



Studies on antibacterial activity of pomegranate (*Punica granatum L.*) plant extracts against clinical pathogens

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Abstract

Pomegranate (*Punica granatum L.*) has long been recognized for its medicinal properties, including its antimicrobial activity. In the study, the pomegranate plant materials stem, leaves, peels, and fruit juice were utilized for checking its antibacterial potential. Methanol, Ethanol, Acetone, Petroleum ether, Aqueous extracts were prepared and fresh pomegranate fruit juice were tested against clinical pathogens. In the study it was found that each part of Pomegranate stem, leaves, peels and fruit juice possesses antibacterial potential. The methanol, ethanol, and acetone extracts of stem and leaves showed prominent antibacterial activity against test bacteria as compared to peel extract, while petroleum ether, and aqueous extract of leaves showed prominent activity than other. The fresh juice also found to exert considerable antibacterial activity against test clinical pathogens.

Keywords: Pomegranate (*Punica granatum L.*), antibacterial activity

Introduction

The Pomegranate is an important source of bioactive compound and has been used in folk medicine for many countries. Most pomegranate fruit parts are known to possess enormous antioxidant activity. In India pomegranate arils are used as such or are made into juice. Pomegranate juice has been demonstrated to be high in antioxidant activity and is effective in prevention of atherosclerosis, low-density lipoprotein oxidation, prostate cancer, platelet aggregation and various cardiovascular disease (Adhani and Mukhta, 2006).

Pomegranate is a fruit -bearing deciduous shrub or small tree, native to Asia and belongs to the family *Lythraceae*. The leaves are shiny and about 7.6 cm long. Different parts of the plant such as bark, leaves, immature fruits and fruit rind have medicinal significance. *P. granatum* has been extensively used as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies. Additionally, this plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties. Numerous phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious (Ravva, 2018). Antimicrobial drug resistance in human bacterial pathogens is a worldwide issue and as a consequence, effective treatment and control of such organisms remain an important challenge natural herbal components from plants could be one of the alternative for controlling such antibiotic resistant bacterial strains.

Material and Methods

Collection of plant material

The plant material stem, leaves, peel and fruits were utilized into the study. The stem, leaves were collected from plants washed properly with distilled water. Several times to remove dirt and dust. The plant material were cutted into pieces and air and sun dried. The material was finely powdered by mixer grinder and the dried powder was stored into air tight containers and stored at 4°C for untill further

use. Similarly, peels from fruits purchased from local market was collected, dried and powdered and stored for further use. While for the fresh fruit juice activity fruits were collected washed and juice prepared freshly was used for the antibacterial activity.

Preparation of plant material extracts

The 10 gm of power of leaves, stem and peela of Pomegranate was weighed in with different conical flasks and was extracted with different solvents as methanol, ethanol, acetone, petroleum ether and water (100 ml each) The conical flask were placed in a shaking incubator over night at room temperature. The extracts were filtered separately through Whatman No.1 filter paper and the residue was reextracted with fresh solvent for three consecutive days. The combined extracts were filtrated and evaporated at 40 °C to get a concentrate which was dired and extract in a refrigerator at 4°C were stored in until use.

Determination of antibacterial activity of extracts against clinical pathogens

The agar well diffusion method was used. The dried extract was reconstituted with DMSO and for juice fresh juice was prepared used directly. Overnight broth culture of the respective bacteria strains were use for incubation to the nutrient agar plates were swabbed using a sterile cotton swabs with the made equidistance in each of the plates using a sterile cork borer. Up to 100 µl (0.1 ml) of the extract were respectively introduced into the wells using sterile pipettes. It was allowed to diffuse for 30 min at a room temperature. The plates were incubated at 37°C for 24 hours room temperature. The zone of inhibition were measured.

Phytochemical analysis

Phenols

To test the Phenol presence, in a test tube 1ml of extract and 2 ml of distilled water were added followed by few drops of 10% ferric chloride (FeCl₃). Appearance of blue or green colour indicates presence of phenols.

