Phytochemical investigations of Pomegranate (*Punica granatum*) rind extracts and their antibacterial activity

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Abstract-Pomegranate (*Punica granatum* L.) is an ancient fruit rich in phytochemical bioactive compounds. *Punica granatum* L belonging to the family Punicaceae is described as an ingredient in remedies and is a widely used plant having medicinal properties. Having served as a symbolic fruit since ancient times, pomegranate (*Punica granatum* L) gained considerable recognition as a functional food in the modern era. Present study reports the phytochemical analysis which has been carried out on the peel (rind) extract. Rind of pomegranate (*Punica granatum*) was extracted using Methanol as solvents. Phytochemical investigations included qualitative detection of phytochemicals .This phytochemical investigation results shows the presence of phenols , tannins, flavonoids, coumarins, quinones, steroids, triterpenoids, and alkaloids in Methanol extracts.The antibacterial activity of extracts were also evaluated by agar diffusion method against *Staph aureus*, *E.coli* and *Pseudomonas aeruginosa*.Methanolic extracts of pomegranate rind was most effective in inhibiting the growth of *Staph.aureus* showing 22 mm of diameter of zone of inhibition followed by Ethanolic extract against *Staph.aureus* with 19 mm diameter of zone of inhibition followed by *E.coli* with 21 mm diameter of zone of inhibition by methanol extract and 17 mm of diameter of zone of inhibition by ethanol extract. Work is under progress on new emerging AI technologies for detailed analysis of phytochemicals in *Punica granatum* rind. AI provides new approaches for screening the main components and pathways of single herb or prescriptions, and predicting the mechanism of action.

Key words: Pomegranate peel/Rind; Phenols; Flavonoids; Phytochemical; Antibacterial

Introduction

Pomegranate is an ancient fruit native to Persia which has been cultivated in the Mediterranean region through years[1 and 2]. Pomegranate, which belongs to the family of Punicaceae, is botanically named *Punica granatum*[3]. Pomegranate trees are considered as shrubs or small trees which grow 5 to 10 meters high[2]. Its flowers are red and can occur either as single blossoms

or clusters of several blossoms[4] and [5].Pomegranate is rich in bioactive molecules, it has shown myriad medicinal properties due to its high phenolic content [1,6]. Pomegranate, as a fruit rich in antioxidants, can intensively and positively contribute in humans' health. Pomegranates strong historical, cultural and religious significances, besides to the researches determining the phytochemicals present in pomegranate triggered analysing and evaluating pomegranate rind.

Resistance to antimicrobial drugs in pathogenic bacteria is a global concern.[7-9]*Pseudomonas aeruginosa* and *Staphylococcus aureus* are important nosocomial pathogens, which both frequently cause multidrug resistance.[7,10] Infections caused by commercial antibiotic resistance isolates have increased greatly during the last decades in hospitals.[8] The spread of these organisms in healthcare settings are often difficult to control, due to the presence of multiple intrinsic and acquired mechanisms of antimicrobial resistance.[8,11] In recent years, study of antibacterial properties of plant extracts is of interest.[12] Pomegranate (*Punica-granatum* L.) is a native fruit in Iran it has a rich history of traditional use in medicine.[13] Pharmacological effects of pomegranate have been mentioned anciently and research on pomegranate is increasing due to its great nutritional values and medicinal uses.[14] In several studies it had been found that pomegranate extracts have many potential effects including antibacterial[15], antifungal[16], antiviral[17]and some other activities.As an emerging discipline, artificial intelligence (AI) technologies have been enthusia-stically explored by Traditional Medicine(TM) researchers in recent years. AI-powered methods, such as machine learning, deep learning, network pharmacology, bioinformatics, systems biology, chemical informatics, and computer vision, can link chemical composition, herbal medicine, drugs, targets, symptoms, and diseases. In other words, AI provides new approaches to exploring ancient literature on TM, enabling the screening of major components of herb or formula, revealing the mechanism of action, and guiding the precise use of TM. The present Research Topic aims to discover novel approaches and strategies for developing and evaluating medicines *via* AI technologies[20.]

The aim of this study was to assess the potential antibacterial activity of *Punica granatum* L. peel in alcoholic extracts against *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *E.coli.*

Material and Methods

Collection and Sample preparation

Fresh pomegranate fruit was bought from the local market and washed thoroughly using water. The rind of the pomegranates was separated, washed with distilled water and dried. The dried rind was powdered using commercial grinder. Samples were kept in sealed plastic bags and stored at low temperature in dark, until use.

