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Antibacterial Activity of *Punica granatum* L. against Gastro Intestinal Tract Infection Causing Organisms

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Abstract

The pericarp of *Punica granatum* Linn. has been commonly employed as a crude drug in Indian traditional medicine for the treatment of diarrhoea as well as for use as an astringent, antihelminthic, asphrodisacs, laxative, diuretic, stomachic, cardiotonic and refrigerant. Antibacterial activity of *P. granatum* pericarp extracts was evaluated against ten Gastro Intestinal Tract (GIT) infection causing bacterial strains using paper disc agar diffusion method. The result indicated that the extracts obtained from *P. granatum* pericarp exhibited antimicrobial activity against all organisms except the crude extract used against *Pseudomonas aeruginosa.* The methanol extract has exhibited maximum antibacterial activity against *Salmonella typhimurium*, *Salmonella typhi* and *Shigella dysenteriae* Serotype 1. Methanol extract shows significant activity against tested bacterial strains when compared to other extracts used in the study. Our findings suggest that an appropriate bioactive compound(s) may be developed from *P. granatum* pericarp as complementary alternative medicine for the treatment of GIT infection causing bacterial strains.

Key words: Medicinal plant, Antibacterial activity; *Punica granatum*; Gastrointestinal Tract Infections

Introduction

Ever since the dawn of civilization man has used plants for his food, shelter, and fodder for his animals. Plants were also identified for use to cure him from innumerable ailments which struck his physical being. They designated these plants as 'medicinal plants'. In India, Ayurvedic system of medicine has existed for over four thousand years. From ancient literature it is evidence that the various parts of the plants were used in Siddha, Ayurvedha and Unani medicine for the treatment of disease of human beings (Palaniswamy *et al*., 2008).

Punica granatum Linn (Pomegranate) belonging to family punicaceae, has long been esteemed as food and medicine, and is a diet in convalescence after diarrhoea (Nadkarni, 2000). It is used in Siddha, Ayurvedha and Unani medicine especially for the treatment of Gastro-Intestinal (GI) diseases. Pomegranate is a fruit of great antiguity and is known to have been cultivated in the Middle East more than 5,000 years ago. The plant is found all over India. Pomegranate has been considered important since prehistoric times as an agency of longevity (Ram, 1998). The fruit is good for dysentery, diarrhoea and gastralgia (Warrier, *et al.,* 2002). Hindoo physicians use the rind of the fruit and

flowers, combined with aromatics, such as cloves, cinnamon, coriander, pepper etc as bowel astringent in diarrhoea (Blatter, *et al.,* 2001). In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine the pomegranate is considered "a pharmacy unto itself" and is used as an antiparasitic agent, a "blood tonic," and to heal aphthae, diarrhea, and ulcers (Jurenka, 2008).

The fresh rind of the fruit contains: wax,0.8; resin, 4.5; mannitol, 1.8; non-crystallized sugars, 2.7; gums, 3.2; inulin,1.0; mucilage, 0.6; tannin, 10.4; gallic acid, 4.0; and calcium oxalate, 4.0%. Pectin occur to the extent of 2-4 % (Ram, 1998). Pomegranate peel combined with optimum level of aromatic such as cloves is a most useful remedy in chronic dysentery as well as diarrhoea. The rind is an antihelmintic and an astringent and useful in treating diarrhoea, dysentry and gastralgia (Prashanth, *et al.,* 2001). Commonly used as febrifuge and part of the diet in convalescence after diarrhoea. Wet and dry fruit is good for heart, stomach and enhances the production of hemoglobin. It is a good diuretic agent and gives strength. Pulp is a good anti-diarrhoeal agent (Chaterjee and Pakrashi, 1991; Hussain, *et al.,* 1992). Duraipandiyan *et al.,* (2006) reported that dried fruit coat is grounded and mixed with water and taken internally to treat stomachache and diarrhoea. Extract of different parts of the fruit exhibited antibacterial activity. Extracts of the whole fruit were highly active against *Micrococcus pyogens*, *S. aureus, E.coli,* and *Pseudomonas aeruginosa*. They were also very effective against intestinal pathogenic bacilli such as *Salmonella paradysenteriae* III-Z, *S. typhi, S. monetevideo, S. scholtmuelleri* and *Shigella paradysentriae* B.H. Alcoholic extracts of the fruit rind and root bark showed activity against *Micrococcus pyogens* 60% (Ram, 1998).

Ingestion of pathogens can cause many different infections. These may be confined to the GIT or initiated in the gut before spreading to other parts of the body. A syndrome characterized by GI symptoms including nausea, vomiting, diarrhoea and abdominal discomfort. Worldwide, diarrhoea diseases are the second leading cause of death; about 25 million enteric infections occur each year. These infections cause significant morbidity and death, particularly in elderly people and children younger than age 5. It has been estimated that 4 to 6 million children die each year from diarrhoea, particularly in developing countries in Asia and Africa. Even in developed countries, significant morbidity occurs as a result of diarrhoea illness, although acute diarrhoeal syndromes are usually self-limited, some persons with infectious diarrhoea will require diagnostic studies and treatment. The last decade has seen a resurgence of global interest in medicinal plants as therapeutic agents.