Flavonoids

To test the flavonoid presence, in a test tube 1 ml of extract a few drops of dilute sodium hydroxide (NaOH) was added. An intense yellow colour was produced in the plant extract which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoid.

Quinones

To test the quinone presence, in a test tube 1ml of extract and 1ml of concentrated sulphuric acid (H₂SO₄) was added. Formation of red colour is an indication for the presence of quinones.

Tannins

To test the tannin presence, in a test tube 1 ml of 5% ferric chloride was added to solvent free extract. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

Saponins

To test the saponin presence in the extract, the extract was diluted with 20 ml distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponin.

Cardiac Glycosides

To test the cardiac glycoside presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride (FeCl₃) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H₂SO₄). brown ring of the interface indicates a de-oxy sugar characteristic of cardenolites.

Steroids

To test the presence of steroid, 1 ml of extract was dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid (H₂SO₄) added by the sides of test tube. The upper layer turns red and sulphuric acid layer shown yellow with green fluorescence. This indicated the presence of steroids.

Terpenoids

To test the presence of terpenoid, in a test tube 5 ml of each extract was mixed with 2 ml of chloroform. 3ml of concentrated sulphuric acid (H₂SO₄) was then added to form a layer. A reddish brown precipitate colouration at the interface formed indicated the presence of terpenoids.

Results and Discussion

In the present study the antibacterial activity of the methanol extract of the stem, leaves and peel extract was checked (Fig1). It was found that extract from stem showed highest zone of inhibition 30 mm. against *P.aeruginosa*, followed by 28 mm against *S.aureus* and *S typhi* 26 mm against *Enterobacter spp* and 23 mm against *E.coli* For leaves extract highest zone of inhibition was also shown against *P.aeruginosa* (32mm) followed by *Enterobacter spp* (30mm) *Salmonella typhi* (29 mm) against *S.aureus* and *E.coli* (28 mm). Methanol peel extract also showed highest zone of inhibition 15mm against *P.aeruginosa*, followed by *S.aureus* (14mm), *E.coli* & *Enterobacter spp* (13mm), and least zone against *S. typhi* (12 mm).

There are *in vitro* studies evaluating the efficacy of *P granatum L.* extract against several microorganisms with

caricogenic activity (Gram-positive bacteria, Gram-negative bacteria, and yeasts). One of these studies was performed by Abdollahzadeh *et al.*, (2011) [3], in which different concentrations of methanolic extract of *P. granatum L.* promoted high inhibition of *Staphylococcus aureus* and *Staphylococcus epidermidis*, suggesting an antimicrobial action of this extract. This activity is also related to the presence of hydrolyzable/gallic tannins and polyphenols in the *P. granatum L* extract, such as punicalagin and gallic acid. These tannins can act on the bacteria's cell membrane, precipitating proteins and suppressing some enzymes. In turn, the polyphenols can affect the cellular wall by making some enzymes interact with proteins, thus impairing congregation of microorganisms (S. Abdollahzadeh *et al.*, 2011) [3].

In study the antibacterial activity of the Ethanol extract of the stem, leaves and peel was also checked (Fig 2). It was found that extract from stem showed highest zone of inhibition against *E.coli* (30mm) followed by *Enterobacter spp* (24mm), *S.aureus* (23 mm) *Pseudomonas aeruginosa* and *Salmonella typhi* (20 mm). Highest activity for leaves extract (30 mm) was shown against *E.coli*, *Salmonella typhi* and *Enterobacter spp* followed by *S.aureus* (20 mm), *Pseudomonas aeruginosa* (19 mm). The Ethanol peel extract also showed highest zone of inhibition against *S.aureus* (20 mm) followed by *E.coli* (18mm) and *Pseudomonas aeruginosa* (15 mm) and the least zone against *Salmonella typhi* and *Enterobacter spp* (12 mm).

