

Original Research Article

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Antibacterial Activity of Silver nanoparticles and Pomegranate (*Punica granatum L.*) Extracts

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ABSTRACT

Water and ethanol extracts of *Punica granatum* peels were prepared at the concentration 0.5 and 1.0mg/10ml, silver nanoparticles (4000mg/l) prepared at the same concentrations (0.5 and 1.0mg/10ml). Bacterial samples used in the experiment consisted of two gram-negative bacteria (*Escherichia coli* and *Pseudomonas erogenous*) and two gram-positive bacteria (*Staphylococcus aurous* and *Streptococcus pyogenes*). All bacterial types were treated either with plant extracts alone or mixed with the same concentrations of silver nanoparticles. Results showed that there was a significant decrease recorded in the mean inhibition zone at 0.5mg/10ml peel water extract (B) (27.5mm), 0.5mg/10ml peel ethanol extract (C) (25.5mm) and 1.0mg/10ml peel ethanol extract (H) (27.7mm) in comparison to the control (A) (32.5mm). According to the type of bacteria the highest mean inhibition zone observed in *Staphylococcus aurous* bacteria (34.8mm) and the lowest mean obtained in *Pseudomonas erogenous* bacteria (16.3mm). The interaction between the type of bacteria and treatment types indicated that the highest mean inhibition zone recorded in 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and ethanol peel extract (F), and 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and ethanol peel extract (K) with *Staphylococcus aurous* bacteria (39.0mm). While there was a significant reduction recognized in the mean inhibition zone with other types of bacterial strains used in the experiment in comparisons to the control (250mg/10ml cefotaxime).

Keywords

Punica granatum,
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activity,
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nanoparticles.

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Introduction

P. granatum is a shrub or small tree growing 6 to 10m high, pomegranate has multiple spiny branches, its long-lived, the edible fruit is a berry, intermediate in size between a lemon and a grapefruit, 5–12 cm in diameter with a rounded shape and thick, reddish skin (singh *et al.*, 2002). The seeds are exarillate and unlike some other species in the order, Myrtales, no aril is present. The

sarcotesta of pomegranate seeds consists of epidermis cells derived from the integument. The seeds are embedded in a white, spongy, astringent membrane (Jayaprakasha *et al.*, 2006). Pomegranate seed oil contains punicic acid (65.3%), palmitic acid (4.8%), stearic acid (2.3%), oleic acid (6.3%), and linoleic acid (6.6%) (Shay *et al.*, 1999). The major class of pomegranate

phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups) that predominate in the fruit. Pomegranate polyphenols include flavonoids, condensed tannins (proanthocyanidins) and hydrolysable tannins (Passamonti *et al.*, 2003). *P. granatum* has been shown to have antimicrobial properties against harmful bacteria that can exist in the stomach, such as *Escherichia coli* (E. coli) or *Bacillus subtilis*, both of which can cause painful infections and serious stomach conditions (Debjit *et al.*, 2013).

Silver nanoparticles have various and important applications and they have been known to have a disinfecting effect and have applications ranging from traditional medicines. It's been reported that silver nanoparticles (AgNPs) are non-toxic to humans and are effective against microbes at low concentrations and have no side effects. but toxic for microorganisms (Mohanpuria *et al.*, 2008). Nanosilver is an effective killing for bacteria, fungus, and viruses (Ranganathan *et al.*, 2012).

Nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance (Rai *et al.*, 2012). The potent antibacterial and broad-spectrum activity against morphologically and metabolically different microorganisms seems to be correlated with a multifaceted mechanism by which nanoparticles interact with microbes (Dos *et al.*, 2014).

AgNPs are able to physically interact with the cell surface of various bacteria (Lara *et al.*, 2011). This is particularly important in the case of Gram-negative bacteria where numerous studies have observed the adhesion and accumulation of AgNPs to the bacterial surface. This effect is highly influenced by the nanoparticles size, shape

and concentration and a study using *Escherichia coli* confirmed that AgNPs accumulation on the membrane cell creates gaps in the integrity of the bi-layer which predisposes it to a permeability increase and finally bacterial cell death (Dos *et al.*, 2014). Nanoparticles have a higher antibacterial activity than the free ions of silver, whereby the antibacterial properties are attributed to both the physical properties of nanoparticles and the elution of silver ions (Choi *et al.*, 2008). The AgNPs also damage membranes and induce the release of reactive oxygen species (ROS), forming free radicals with a powerful bactericidal action (Wu *et al.*, 2014). Silver ions could easily enter the microbial body causing the damage of its intracellular structures. As a consequence ribosomes may be denatured with inhibition of protein synthesis, as well as translation and transcription can be blocked by the binding with the genetic material of the bacterial cell. Protein synthesis has been shown to be altered by treatment with AgNPs and proteomic data have shown an accumulation of immature precursors of membrane proteins resulting in destabilization of the composition of the outer membrane (Mirzajani *et al.*, 2014).

