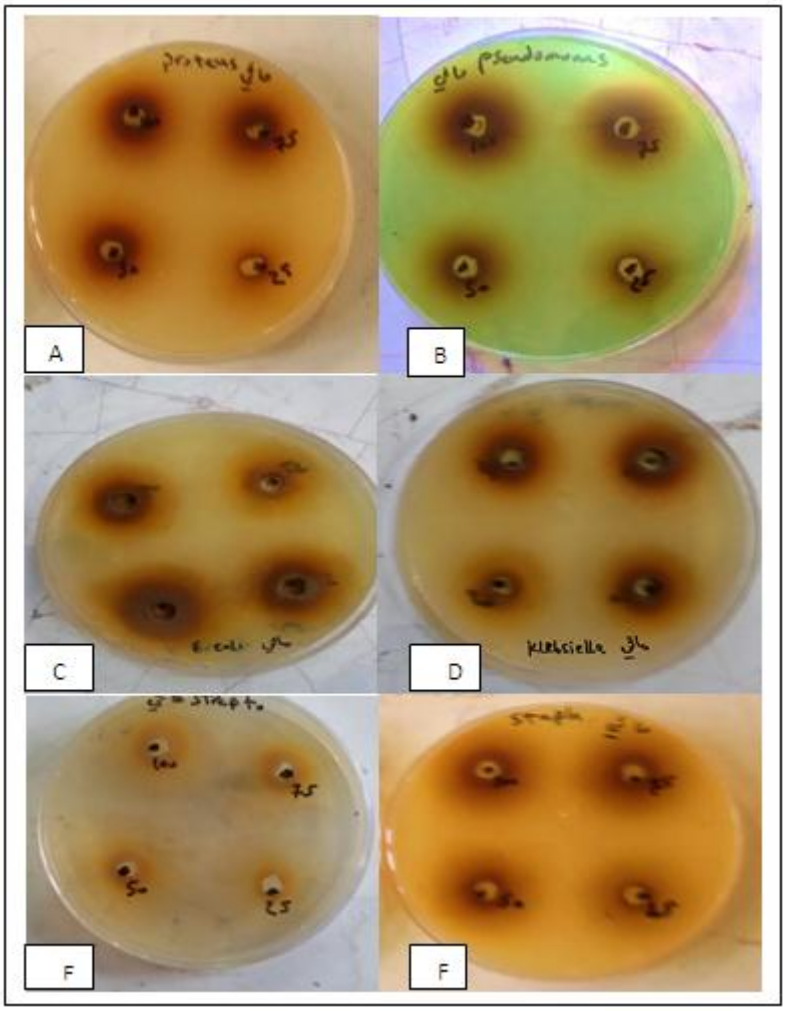

Fig. 2: Inhibition zone of bacterial isolates against various concentrations (25,50,75 and 100 mg/ml) from Aqueous extracts, A: *Ps. aeruginosa*, B: *P. mirabilis*, C: *K. pneumonia*, D: *E.coli*, E: *S. aureus*, F:*St. pneumonia*.

Table 1: Antimicrobial activity of Methanol Peel extract of *Punica granatum* against different bacterial isolates.

Bacterial isolates	Concentration Of peel extract (mg/ml)				Positive Control (tetracycline) (mg/ml)
	25%	50%	75%	100%	
S. aureus	15.5±0.57^{d}	19±1^{a}	21±1^{e}	22.33±0.57^{c}	21.5±0.57
E.coli	12.33±0.28^{d}	14.1±0.28^{a}	16.5±0.5^{e}	17.5±0.5^{c}	19.6±0.57
St. pyogenes	13.33±0.57^{d}	17.6±0.57^{a}	20.3±0.57^{e}	21±1^{c}	20.33±0.57
P. mirabilis	13.33±0.57^{d}	15.6±0.57^{a}	18±0.57^{e}	20.7±0.57^{c}	21±1
K. pneumonia	10.6±0.57^{d}	12.33±0.57^{a}	15±0.5^{e}	17.5±0.57^{c}	15.66±0.57
Ps. aeroginosa	6.33±0.57^{d}	8.33±0.57^{a}	9.66±0.57^{e}	12.16±0.57^{c}	13±0.57

Means and standard deviation for n = 3. For each bacteria, ^{a}, ^{c} ,^{d} and ^{e} means in each raw with different superscripts are different significantly ($p< 0.05$).

Table 2: Antimicrobial activity of Aqueous Peel extract of *Punica granatum* against different bacterial isolates.

