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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL ACTIVITY IN PUNICA GRANATUM

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Abstract:

Pomegranate (Punica granatum) is most important plant belonging to family Lythraceae. A symbol of fecundity and divine femininity emerges, whose fruit rinds, bark and roots are used worldwide astaenicides, owing to alkaloids, and treatment of diarrhea and oral and genital lesions, owing to tannins and astringency.

Both the juice and the oil contain numerous and diverse bioflavonoid, which have been shown to be both potently antioxidant and inhibitory of one or both of the enzymes cyclooxygenase (catalyzing arachidonic acid to prostaglandins) and lipoxygenase (catalyzing arachidonic acid to leukotrienes). Extracts of the rinds have been shown to be bactericidal, antiviral, antitumor and use of pomegranates in the treatment of Acquired Immune Deficiency Syndrome (AIDS) owing to their antioxidant properties and botanical uniqueness. This present study is designed to evaluate the phytochemical and pharmacological profile of different extract of Punica granatum

Key words: Pomegranate, astaenicides, tannins, cyclooxygenase, lipoxygenase, phytochemical.

INTRODUCTION

According to World Health Organisation, medicinal plants are the best source to obtain a variety of newer herbal drugs. Medicinal plants are the local heritage with global importance and they are eco-friendly. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases.[1-3]

The constituents of pomegranate have been reported to have antioxidant, anticarcinogenic and anti-inflammatory properties, focussing on the treatment and prevention of various diseases.[2] The pericarp of Punica granatum has been commonly employed as a crude drug in Indian traditional medicine for treatment of diarrhoea as well as for use as an astringent, anti-helminthic, laxative, diuretic, stomachic, cardiotonic and refrigerant.[4]

Punica granatum is high in antioxidants and other nutrients it has been proposed that drinking the juice regularly may help prevent cancer. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Natural products are the most consistently successful source of drug leads. Any substance that reduces oxidative damage (damage due to oxygen) caused by free radicals is called an antioxidant. Free radicals are highly reactive chemicals which attack molecules by capturing electrons and thus modifying chemical structures.[3, 5-8]

Commercial juice shows potent antioxidant and anti-atherosclerotic properties attributed to its high content of polyphenols including ellagic acid. Extracts of the whole fruit (pomegranate) were highly active against Micrococcus pyogens, Staphylococcus aureus, E. coli and Pseudomonas aeruginosl.[1,9]

Although study of Pomegranate (Punica granatum) for antibacterial efficacy in the context of growing drug-resistance of virulent pathogens is therefore felt to be timely important. The various aspects and steps that such a study involves crystallised in terms of objectives elaborated in the next section. These objectives were formed in the light of the information thrown up by literature pertaining to the prevalence and advance of nosocomial infection, the growing drug-resistance and the mechanisms of such resistance including the virulence factors, the antibacterial activity of natural extracts, their antibacterial action in forms of different extracts, their phytochemical components and their therapeutic activity, etc.[10-12]

Punica granutam is also well known by different local name like dalim, anar, pomegranate. It belongs to the family of Punicaeae.[1] Punica granatum are widely available in Mediterranean basin and Southern Asia in warm environment.[2] The chief production of

pomegranates is carried out at Alicante and Murcia provinces of India.[2] Different part of pomegranate like bark, leaves, immature fruits, and fruit rind have some medicinal importance. Various investigations were carried out to determine antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents.[3-7] Various studies focuses on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage, infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity using various extract from plant.[6-8] This study was devised to study the phytochemical and pharmacological activities of the plant Punica granatum (Pomegranate) for therapeutic benefits.

PHYTOCHEMICAL PROFILE

SYNONYMS

Hindi : Anar, Sanskrit : Dadimah, English : Pomegranate, Marathi : Dalimba, Gujarati : Dalimba, Bengali : Dadim, Tamil : Madalai, Telgu : Danimma, Malayalam : Talimatatalum, Pharsi : Anar tursa, Arabi : Roman Hamiz, German : Granatapfels.

BOTANICAL CLASSIFICATION

Botanical name- Punica granatum

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Myrtales

Family : Lythraceae

Genus : Punica

Species : granatum

COMPONENTS OF PUNICA GRANATUM

The pomegranate (Punica granatum) tree/fruit can be compartmented:

1. Seed,
2. Juice,
3. Peel,
4. Leaf,
5. Flower,
6. Bark, and
7. Roots.

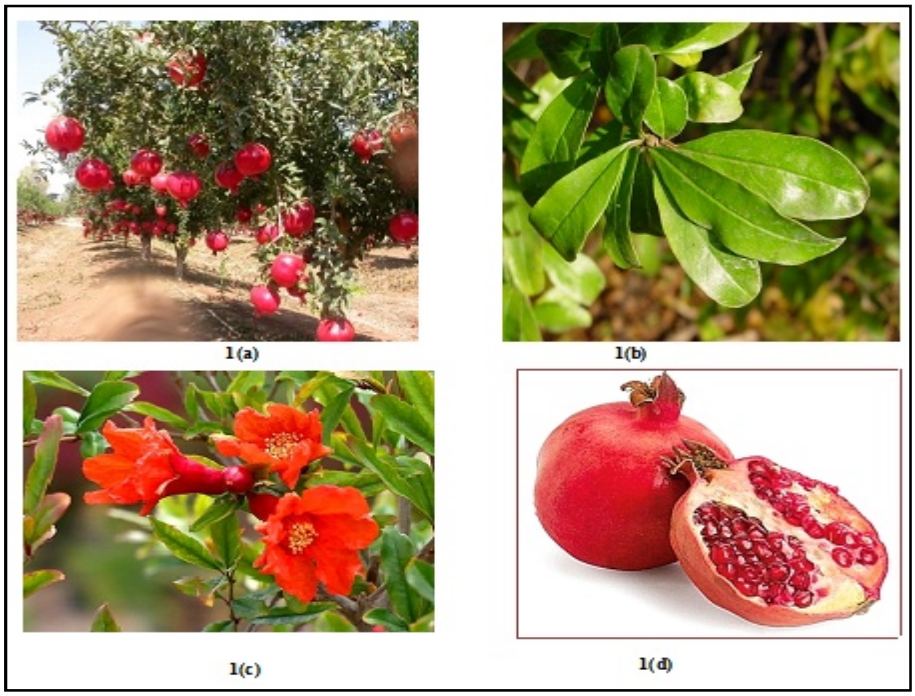

Figure 1(a,b,c,d,) representing the Whole plant, Leaf, Flowers, fruit and Seeds of Punica granatum

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 occurs to the extent of 2-4 %. The root and bark contain tannin (20-22%) and alkaloids (0.5-1%). The seeds contain steroidal estrogen. The fruit pulp contains protein, carbohydrate, fat, fibre, minerals, oxalic acid and vitamins A, B and C [13-15]. Edible parts of the pomegranate fruit (80% of total fruit weight) are comprised of 80% juice and 20% seed (Gil et al., 2007). The fresh juice contains 85% water, 10% total sugars, and 1.5% pectin, ascorbic acid, and polyphenolic flavonoids. In pomegranate juice, fructose and glucose are present in similar quantities; calcium is 50% of its ash content; and the principal amino acids are glutamic and aspartic acids. [16]

MATERIALS AND METHODS

- **Collection of Plant Material**

  Fresh peel of *Punica granatum* were purchased from local market of Ujjain, M.P, India during Dec 2014 and the specimen samples was identified by the expert at Department of Botany, Vikram University,Ujjain (M.P). Collected materials was washed in running tap water, rinsed properly in distilled water and then subjected to drying at room temperature for about 5 days in open air. These air dried material was grind into powdered and stored under refrigeration until their further utilization.

- **Preparation of Plant Extracts**

  Aqueous extraction was prepared as well as soxhlet extraction was carried out using different solvent such as methanol, ethanol, acetic acid and petroleum ether. The filtrates were evaporated to get concentrated residue. This residue treated as experimental drug for the present study. The extract was stored at 4^{o}C until assay was completed. [18-19]

- **Test organisms**

  Four strains of Gram-positive bacteria – *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Bacillus megaterium* and six strains of Gram negative bacteria - *Escherichia coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Proteus vulgaris* and *Pseudomonas aeruginosa* were used for antibacterial activity. All bacterial cultures were grown and maintained on nutrient agar plates and were stored at 4^{o}C. The bacterial cultures were periodically sub-cultured.[20-22]

- **Preliminary Antimicrobial Activity Screening**

  The screening of different solvent extraction of peel of *Punica granatum* was carried out using agar well diffusion method. The test organisms were grown on nutrient agar plates and subculture prior to start the screening. The subculture plate containing test organism was kept in incubator at 37°C for 24 hours. The inoculums for each test microorganism were prepared which have approximately 105 CFU/ml. The plant extracts were dissolved in DMSO for conducting antimicrobial activity. Antibiotics such as Tetracycline (1mg/ml) were used as positive controls, while DMSO was used as negative controls. The plates for antimicrobial activity were incubated at 37ºC for 24hrs. After 24 hours the plates was examined for zone of inhibition (Table 1). [12, 23]

- **Phytochemical screening**

  Phytochemical analysis of the extract was carried out using various procedures describe by various authors.[10-13] Phytochemical screening was performed to detect the presence of several phytochemicals like Alkaloids, Flavonoids, Steriods, Saponins, Cardiac glycoside, Tannins, Terpenoids and free Amino acid (Table 2). [21, 24]

- **Test for Alkaloids [10]**

  Dragendroff's reagent test was conducted for detection of alkaloids. 0.5 g of peel extract was dissolved in 5 ml of 1% HCl and the mixture was kept for 2 minutes in

water bath. 1 ml of filtrate is treated with dragendroff's reagent. Turbidity or precipitation was indication for presence of alkaloids.

