



ANTIBACTERIAL PROPERTIES OF PEEL EXTRACTS OF *PUNICA GRANATUM* AND *SOLANUM TUBEROSUM* AGAINST HUMAN PATHOGENS

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ABSTRACT

An attempt was made to screen the antibacterial activity of peel extracts of *Punica granatum* (Pomegranate) and *Solanum tuberosum* (Potato) against human pathogenic bacteria. Aqueous and ethanolic extract of two peels were prepared by hot extraction method. The antibacterial activity of peel extracts and standard antibiotic assay was evaluated against two bacterial pathogens viz., *Escherichia coli* and *Staphylococcus aureus* by Agar well diffusion method and concentration was 100 mg/10 µl. Distilled water and alcohol was used as negative and positive control. Standard antibiotic (Amphilox) was used for the assay. Minimum Inhibitory Concentration (MIC) was found out using Broth Dilution Method. The result of study showed the presence of wide spectrum of antibiotic activity against human pathogens. Alcoholic extract of pomegranate peel was found to be much efficient than the standard antibiotic used. Peel extracts showed the presence of essential phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, tannin and saponin which are responsible for antibacterial activity.

KEYWORDS: Antibacterial, Human pathogens, *Punica granatum*, *Solanum tuberosum*.

INTRODUCTION

Medicinal plants contain several pharmacological active compounds that may act individually or in synergy to improve health.^[1] These plants have been identified and used from pre-historic times. *Solanum tuberosum* known as potato is commonly found in India. It is a starchy crop and a staple food in many parts of the world. This tuber peel is said to possess medicinal properties and is widely used in medicinal and beauty industry.^[2,3] *Punica granatum* commonly known as pomegranate is said to be a cure for all illness. The peel of this fruit is valuable in the treatment of diarrhea and acidity. It has gained importance in beauty industry and is used in the manufacture of beauty creams and oils.^[4] Due to the wide applications of these two peels a study was made to test the antibacterial properties of these against human bacterial pathogens like *Escherichia coli* and *Staphylococcus aureus*. *E. coli* is Gram negative microorganism that mostly thrives in the intestine. Most *E. coli* are harmless but some possess pathogenicity that can make us sick and cause diarrhea, sometimes kidney failure and even death.^[5] In specific patient population *S. aureus* can be a primary urinary pathogen. It can also cause skin and respiratory problems.^[6] These organisms have become resistant to antibiotics over a period of time which becomes difficult for recovery among patients suffering from this disease. These antibiotics also cause certain side effects such as nausea, vomiting, hypersensitivity reaction, kidney failure and even

death.^[7] Hence there is a need to find natural remedies for these pathogens which is effective and has no side effects. The peel extracts of these two fruits possess wide range of antibacterial property and pomegranate peel also enhances the activity of immune system.^[8]

MATERIALS AND METHODS

Samples were purchased from the local market of Mangalore and brought to the laboratory, washed thoroughly under running tap water and rinsed with distilled water. Peels were separated, shade dried and placed in a hot air oven at 40 °C for 3 days. After complete removal of moisture, peels were taken out and powdered using a mixer grinder and powdered sample was stored in an air tight container for further use.

10 g of the powdered material was dissolved in 40 ml of the respective solvent and left undisturbed for 24 hours. Aqueous and ethanolic extract was obtained by Hot Extraction Method and the extracts prepared were immediately evaluated for their Antibacterial Property.^[9,10] Minimum Inhibitory Concentration of the two extracts was found out using Broth Dilution Method.^[11]

Qualitative analysis for Phytochemical Test

Phytochemical screening was made to detect the presence of essential phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, tannin and saponin.^[12,13]

Test for Alkaloids: To the extract 6 drops of 1% HCl, Dragendroff's reagent and Mayer's reagent were added. An orange precipitate indicates the presence of Alkaloids.

Test for Flavonoids: To the extract 5 ml of dilute ammonia and 3 drops of Conc. Sulfuric acid were added. A yellow precipitate indicates the presence of Flavonoids.