Bacterial isolates	Concentration Of peel extract (mg/ml)				Positive Control (tetracycline) (mg/ml)
	25%	50%	75%	100%	
S. aureus	13.33±0.57^{x}	16.33±0.57^{a}	18±1 ^{e}	20.66±0.57^{c}	21±1
E.coli	10.5±0.5^{x}	12.33±0.57^{a}	15.6±0.57 ^{e}	17.1±1^{c}	19.6±0.57
St. pyogenes	12.5±0.57 ^{d}	16±1^{a}	17.66±0.57 ^{e}	19.66±1.1^{c}	20.33±0.57
P. mirabilis	11±1^{d}	12.33±0.57^{a}	14.33±0.57 ^{e}	17±1^{c}	21±1
K.pneumonia	7.66±0.57^{d}	10.66±1.1^{a}	13.33±1.1 ^{e}	16±1^{c}	15.66±0.57
Ps. aerognosa	5.5±0.5^{d}	6.33±0.57^{a}	8.5±0.5 ^{e}	10.13±0.57^{c}	13±0.57

Means and standard deviation for n = 3. For each bacteria, ^{a}, ^{c} ,^{d} and ^{e} means in each raw with different superscripts are different significantly ($p< 0.05$) while ^{x} means are not significantly different.

DISCUSSION

Increase of antibiotic resistance in addition to unwanted side effects of artificial drugs have triggered great attention of the search to new antimicrobial agents of plant origin.[19]

Most of the world populations depends on the natural products for main Health care[20]. Researchers have widely studied the biological properties of Punica granatum and their results revealed that this plant is strongly have therapeutic property.[21] This study showed the antimicrobial activities of pomegranate of methanol and aqueous peel extracts against six types of bacteria. These results are agreement with other studies Naziri *et al.*[22] who determined that methanolic and aqueous peel extracts of *Punica granatum* in different concentrations were effective against all bacteria tested in their study and Prashanth *et al.*[23] who determined methanolic extracts of *Punica granatum* fruit peel to be effective against all positive and Gram-negative bacteria tested of their study. Also, Similarly to Melendez and Capriles[24] who described that extracts from pomegranate peels possess strong antibacterial effectiveness against serveral bacteria.

Al-Zoreky[16] who stated that methanol extract of peels have marked inhibition zones against eleven microorganisms tested in their study. McCarrell *et al.*[25] reported that *Staphylococcusaureus* and *Ps. aeroginosa* have the highest sensitivities to the aqueous extract of pomegranate peel. This antibacterial activity of *Punica granatum* fruit rind perhaps linked to the found of hydrolysable tannins and polyphenolics of the pomegranate extract precisely punicalagin and gallagic acid,which has the highest anti-bacterial influence against strains tested even at low concentrations.[26,27]

Effects of tannins on bacterial metabolism are identified by their effect on bacterial membrane, because tannins can pass through cell walls, which contain polysaccharides and proteins and bind to their surface, preventing their normal activity.[28,29] The phenolic compounds of pomegranate peel can causes cell damage during damage the cell wall, degrade the cytoplasmic membrane, disrupt membrane proteins and interfere with membrane integrated enzymes, which may finally lead to cell death.[30]

The result showed that methanol extract was more effectiveness of aqueous extract against of bacteria. This result Similarly, Ali *at el*.[19] who described that metanolic extract of pomegranate fruit peels more effective than aqueous extract. Also, it is reasonable to assume that the principal chemical constituents with antimicrobial activity were concentrated in the alcoholic portion.[19] The methanol extract was more effectiveness of aqueous extract may be due to the quantity and quality of active substances released and solvated in alcohol is greater compared to water. so, alcohol is an excellent solvent for most medicinal herbs to be studied the effect of the extract of alcohol in most researchers in the field of biological effectiveness of water extracts that the active substance that affects the bacteria melt in Organic solvents are more than soluble in water.[31] Also, this result agreement with Ashwaq et al.[32] who show that methanol extract was given a high inhibition rate compared with aqueous extract against of pathogenic bacteria. While the results disagree with Khan and Hanee[33] who found that the aqueous and the alcohol pomegranate peel extracts had the same effect.

The result show Gram-positive bacteria more sensitive than Gram-negative bacteria to *Punica granatum* fruit peel extract. This result agreement with Abdullah *et al*.[34] who report that the antibacterial effect of pomegranate peels extracts was more effectiveness against Gram-positive bacteria than Gram-negative bacteria. Naziri *et al*.[22] who show that a stronger effect of pomegranate peel extract on Gram positive bacteria related to that against Gram-negative bacteria. Ali *et al*.[19] who found that the aqueous extract of pomegranate peel lower effective for Gram negative than for Gram positive bacteria. This is mostly related to variances in the cell wall structure between Gram positive bacteria and Gram-negative bacteria, cell wall in Gram-negative bacteria contain lipopolysaccharides which perhaps stop active constituents of reaching the cytoplasmic membrane.[35] We concluded that the pomegranate peel extracts showed to have possess strong antimicrobial activity against six bacteria and may be used as medicine for humans diseases caused by multi-drug resistant

bacterial pathogens. The methanol extracts of pogmenate are more active against bacteria than the aqueous extracts.

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