Materials and Methods

Collection of plant materials

The fruit peels of *P. granatum* were collected from nearby fruit shop in a local markets. The fruits peels were cut away from fruit seeds, thoroughly washed in deionized water and cut into small pieces (Mahendran *et al.*, 2013).

***P. granatum* peel Drying**

P. granatum peel was dried under shade for 3-4 days and coarsely powdered (Bharani *et al.*, 2015).

Preparation of extracts

50g of each fruit peel powder was extracted with 250ml of sterilized distilled water and 250ml of 70%Eethanol using magnetic stirrer for 24 hours at room temperature. Extract were filtered through Whatman filter paper No.1 then it was concentrated in vacuum at 40°C using a rotary evaporator. The yield of fruit extract was calculated. Then the dried extracts was stored properly (Piyush *et al.*, 2015).

Sterilization of extracts

P. granatum peel extracts were sterilized using Millipore filter in a laminar air flow cabinet.

Preparation of media for bacterial cultures

Muller Hinton agar medium was prepared by Suspension 38g of Muller Hinton agar medium in 1000ml sterilized D.W, heat till boiling to dissolve the medium completely. Sterilized by autoclaving at 15lbs pressure and 121°C for 15min, mix well before poring. Then the media was poured into a disposable Petri dishes in a depth of 3 to 4mm, the final pH the medium was adjusted to 6.8 by using pH-meter. After solidification, all prepared plates containing medium were kept at 4°C till use.

Measurement of bacterial cultures concentration

Bacterial isolates were supplemented from bacterial isolates bank in Microbiology Lab., Post graduate laboratories, College of Applied Science, Al-Nahrain university, at which single colonies from cultures grown on nutrient agar for 18-24hrs were transferred to test tubes containing 5ml of normal saline and mixed well by vortex,

then bacterial growth was compared with McFarland tube No.0.5 turbidity standard solution, which was equivalent to a bacterial inoculums concentration of 1.5×10^8 cell/ml.

Determination of inhibition zones of treatments

Using cotton swab, a touch of bacterial culture (broth) was transferred to Muller Hinton agar medium and streaked three times by rotating the plate approximately 60° between streaking to ensure even distribution of the inoculums, the inoculated plates were placed at room temperature for 10 min to allow absorption of excess moisture (Atlas *et al.*, 1995).

Then, using sterilized pasteur pipette for making wells (the wells were arranged so as to avoid the development of overlapping of inhibition zones) which were then filled with 25µl of the sterilized *P. granatum* peel water and ehanolic extracts alone (0.5 and 1.0mg/ml for water and ethanol extracts) or mixed with two different concentrations of copper nanoparticles (0.5 and 1.0mg/ml).

The plates were incubated at 37°C for 18-24 hrs. After incubation, inhibition zones were measured using ruler for determination their diameters in millimeters.

The same procedure was made for antibiotic standard by using Cefotaxime 250mg /10ml D.W. Adding 25microletter of antibiotic to the wells in plates of Muller Hinton agar medium then the plates were incubated at 37°C for 18-24hrs.

After incubation, inhibition zones were measured by ruler also and the results were compared with the standards as in NCCLs, (2007).

Results and Discussion

Antibacterial activity of *P. granatum* peel extracts water and ethanol extract alone or mixed with silver nanoparticles against *Escherichia coli*, *Pseudomonas erogenous*, *Staphylococcus aureus* and *Streptococcus pyogenes*.