Test for Steroids and Terpenoids: To the extract 1 ml of chloroform was added and filtered. 1 ml of acetic acid was added and mixed. Few drops of Conc. Sulfuric acid were added slowly. Appearance of pink or pinkish brown ring indicates the presence of terpenoids. Blue or bluish green indicates the presence of steroids. Amalgamation of pink and blue color indicates the presence of both terpenoids.

Test for Tannin: To the extract 2 ml of ferric chloride was added. Dark green indicates positive test for Tannin.

Test for Saponin: 2 ml of the extract was taken and diluted with distilled water and shaken. Kept aside for five minutes. Development of foam on the surface of the mixture indicates the presence of saponin.

Test Organisms

E. coli (MTCC- 725) and *S. aureus* (MTCC-7443) were used to assess the antimicrobial potential. The isolates were acquired from Department of Biosciences, Mangalore University.

Antibacterial Activity

The efficiency of extracts of *Punica granatum* and *Solanum tuberosum* were evaluated against *S. aureus* and

E. coli, using disc diffusion method. Muller-Hinton Agar plates were inoculated with different bacterial strains and sterile Whatman filter paper discs (3 mm) containing extracts of the samples with different concentrations were placed on the plates and incubated at 37 °C for 24 hours in an incubator to observe zone of inhibition.^[14]

RESULTS AND DISCUSSION

Aqueous and ethanol extracts of the peels were tested for the presence of phytoconstituents such as alkaloids, terpenoids, tannin, saponin, steroids, flavonoids using standard procedure and the results are shown in Table 1.

Alkaloids are a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. They have a wide range of pharmacological activities like anticancer, antiasthma, antimalarial and vasodilator.^[15,16] Tannin is an astringent polyphenolic biomolecule that binds to proteins and play a role in protecting the plants from predation and helps in regulating plant growth.^[17] Saponin are amphipathic glycosides and form foam when they are shaken in aqueous solution. In plants saponin serves as antifeedants and protects the plant against microbes and fungus.^[18] Terpenoids for their aromatic qualities are used extensively in traditional herbal remedies and manufacture of perfumes. Widely used for the synthesis of vitamin A.^[19,20] Steroids are group of polycyclic chemical compounds used for the manufacture of drugs.^[21] Flavonoids are plant pigments for flower coloration, producing yellow, red or blue pigments in petals designed to attract pollinator animals. Flavonoids are reported to possess many useful properties, including anti-inflammatory, antimicrobial, enzyme inhibition, estrogenic, anti - allergic, antioxidant and anti-tumor activity.^[22]

Table 1: Phytochemicals in peel extracts of *Punica granatum* and *Solanum tuberosum*.

Peel extracts	Phytochemicals					
	Alkaloid	Flavonoid	Steroid	Tannin	Saponin	Terpenoids
<i>P.granatum</i> (aqueous)	-	-	-	-	-	+
<i>P.granatum</i> (ethanolic)	-	-	-	+	-	+
<i>S. tuberosum</i> (aqueous)	+	-	-	+	+	+
<i>S.tuberosum</i> (ethanolic)	+	-	-	+	-	-

+: Present, -: Absent

Antibacterial Activity

The peel extracts of *S. tuberosum* and *P. granatum* were tested for their antibacterial property using Agar Well Diffusion Method. Results of the study showed the presence of wide spectrum of antibacterial activity against human pathogens. Zone of Inhibition was measured in mm and the results are summarized in Table 2.