In a study carried out by Malviya *et al.*, (2006) methanolic and ethanolic extracts of pomegranate showed inhibition zone of 24.5 ± 0.53 mm and 20.0 ± 0.31 mm, respectively against *S. aureus*. Reported that peel extracts of sour and sweet pomegranate cause zone inhibition of 15.3 to 25.7 mm and 16.0 to 25.3 mm, respectively, against *S. aureus* at 1 to 8 mg/disc. Melendez and Capriles demonstrated that Pomegranate extract produced inhibition zone sizes of 22 mm. for *S. aureus*. Mathabe *et al* (2006). showed that methanol, ethanol, acetone, and water extracts of pomegranate had activities against *S. aureus*, showing inhibition zones of 27.0 to 29.3 mm.

In the present study the antibacterial activity of Acetone extract of stem, leaves and peels also checked (Fig 3). It was found that extract from stem showed highest zone of inhibition against *Enterobacter spp* (37 mm) followed by *Salmonella typhi* (32 mm) *Pseudomonas aeruginosa* (30 mm), *S.aureus* (27 mm). For leaves extract highest zone of inhibition was showed against *Enterobacter spp* (32 mm) followed by *Pseudomonas aeruginosa* and *Salmonella typhi* (29 mm), *S. aureus* (25 mm), *E.coli* (25 mm). The Acetone peel extract also showed highest zone of inhibition 15 mm against *S.aureus* followed by *Pseudomonas aeruginosa* (14 mm), *E.coli* and *Salmonella typhi* (13 mm) and least zone against *Enterobacter spp* (11 mm).

Nozohour *et al.*, (2018) [15] reported the MIC and MBC values showed the antibacterial activity of pomegranate extract against both *P. aeruginosa* and *S. aureus*, while the highest effect belonged to peel extract. This finding was consistent with previous report. Dahkam *et al* (2018) assessed the antimicrobial effect of alcoholic extract of seed, fruit, peel, and juice of pomegranate on specific bacteria including *S. aureus* and reported that the pomegranate peel extract have the greatest antimicrobial activity.

Naziri, *et al.*, (2012) [10] reported that methanolic pomegranate peel extract has a stronger effect on gram-

positive bacteria than gram-negative bacteria. So that *S. aureus* has highest sensitivity to the methanolic extracts of sour and sweet pomegranate peels. Mathabe *et al.*, (2006) also showed that antibacterial activity of acetone extract.

In the present study the antibacterial activity of Aqueous extract of stem, leaves and peel was checked (Fig 4). It was found that extract from stem showed highest zone of inhibition *Enterobacter spp* (25 mm) followed by *E.coli* (23 mm), *Pseudomonas aeruginosa* (19 mm), *S.aureus* and *Salmonella typhi* (18 mm). For leaves extract highest zone of inhibition was showed highest zone *E.coli* (26 mm), followed by *S.aureus* and *Enterobacter spp* (25 mm), *Pseudomonas aeruginosa* and *salmonella typhi* (20 mm). The Aqueous peel extract showed highest zone of inhibition (25 mm) *S.aureus* (15 mm), *Salmonella typhi* (13 mm) against *E.coli* (12 mm) and least zone against *Enterobacter spp* (11mm). Dastjerdi *et al.* (2011), who evaluated the effect of *P. granatum* water extract on 5 common oral bacteria (i.e., *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus sobrinus*, and *Enterococcus faecalis*) and then the magnitude of the inhibitory effect of *P. granatum L.* on bacterial biofilm formation on orthodontic wire.

In the present study the antibacterial activity of petroleum ether extract of stem and leaves was checked (Fig 5). It was found that the extract from stem showed highest zone of inhibition 19 mm against *Pseudomonas aeruginosa* followed by *E.coli* and *Salmonella typhi* (17 mm), *S.aureus* and *Enterobacter spp* (15 mm). For the leaves extract zone of inhibition was showed against *S.aureus*, *E.coli*, *Pseudomonas aeruginosa* and *Enterobacter spp* (20 mm) and least zone against *Salmonella typhi* (17 mm). Devi *et*

al., (2011) [6] have reported the Ethanol and Petroleum ether extract on *Staphylococcus* and other dental bacteria. Petroleum ether extract showed 24 mm of zone against *S.aureus*. while ethanol extract showed 26 mm of zone.

