

**ANTIFUNGAL AND ANTIMICROBIAL ACTIVITY OF
POMEGRANATE PEEL POWDER****Anjum Patel^{1*}, Khan Shadab² and Bhise K. S.³**¹M. C. E. S Institute of Pharmacy, Pune.^{2,3}Department of Pharmaceutics, M.C.E. Society's Allana College of Pharmacy, Pune-411001, Maharashtra, India.Article Received on
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Society's Allana College of
Pharmacy, Pune-411001,
Maharashtra, India.**ABSTRACT**

Punica Granatum belongs to family Punicaceae. The objective of the present work was to identify the phytochemical constituents and to study of antibacterial and antifungal activity of pomegranate peel powder suspension. Punicagranatum are utilized by local people as the part of their meal and treat health diseases. Peoples supposed to consume Punicagranatum seed and their peel was thrown as waste. Recently, natural products have been evaluated as sources of antimicrobial and antifungal agents with efficacies against a variety of microorganisms. Present study was designed to evaluate the antibacterial and antifungal activity of Punicagranatum peel against human pathogens. The powder suspension was prepared using water.

Antimicrobial activity was tested against gram positive bacteria and gram negative bacteria while antifungal activity was tested against fungi. The various Concentrations of powder suspension were tested. Evaluations were based on the zone of inhibition using Agar well diffusion assay. The inhibitory activity was found to be dose dependent. This study represents that pomegranate extracts of waste material (peel) of Punicagranatum may be utilize as a potential source of antimicrobial and antifungal agents.

KEYWORDS: Punicagranatum, Sabouraud agar, Antifungal, antimicrobial.**1. INTRODUCTION**

Medicinal plants play an important role in the human health care and are one of the oldest forms of health care known to mankind. In India, over 2600 plant species has been considered useful in the traditional system of medicines like Ayurveda, Unani and Siddha

(Khandelwal, 1999). A country like India is much suited for development of drugs from plant origin. Many naturally occurring compounds found in dietary and medicinal plants and fruits have been shown to possess antimicrobial and antifungal activities. Antimicrobials of plant origin have enormous therapeutic potential and have been used from ancient times. *Punicagranatum* belonging to family *Lythraceae*, is deciduous shrub. Fruits are consumed fresh or used for the preparation of fresh juice, jelly and jam, and beverage products. In many systems of medicine *Punicagranatum* fruit is used for variety of ailments. Its fruit juice have various phytoconstituents whose functional and medicinal effects such as hepatoprotective, antibacterial, antioxidant, anticancer, antidiabetic, anti-atherosclerotic effects, estrogen-like activity had been confirmed. The peels has wide range of therapeutic properties and can be used in treatment of diabetes, cancer, cardiovascular disease, dental conditions, erectile dysfunction and male infertility, infectious diseases, Alzheimer's disease and dermal wounds. pomegranate fruits peel powder (PPP) protein contained a much higher content from lysine, leucine, aromatic fatty acids (phenylalanine and tyrosine), threonine and valine than the reference protein pattern and therefore the amino acid score of these IAAs was higher than 100. On the other hand, the PPP had slight lower contents of amino acids containing sulphur (methionine and cysteine) and isoleucine which having amino acid score of 95.7 and 93.2; respectively. Therefore, the incorporation of available inexpensive pomegranate by-products; peel, powder in Egypt into the foodstuff; especially which deficient in amino acids containing sulfur, aromatic amino acids, leucine and isoleucine has a great economic value and a good standpoint in food technology and human.

- Ancient time in Greek mythology, pomegranate known as “fruit of the dead”, the substances available in Hades for its residents.
- Fruits are one of the oldest forms of food known to man. There are many references to fruits in ancient literature.
- Vedas state that fruits form the base of the Food of Gods. According to Qur'an, the fruits like grapes, date, fig, olive and pomegranate are gifts and heavenly fruits of God.
- The people in ancient times regarded fruits to be endowed with magic or divine properties.
- It was found in the Indus Valley so early that there is a word in Sanskrit for pomegranate. The pomegranate is also significant in Jewish, Christian and Muslim traditions.

The pomegranate is native of Iran and Afghanistan known in ancient Egypt. Pomegranate belongs to punicaceae family. It is one of the important horticulture fruit to the Mediterranean climate. The edible part of fruit contains considerable saccharides, polyphenol and important minerals.

The objectives of the present study were to evaluate the antibacterial and antifungal activity of pomegranate peel on selected bacterial and fungal cultures.^[1,2,3,4]

2. MATERIAL AND METHOD

A. MATERIAL

Pomegranate peel (Pomegranate fruit (*Punicagranatum*) were collected from the local markets, Peels were removed and dried in an oven by hot air (50°C) for 48hr. Dried peels were powdered to get 60-mesh size using a mixing grinder. then dissolved in particular amount of distilled water to make required concentrated solutions.), Sabouraud agar, subouraud broth.^[5]

• TEST MICROORGAINSMS

The microorganisms selected forthis study were

- Gram positive bacteria -*Staphylococcus aureus* (ATCC 29213), *Salmonella typhii* (MTCC 3214),
- Gram negative bacteria- *Escherichia coli* (ATCC 25922)
- Yeast- *Candida albicans* (ATCC10231).