- **Test for Flavanoids [12]**

  Yellow precipitation was observed when the test solution was treated with 10% lead acetate solution indicates presence of Flavanoids.

- **Test for Tannins [11]**

  For detection of tannin, the method describes by E. Y. Qnais et al, 2007 was utilized with certain modification. About 0.5 g of peel extract was dissolved in 10 ml of boiling water. The solution was filtered and to filtrate few ml of 6% FeCl3 was added. Development of deep green colour shows presence of Tannin.

- **Test for Saponin [25]**

  The test solution was mixed with water in the test tube and shaken properly. Foaming arises suggest presence of saponin.

- **Test for Cardiac glycoside [26]**

  About 0.5 g of the extract was dissolved in 2ml of glacial acetic acid containing 1 drop of 1% FeCl3. This was under laid with conc. H2SO4. A brown ring obtained at the interphase indicates the presence of deoxy-sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

- **Test for Free Amino Acids [27]**

  Ninhydrin Test was utilizing to detect free amino acid. Peel extract solution boiled with 0.2% Ninhydrin solution. Purple colour formation indicates positive result.

- **Test for Steroids and Triterpenoids [28]**

  The Salkowaski method was used as describe by Agarwal et al (2011) with certain modifications. About 0.5 g of extract was dissolved in 3 ml of chloroform and filtered. Concentrated H_2SO_4 was added to the filtrate to form a lower layer. Reddish brown color develop was considered as positive results for the presence of steroids ring.

Dose dependent Antimicrobial Activity Test

The dose dependent studies were conducted for different solvent extraction of peel of Punica granatum on microorganisms showing positive screening for antimicrobial activity. Different doses of 1 mg/ml, 2 mg/ml, 5 mg/ml and 10 mg/ml was tested for each peel extract for their antimicrobial activity.[28] The dose dependent antimicrobial activity was carried out using agar well diffusion method (Table 4). The plates for antimicrobial activity were incubated at 37ºC

for 24hrs. After 24 hours the plates was examined for zone of inhibition and compared with the Tetracycline control (Table 3). The zone was measured in mm. All the tests were conducted in triplicate.[22-26]

RESULTS

Table 1. Preliminary Screening for Antimicrobial Activity of *Punica granatum* Peel

Pathogenic Organisms	Zone of Inhibition				
	Aqueous Extracts	Ethanolic Extracts	Methanolic Extracts	Petroleum Ether Extracts	Acetic Acid Extracts
Bacillus megaterium	+	+	+	+	+
Bacillus subtilis	+	+	+	-	-
Escherichia coli	-	+	-	+	-
Proteus vulgaris	+	+	+	+	+
Salmonella typhi	+	+	+	+	-
Salmonella paratyphi A	+	+	+	+	-
Salmonella paratyphi B	-	-	-	-	-
Bacillus cereus	+	+	+	+	-
Pseudomonas aeruginosa	-	+	-	+	-
Staphylococcus aureus	+	+	+	-	-

+ indicates = Zone of Inhibition, - indicates = No Zone of Inhibition

Table 2. Phytochemical Analysis of *Punica granatum* Extracts

Phytochemical Parameters	Solvent systems				
	Aqueous	Ethanol	Methanol	Petroleum Ether	Acetic Acid
Steroids	+	-	+	-	-
Alkaloids	-	-	-	-	+
Flavonoids	+	+	+	-	-
Saponins	-	-	-	-	-
Tannins	-	-	+	-	+
Cardiac glycosides	+	-	+	-	-
Terpenoids	+	-	-	-	-
Amino acids	-	-	-	-	-

+ indicates = Positive results, - indicates = Negative results.

Table 3. Antimicrobial Activity of Tetracycline Control

Pathogenic Organisms	Zone of Inhibition (m. m)
Bacillus	1
Bacillus	1
Escherichia	1
Proteus	5
Salmonella	6
Salmonella paratyphi A	1
Salmonella paratyphi B	1
Bacillus	1
Pseudomonas aeruginosa	7
Staphylococcus aureus	2

