

**Study on antibacterial activity of pomegranate (*Punica granatum L.*) juice, peel and membrane with understanding through Islamic ideology**

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**Abstract:**

Known from its many health benefits, Pomegranate (*Punica granatum L.*) is also one of the Quranic plant and credited as a heaven fruit in Islamic literature. Pomegranate pulp, membrane and peel have biologically active compounds which are very potent for good health. The active compounds from the peel, arils and membrane were obtained using double distilled water as extracting solvent. Antimicrobial activity of pomegranate peel extract [PPE], pomegranate membrane extract [PME], pomegranate juice extract [PJE] were studied on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* by agar well spreading methods [diffusion method]. All organisms were sensitive to the extract with inhibitory concentration of 100 mg/ml. In this study, effects of PPE, PME, and PJE were analyzed under aseptic conditions and Islamic commentaries are added to understand its importance in human life.

**Keywords:** Phytochemical, Membrane, Extract, Pathogen

**Introduction**

The word 'pomegranate' is the Latin combination of the words *ponum*, meaning apple and *granatum* meaning 'grainy' or 'seeded'. The pomegranate (rumman in Arabic) is one of the oldest known fruits and is mentioned in the Qur'an as a fruit from Paradise.

***"In them both (gardens) will be fruits, and date palms and pomegranate"*(Quran, 55:68).**

Pomegranate (*Punica granatum L.*) belongs to the *Punicaceae* family and is nutrient dense food source and rich in phytochemical compounds. The ripe pomegranate fruit can be up to five inches wide with a deep red, leathery skin. It is grenade-shaped, and crowned by the pointed calyx. The fruit contains many seeds (arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red

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juice. The pomegranate is native of the Himalayas in northern India to Iran. However, it has been cultivated and naturalized since ancient times over the entire Mediterranean region. It is also found in India, in the more arid regions of Southeast Asia, the East Indies, and tropical Africa. The tree is also cultivated for its fruit in the drier regions of California and Arizona<sup>1</sup>. Along with dietary sugars, pomegranate also contains phytochemical compounds. Phytochemical compounds are low molecular weight compounds with several health benefits. Pomegranates are popularly consumed as fresh fruits, beverages, food products [jams and jellies]. Its extracts are used as botanical ingredients in herbal medicine and dietary supplements. Several studies reported that phytochemicals have been identified from, various parts of pomegranate tree and from pomegranate fruit i.e. peel, juice, and seeds<sup>2</sup>. The pomegranate extracts act as natural inhibitors of pathogens, bacteria and fungi<sup>3</sup>.

### **Review of literature**

Pomegranate has long decorative ancient history in various civilizations. According to Ebers Papyrus (1500 BC), Egyptian used the pomegranate for treatment of tapeworm and other infections. Pomegranate is also prominent at Greek weddings and funerals. Jews consumes pomegranates on Rosh hashana (Jewish new year) as its numerous seeds symbolizes fruitfulness. Azerbaijan

celebrates pomegranate festival in October annually. Pomegranates were symbol of fertility in ancient Persian culture. In Hinduism it symbolizes prosperity and fertility<sup>4</sup>.

Wide range of literature in Islamic studies had been quoted the importance of pomegranate, both nutritionally and medicinally. There are several *ahadith* which states the miraculous importance of pomegranate and advices its consumption. Although some *Rijal* studies are required for the authenticity of *ahadith*, some of very famous and important *ahadith* have been quoted here under.

A number of our people have narrated from Shal ibn Ziyad Ja'far ibn Muhammad al - Ash'ariy from ibn al-Qaddah who has said,

*Abu 'Abd Allah, 'Alayhi al -Salam, has said, 'Eat sour-sweet pomegranate with its flesh; it tans [cleanses] the stomach<sup>5</sup>.*

*Ali ibn Ibrahim has narrated from his father from & Muhammad ibn 'Isma'il has narrated from al Fadi ibn Shadhan from ibn abu Umayr from Ibrahim ibn' abd al- Hamid who has said,*

*I once heard abu 'Abd Allah 'Alayhi al -salam, saying, You must eat pomegranate, it sufficiency the hungry person and it is pleasant to satisfied ones<sup>6</sup>.*

*Ali ibn Muhammad ibn Bandar has narrated from his father*

*Muhammad ibn Ali al- Hamadaniy from sa'id al- Raqqam from Sahil ibn ' Uqbah who has said, I once heard abu 'Abd Allah , 'Alayhi al - salam, saying , ' Eat pomegranate with its flesh; it tans (cleans) the stomach and increase the brains ability<sup>7</sup>.*

From above we can understand the rich Islamic literature which convinces various aspects of pomegranate.

