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Antibacterial activity of *Punica granatum* peel extract against selected ATCC pathogens

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Abstract

The *Punica granatum* peel samples were collected from Panruti District, Tamilnadu. The fruit peel were washed with water, shade dried and grinded. Powdered peel samples were individually extracted with solvents such as ethanol, methanol, acetone and water. Antibacterial activity of the extract was done by disc diffusion method. The solvent extracts (ethanol, methanol, acetone and water) of peel samples were prepared and tested against pathogens such as *Escherichia coli* (ATCC-8739), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Salmonella typhimurium* (ATCC-14028). *Punica granatum* yellow peel ethanol extract showed maximum inhibition against *Staphylococcus aureus* (ATCC-25923) (33mm) and *Punica granatum* red peel acetone extract showed maximum inhibition against *Staphylococcus aureus* (ATCC-25923) (30mm). GC/MS analysis was carried out for the yellow peel ethanol extract of *Punica granatum* in Indian Institute of Food Processing Technology (Tanjore) and seventeen major compounds were identified such as 5-Hydroxymethylfurfural, β -copaene, 7-Octadecyn 2-methyl, 17-Octadecynoic acid, n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoyl chloride, Octadecanoic acid, cis-13-Eicosenoic acid, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, 1,2-Benzenediol, O,O'-di(2-methoxybenzoyl)-, Retinal, 9-cis-, Piperine, 6,9,12,15-Docosatetraenoic acid, methyl ester, Piperidine, 1-[5-(1, 3-benzodioxol-5-yl)-1-oxopentyl], Benzoic acid 3-methyl-4-(1, 3, 3, 3-tetrafluoro-2 methoxycarbonyl - propenylsulfanyl)-phenyl ester and β -Sitosterol.

Keywords: *Punica granatum*, ethanol, methanol, acetone and gas chromatography-mass spectrometry

1. Introduction

The pomegranate peel is otherwise called as fruit pericarp. Pomegranate bark, leaves, immature fruits and fruit rind have medicinal value. *Punica granatum* seed oil, peel and juice have anti-cancer properties that inhibit proliferation, cell cycle, and angiogenesis. Studies revealed its anti-cancer activity in several human cancers (Adhami, 2009) [2]. Pomegranate peel extract delays the proliferation of human breast cancer and prostrate cancer cell line (Mehta and Lansky, 2004) [7].

The anti-inflammatory compounds were screened from the peels. Fatty acids and polyphenols have anti-inflammatory properties (Bachoual *et al.*, 2011) [5]. Flowers, seeds, and juice have hypoglycemic activity. Oleanolic, ursolic, and Gallic acids have anti diabetic activity. In Unani medicine Pomegranate flowers were used as a supplement in the diet to control diabetes in many countries. The flowers are used to control type II diabetes with mechanisms such as enhancement of mRNA expression, increment of peripheral glucose utilization and improvement of insulin receptor sensitivity, etc.

Punica granatum has many potential effects including antibacterial, antifungal and antiviral activities. Antibacterial properties against oral pathogens (Abdollahzadesh *et al.*, 2011) [1] were noted. Antiviral effects were noted against influenza virus, herpes virus, poxviruses, human immunodeficiency (HIV-1) virus and adeno virus (Moradi *et al.*, 2015) [8]. The rind extract has the ability to control enteric pathogens such as enterohemorrhagic *E. coli* which cause hemorrhagic diarrhea. Gallic acid and catechin are the major components of *Punica granatum* which are responsible for the wound healing activity. The peel preparation hold promise in increasing the rate of wound healing.

Punica granatum extracts control dental bacteria and reduce the risk of plaque, gingivitis and periodontal disease. It can be used to treat dyspepsia, ulcers, sores and mouth lesions (Devi *et al.*, 2011) [6].

Bacterial and fungal infections caused by multidrug resistant pathogens are a major concern for health issues in developing countries. Drug resistance cause clinical trouble in the treatment of infectious diseases. Pomegranate peels have antimicrobial activity against many drug resistant pathogens which cause diseases (Alka Chaudhary and Siddarth Nandan Rahul, 2017) [3]. In the present study the solvent extracts of *Punica granatum* were studied for the antibacterial activity against the human ATCC cultures *E. coli* ATCC (8739), *Salmonella typhimurium* ATCC (14028), *Staphylococcus aureus* ATCC (25923) and *Pseudomonas aeruginosa* ATCC (27853).

