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STUDY OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF PUNICA GRANATUM PEEL AND ITS PHYTOCHEMICAL SCREENING

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ABSTRACT

Punica Granatum belongs to family Punicaceae. The objective of the present work was to identify the phytochemical constituents and to study of antibacterial and antifungal activity of pomegranate peel extract. *Punica granatum* are utilized by local people as the part of their meal and treat health diseases. Peoples supposed to consume *Punica granatum* seed and their peel was thrown as waste. Recently, natural products have been evaluated as sources of antimicrobial and antifungal agents with efficacies against a variety of microorganisms. Present study was designed to evaluate the antibacterial and antifungal activity of *Punica granatum* peel against human pathogens. The extract was prepared using ethanol. Antimicrobial activity was tested against one gram positive bacteria and three gram negative bacteria while antifungal activity was tested against two fungi. The various Concentrations (30, 60, 100mg/ml) of the ethanolic extract were tested. Evaluations were based on the zone of inhibition using Agar well diffusion assay. The inhibitory activity was found to be dose dependent. This study represents that ethanol extracts of waste material (peel) of *Punica granatum* may be utilize as a potential source of antimicrobial and antifungal agents. Ciprofloxacin (10µg/ml) used as standard for antibacterial activity while fluconazole (10µg/ml) used as standard for antifungal activity. The phytochemical investigation showed the presence of active chemical constituents such as alkaloids, tannins, flavanoids, steroids, cardiac glycosides and terpenoids.

KEYWORDS: Punica Granatum, Antibacterial, Antifungal, Phytochemicals, Extraction.

INTRODUCTION

Since ancient times herbal drugs are used for the treatment of infectious diseases in human without any side effects. To eradicate the side effects of the present allopathic drugs now scientist are moving towards the herbal drugs what our ancient peoples used.[1] Herbal medicine also known as botanical medicine or phytomedicine refers to using plants seeds, flowers, roots for medicinal purpose. Herbals have a long tradition of use of outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease.[2] In this way to create the scientific evidence for the natural Punica Granatum selected for the antibacterial and antifungal activity and taken for Phytochemical screening. *Punica granutam* is also well known by different local name like dalim, anar, and pomegranate. It belongs to the family of Punicaeae.[3] *Punica granatum* are widely available in Mediterranean basin and Southern Asia in warm environment.[4] The phytochemical constituents were studied by qualitative analysis for performing various chemical tests. Punica Granatum Linn (Pomegranate) belonging to family Punicaceae. The medicinal parts are the root, the bark, the fruits, the peel of the fruit and the flowers.[5] Various parts of Punica Granatum have been used for various medicinal purposes. Although the global studies pointed to the impacts of the flowers, as decreases the blood glucose, reducing the cholesterol, and anti-allergic effect, but it did not showed antibacterial activity. With regard to the popular therapeutic uses of pomegranate, it has known as an anti-diarrhoea, antiparasitic agent, ulcers, diuretic, and an antibacterial activity. The pharmacological functions of pomegranate include antioxidation, anti-tumour anti-hepatotoxicity, antilipoperoxidation and anti- inflammatory. Although many studies have reported the antibacterial activity of pomegranate but it did not revealed enough studies about its effect on bacterial resistance, and did not determined the most effective part of the plant in dealing with bacteria, whether peel of the fruit, leaves, flowers, or seeds of the pomegranate.[6] The objective of present study were to evaluate the antibacterial and antifungal activity of ethanolic extract of Punica Granatum peel on selected bacterial gram positive and gram negative and fungal cultures.

MATERIAL AND METHOD

Collection of Plant Material

Fresh Punica granatum was purchased from local market of Jaysingpur, Maharashtra, India 416101 on date 02.04.2014. The sample was identified and authenticated by Dr. (Miss.) K. R.

Datar, Head Dept. Of Botany, Deccan Education Society, Pune Willingdon College, Sangli. Collected material was subjected to drying at room temperature for about a week in open air. These air dried material was grind into powdered and stored under refrigeration until their further utilization.

Preparation of Extracts

The extract of Punica Granatum peel powder was prepared in 5% concentration (5gm of weighed Punica Granatum peel powder added in 100ml of solvent separately). After extract preparation coarse suspended particles were removed by passing through strainer. Ethanolic extraction was carried out by using soxhlet apparatus. The extract was stored in refrigerator for further use. [7,8,9,10,11]

Assessment of Antimicrobial and Antifungal Activity

The screening of ethanolic extraction of peel of *Punica granatum* was carried out using agar well diffusion method. The bacterial and fungal strains for the study were obtained from Govt. Medical college, (Microbiology and bacteriology department) Miraj. The Gram negative bacterial strains used for study are Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Gram positive Staphylococcus aureus. Fungal strains used in the study are Candida albicans and Aspergillus flavus.