The chemical composition of the pomegranate fruits differs depending on the cultivar, growing region, maturity, cultivation practice, climate, and storage circumstances<sup>8</sup>. Pomegranate is an important source of bioactive compounds such as phenolics, flavonoids [flavanols, flavonols, anthocyanins], condensed tannins [proanthocyanidins] and hydrolysable tannins [ellagitannins and gallotannins]. Other Phytochemicals identified from the pomegranate are organic and phenolic acids, sterols, and triterpenoids, fatty acids, triglycerides, alkaloids, ellagic, caffeic, and punicic acid. These phenolic components belong to different representative chemical classes with known bioactivities. Several compounds identified as minerals, mainly potassium, nitrogen, calcium, phosphorus, magnesium, and sodium and complex of polysaccharides .The edible parts of pomegranate fruit [50%] consist of 40% arils, 10% seeds. Arils contains 85% water, 10% total sugar mainly fructose and glucose, 1.5% pectin ,organic

acid such as ascorbic acid, citric acid, and malic acid<sup>9</sup>.

Ellagitannin is a type of tannin; it can be broken down into hydroxybenzoic acid such as ellagic acid. It is widely used in plastic surgeries, which prevents skin flap's death due to it's antioxidant activity<sup>10</sup>. Anthocyanins have known pharmacological properties and are used by humans for therapeutic purpose. Anthocyanin are water soluble pigments responsible for the bright red color of pomegranate juice. Several anthocyanin compounds identified in pomegranate include pelargonidin-3-glucoside, cyanidin-3-glucoside, delphinidin -3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3,5-diglucoside and delphinidin-3,5-diglucoside<sup>11</sup>. Anthocyanin can be insect attractants in flowers but can be also be insecticidal and antimicrobial at the same time. Tannins as well as anthocyanins have signification antiproliferative and proapoptotic effects in several different types of cancer cell in vitro, including colon cancer, prostate cancer, head and neck cancer, skin cancer, cardiovascular diseases, Rheumatoid arthritis, Dental effects, Alzheimer, Malaria, HIV and wound healing.<sup>12</sup>

Recent study indicates that both pomegranate aril and peel extracts have an effective antimicrobial activity, including *Staphylococcus aureus* and *Escherichia coli*, often involved in food borne illness<sup>13</sup>. In addition, several research data strongly support the antibacterial activity of

extracts against oral pathogen such as *S. mutans*<sup>14</sup>.

Peak levels of these bioactives are found in fruit peel then in pulp. The peels of fruits discarded there by increasing solid waste production, even though they are actually rich in polyphenols. Thus, this waste which is mine of nutraceuticals can be harnessed for its fullest potential. The peels of pomegranate contain 249.4mg/ml of phenolic compounds as compared to only 24.4 mg/ml phenolic compounds found in the pulp of pomegranate<sup>15</sup>.

## **Materials and Methods**

### **Preparation of Extracts for Microbiological Assay**

Pomegranates (*P. granatum* L.) were procured from the local market of Sidhpur. The fruits were washed, peeled. Membrane and the arils, without seeds, were hand-crushed and then squeezed in order to obtain the juice. The peel and membrane were air dried for a few days and then pulverized in water. Fresh aqueous extract is used for further antimicrobial analysis. The same procedure was carried out for the membrane powder. Each sample was mixed properly and then the extracts were filtered.

### **Microorganisms and Growth Conditions**

The antimicrobial activity of the pomegranate extracts was evaluated against the strain *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Bacteria were

cultured aerobically in broth and nutrient agar media at 37 C. The nutrient media was used for cultivation of bacteria. Microbial strains were maintained at 4 C on agar media. The isolates were stored at 4 C in nutrient broth supplemented with 10% glycerol (v/v) until use and the working cultures were activated in the respective broth at 37 C for 15–18 h.

### **In Vitro Antibacterial Activity Assays**

The susceptibility of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* to extracts of peel, membrane and juice of *Punica granatum* L. was determined. Firstly, all organisms were cultured separately on agar plate by spreading. By using cup borer method, hole on solidified agar was made for analysis. 100µl of different juice extracts ([PPE], [PJE], [PME]) were added to each hole of different bacterial strains.

To verify the effect of pomegranate juice, membrane and peel double distilled water extracts on the growth *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were performed in presence of increasing concentrations of the extracts. All the plates were incubated at 37 C temperature under sterile incubator. To evaluate the effect of extracts on growth of bacteria, plate analysis is done at every 24 hour. All experiments were performed in triplicate, with three independent cultures; the results obtained were analyzed.

## Results

### In Vitro Antibacterial Activity of Pomegranate Extracts

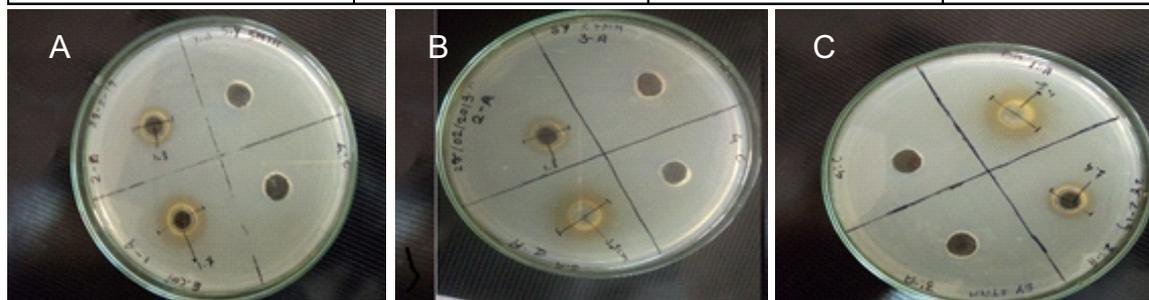
The antimicrobial activity of pomegranate extracts against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* isolates were evaluated by spread plate-cup borer method. Growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* isolate were inhibited with a concentration of pomegranate juice, membrane and peel extract equal to 100mg/ml respectively. The mean zone of inhibition (cm) of double distilled water extract of

pomegranate (*Punica granatum* L.) peel, membrane and juice giving in table 1.