Preparation of extracts

Dried and powdered pomegranate peels were kept in air tight plastic containers and stored at freezing temperature until used for extraction. Methanol (100 mL) was added to 0.5 g of dried sample in conical flasks and was stirred for 3h at room temperature (20°C). Methanol extract was capable of recovering many compounds thus Methanol was chosen for the extraction of functional components from

pomegranate peels[16]. The extractions were performed for 48 h and concentrated by slow evaporation process[18]. The obtained extract was kept in moisture free container and used for phytochemcial analysis. Aqueous extract was also prepared using the same method.

Phytochemical screening

Primary phytochemical screening of pomegranate rind

Preparation of extracts- Aqueous extracts of pomegranate rind was prepared by soaking 3.0 g of dried rind in 80 mL distilled waterfor 24 hours, followed by filtration. Alcoholic extracts of pomegranate rind was prepared by soaking 3.0 g of dried rind in 80 mL methanol, separately, for 24 hours, followed by filtration. Chloroform extracts of pomegranate rind was prepared by soaking 0.5 g of dried rind in 5 mL chloroform, for 24 hours, followed by filtration.

Test for phenols and tannins- To 1 mL of aqueous extract, 1 mL of 10% aqueous ferric chloride was added. The presence of phenols is indicated by formation of blue or green colour. The presence of hydrolysable tannins is indicated by formation of dark blue colour, while the presence of condensed tannins is indicated by formation of green colour[8,9].

Test for flavonoids

Test (a)-To 1 mL of aqueous extract, few magnesium turnings were added followed by few drops of concentrated hydrochloric acid. He presence of flavones is indicated by the formation of red colour[8].

Test (b)-To 1 mL of aqueous extract, 1 mL of 10% sodium hydroxide was added. He presence of flavonoids is indicated by the formation of yellow to orange colour[8,9].

Test (c)-To 1 mL of alcoholic extract, 1 mL of concentrated sulphuric acid was added. He presence of flavanones is confirmed by the formation of orange to crimson red[8].

Test for anthocyanins: To 1 mL of alcoholic extract, 1 mL of 10% sodium hydroxide was added and heated for 5 minutes in 100°C water bath. He presence of anthocyanins is indicated by the formation of blue colour[8,9].

Test for coumarins: To 1 mL of alcoholic extract, 1 mL of 10% sodium hydroxide was added. He presence of coumarins is indicated by the formation of yellow colour which fluorescence under ultraviolet light[8,9].

Test for quinones: To 1 mL of alcoholic extract, 1 mL of concentrated sulphuric acid was added. He presence of quinones is indicated by the formation of red colour[8].

Test for saponins: To 1 mL of aqueous extract, 5 mL of distilled water was added, the tube was vortexed for 2 minutes. He presence of saponins is indicated by lather formation[8,9].

Test for steroids: To 2 mL of chloroform extract, 2 mL of concentrated sulphuric acid was added slowly on the tube's wall. The presence of steroids is indicated by formation of two layers, an upper red layer and a lower yellowish-green layer[10].

Test for triterpenoids: To 2 mL of chloroform extract, 1 mL of acetic anhydride was added, followed by the slow addition of 1 mL of concentrated

sulphuric acid. The presence of triterpenoids is indicated by formation of **reddish white colour**[10].

Test for alkaloids: Test (a): Dragendroff Test-To 2 mL of chloroform extract, few drops of Dragendroff reagent was added. The presence of alkaloids is indicated by formation of orange colour[8]

Test (b): Mayer's Test-To 2 mL of chloroform extract, 2 mL of Mayer's reagent was added. The presence of alkaloids is indicated by formation of white precipitate[8].

Antibacterial assay

Agar well diffusion method was used for the antibacterial assay[19]. The bacteria chosen for the antibacterial assay were obtained from American Type Culture Collection (ATCC) *Staphylococcus aureus* (ATCC 6538) [gram positive], and *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027) [gram negative]. The cultures of the chosen bacteria were grown on 25 mL nutrient agar (Hi Media) plates . Overnight nutrient broth cultures of bacteria (10^5 per 0.5 mL) were aseptically mixed with 20 mL of nutrient agar cooled down to 50°C in Petri dishes following that, wells of 7 mm diameter were made in the solidified agar medium using a sterilised steel cork borer. A volume of 100 µL of each extract was slowly loaded into the wells using micropipettes of sterilised tips, the plates were then incubated for 24 hours at 37°C. The diameter of inhibition zone surrounding the agar well for each plate was measured. Triplicate tests were done for each extract[19].

