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Research Article

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Antimicrobial Effect of *Punica granatum* on Pyogenic Bacteria

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Abstract

An *in-vitro* antibacterial study was done using methanolic extract from the pericarp of *Punica granatum* against pathogens isolated from pus samples of human hosts by disc diffusion method. The study showed that more than 84% of the tested pathogens were susceptible to the methanolic extract. Among the tested strains, 96% of *Staphylococcus aureus*, 57% of *Escherichia coli*, 83% of *Klebsiella pneumoniae*, 75% of *Proteus mirabilis*, 81% of *Proteus vulgaris* and 75% of *Pseudomonas aeruginosa* were prominently inhibited by the extract. Results of the study indicate that the methanolic extract from the pericarp of pomegranate has a potential broad spectrum of antimicrobial activity against pathogenic strains isolated from human pus samples

Key words: Antibacterial activity, Pomegranate, *Punica granatum*, Pyogenic bacteria.

Introduction:

Pyogenic bacteria are responsible for most of the nosocomial infections. The emergence of multi drug resistant strains of such pathogens is a serious threat and makes chemotherapy more difficult. The toxicity of new generation antibiotics discourages their use in treatment. Moreover, the current cost of most of the chemotherapeutic agents is unaffordable to the public especially in developing countries like India. Therefore attempts must be directed towards the development of effective natural, non-toxic drugs for treatment.

Human infections, particularly those involving the skin and mucosal surfaces may lead to serious complications, especially in tropical and subtropical developing countries. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* are the most frequent pathogens causing hospital-acquired infections. [1] Many infections that produce pus are derived from various infectious microorganisms including bacteria. Pus is a viscous substance, usually yellowish to green in colour. It is composed of pus cells, bacteria and cellular debris. Infectious microorganisms such as *Staphylococcus, Streptococcus, Neisseria, Klebsiella, Proteus, Pseudomonas* and some other species of pyogenic bacteria usually produce pus. This is usually a source of infection to others and the transmission can be either due to direct contact or through fomites. Young children and elderly are especially vulnerable to infections by *Staphylococcus aureus*. Drug-resistant bacteria and the fungal pathogens may complicate the treatment of suppurative infectious diseases. This has necessitated researches for new broad spectrum and relatively non-toxic antimicrobial substances from natural sources including plants. [2]

The active principles of many drugs are found in plants in the form of secondary metabolites. During the last few years these secondary metabolites have been used widely for curing many diseases. An added advantage for plant products is that development of drug resistance by microorganisms towards these products is very rare. Moreover their toxicity is very low compared to that of the currently available commercial antibiotics.

In this study, methanolic extract of the pericarp of *Punica granatum* were screened for antibacterial activity against pathogens isolated from pus and pus swab samples. *Punica granatum* L. is a shrub belonging to the family *Punicaceae*. Even though many parts of this plant is found to have medicinal properties, the fruit commonly known as pomegranate, possesses a vast ethno medical history and represents a phytochemical storage for many medicines. Many studies reveal that the plant and its parts are useful to cure various infectious diseases of bacterial, viral and fungal origin. Recent report shows that they have anti tumor activity. The present study has extensively investigated on the activity of its pericarp against pus forming clinical bacterial strains.

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Materials and methods:

Preparation of the extract

Fresh fruits were collected from shop and washed thoroughly first in tap water and then in saline water and finally with distilled water. The pericarp was separated from the fruits and was dried in shade. It was powdered and utilized for extraction. 100 ml of solvents were added to 5g powders in the order petroleum ether, acetone, methanol and water. Solvents were changed at every 48-hour duration. Intermittent shaking was given for maximum dissolution of the compounds in the

solvent. After 48 hours in appropriate solvent, the extract was filtered, and from this stock 1 ml was taken which approximately contains 50 mg of crude sample. It was used for loading 100 discs. Therefore each disc contains 0.5 mg of the crude extract. It was used for testing the antimicrobial property by disc diffusion method. Only the methanolic extract was used for the study because all the other extracts showed limited activity in preliminary screening.

Bacterial Strains

Different strains of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris* and *Pseudomonas aeruginosa*, isolated from pus and pus swab samples of patients were used for screening study. The pathogens were collected from the Department of Doctors' Diagnostic Centre, Kottayam, Kerala (India).

Culture media

The dehydrated Muller - Hinton Agar (MHA) medium purchased from Hi-media Laboratories Pvt.Ltd. Mumbai was used. The medium was rehydrated, sterilized in an autoclave at 121° C for 15 minutes and was poured into sterilized Petri dishes and allowed to set. The plates were stored at 4 - 10° C in refrigerator. Before inoculation the surface of the media was dried in an incubator.