In the study the antibacterial activity of fresh pomegranate juice was also checked (Fig 6). It was found that highest zone of inhibition was observed against *Salmonella typhi* (33 mm) followed by *Enterobacter spp* (23 mm), *P.aeruginosa* and *S.aureus* (19 mm) and least zone of inhibition against *E.coli* (12 mm). Shivsharan and Ravva (2018) have also reported promising antimicrobial activity of juice against *E.coli*. They have also reported 12 mm of zone.

The phytochemical analysis of extract was also performed. It was found that stem extract showed presence of flavonoids, Quinones, Tannins, Cardiac Glycosides, Terpenoids. while leaf extract showed presence of Flavonoids, Quinones, Tannins, Cardiac Glycosides, Terpenoids. As per various literature reports, *P. granatum* contains chemical constituents like, ellagitannins, phenols, tannins, punicic acid, flavonoids, anthocyanins, estrogenic flavonoids and flavones. 3 Some of these compounds have been found to own antibacterial activity. The tannin-rich ellagitannins Duman *et al.*, (2009) and phenolic acids of pomegranate may have antibacterial activity 8.29.32 It has been suggested that phenolic compounds, especially gallic acid, were the most important and active compounds against bacteria. Pomegranate contains large amounts of tannins (25%) and antibacterial activity may be indicative of the presence of secondary metabolites Malviya S., *et al* (2014) [18].