Results and Discussion

Due to the presence of a wide range of phytochemicals in pomegranate rind, it can be predicted that they have significant medicinal values. Phytochemical screening of pomegranate rind revealed that pomegranate rind contained phenols, tannins, flavonoids, quinones, coumarins, steroids, triterpenoids and alkaloids. (**Table-1**).

Table-1 Primary phytochemical screening of Pomegranate Rind.

S.No.	Phytochemicals tested	Pomegranate Rind
1.0.	Phenols	+
2.0.	Tannins	+
3.0.	Flavonoids	+
4.0.	Anthocyanins	-
5.0.	Coumarins	+
6.0.	Quinones	+
7.0.	Anthroquinones	-
8.0.	Saponins	-
9.0.	Steroids	+
10.0.	Triterpenoids	+
11.0.	Alkaloids	+
Key:(+)=Present,(-)= Absent		

Antibacterial activity

Table-2 Antibacterial activity of solvents extract of *Punica granatum* rind

S.No.	Solvent extracts	Diameter of zone of inhibition(mm)		
		Staph.aureus-ATCC 6538	***E.coli ATCC 8739***	***Psuodomonasaeruginosa ATCC 9027***
1.0.	Methanol extracts	22	21	17
2.0.	Ethanolic extract	19	17	16
3.0.	Aqueous Extracts	15	12	NAD
4.0.	Ciprofloxacin (Positive control)	38	36	37
5.0.	Solvents(Negative control)	NAD	NAD	NAD
Key : NAD= No Activity Detected				

Antibacterial activity of pomegranate rind extracts was determined Well agar Diffusion method. The methanolic extract of pomegranate rind was the most effective in inhibiting the growth of *Staph.aureus* ,*E. coli* and *Pseudomonas aeruginosa* by 22 mm ,21mm & 17 mm respectively (Table-2) followed by Ethanolic extract. Nevertheless, the pomegranate rind aqueous extract was also significantly effective in inhibiting the growth of *Staph aureus* and *E.coli* by 15 mm& 12 mm. The growth of *P. aeruginosa* was again only inhibited by the methanolic and Ethanolic extracts of pomegranate rind. Overall, the most effective extract in inhibiting the growth of the chosen bacteria was the methanolic extract of pomegranate rind.

When a new drug to be discovered, qualitative phytochemical analysis is a very important step as it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. The present study showed interesting preliminary phytochemical constituents in solvent peel extracts of *Punica granatum*. Further characterization and quantitative assay may be carried out to test the peel extracts for various therapeutic and pharma-cological activity. In any case, if any significant bioactive natural product is present, it is necessary to separate that compound from the mixture of compounds by using suitable chromatographic technique[21].

Conclusion

The present study provides more evidence on the importance and value of pomegranate fruit, especially pomegranate's rind which is usually considered as a waste product. According to the phytochemical screening done pomegranate rind contain phenols , tannins, flavonoids, quionones, coumarins, steroids, triterpenoids and alkaloids. The methanolic and s extract of pomegranate rind was the most effective in inhibiting the growth of a number of bacteria according to the well agar diffusion method. Further studies could be conducted on the antibacterial capacity of pomegranate rind components, which may led to the discovery of new antibacterial agents.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

References

1. Viuda-Martos, M.; Fernández-López, J. and Pérez-Alvarez, J. Pomegranate and its many functional components as related to human health: a review. Comprehensive Reviews in *Food Science and Food Safety*, 2010, 9: 635-654.

2. Silva, J.; Rana, T.; Narzary, D.; Verma, N. and Meshram, D. et al. Pomegranate biology and biotechnology: A review. *Science Horticulturae*, 2013, 160: 85-107.

3. Chandra, R.; Babu, K.; Jadhav, V. and Silva, J. Origin, History and Domestication of Pomegranate. Fruit, Vegetable, Cereal Science and Biotechnology, 2010, pp. 1-6.

4. Baliga, M.; Shivashankara, A.; Shetty, C.; Hilakchand, K. and Periera, N. et al. AntidiabeticEffects of *Punica granatum* L (Pomegranate): A Review. *Bioactive Food as Dietary Inventions for Diabetes*, 2013, pp. 355-369.

5. Stover, E. and Mercure, E. He Pomegranate: A New Look at the Fruit of Paradise. *Hort. Science*, 2007, 42: 1088-1092.

6. Akhtar, S.; Ismail, T.; Fraternale, D. and Sestili, P. Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, 2015, 174: 417-425.