From bacterial and fungal cultured slants, several colonies were transferred to 5ml of sterile distilled water. It is mixed for some seconds to ensure homogenicity and further diluted to match the turbidity with 0.5 McFarland standard solutions which is roughly equivalent to 150 million cells per mL. (Corresponding to 1.5 × 10^8CFU/ml).[12, 13] Punica granatum peels ethanolic extract was prepared at 30mg/ml, 60mg/ml & 100mg/ml concentrations in sterile water. Ciprofloxacin 10µg/ml and fluconazol 10µg/ml were taken as standard for antibacterial and antifungal activity respectively.

Nutrient agar was prepared as bacterial media and Sabouraud Dextrose agar is prepared as fungal media and sterilized. [14] All glassware's, borer, petriplates, extract dilutions were sterilized in autoclave. In aseptic technique, using sterile swab a bacterial lawn is made on sterile petri plates from microbial inoculums suspension. Swab is made in one direction by rotating plate at 90º. An agar-well diffusion method was employed for determination of antibacterial and antifungal activities.[15] Wells (4.6mm in diameter) were cut from the agar with a sterile borer and 60µL extract solutions were delivered into them. Sterile water is kept

as control. The inoculated plates were incubated at 37°C for 24 h. Antibacterial and antifungal activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. The inhibitory DIZ was expressed in millimeters. All tests were performed in triplicates. After 24 hours the plates was examined for zone of inhibition.

Phytochemical Screening

Phytochemical analysis of the extract was carried out using various procedures describe by Various authors. [16,17] Phytochemical screening was performed to detect the presence of several phytochemicals like Alkaloids, Flavonoids, Steroids, Saponins, Cardiac glycoside, Tannins, Terpenoids and free Amino acid etc "Table 1"

Table 1. Phytochemical Analysis of Punica Granatum Peel Extract.

S. No.	Test For	Chemical Tests	Peel Ethanolic extract
1	Test for alkaloids	A. Hager's test	Positive
		B. Wagner's test	Positive
		C. Dragandroffs test	Positive
2	Test for flavanoids	A. Lead acetate test	Positive
3	Test for saponins	A. Foam test	Negative
4	Test for steroids	A. Salkowski test	Positive
5	Test for cardiac glycosides	A. Keller-killiani test	Positive
		B. Legal's test	Positive
6	Test for tannins	A. 5% w/v FeCl3 solution	Positive
		B. Acetic acid solution.	Positive
		C. Dil. KMnO4 solution.	Positive
7	Test for amino acids	A. Ninhydrine test	Negative

RESULT AND DISCUSSION

The phytochemical screening of ethanolic extract of *Punica granatum* peels show presence of flavanoids, steroids, cardiac glycosides, alkaloids, Tannin. The extract of peel shows absence of saponins and amino acids. Ethanolic extract shows significant antibacterial and antifungal activity towards clinically significant microbes "Table 2 and 3".

Nearly 80% of the world populations depend on the traditional medicine for primary health care, mainly including the use of natural products. Researchers have extensively studied the biological properties of *Punica granatum* and their results showed that this plant is ethno medically valuable. The study shows the pharmacological importance of peel of *Punica granatum*, thereby exploring bioactive phytochemicals from waste material (peel) showing antimicrobial and antifungal activity and thus substantiates traditional medicinal use. The

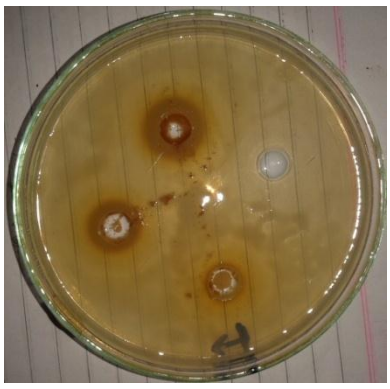
separation and further activity mediated approach was emphasize to conduct in future to demonstrate active phytochemicals to be utilize as lead compounds for antimicrobials. Thus, the study provides a strong direction for proper investigation of various plants to explore molecules having antimicrobial and antifungal properties against human pathogens using waste sources.