The microbial cultures of these microorganisms were obtained from Pune.^[6,7]

• CULTURE MEDIA AND PREPARATION OF INOCULUM

The stock cultures were prepared by incubating pure cultures of bacteria on nutrient agar for 48 hours at 37±2°C and pure culture of *Candida albicans* on Sabouraud dextrose agar for 72 hours at 25±2°C. The stock cultures were maintained at 4°C on agar slant. Culture inoculum was prepared by suspending in broth.^[8,9]

• APPARATUS

Zone reader, oven memmert, pipette, mixer, Balances, Homogenizer, Mixer, Incubator, Ultrasonic (soniprep 150 HSE) at 20 KHZ. Centrifuge, Autoclave, Water bath, Rotary evaporator, Magnetic stirrer and Shaker.

B. METHODS

• PREPARATION OF AQUEOUS SUSPENSION

The required amount of the powder of pomegranate peels are weighed to make different concentration then dissolved in distilled water. ultrasonication is used to make clear solution.^[10,11]

• ANTIBACTERIAL ACTIVITY

The agar diffusion cell method was used to check antibacterial activity. The agar diffusion method was used to study the effect of pomegranate peel powder suspension on growth of *Staphylococcus aureus*, *Escherichia coli*, *B.Subtilis* by measuring of the diameter of the inhibition zone. 10ml sterile assay agar was added to each of two Petri dishes with slow shaking.

Warmed agar was inoculated with 0.5ml active bacterial culture and allowed to harden in a refrigerator. afterwards equidistant holes were made in the agar using sterile cork borers and 100µl of suspension were added at different concentration (100,300,500,700µg/ml) was added at the top of the inoculated agar layer then dried at 25°C for 30 min. Plates were kept at 4°C for 1 h for harden in a refrigerator then incubated at 37°C for 48-72h. At the end of this period, inhibition zones formed on medium were accurately measured in mm.^[12,13]

• ANTIFUNGAL ACTIVITY

The agar diffusion cell method was used to check antifungal activity. The agar diffusion method was used to study the effect of pomegranate peel powder suspension on growth of *candida albicans* by measuring of the diameter of the inhibition zone. 10ml of sterile agar solution was added one Petri dish with slow shaking. Warmed agar was inoculated with 0.5ml active *candida albicans* culture and allowed to harden in a refrigerator. afterwards equidistant holes were made in the agar using sterile cork borers and 100µl of suspension were added (at different concentration (100,300,500,700µg/ml) was added at the top of the inoculated agar layer then dried at 25°C for 30 min. Plates were kept at 4°C for 1 h for harden in a refrigerator then incubated at 37°C for 48-72 h. At the end of this period, inhibition zones formed on medium were accurately measured in mm.^[14,15]

3. RESULTS

A. ANTIMICROBIAL ACTIVITY

The antimicrobial activity of different prepared concentration of pomegranate peel powder suspension against microorganisms including (*Staphylococcus aureus*, *Escherichia coli*) was recorded after 24hr of incubation. The result was analyzed and compared with the control of each of selected microorganisms to find out the significant differences in the microbial growth after treatment with pomegranate peel powder suspension .^[16]

Table 1: Antimicrobial Activity

concentration µg/ml	Zone Of Inhibition (mm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
100	7	5	8
300	9	11	10
500	11	14	11
700	13	17	13
900	25	20	16

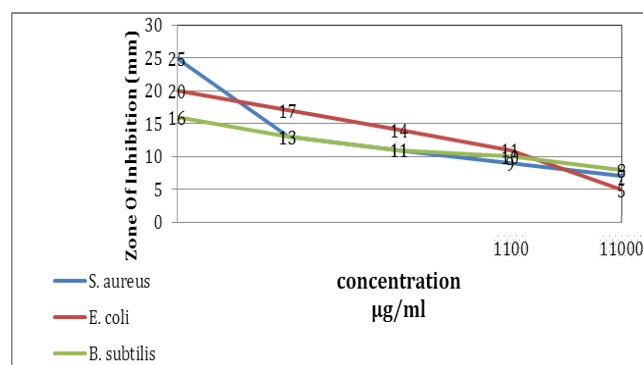


Figure 1: Antimicrobial Activity

B. ANTIFUNGAL

The minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) of pomegranate powder suspension against *C. albicans* ATCC 10231 were determined according to reference procedure.