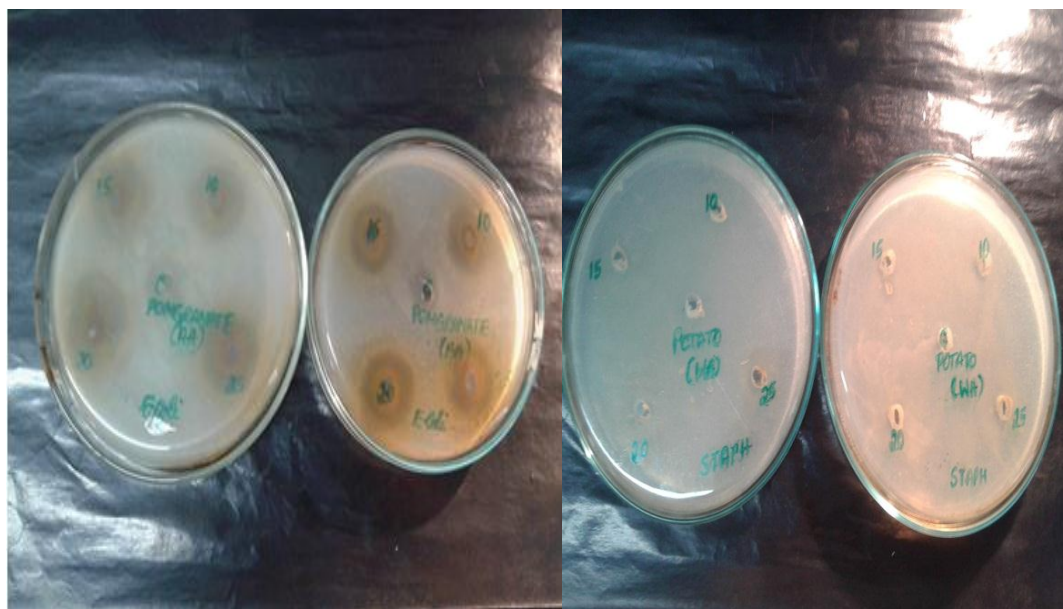


Fig. 1: Showing zone of inhibition of Antibacterial activity of peel extracts of *S. tuberosum* and *P. granatum* against human pathogens (*S. aureus* and *E. coli*).

Table 2: Antibacterial activity of peel extracts of *P. granatum* and *S. tuberosum* against *S. aureus* and *E. coli*.

		Zone of Inhibition (mm)							
Concentration/well		10 μ l		15 μ l		20 μ l		25 μ l	
Test organism		<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
Solvents	Sample								
Aqueous	<i>P. granatum</i>	19 $\pm$ 0.424	19.5 $\pm$ 0.14	20 $\pm$ 0.6	20 $\pm$ 0.7	22 $\pm$ 0.6	21 $\pm$ 0.35	24 $\pm$ 0.35	22 $\pm$ 1.06
	<i>S. tuberosum</i>	0 $\pm$ 0	4 $\pm$ 0.353	0 $\pm$ 0	10 $\pm$ 0.7	0 $\pm$ 0	12 $\pm$ 0	0 $\pm$ 0	15 $\pm$ 0.31
Ethanollic	<i>P. granatum</i>	20 $\pm$ 0.424	22 $\pm$ 0.353	20 $\pm$ 0	23 $\pm$ 0.4	23 $\pm$ 0.1	29 $\pm$ 0.34	28 $\pm$ 0.21	32 $\pm$ 0.31
	<i>S. tuberosum</i>	7 $\pm$ 0.145	0 $\pm$ 0	9 $\pm$ 0.21	0 $\pm$ 0	13 $\pm$ 0.2	0 $\pm$ 0	16 $\pm$ 0.58	0 $\pm$ 0
-	Amphilox (Standard)	18 $\pm$ 0.12	19 $\pm$ 0.564	20 $\pm$ 0.4	22.5 $\pm$ 0.4	22 $\pm$ 0.6	28 $\pm$ 0.46	25 $\pm$ 0.65	29 $\pm$ 0.68

Values are the mean inhibition zone (mm) mean $\pm$ S.D of three replicates.

The peel extracts of *P. granatum* and *S. tuberosum* were screened for their antibacterial activity. It was found that the peel extracts of *P. granatum* showed a wide range of antibiotic spectrum when compared to the peel extracts of *S. tuberosum*. The antibacterial activity of peel extracts were compared to the Standard drug (Amphilox). Ethanolic extract of *P. granatum* was found to be much more efficient than the standard drug used. The Minimum Inhibitory Concentration (MIC) of ethanolic extract of *P. granatum* was determined by Broth Dilution Method. It was found to be 7 μ l for both the pathogens *E. coli* and *S. aureus*.

CONCLUSION

The present study indicate that selected peel extracts of *Punica granatum* (Pomegranate) and *Solanum tuberosum* (Potato) are the potential sources of phytochemicals. The antibacterial properties of the peel extracts may vary against human pathogens used depending on the nature and type of the phytochemicals.