Data in table 1 indicated that there was a significant decrease recorded in the mean inhibition zone at 0.5mg/10ml peel water extract (B) (27.5mm), 0.5mg/10ml peel ethanol extract (C) (25.5mm) and 1.0mg/10ml peel ethanol extract (H) (27.7mm) in comparison to the control (A) (32.5mm). According to the type of bacteria,

the highest mean inhibition zone obtained at *Staphylococcus aureus* bacteria (34.8mm) and the lowest mean was in *Pseudomonas erogenous* bacteria (16.3mm); The interaction between the treatment type and the type of bacteria shown that the highest mean inhibition zone recorded at 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and water peel extract (F), 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and ethanol peel extract (K) in *Staphylococcus aureus* bacteria (39mm), mean inhibition zone significantly reduced with *Escherichia coli* bacteria at 0.5mg/10ml peel ethanol extract (C) (29.0mm) and 1.0mg/10ml peel ethanol extract (H) (26.0mm);

Table.1 Inhibition zones diameters (mm) as a results of treatment four types of bacteria with *P. granatum* peel extracts water and ethanol extract alone or mixed with silver nanoparticles at the different concentration

Type of bacteria \ Type of treatment	<i>Escherichia coli</i>	<i>Pseudomonas erogenous</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	Mean
A	37.0	19.0	35.0	39.0	32.5
B	34.0	15.0	33.0	28.0	27.5
C	29.0	13.0	29.0	31.0	25.5
D	35.0	13.0	31.0	33.0	28.0
E	35.0	17.0	30.0	32.0	28.5
F	37.0	15.0	39.0	37.0	32.0
G	31.0	17.0	37.0	29.0	28.5
H	26.0	17.0	37.0	31.0	27.7
I	33.0	16.0	36.0	34.0	29.7
J	35.0	17.0	37.0	31.0	30.0
K	35.0	21.0	39.0	33.0	32.0
Mean	33.3	16.3	34.8	32.5	
L.S.D 0.05	Type of treatment = 5.11; Type of bacteria=1.60; Type of treatment * Type of bacteria =4.70				

Fig.1 Antibacterial activity of *P. granatum* peel extracts water and ethanol extract alone or mixed with silver nanoparticles against *Escherichia coli*. (A) control, (B) 0.5mg/10ml peel water extract, (C) 0.5mg/10ml peel ethanol extract, (D) 0.5mg/10ml silver nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and water peel extract, (F) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and ethanol peel extract, (G) 1.0mg/10ml peel water extract, (H) 1.0mg/10ml peel ethanol extract, (I) 1.0mg/10ml silver nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and water peel extract, (K) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and ethanol peel extract.

