ANTIFUNGAL AND ANTIMICROBIAL ACTIVITY OF POMEGRANATE PEEL POWDER

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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. Punica granatum are utilized by local people as the part of their meal and treat health diseases. Peoples supposed to consume Punica granatum 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 Punica granatum 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 Punica granatum may be utilize as a potential source of antimicrobial and antifungal agents.

KEYWORDS: Punica granatum, 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. Punica granatum 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 Punica granatum 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 MICROORGANISMS**
  The microorganisms selected for this 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 weigh to make different concentration then dissolved in distilled water. ultrasonication is used 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 with0.5ml 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 dishe 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**

<table>
<thead>
<tr>
<th>concentration µg/ml</th>
<th>Zone Of Inhibition (mm)</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>B. subtilis</th>
</tr>
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<tr>
<td>100</td>
<td>7</td>
<td>5</td>
<td>8</td>
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<td>700</td>
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<td>17</td>
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<td>900</td>
<td>25</td>
<td>20</td>
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**Figure 1: Antimicrobial Activity**

B. ANTFUNGAL

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**

<table>
<thead>
<tr>
<th>concentration µg/ml</th>
<th>Zone Of Inhibition (mm)</th>
<th>C. albicans</th>
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<tr>
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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 Punica granatum against Candida spp. and this finding was also reported.\textsuperscript{[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.\textsuperscript{[18]}

5. REFERENCE
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