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REFERENCES

- Viji M, Murugesan S. Phytochemical analysis and antibacterial activity of medicinal plant *Cardiospermum halicacabum* Linn. Pytopharmacol, 2010; 2: 68-77.
- Parekh J, Chanda SV. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol, 2007; 4: 3153-58.
- Harborne JB. Phytochemical methods, 2nd Edition, Chapman and Hall publications London, New York, 1984; 288.
- Srinivashan V, Durairaj B. Effects of essential oils from *Citrus aurantium* L. International Journal of Pharmacy and Pharmaceutical science, 2014; 6(4): 44-49.
- Braide W, Odiong IJ, Oranusi S. Phytochemical and antibacterial properties of the seed of Watermelon (*Citrullus lanatus*). Prime J Microbiol Res, 2012; 2(3): 99-104.

6. Baquero F. Gram – positive resistance: Challenges for the development of new antibiotics. *J. Antimicrob. Chemother*, 1997; 39: 1-6.
7. Chopra I, Hodgson J, Metcalf B, Poste G. New approaches to the control of Infectious diseases caused by antibiotic resistant bacteria - An industry perspective approach. *J Med Assoc*, 1996; 275: 401–403.
8. Rasdi NHM, Samah OA, Sule A, Ahmed QU. Antimicrobial studies of *Cosmos caudatus* Kunth (Compositae). *J Med Plant Res*, 2010; 4: 669–673.
9. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to Antibiotics using the agar diffusion method. *J Antimicro Chemo*, 2008; 61(6): 1295-1301.
10. Shukla A, Lalit A, Sharma V, Vats V, Alam A. Pearl and Finger Millets. The Hope of Food Security. *App Res Journal*, 2015; 1(2): 59-66.
11. Ali NAA, Julich WD, Kusnick C, Lindequist U. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharm*, 2001; 74: 113-115.
12. Trease GE, Evans WC. *Pharmacology* 11th Edn, Bailliere Tindall Ltd; London, 1989; 60-75.
13. Laveena KB, Chandra M. Qualitative and quantitative phytochemical analysis of primary and secondary metabolites of *Mussaenda bellila* L. from Western Ghats of Karnataka. *World J Pharm Res*, 2017; 6(16): 1155-1166.
14. Srinivasan D, Nathan S, Suresh T, Perumalsam O. Antimicrobial activity of certain India medicinal plants used in folkloric medicine. *J Ethnopharm*, 2001; 6(74): 217-220.
15. Kumarasamy Y, Cox P, Jaspars M, Nahar L, Sarker S. Screening seeds of Scottish plants for antibacterial activity. *J Ethnopharm*, 2002; 83: 73-77.
16. Adewuyi A, Oderinde R, Ademisoje A. Antibacterial activities of Nonionic and anionic surfactants from *Citrullus lanatus* seed oil. *Jundishapur J Microbiol*, 2013; 6(3): 205-208.
17. Evans WC. General methods associated with the phytochemical investigation of herbal products. In: Trease and Evans *Pharmacognosy*, (15 Ed.). New Delhi: Saunders (Elsevier), 2002; 137-148.
18. Ramya and Chandra M. Preliminary phytochemical study of *Nyctanthes arbor-tristis* L. and *Santalum album* L. from Western Ghats of Karnataka. *Euro J Biomed Pharm Sci*, 2018; 5(01): 644-650.
19. Gayathri Pai and Chandra M. Screening of phytochemicals and isolation of endophytic fungi from medicinal plants *Helicteres isora* L. *IOSR J Pharm*, 2017; 7(12): 01-05.
20. Sazada S, Verma A, Rather AA, Jabeen, Meghvansi MK. Preliminary Phytochemicals analysis of some important medicinal and aromatic plants. *Adv Biol Res*, 2009; 3: 188-195.
21. Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. *Int J Appl Nat Sci*, 2012; 1(1): 8-26.
22. Harborne JB, Williams CA. Advance in flvonoids research since 1992. *Phytochem*, 2000; 55: 481-504.