7. Palavutitotai, N.; Jitmuang, A.; Tongsai, S.; Kiratisin, P. and Angkasekwinai, N. Epidemiology and risk factors of extensively drugresistant Pseudomonas aeruginosa infections. PLoS One, 2018, 13(2):e0193431. doi:10.1371/journal.Pone .0193431

8. Hosseini, M. J.; Kiarsi, M.; Golmohammadi, R. and Sadripour, R. Antibiotic Resistance pattern of bacteria isolated from nosocomial infection in internal surgery and neurosurgery intensive care unit (NICU) at a tertiary care hospital in Tehran, Iran. *Biosci. Biotech. Res. Asia*, 2017, 14(3):1095-1102. doi:10.13005/bbra/2547

9. Ranjbar, R.; Tolon, S. S.; Sami, M. and Golmohammadi, R. Detection of plasmid-mediated qnr genes among the clinical quinolone-resistant Escherichia coli strains isolated in Tehran, Iran. *Open Microbiol. J.*, 2015.

10. Choopani, A.; Golmohmmadi, R.; Rafati, H. and Imani Fooladi, A. A. prevalence of *Staphylococcus aureus* strains isolated from wound infection and drug sensitivity pattern, Tehran-Iran (2006-07). *J. Gorgan. Univ. Med. Sci.*, 2012, 14(3):135-140.

11. Kian, B.; Mirnejad, R.; Mirkalantari, S.; Moradli, G. and Golmohammadi, R. Molecular genotyping of Acineto bacterbaumannii species isolated from patients in Tehran, Iran, by repetitive element PCR fingerprinting. *Iran. J. Pathol.*, 2018, 13(2):144-150.

12. Alanis, A.D.; Calzada, F.; Cervantes, J.A.; Torres, J. and Ceballos, G. M. Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. *J. Ethnopharmacol.*, 2005, 100(1-2):153-157. doi:10.1016/j. jep.2005.02.022

13. Derakhshan, Z.; Ferrante, M. and Tadi, M. et al. Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food Chem. Toxicol.,* 2018, 114:108-111. doi: 10.1016/j.fct.2018.02.023

14. Reddy, M. K.; Gupta, S. K.; Jacob, M. R.; Khan, S. I. and Ferreira, D. Anti-oxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. *Planta. Med.,* 2007, 73(5):461-467. doi:10.1055/s-2007-967167

15. Devatkal, S. K.; Jaiswal, P.; Jha, S. N.; Bharadwaj, R. and Viswas, K. N. Antibacterial activity of aqueous extract of pomegranate peel against Pseudomonas stutzeri isolated from poultry meat. *J. Food Sci. Technol.,* 2013, 50(3):555-560. doi:10.1007/s13197-011- 0351-y

16. Vasconcelos, L. C.; Sampaio, M. C.; Sampaio, F. C. and Higino, J. S. Use of *Punica granatum* as an antifungal agent against candidosis associated with denture stomatitis. *Mycoses.,* 2003, 46(5-6):192-196.

17. Houston, D. M. J.; Bugert, J. J.; Denyer, S. P. and Heard, C. M. Potentiated virucidal activity of pomegranate rind extract (PRE) and punicalagin against Herpes simplex virus (HSV) when co-administered with zinc (II) ions, and antiviral activity of PRE against HSV and acyclovir resistant HSV. PLoS One, 2017, 12(6):e0179291. doi:10.1371/journal.Pone .0179291

18.Elswijk, D.; Schobel, U.; Lansky, E.; Irth, H. and Greef, J. Rapid dereplication of estrogenic compounds in pomegranate (*Punica granatum*) using on-line biochemical detection coupled to mass spectrometry. *Phytochemistry,* 2004, 65(2):233-24.

19. Mandeel, Q.; Hasan, A.; Al-Nafea, H. and Abbas, A. Antibacterial Activity of Extracts from Selected Marine Algae in Bahrain. *Arab Gulf Journal of Scientific Research*, 2010, 28: 147-162.

20.Chaoyong, Wu.; Chen, Jianxin; Lai-Han Leung, Elaine; Chang, Hang and Wang, Xu. Editorial: *Artificial Intelligence in Traditional Medicine Front Pharmacol*., 2022, 13. doi: 10. 3389/fphar.2022.933133PMCID: PMC938 6475

21. Jayaprakash, A. and Sangeetha, R. Phytochemical Screening of Punica-granatum Linn. Peel Extracts, *Journal of Academia and Industrial Research*, 2015, 4 (5): 160-162.