Highest zone of inhibition by PME were seen in *Escherichia coli* (2.6 cm) followed by *Pseudomonas aeruginosa* (2.6 cm) followed by *Staphylococcus aureus* (1.9 cm). Whereas highest zone of inhibition in PPE were detected in *Escherichia coli* (1.3 cm) followed by *Staphylococcus aureus* (1.3 cm) followed by *Pseudomonas aeruginosa* (1.4 cm). Zone of inhibition in PJE were same seen in given order *Staphylococcus aureus* (1.9 cm), *Pseudomonas aeruginosa* (1.7cm), *Escherichia coli* (1.65 cm).

**Table 1:** Antibacterial activity of pomegranate fruit extracts against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in terms of Zone of inhibition in cm at 24 hour.

Bacterial isolates	Peel extract (10 <sup>-2</sup> dilution )	Membrane e xtract	Juice extract
<i>Escherichia coli</i>	1.3cm	2.6cm	1.65cm
<i>Pseudomonas aeruginosa</i>	1.4cm	2.6cm	1.7 cm
<i>Staphylococcus aureus</i>	1.3cm	1.9cm	1.9 cm



**Fig. 1.** Antibacterial effects of peel on 3 different bacterail organisms at 24 hr.

- |                                   |  |
|-----------------------------------|--|
| A) <i>Escherichia coli</i> ,      | 2) Hot air oven dried peels of pomegranate |
| B) <i>Staphylococcus aureus</i> , | 3) Hot air oven dried seeds of pomegranate |
| C) <i>Pseudomonas aeruginosa</i>  | 4) Control with distilled water            |
- 1) Sun light dried peels of pomegranate



**Fig. 2.** Antibacterial effects of juice, membrane and peel on 3 bacterail organisms at 24 hr.

A) *Escherichia coli*,

B) *Staphylococcus aureus*,

C) *Pseudomonas aeruginosa*

- 1) Fresh juice of pomegranate
- 2) Hot air oven dried peels of pomegranate
- 3) Hot air oven dried membrane of pomegranate
- 4) Control with distilled water

The results are in agreement with earlier investigations reported by other authors to inhibit different microorganism. Many food borne bacteria cause serious gastrointestinal infection, such as *Escherichia coli O15:H7* which can lead to hemorrhagic diarrhoea. These infections can be life-threatening to young children and the elderly. There is an incentive to find alternative control measures, such as plant and herbal extracts, especially in lesser-developed countries where traditional antibiotic may not be readily available. In Thailand, a study was undertaken in which, extracts of pomegranate were tested for their antibacterial activity against different strains of *E.coli*, including 3 strains of *E.coli O157:H7*<sup>16</sup>. Such research supports our findings and provides future thrust for research in this area.

As documented in the earlier studies the effect of methanol peel extract

is done by major researcher. With little work on double distilled water extract, here we are reporting double distilled water pomegranate extract of peel, membrane, juice are able to inhibit both Gram positive and Gram negative bacteria. Studies have been reported that alcoholic extract of pomegranates have positive effect against pathogenic bacteria. Some reports stated that hydro-alcoholic extracts also have negative effects on pathogenic bacteria. Here, only double distilled sterile water extracts of pomegranate is used to analyze antibacterial effects. Water extract is used for two reasons. First, Alcohol extracts may have effects as it may interfere with antimicrobial activity. Second, alcohol is prohibited to use in Islam.

### **Conclusion and discussion**

From the study undertaken, it can be concluded that double distilled water extract of pomegranate peel, membrane, juice have antibacterial properties. In this experiment, extract of pomegranate membrane gave a highest zone of inhibition compare with pomegranate peel and juice. In terms of effect of these extract on gastrointestinal pathogenic organisms such as *Escherichia coli*,

*Pseudomonas aeruginosa* and *Staphylococcus aureus* have been analysed. The experiment was undertaken based on Hadith of *abu 'Abd Allah, Imam Jafar* (a.s.) for pomegranate, which clearly mentions to eat pomegranate with its membrane. Certainly this fruit have Islamic importance and used by humanity from ancient period by knowing its preventive and curative effects on diseases. These three bacteria selected on the basis that they are causing some routine gastrointestinal disorder like food poisoning, colitis, diarrhoea and infection such as impetigo, boils, gastrointestinal cancer etc. It also shows the intelligence of nature, as these white membranes around arils might naturally protects against various degradative organisms during its development as a fruit. In conclusive remarks, it should be understood from above research that rich Islamic literature advices to use pomegranate and also pomegranate should be ate along with their white membranes which have protective effects against some routine bacterial infections.

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