Table 2: Antifungal Activity

concentration µg/ml	Zone Of Inhibition (mm)
	<i>C. albicans</i>
100	5
300	7
500	10
700	15
900	25

Ellagic acid and punicalagins constituent from pomegranate revealed antifungal activity against *Aspergillus fumigatus*. Variable activity of pomegranate peel also was observed in an earlier study and some researchers, Our finding exhibited the existence of antifungal activity of selected *Punicagranatum* against *Candida spp.* and this finding was also reported.^[17]

4. CONCLUSION

Pomegranate peel extract was active and effective against the growth of tested microorganisms. Whereas, the inhibition zones ranged from 9.6 to 25.7 mm depend on type of microorganism. These results provide evidence for the presence of antimicrobial phenolic compounds. These compounds can degrade the cell wall, disrupt the cytoplasmic membrane, damage membrane proteins and interfere with membrane-integrated enzymes, which may eventually lead to cell death.^[18]

5. REFERENCE

1. Adhami, V.M., and Mukhtar, H. 2006. Polyphenols from green tea and pomegranate for prevention of prostate cancer. *Free. Rad. Res.* 40(10): 1095-104.
2. Ahmad, I., Z. Mehmood and Mohammad, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.* 62: 183-93.
3. Faria, A., R. Monteiro, N. Mateus, I. Azevedo and Calhau, C. 2007. Effect of pomegranate (*Punicagranatum*) juice intake on hepatic oxidative stress. *Eur. J. Nutr.* 46(5): 271-8.
4. Rowayshed, G., A. Salama, M. Abul-Fadl, S. Akila-Hamza and E.A. Mohamed, 2013. Nutritional and Chemical Evaluation for Pomegranate (*Punicagranatum L.*) Fruit Peel and Seeds Powders By Products. *Middle East Journal of Applied Sciences*, 3(4): 169-179.
5. Eliana Harue Endo , Tânia Ueda-Nakamura , Celso Vataru Nakamura, Benedito Prado Dias Filho ; Activity of Spray-dried Microparticles Containing Pomegranate Peel Extract against *Candida albicans*; *Molecules* 2012; 17: 10094-10107.
6. Sachin Annasaheb Nitave*, Vishin Ashish Patil; study Of antibacterial and antifungal activity of *punicagranatum* peels and its phytochemical screening; *World Journal of Pharmaceutical Research*; 2014; 3(10): 505-512.
7. M.I. Ibrahim; Efficiency of Pomegranate Peel Extract as Antimicrobial, Antioxidant and Protective Agents; *World Journal of Agricultural Sciences*, 2010; 6(4): 338-344.

8. Mahsa Shafighi , Leila Amjad, Mahboubeh Madani; Effect of Fungal Growth Inhibition from Pomegranate Flower and Peel Extracts; International Conference on Applied Life Sciences (ICALS2012) Turkey, September 2012; 10-12: 377-380
9. Ibrahim.S. Abaas and Mohammed. J. Hamzah, Ali.J.Ali; Extraction, Identification And Antifungal Studies Of Leaves Extract Of Punica Granatum L.; World Journal of Pharmacy and Pharmaceutical Sciences; 2014; 3(3): 190-193.
10. SaadSabbar Dahham, Mir Naiman Ali, Hajera Tabassum and Mazharuddin Khan; Studies on Antibacterial and Antifungal Activity of Pomegranate (Punicagranatum L.); American-Eurasian J. Agric. & Environ. Sci., 2010; 9(3): 273-281.
11. Alanowd Omar Ali Mehder, Pomegranate Peels Effectiveness In Improving The Nutritional, Physical And Sensory Characteristics Of Pan Bread; Current Science International 2013; 2(2): 8-14.
12. K. Ashok Kumar and K. Vijayalakshmi; In vitro anti-microbial activity and phytochemical analysis of selected fruit wastes; Int. J. Curr. Microbiol. App. Sci 2013; 2(5): 196-204.
13. Trease, G. E. and Evans, W. C. 1989. Pharmacognosy, 14th Edition, W.B.
14. Scandars Company, Ltd. London .pp. 269-300.
15. Ashok Kumar, K., and Vilayalakshmi K. 2011. GC-MS Analysis of phytochemical constituent in Ethnolicextract of Punicagranatum peel and Vitisvinifera seeds. Int. J. Pharma. Bio sci. 2(4): 461 468.
16. Mahajan DC, Satyapal US, *Tatke PA, Naharwar V; Antimicrobial and Anthelmentic Activity of Punicagranatum Fruit.
17. Peel Extracts; International Journal of Pharmacognosy and Phytochemical Research 2014; 6(3): 482-487.
18. Singh RP, Chidambara Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate (Punicagranatum) peel and seed extracts using in vitro models. Journal of Agricultural and Food Chemistry 2002; 50(1): 81–86.
19. Khandelwal KR. Practical Pharmacognosy- Techniques and experiments. 19th ed., Nirali Prakashan, Pune, 2008; 149-156.
20. L. Vasconcelos, F. C. Sampaio, M. C. Sampaio, M. S. Vieira Pereira, J. S. Higino, M. P. Peixoto, 2006. Minimum Inhibitory Concentration of Adherence of Punicagranatum Linn (pomegranate) Gel against S. mutans, S. mitis and C. albicans. Braz Dent J, 17(3): 223-227.