Table 4. Dose Dependent Antimicrobial Activity of *Punica granatum* Extracts

Bacterial strains	Doses of Peel Extract of *Punica granatum*																			
	Aqueous				Ethanol				Methanol				Acetic acid				Petroleum ether			
	1 mg/	2 mg/	5 mg/	10 mg/	1 mg/	2 mg/	5 mg/	10 mg/	1 mg/	2 mg/	5 mg/	10 mg/	1 mg/	2 mg/	5 mg/	10 mg/	1 mg/	2 mg/	5 mg/	10 mg/
Bacillus megaterium	2	2	4	9	1	2	9	12	4	5	5	9	2	3	4	8	-	1	2	5
Bacillus subtilis	1	4	5	8	1	2	5	12	3	2	4	9	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	-	4	6	11	12	-	-	-	-	-	1	2	6
Proteus vulgaris	6	11	12	13	1	2	5	12	5	7	9	11	1	2	4	6	2	4	6	7
Salmonella typhi	3	5	10	12	1	2	5	11	4	4	5	10	-	-	-	-	1	3	5	6
Salmonella paratyphi A	-	-	-	-	1	2	3	12	6	5	3	11	-	-	-	-	1	2	5	6
Salmonella paratyphi B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus cereus	-	-	-	-	2	7	8	11	2	3	6	12	-	-	-	-	1	2	4	8
Pseudomonas Aeruginosa	-	-	-	-	-	-	-	-	9	10	11	12	-	-	-	-	1	2	3	5
Staphylococcus aureus	4	8	10	11	1	2	10	10	1	1	2	10	-	-	-	-	-	-	-	-

DISCUSSION

The peel of Punica granatum shows various therapeutic applications as Antibacterial, antifungal, antioxidant, antitumor, antiviral, antimalarial and antimutagenic effects as reported by different authors.[3-6] Much work has been carried out to demonstrate ethno-medicinal value of various plants in India because traditional natural products are widely used to cure certain diseases. The present investigated focuses on peel extract using various solvent systems to show the antimicrobial activity of each extract. Antimicrobial activities were measured with respect to pathogenic microorganism's reveals that each peel extracts from Punica granatum show significant antimicrobial activity as reported by different researchers.[11,14,15]

During screening for antimicrobials, Punica granatum aqueous and methanolic extract was shown to be effective against B. subtilis, B. megaterium, B. cereus, S. typhi, S. paratyphi A, P. vulgaris and S. aureus. However, unlike aqueous and methanolic extract, ethanolic extract also show significant antimicrobial activity against P. aeruginosa. Acetic acid extract show antimicrobial activity against B. megaterium and P. vulgaris, whereas petroleum ether shows antimicrobial activity against E. coli, B. megaterium, B. cereus, S. typhi, S. paratyphi A, P. vulgaris and P. aeruginosa.

The dose dependent study using aqueous extract show significant antimicrobial activity against S. typhi and P. vulgaris at the concentration of 10mg/ml. The dose dependent study using methanolic extract show antimicrobial activity against S. typhi, P. vulgaris, B. cereus, B. megaterium, S. paratyphi A at extract concentration of 10mg/ml. The ethanolic extract shown to be effective against B. cereus, P. vulgaris, S. typhi, E. coli and P. aeruginosa at extract concentration of 10mg/ml. Punica granatum peel Acetic acid extract shows that zone of inhibition with P. vulgaris, B. megaterium, but dose dependent study show significant antimicrobial activity against only P. vulgaris at 10 mg/ml extract concentration. Punica granatum peel Petroleum ether extract shows that zone of inhibition with E. coli, B. megaterium, B. cereus, S. typhi, S. paratyphi A, P. vulgaris and P. aeruginosa but dose dependent study show significant antimicrobial activity against S. tyhphi and P. vulgaris only at 10 mg/ml extract concentration. Tetracycline was used as positive control and DMSO as negative control for each tested extract.

The phytochemical screening of Punica granatum peels show presence of flavanoids, steroids, cardiac glycosides and terpenoids in aqueous extract. The ethanolic extract show presence of flavanoids, steroids, cardiac glycosides and Tannin. The Methanolic shows presence of only flavanoids whereas acetic acid extracts show presence of only alkaloids.

The petroleum ether extraction show absent of above tested phytochemicals. Hence the extractions of bioactive compounds responsible for antimicrobial activity are solvent dependent.

In the present study, the antibacterial activity of Punica granatum peel extracts towards clinically significant microbes are reported and it was observed that the active constituents in the plant material was extracted in polar as well as non-polar solvent system. However, antimicrobial activity was demonstrated effectively in polar solvent as compared to non-polar one. The study shows the pharmacological importance of peel of Punica granatum, thereby exploring bioactive phytochemicals from waste material (peel) showing antimicrobial activity and thus substantiates traditional medicinal use. The separation and further activity mediated approach was emphasize to conduct in future to demonstrate active phytochemicals to be utilize as lead compounds for antimicrobials. Thus, the study provides a strong direction for proper investigation of various plants to explore molecules having antimicrobial properties against human pathogens using waste sources.

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