Antibacterial Test by Disc Diffusion Method

The antibacterial activity was tested using Disc Diffusion Method. [3] The dried plates were inoculated by test strains uniformly over the surface using a sterile cotton swab. A sterile 6 mm Whatmann No.1 filter paper loaded with the methanolic extract was applied on the surface of the inoculated plate and gently pressed using a sterile forceps. Discs loaded with the solvents were used after drying as the control. The plates were incubated at 37° C for 24 hours. The zone of inhibition of bacterial growth around the disc was measured in millimeters and recorded. The tests were repeated for three times and the average diameter of inhibition zone was recorded.

Results:

Table 1 shows the antibacterial effects of the methanolic extract from the pericarp of *Punica granatum* on pathogens isolated from pus and pus swab samples.

Table 1: Antibacterial effects of methanolic extract of Punica granatum pericarp against pathogens isolated from pus and pus swab samples

Bacterial species	No. of Strains tested	No. of Strains showing inhibition zone to methanolic extract		
		Low inhibition (0-9mm)	Moderate inhibition (10-19mm)	High inhibition (20-25mm)
S. aureus	84	3	***17***	***11***
E.coli	21	***9***	***12***	***0***
K.pneumoniae	18	3	***15***	***0***
P.mirabilis	24	***6***	***16***	***2***
P.vulgaris	11	***2***	***9***	***0***
Ps.aeruginosa	20	***5***	***13***	***5***
Total	178	28	135	15

S. aureus :Staphylococcus aureus , E. coli : Escherichia coli , K. pneumoniae : Klebsiella pneumoniae, Pr.mirabilis : Proteus mirabilis , Pr. vulgaris : Proteus vulgaris , Ps.aeruginosa : Pseudomonas aeruginosa

The results indicate that the methanolic extract showed effective inhibition against the tested strains. A total of 178 stains belonging to six species were tested. Of them 84% showed moderate and high inhibition with an inhibition zone ranging from 10-25mm. Among the strains of *Staphylococcus aureus*, 13% (11 strains) showed high inhibition, 83% (70 strains) showed moderate inhibition and 3.5% (3 strains) showed less inhibition. Among *Escherichia coli*, 57% (12 strains) of tested strains showed moderate and 42 % (9 strains) showed low inhibition. Among *Klebsiella pneumoniae*, 83% (15 strains) showed moderate and 16.5% (3 strains) showed limited inhibition. Among *Proteus mirabilis*, 8% (2 strains) showed high inhibition and 66.5% (16 strains) showed moderate inhibition. Only 25 % (6 strains) showed negligible effect. Out of 11 tested strains of *Proteus vulgaris*, 82 % (9 strains) showed moderate inhibition and 18 % (2 strains) showed low inhibition. Among *Pseudomonas aeruginosa* 10% (2 strains) showed high inhibition and 65 % (13 strains) showed moderate inhibition. Only 25 % (5 strains) showed less inhibition.

Discussion:

The use of medicinal plants still plays a vital role to cover the basic health needs in developing countries. Nearly 80% of the world populations rely on traditional medicine for primary health care, most of which involves the use of natural products. [4] In this connection, plants continue to be a rich source of therapeutic agents.Many researchers made an extensive study on the biological properties of *Punica granatum* and their results showed that this plant is ethno medically valuable. [5] Punica *granatum* peel extracts are currently used for treatment of respiratory diseases and in the preparation of tinctures, cosmetics and other therapeutic formulae. The tannin-rich ellagitannins and phenolic acids of *Punica granatum* have antioxidant [6], antimalarial [7] antibacterial, antifungal and antiprotozoal activities. [8-11] The methanolic extract of dried pomegranate peel possesses wound-healing property. [12] *Punica granatum* flower has been used as an anti-diabetic medicine. The extracts of *Punica granatum* prevent the growth of human breast cancer cells and are effective against inflammations. [13-14]This study proved its antibacterial property against clinical strains.

Conclusion:

In this study, crude methanolic extract of the pericarp of *Punica granatum* was tested for antibacterial activity against clinical strains isolated form pus and pus swab samples of patients. Study showed that the pericarp extract possesses some broad-spectrum antibacterial activity, which is sufficient to inhibit the growth of more than 84 % of the tested pus producing pathogens. Although many reports on the antimicrobial activity of pomegranate exist in the literature, none of them relates such activity with pathogens isolated from pus of human origin. After purification and characterization of the active principle followed by a detailed study of toxicity and pharmacological effects of the compound, the pericarp extract of pomegranate can be used as a remedy for pyogenic diseases especially against skin infections.

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