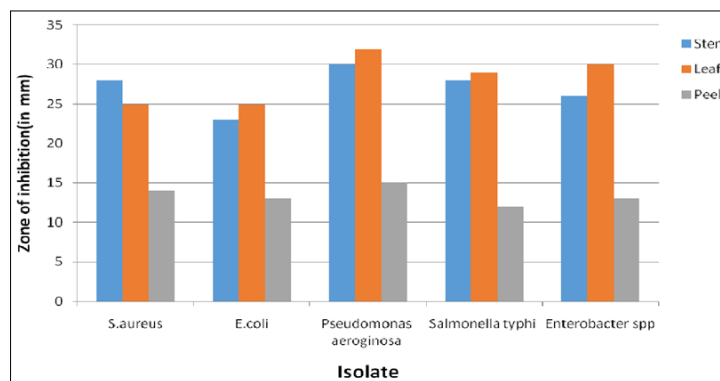


Fig 1: Antibacterial activity of Methanol extracts

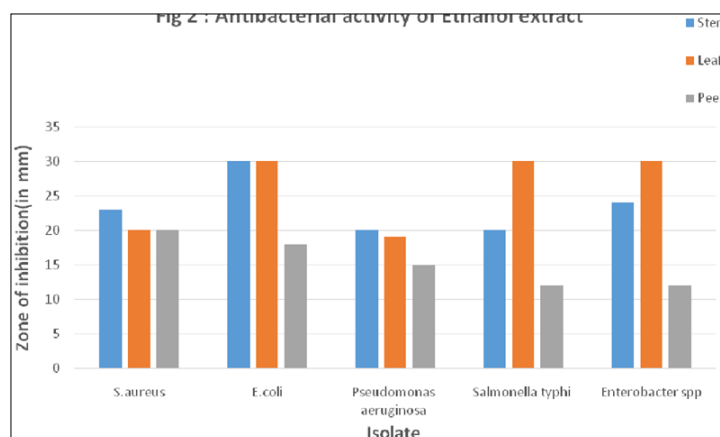


Fig 2: Antibacterial activity of Ethanol extracts

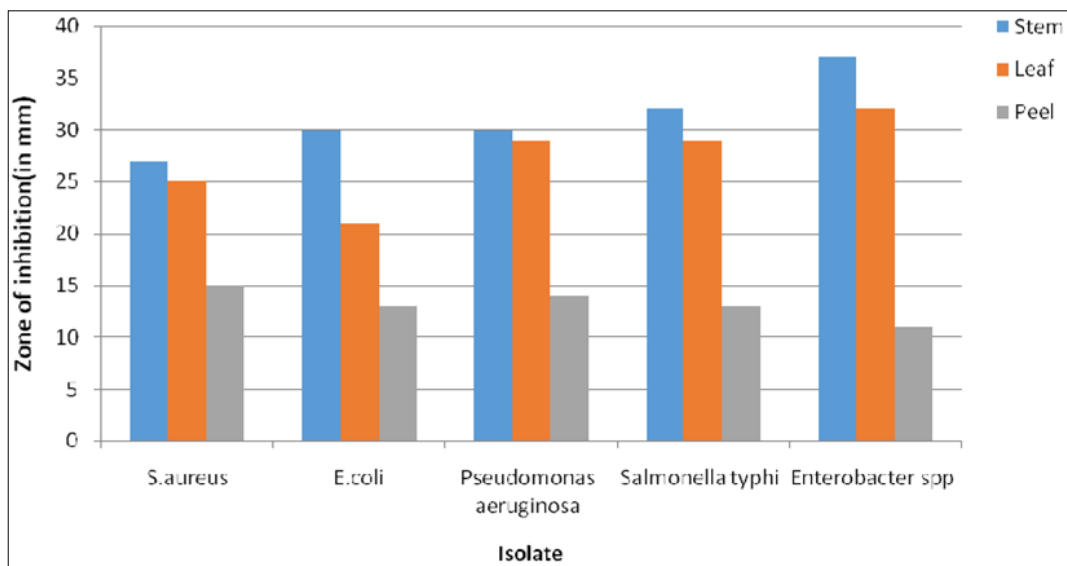


Fig 3: Antibacterial activity of Acetone extracts

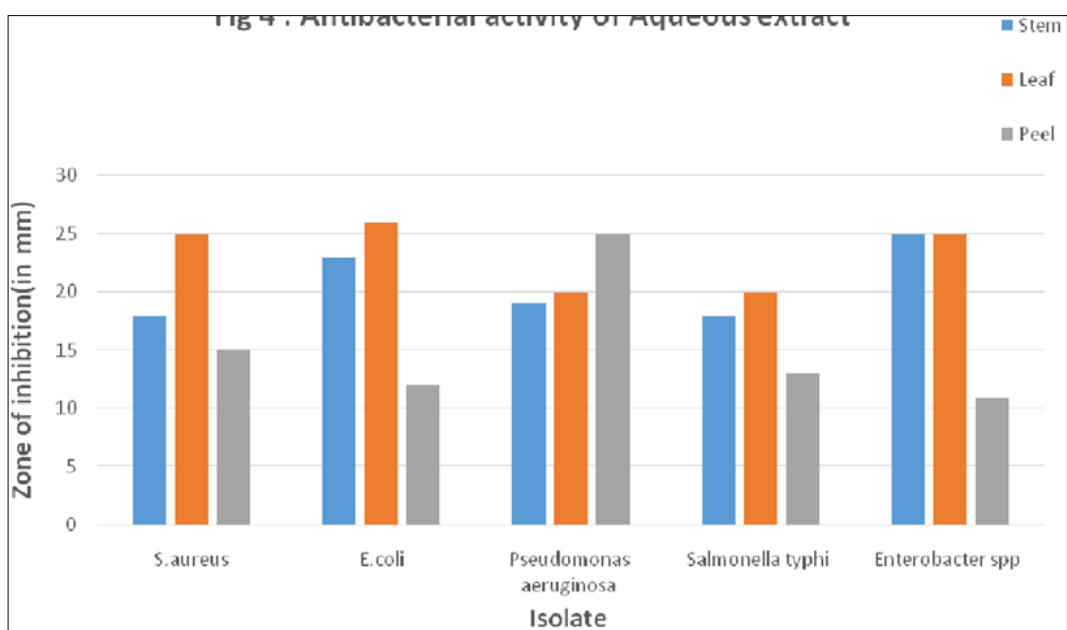


Fig 4: Antibacterial activity of Aqueous extracts

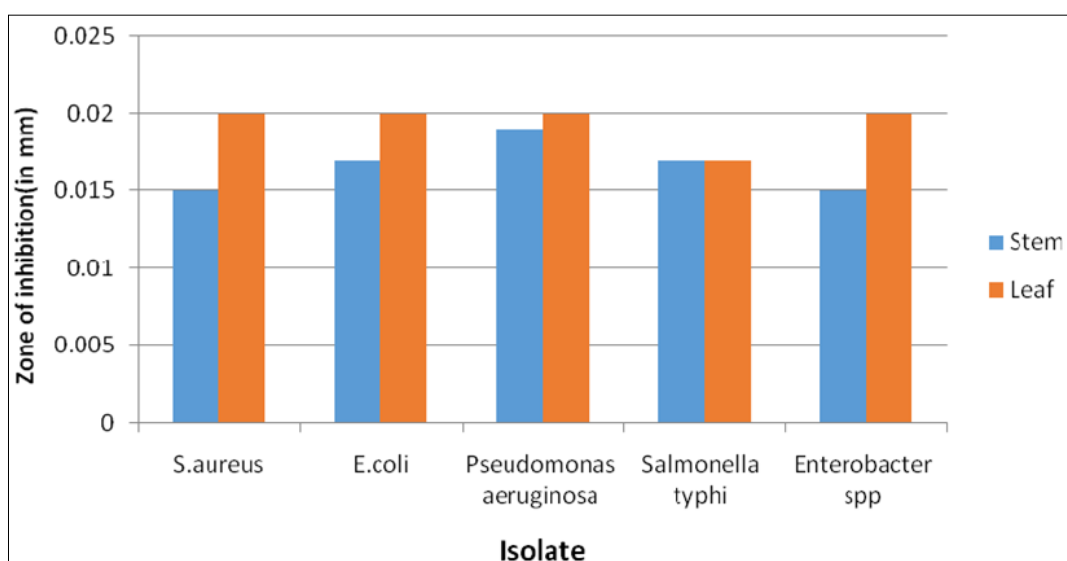