A) *Escherichia coli*

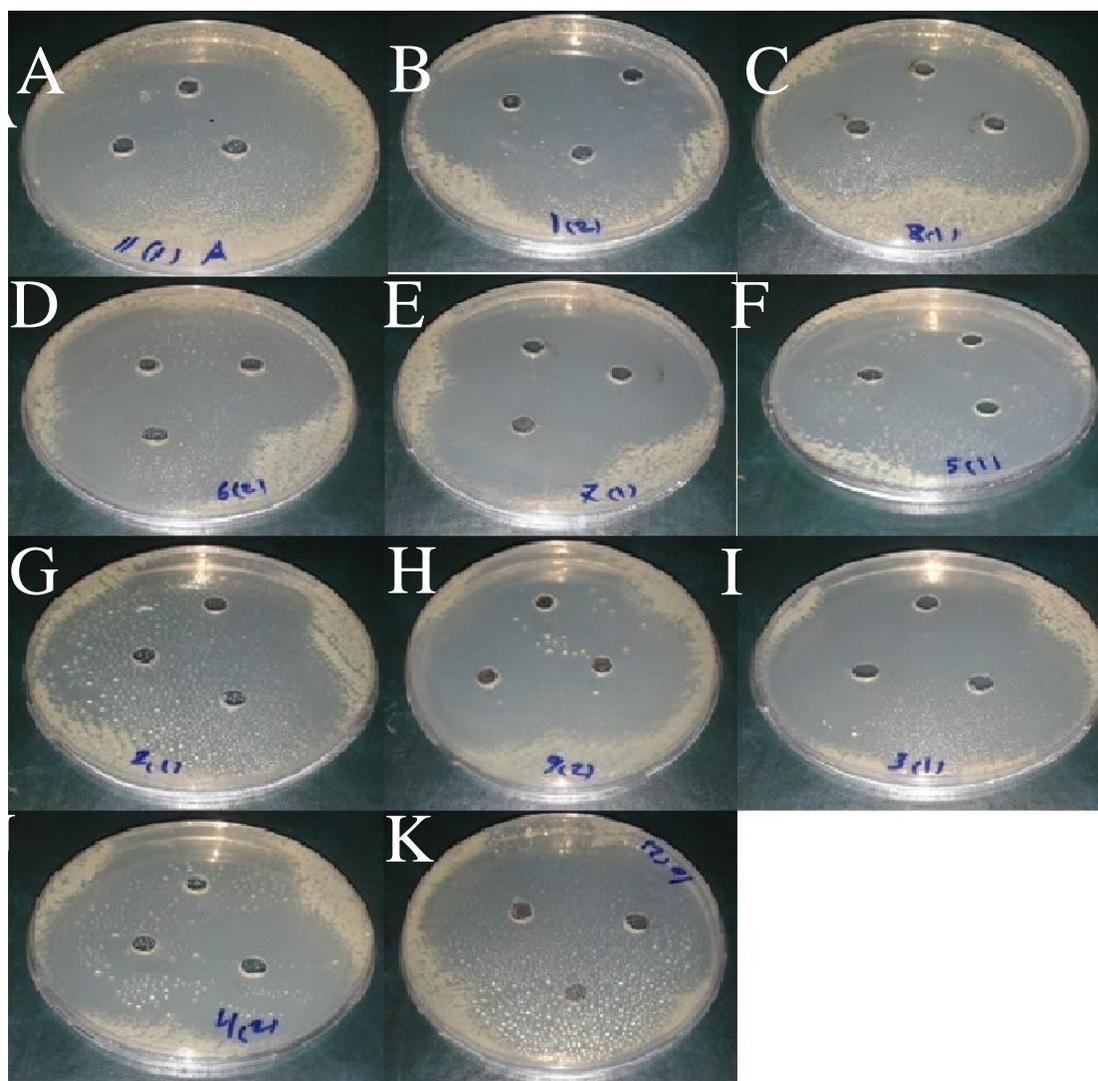


Fig.2 Antibacterial activity of *P. granatum* peel extracts water and ethanol extract alone or mixed with silver nanoparticles against *Pseudomonas erogenous*. (A) control, (B) 0.5mg/10ml peel water extract, (C) 0.5mg/10ml peel ethanol extract, (D) 0.5mg/10ml silver nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and water peel extract, (F) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and ethanol peel extract, (G) 1.0mg/10ml peel water extract, (H) 1.0mg/10ml peel ethanol extract, (I) 1.0mg/10ml silver nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and water peel extract, (K) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and ethanol peel extract.