Materials and methods

Collection and processing of *Punica granatum* peel samples

The *Punica granatum* fruit peel samples (red peel and yellow peel) were collected from Panrutti District, Tamilnadu. The peel samples were cleaned with water to remove the dust particles over the surface of the samples. The peel samples were shade dried at room temperature for 10-15 days (Plate-1). The dried peel samples were ground into a fine powder and stored in clean airtight plastic containers for further use.

Preparation of peel extracts

The prepared powder sample of *Punica granatum* peels were mixed with different solvents like ethanol, methanol, acetone and water individually. Then they were kept at room temperature for 72 hours. Each mixture was stirred using glass rod. These mixtures were then filtered through Whatmann no.1 filter paper. Exrtaction procedure was done further twice for extraction of the bioactive compounds. The filtrate was collected in a separate container and was evaporated. Stock solution of the peel extract were prepared (100 mg/ml) and the sterile disc were dipped in the extracts for proper absorption. Then they were dried at room temperature. After drying they were used for antimicrobial studies.

Antibacterial activity of *Punica granatum* peel samples

The disc diffusion technique was used to determine the antibacterial activity of the extract. The solvent extracts (ethanol, methanol, acetone and water) of yellow peel and red peel samples of *Punica granatum* were tested against pathogenic organisms *E. coli* ATCC (8739), *Salmonella typhimurium* ATCC (14028), *Staphylococcus aureus* ATCC (25923) and *Pseudomonas aeruginosa* ATCC (27853). Muller Hinton agar was poured into the sterile Petri plates and allowed to set. The test isolates were swabbed aseptically on the agar plates. The *Punica granatum* peel extract loaded discs were then gently placed on the plates at equal interval. Then the plates were incubated at 37° C for 24 hours. The diameter of the zone of inhibition was measured in mm after 24 hours.

Analysis of the phytocomponents in *Punica granatum* yellow peel extract using GC-MS technique:

GC/MS analysis was carried out for the *Punica granatam* yellow peel extract in Indian Institute of Food Processing Technology (Tanjore). One micro litre of the filtrate was injected into the GC-column. Then the sample get evaporated and carried away by the carrier gas, helium and it got segregated into individual fractions. The sample fraction coming out of the column was let into the mass detector and the mass spectrum of each component was recorded. The mass spectrum of the unknown component was compared with the known spectrum using data base dictionaries. The database in the National Institute of Standards and Technology (NIST) has been used for the interpretation on GC – MS. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. Then the structure, molecular formula and molecular weight of components were identified accordingly.

Result and discussion

The ethanol extract of *Punica granatum* yellow peel showed maximum activity against *Staphylococcus aureus* (ATCC-25923) (33mm) (Table-1). *Punica granatum* red peel acetone extract showed maximum inhibition against *Staphylococcus aureus* (ATCC-25923) (30mm) (Figure-1) (Plate-2).



Punica granatum fruit samples

Yellow peel

Red peel

Plate 1:

Table 1: Antibacterial Activity of *Punica granatum* yellow peel extract against ATCC cultures:

S. No	ATCC Cultures	Zone Of Inhibition In Mm			
		Ethanol	Methanol	Acetone	Water
1	<i>E. coli</i>	T	T	T	NA
2	<i>Staphylococcus aureus</i>	33	31	23	T
3	<i>Pseudomonas aeruginosa</i>	17	22	20	14
4	<i>Salmonella typhimurium</i>	NA	NA	NA	NA

NA-No activity T -Trace activity

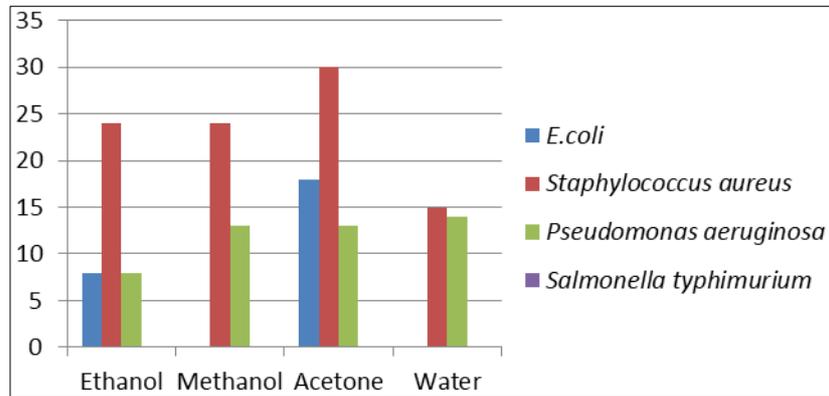


Fig 1: Antibacterial Activity of *Punica granatum* red peel extract against ATCC cultures:

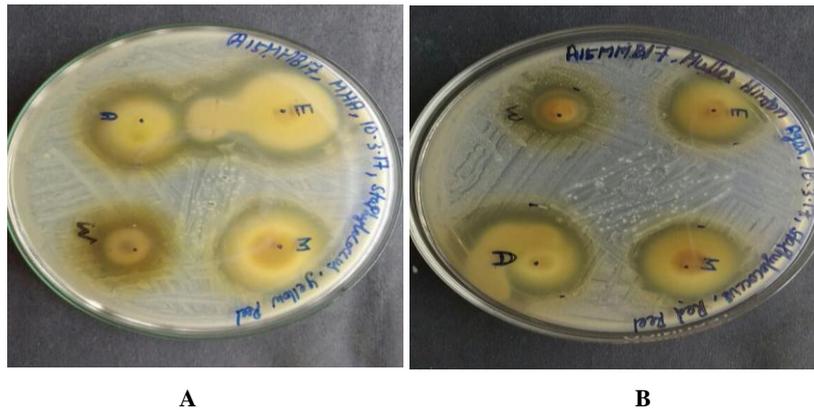


Plate 2: Antibacterial Activity of *Punica granatum* yellow peel (A) and red peel extract (B) against *Staphylococcus aureus*

GC/MS analysis for the yellow peel extract of *Punica granatum*

GC/MS analysis was carried out for the *Punica granatum* yellow peel extract and the mass spectrum (Figure-2) showed seventeen compounds such as 5-Hydroxymethylfurfural, β -copaene, 7-Octadecyn 2-methyl, 17-Octadecynoic acid, n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoyl chloride, Octadecanoic acid, cis-13-Eicosenoic acid, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, 1, 2-Benzenediol, O,O'-di(2-methoxybenzoyl)-, Retinal, 9-cis-, Piperine, 6,9,12,15-Docosatetraenoic acid, methyl ester, Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxopentyl], Benzoic acid 3-methyl-4-(1,3,3,3-tetrafluoro-2 methoxycarbonyl-propenylsulfanyl)-phenyl ester and β -Sitosterol.

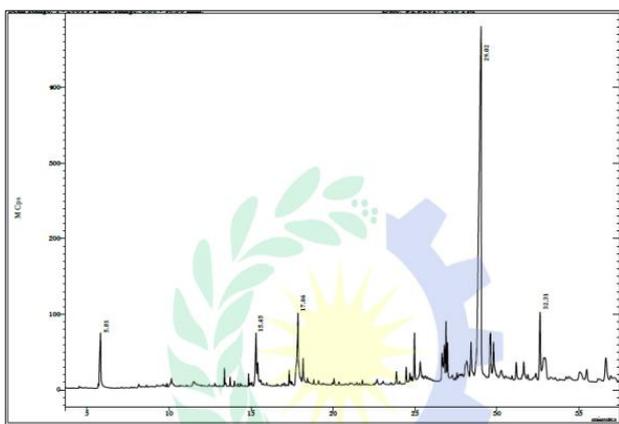


Fig 2: Mass Chromatogram of yellow peel extract of *Punica granatum*

Pai *et al.*, (2011) [9] evaluated the antibacterial activity of alcoholic and aqueous peel extract of *Punica granatum* against enteric pathogens *Vibrio cholerae*, *Vibrio parahemolyticus*, *Shigella* sp, *Salmonella* sp, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteraggressive *E. coli* (EAEC) and *Aeromonas hydrophila*. The ethanol peel extract showed greater inhibition compared to the aqueous extract. The most significant inhibitory effect was seen against *Shigella flexneri* (30mm) and *Aeromonas hydrophila* (23mm). In the current study yellow peel ethanol extract of *Punica granatum* showed maximum inhibitory activity against *Staphylococcus aureus* (33mm) and red peel acetone extract showed maximum activity against *S aureus* (30mm). Ashok Kumar and Vijayalakshmi (2011) [4] analysed the medicinal properties and antioxidant activity of *Vitis vinifera* and *Punica granatum*. The GC/MS analysis was carried out with the ethanolic extract of *Punica granatum* peel and seeds of *V. vinifera*. Twenty six compounds were identified in *Punica granatum*. In the present study GC/MS analysis was carried out for the yellow peel ethanol extract of *Punica granatum* and 17 compounds were identified.

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