Fig 5: Antibacterial activity of Petroleum ether extracts

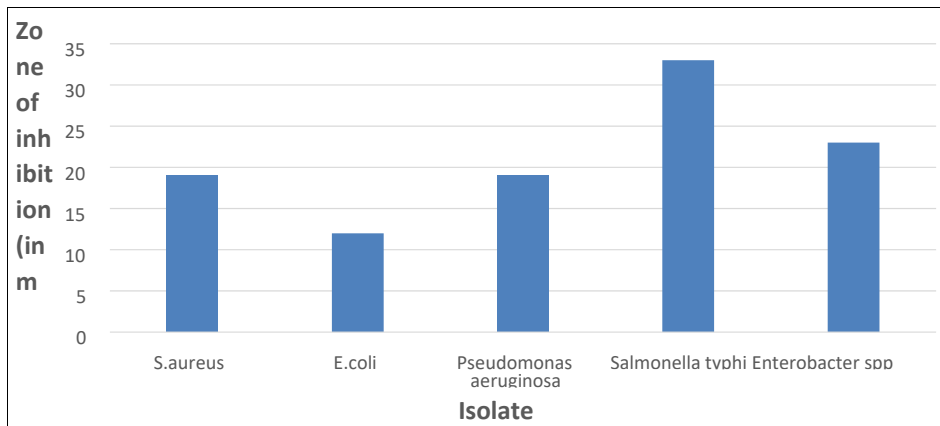


Fig 6: Antibacterial activity of Pomegranate juice

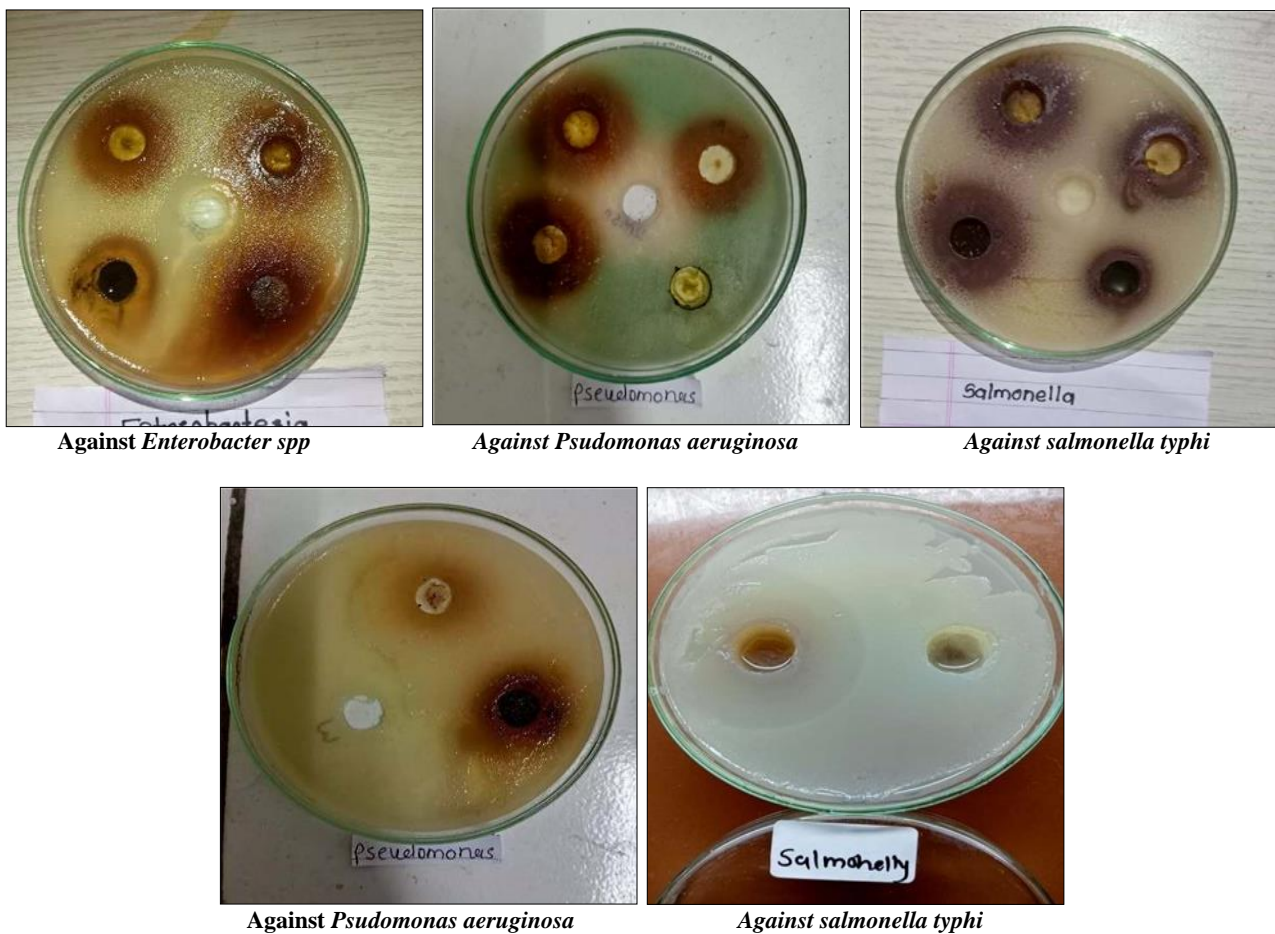


Fig 7: Antibacterial activity of different extracts

Conclusion

In the study it was found that each part of Pomegranate stem, leaves, peels and fruit juice possesses antibacterial potential. The methanol, ethanol, and acetone extracts of stem and leaves showed prominent antibacterial activity against test bacteria as compared to peel extract, while petroleum ether, and aqueous extract of leaves showed prominent activity than other. The fresh juice also found to exert considerable antibacterial activity against test clinical pathogens.

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