Table 2. Dose Dependent Antimicrobial Activity of *Punica granatum* Peel Extracts

S. No.	Name of Pathogens	Extract Conc. In mg/ml	Zone of Inhibition in mm of diameter	Standard Ciprofloxacin 10µg/ml Zone of Inhibition in mm of diameter
01	Escherichia coli	30	25	25
		60	29	
		100	34	
02	Proteus vulgaris	30	22	17
		60	25	
		100	27	
03	Pseudomonas aeruginosa	30	20	22
		60	23	
		100	27	
04	Staphylococcus aureus	30	18	17
		60	23	
		100	30	

Table 3 Dose Dependent Antifungal Activity of *Punica granatum* Peel Extracts.

S. No.	Name of Pathogens	Extract Conc. In mg/ml	Zone of Inhibition in mm of diameter	Standard Fluconazol 10µg/ml Zone of Inhibition in mm of diameter
01	Candida albicans	30	07	11
		60	15	
		100	18	
02	Aspergillus flavus	30	14	09
		60	15	
		100	19	



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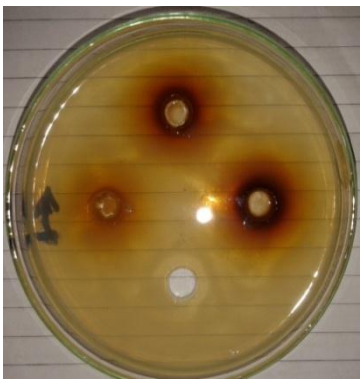

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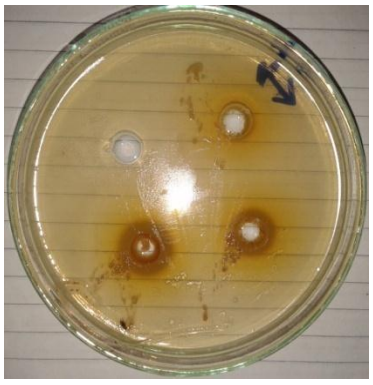
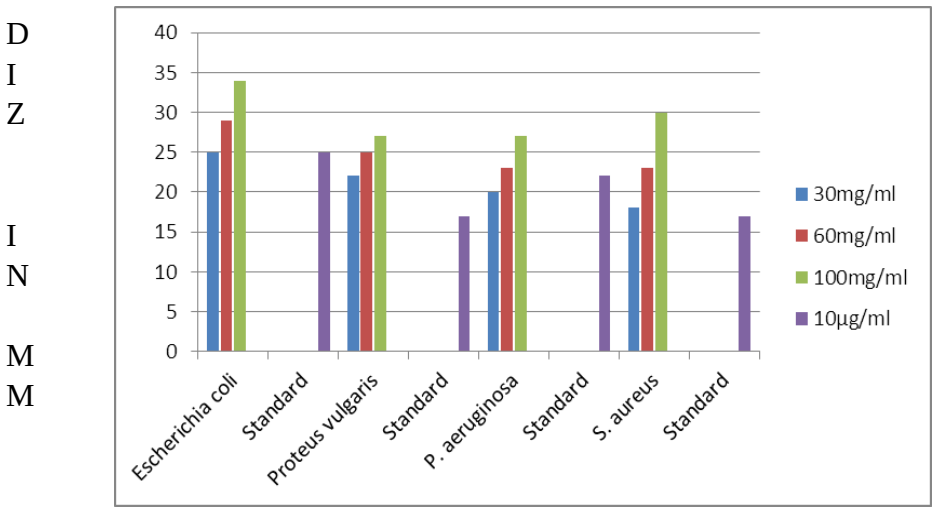