B) *Pseudomonas erogenous*

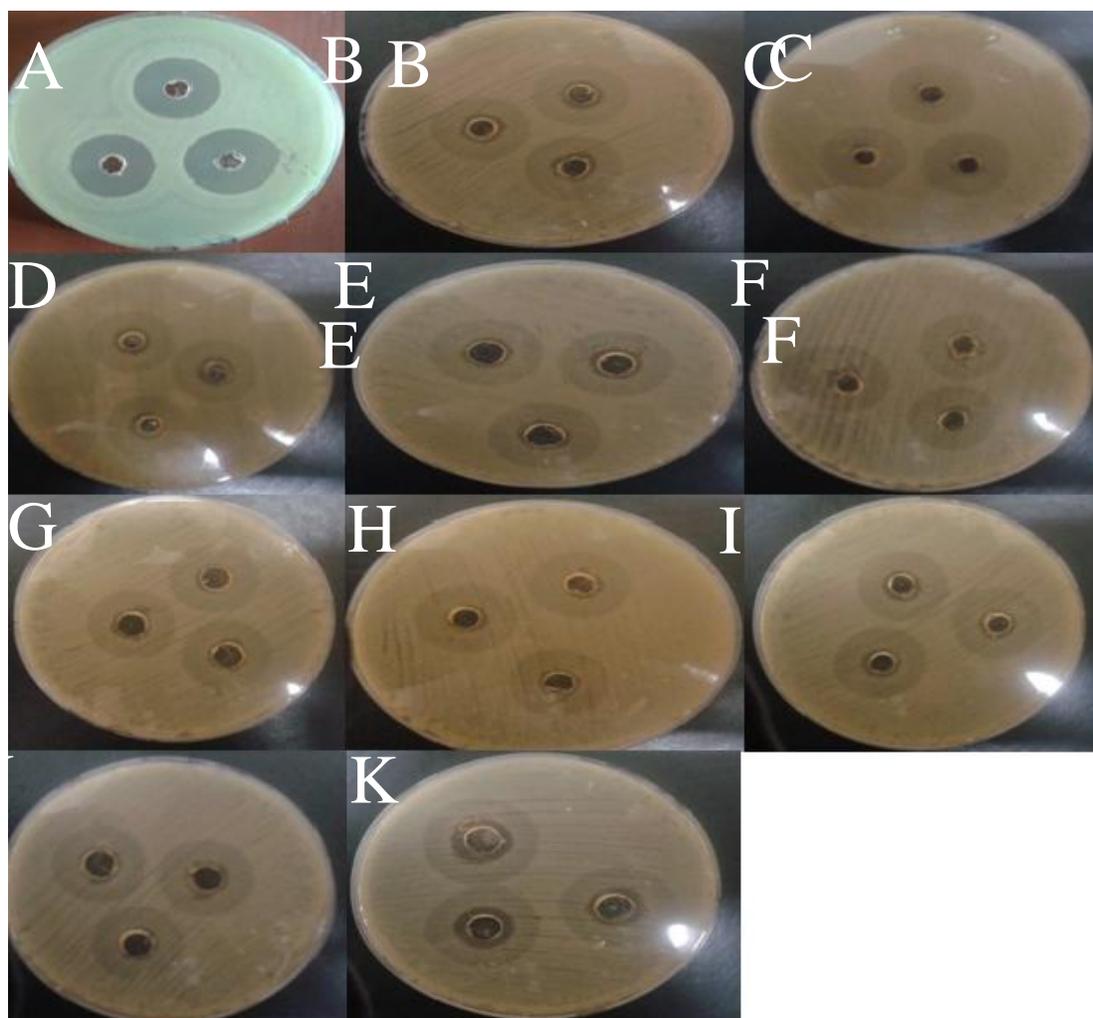


Fig.3 Antibacterial activity of *P. granatum* peel extracts water and ethanol extract alone or mixed with silver nanoparticles against *Staphylococcus aureus*. (A) control, (B) 0.5mg/10ml peel water extract, (C) 0.5mg/10ml peel ethanol extract, (D) 0.5mg/10ml silver nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and water peel extract, (F) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and ethanol peel extract, (G) 1.0mg/10ml peel water extract, (H) 1.0mg/10ml peel ethanol extract, (I) 1.0mg/10ml silver nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and water peel extract, (K) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and ethanol peel extract.

C) *Staphylococcus aureus*.