This traditional treatment approach is of much significance in the world especially in India due to the endemic presence of infective gastrointestinal diseases, which are the major causes of infant and adult mortality. Knowing the activity of *P.granatum* a study has been carried out to know its antibacterial activity, which has been reported in this paper.

Materials and Methods

Plant Material

The pericarp of the ripened and unripened fruit of *P. granatum* Linn. was selected for this study based on their traditional practices by Indians. Fresh fruits both ripened and unripened were collected from the local market, Namakkal, Tamil Nadu, India. Taxonomic identification of the plant was established.

Preparation of crude extracts

Pericarp of ripened and unripened fruit was collected and washed with sterile distilled water. Samples were crushed into parts and squeezed to remove the crude extract. The crude extracts were filtered through sterile musculine

cloth into vials.

Preparation of methanol and acetone extract

The pericarp of ripened fruit was dried under shade and stored into fine powder using electric blender. 50g of dried powder sample was taken and extracted by soxhlet apparatus using methanol and acetone separately. The solvents were removed under reduced pressure in a rotary evaporator until they become completely dry. The residues were stored at 4°C for further use.

Antimicrobial screening

The crude, methanol and acetone extracts of the pericarp of *P. granatum* was screened against a total of ten bacterial strains. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, S. paratyphi A, S. paratyphi B, S. typhimurium, Shigella dysenteriae* Serotype 1, *Shigella flexneri* Serotype 2 and *Vibrio cholerae* were obtained from the Christian Medical College and Hospital , Vellore, Tamil Nadu, India.

Determination of antibacterial activity

The disc diffusion method (Voraruthikunchai *et al., 2005*) was used to screen the antibacterial activity. *In-vitro* antibacterial assay was screened by using Mueller Hinton Agar (MHA) obtained from HiMedia, Mumbai, India. The MHA plates were prepared by pouring 15ml of molten media into sterile petridishes. The Plates were allowed to solidify for 10 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. Sterile paper disc (6mm) were soaked with 10ml of extract residue diluted into corresponding extraction solvents, so that each disc was impregnated with 2.5mg of residue and dried at 37°C overnight. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. Antibiotic discs containing Ofloxacin, Ciprofloxacin and Tetracycline (5-30µg) were used as controls. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the discs.

Results and Discussion

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants and microbes (Erdogrul, 2002). The results on antimicrobial screening of the crude extracts of the *P.granatum* are shown in table 1. The antibacterial activity of the crude extract of both ripened and unripened pericarp extracts resulted in clear inhibition zones of atleast 10mm for all the strains tested except *Pseudomonas aeruginosa* strain. This antibacterial activity may be indicative of the presence of metabolic toxins or broad spectrum antibiotic compounds. This is in agreement with previous reports by the several researchers (Prasanth *et al.,* 2001; Machado *et al.,* 2002; Voravuthikunchai *et al.,* 2005). Methanol extracts exhibited a higher degree of antimicrobial activity as compared with acetone extracts. Both methanol and acetone extracts of *P.granatum* pericarp showed high degree of antibacterial activity tested against GIT infection causing bacterial species which may be due to interesting novel secondary metabolites. Prasanth *et al.,* 2001, reported that, different extracts of *P.granatum* fruit showed some antibacterial activity against *Proteus vulgaris* and *Bacillus subtilis*. Voravuthikunchai *et al.,* (2004) reported that *P.granatum* contains large amount of tannins (25%) and the antibacterial activity may be indicating the presence of some secondary metabolites. The ethanolic extract of *P.granatum* showed some antibacterial

activity against *E.coli* (Voravuthikunchai *et al.,* 2005) and *S.aureus* (Machado *et al.,* 2002).

Table 1. Antibacterial activity of extract of *P. granatum* pericarp (concentration 2.5 mg / disc, inhibition zone in mm).

Test organism	Unripened crude extract	Ripened crude extract	Methanol extract	Acetone extract	Ciprofloxacin (30mcg)	Ofloxacin (5mcg)	Tetracycline (30mcg)
Staphylococcus aureus	21	12	21	16	22	20	20
Escherichia coli	16	20	21	13	26	29	24
Pseudomonas aeruginosa	05	05	14	16	15	20	24
Salmonella typhi	18	22	21	22	20	20	23
Salmonella paratyphi A	10	16	15	15	18	18	19
Salmonella paratyphi B	16	12	18	14	15	18	20
Salmonella typhimurium	19	21	25	17	17	19	20
Shigella dysenteriae Serotype 1	21	20	22	12	20	20	25
Shigella flexneri Serotype 2	19	20	25	12	17	19	21
Vibrio cholerae	22	25	20	14	20	18	21

Conclusion

The antibacterial activity of crude extract of unripened fruit of *P. granatum* is reported for the first time. Further phytochemical elucidations are required to determine the nature of compound(s) responsible for the antibacterial effects. This study is generally considered an effective approach in the discovery of new antibacterial agents from *P. granatum*.

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