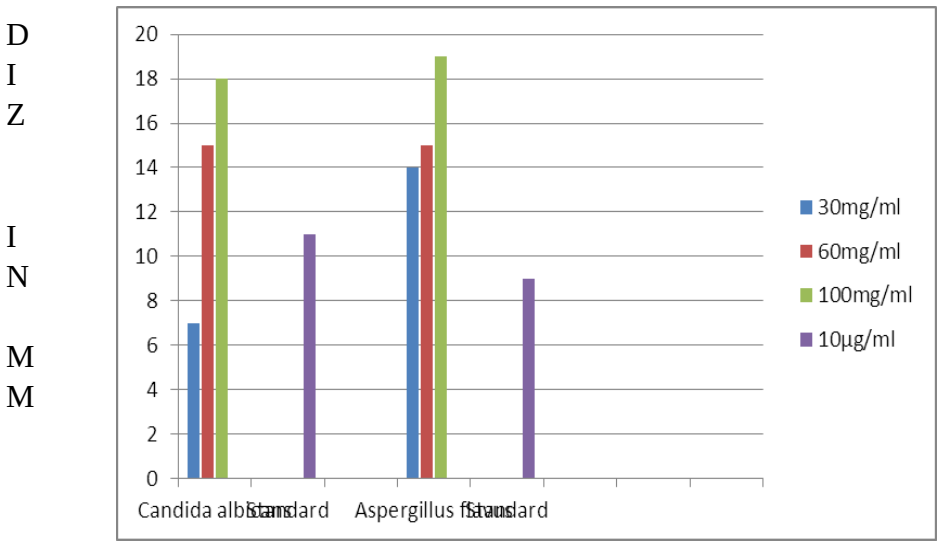
S. AUREUS

Antibacterial activity of Ethanolic Extract of *Punica granatum* peel and Standard drug.



CONCENTRATION IN MG/ML

Antifungal activity of Ethanolic Extract of *Punica granatum* peel and Standard drug .



CONCENTRATION IN MG/ML

CONCLUSION

In the present study an attempt has been made to make out the antimicrobial and antifungal activity of ethanolic extract of peels of *Punica granatum* (which are generally treated as wastes). The extract has antibacterial activity against bacterial strains (E. coli, P. vulgaris, P. aeruginosa, S. aureus) and shows more potency than that of standard ciprofloxacin. The extract also has antifungal activity against fungal strains (Candida albicans and Aspergillus flavus) and shows more potency as that of standard fluconazol. After further purification and characterization of the active metabolites present in *Punica granatum* followed by a detailed study of toxicity and pharmacological effects of the compound, the peel extracts of

pomegranate may be used as remedy against various diseases without any side effects and the plant species can be a good pharmacophore source in future.

REFERENCES

1. Rafi Khan P et al Anthelmintic activity of Nerium Oleander flower extract in Indian adult earth worm, Journal of Natural Product and Plant Resources, 2011; 1(4):40-46.
2. CH. S. D. Phani Deepthi Yadav et al, Phytochemical evaluation of Nyctanthes Arbortristis, Nerium Oleander and Catharathnus Roseus, Indian Journal of Research in Pharmacy and Biotechnology, 2013; 1(3): 333-338.
3. Anonymous. http://www.mpbd.info/plants/*punica-granatum*.php. (Accessed date: 3rd January 2013).
4. Amit Parashar, Ayub Ansari. A therapy to protect pomegranate (*Punica granatum L*) from sunburn. *Pharmacie Globale (IJCP)*, 2012; 5(05):1-3.
5. Neelam Arun, D P Singh. *Punica granatum*: A review on pharmacological and therapeutic properties. *IJPSR*, 2012; 3(5):1240-1245.
6. Shaza Al Laham ea al, The antibacterial activity of various parts of Punica Granatum on antibiotics resistance Escherichia coli, International Journal of Pharmacognosy and Phytochemical Research, 2014; 6(1):79 – 85.
7. Sikandar Khan Sherwani et al, Organoleptic and wormicidal evaluation of various crude extracts of Pome Granate peel, International Research Journal of Pharmacy, 2013; 4(7): 80–82.
8. Jahir Alam Khan et al, Antibacterial properties of Punica Granatum peels, International Journal of Applied Biology and Pharmaceutical Technology, 2011; 2(3): 23–27.
9. Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. Trends in Food Sciences and Technology, 2006; 17(6): 300–12.
10. D. Estherlydia et al, Antimicrobial activities of Punica Granatum extracts against oral microorganisms , International Journal of PharmTech Research , 2013; 5(3): 973 – 977.
11. Saad Sabbar Dahham et al, Studies on antibacterial and antifungal activity of pomegranate, American – Eurasian Journal of Agriculture and Environment Science, 2010; 9(3): 273–281.
12. http//e.m.wikipedia.org/wiki/McFarland-standards
13. http//e.m.wikipedia.org/Agar-diffusion-test
14. http//en. wikipedia.org/Sabouraud-agar

15. NCCLS (National Committee for Clinical Laboratory Standard), Performance Standards for Antimicrobial Susceptibility Testing, 9 International Supplement. M100-S9, Wayne Pa. 1999.
16. M. Hajoori et al, Evaluation of antimicrobial activity of Punica granatum peel extracts using different solvent system, International Journal of Pharmacological screening methods, 2014; 4(1): 26 – 31.
17. C. K. Kokate, A. P. Purohit, S. B. Gokhale, Pharmacognosy, Nirali Prakashan, 42: edition: A1 – A6.