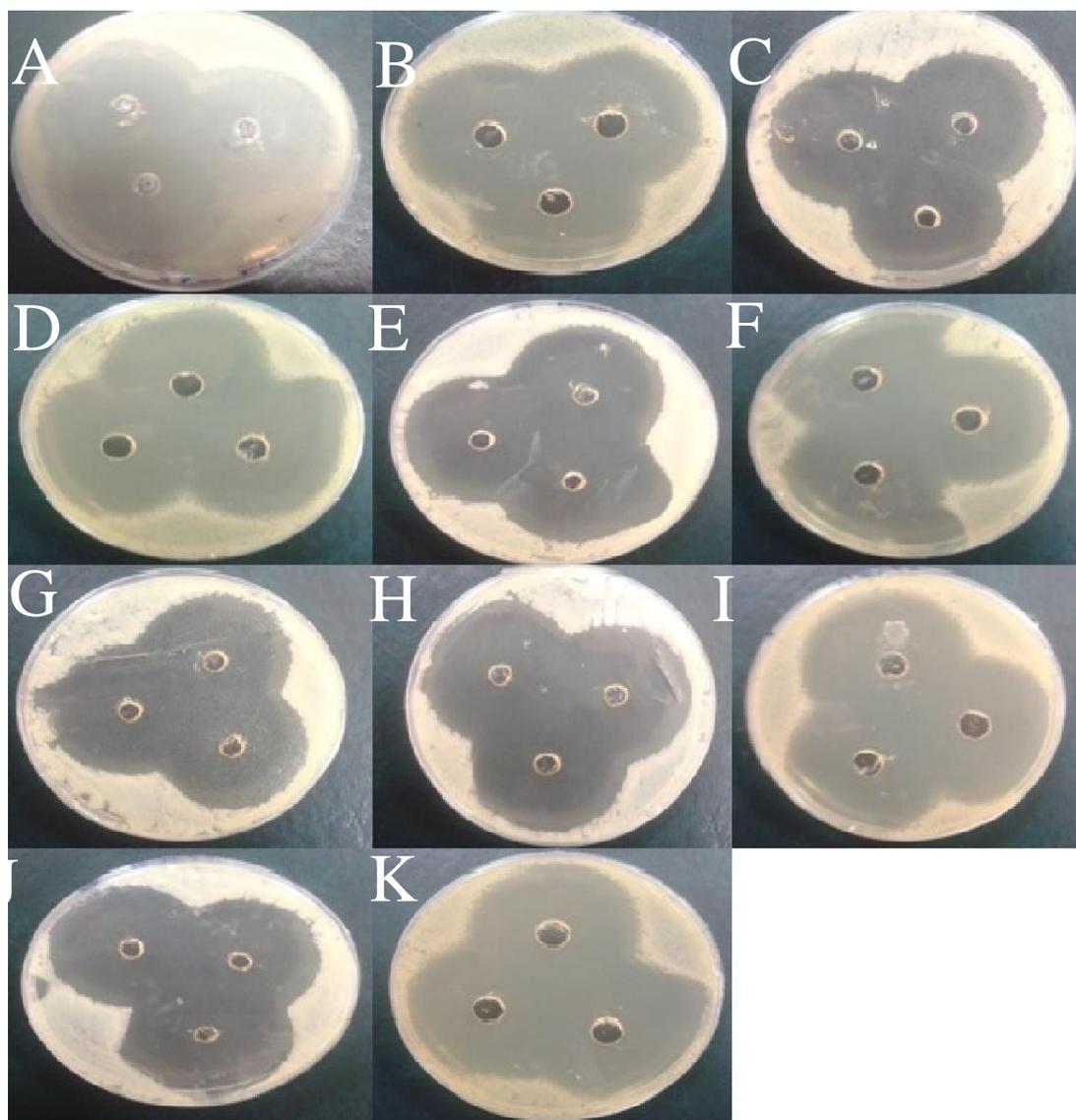
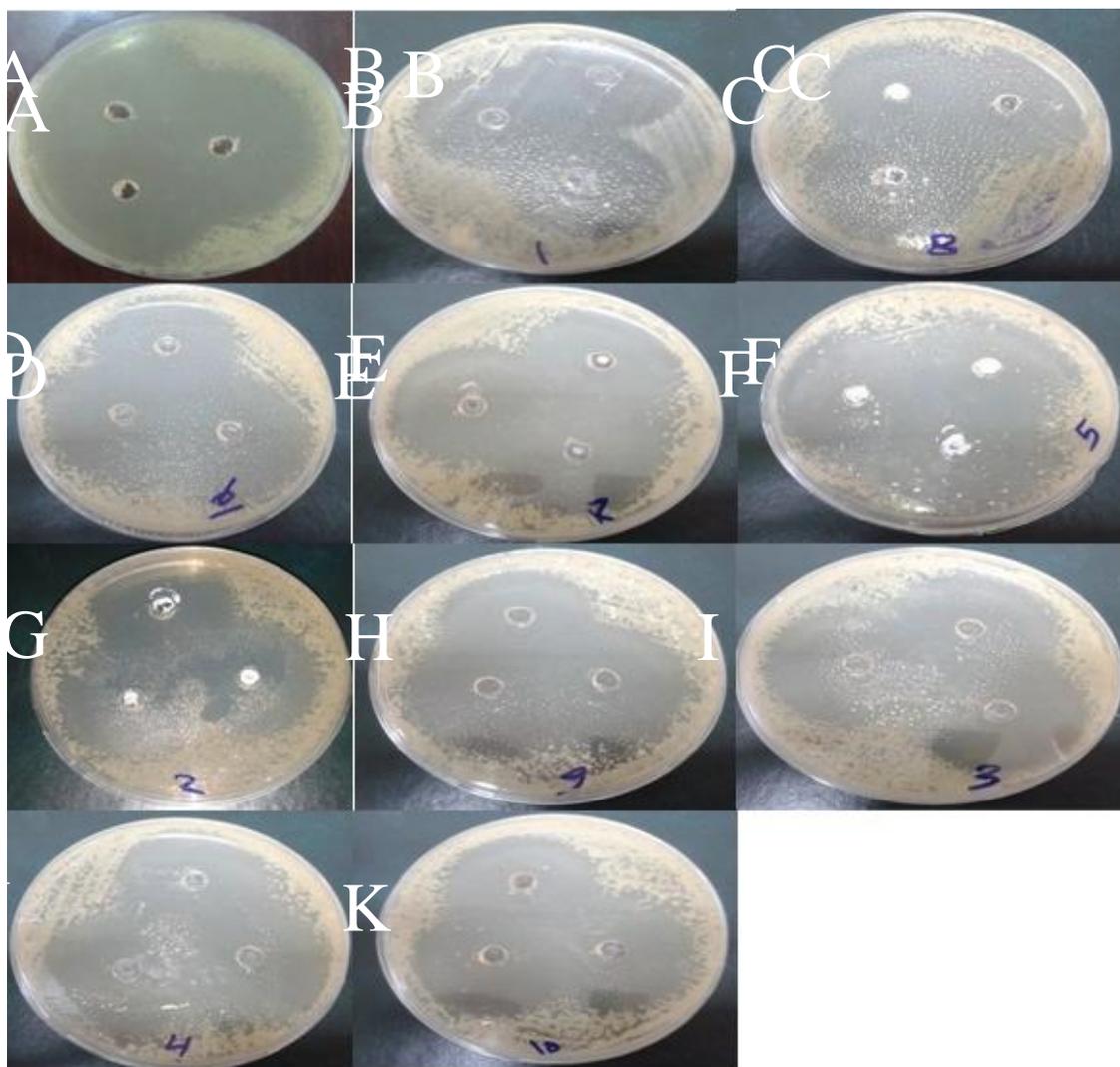


Fig.4 Antibacterial activity of *P. granatum* peel extracts water and ethanol extract alone or mixed with silver nanoparticles against *Streptococcus pyogenes*. (A) control, (B) 0.5mg/10ml peel water extract, (C) 0.5mg/10ml peel ethanol extract, (D) 0.5mg/10ml silver nanoparticles, (E) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and water peel extract, (F) 1:1 (v/v) of 0.5mg/10ml silver nanoparticles and ethanol peel extract, (G) 1.0mg/10ml peel water extract, (H) 1.0mg/10ml peel ethanol extract, (I) 1.0mg/10ml silver nanoparticles, (J) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and water peel extract, (K) 1:1 (v/v) of 1.0mg/10ml silver nanoparticles and ethanol peel extract.

D) *Streptococcus pyogenes*.



Pseudomonas aeruginosa bacteria at 0.5mg/10ml peel ethanol extract (C) and 0.5mg/10ml silver nanoparticles (D) (13.0mm); *Staphylococcus aureus* bacteria at 0.5mg/10ml peel ethanol extract (C)

(29.0mm), finally; *Streptococcus pyogenes* results significantly reduced at all treatment types compared with control (A=39.0mm) except the treatment type F (1:1 (v/v) of 0.5mg/10ml silver nanoparticles and water

peel extract) (37.0mm); In spite of the lower concentration of the plant extract and silver nanoparticles, but it proved its efficiency as bactericidal agent in comparison to the high concentration of the pharmacy in used (Cefotaxime 250mg/10ml). The previous results agreed with those obtained by Debjit *et al.*, (2013) who reported that *P. granatum* have antimicrobial properties against harmful bacteria such as *Staphylococcus aureus*, *Escherichia coli* or *Bacillus subtilis*. Also Saad *et al.*, (2010) explained that highest antibacterial activity of *P. granatum* peel extracts was recorded against *Staphylococcus aureus* bacteria. Other researches as Menezes *et al.*, (2006) demonstrated that *P. granatum* extracts inhibited the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Diplococcus pneumonia* and *Escherichia coli* bacteria. Haniff *et al.*, (2015) investigated that silver nanoparticles have antibacterial activity against *Pseudomonas erogenous*, *Bacillus cereus*, *Staphylococcus albus* and *Proteus* pathogens; figures 1, 2, 3 and 4 describe the Antibacterial activity of *P. granatum* peel water and ethanol extract alone or mixed with silver nanoparticles at specific concentrations against *Escherichia coli*, *Pseudomonas erogenous*, *Staphylococcus aureus* and *Streptococcus pyogenes* respectively.

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