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ANTIMICROBIAL ACTIVITY OF PEELS AND SEEDS OF INDIAN *PUNICA GRANATUM* VARIETIES

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ABSTRACT

Four pomegranate varieties of India were selected to study their antimicrobial activities. The exocarp, mesocarp and seed extractions were obtained through soxhlet procedure and the separated samples were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aspergillus niger* and *Fusarium oxysporum*. The *Minimum Inhibitory Concentration levels* (MIC) of the bacterial strains were also studied. These varieties showed large zone of inhibitions when compared to the standard antibiotic i.e. *ampicillin*. The mesocarp of all the samples showed promising positive results compared to exocarp and seeds. The maximum zone of inhibition was resulted with the mesocarp of Ganesha (34mm) against the fungal strain *Aspergillus niger*. All the parts of pomegranates showed maximum antimicrobial activities with their mesocarp and the minimum activity with the exocarp. This information can be used in the development of novel drugs.

KEY WORDS: Minimum Inhibitory Concentration (MIC), Pomegranate, Antibacterial activity, *E. coli*, *P. aeruginosa*, *P. vulgaris*, Antifungal activity

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INTRODUCTION

Pomegranates belong to *Punicaceae* family and are the important horticulture plants in the Mediterranean regions.[1] The crude extracts of plant leaves for the phytochemical and medical purposes have been tested frequently.[2, 3] At the same time, the use of fruits in treating different diseases has been in practice by humans from ancient times. It is proven that all the parts of a plant can be used as a starting material for medicinal purposes. Medically useful compounds can be obtained from the peel, seeds, flower, bark, juice and roots of pomegranate. The pomegranate peels with antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) has been confirmed recently, indicating its potential to control microbes. The peels of pomegranate can also be used as an antibacterial agent against dental plaque causing bacteria.[4] Nuamsetti *et al*. [5] studied the antibacterial activity of pomegranate fruit peels and arils against four food-related bacteria and showed that Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) were more sensitive to the plant extracts. They also showed that hot-water extracts of peels was the most potent antibacterial agent compared to the arils. Moorthy *et al*. [6] extensively studied 21 microorganisms and studied the antimicrobial activity of ethanolic extracts of pomegranate pericarp and showed that these extracts are highly potent against bacteria than fungi. Chebaibi *et al*. [7] showed bactericidal activity of Moroccan pomegranate peel aqueous extracts against human pathogenic microorganisms. The antimicrobial activity of *E. coli*, *S. aureus* and *S. typhi* were studied by Ahirrao and Suryawanshi.[8] There were some studies resulted in antibacterial activity of pomegranate ethanolic extracts of peels and arils against *S. mutans* and *L. acidophilus* and compared the sensitivity of these organisms against the extracts.[9] The antifungal activity of different Persian pomegranate cultivar extracts were studied against *Candida* species.[10] Bagade *et al*. [11] studied antimicrobial activity of pomegranate peel extract against *S. aureus*, *B. subtilis*, *K. pneumonia*, *P. aeruginosa* and *C. albicans*. Recently, the antimicrobial activity of pomegranate methanolic peel extracts was found against *Shiga* toxin producing *E. coli*.[12] The aqueous extract of pomegranate peel was reported and tested against *P. stutzeri*, which was isolated from poultry meat.[13] These advancements can provide novel pathways for the application of pomegranate extracts as antimicrobial agents. The current study aimed to differentiate the antimicrobial activity of pomegranate exocarp, mesocarp and seeds of four different varieties of pomegranate.

MATERIALS & METHODS

Plant materials

The pomegranate varieties namely, *Aarakta*, *Bhagwa*, *Ganesha* and *Mridula* were collected from the local market of Loni, Maharashtra and brought to the laboratory of Department of Biotechnology, P.V.P. College of Arts, Science and Commerce, Loni. The twenty fruits of each variety brought were washed twice with distilled water and the fruit parts i.e. exocarp, mesocarp and seeds were separated manually. These parts were dried, powdered and processed for soxhlet extraction of the compounds, while methanol is the solvent.[14] The phytochemistry of these methanol extracted samples was studied earlier by the same group.[14,15]

Antimicrobial analysis

The bacterial strains namely, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and the fungal strains *Aspergillus niger* and *Fusarium oxysporum* were obtained from Pravara Institute of Medical Sciences, Loni. These strains were stored in the refrigerator and the working test cultures were prepared from the mother slants to continue further work. The modified agar well-diffusion method was performed to evaluate the antibacterial activities of all extracts. A freshly grown microbial cultures were serially diluted, and 0.1 ml of prepared cells were aseptically spread onto the surface of nutrient agar plates and then left to dry for 30 minutes. Wells (4 mm in diameter) were made in media using a sterilized stainless steel borer. Each well was filled with 20 µl of methanol extracted exocarp, mesocarp and seed samples. Plates were incubated at 37°C for 18– 24 hours. For determining antifungal activity, agar-well diffusion method was followed, where the fungal suspensions were inoculated with potato dextrose agar at 45℃ and allowed to set. Wells (4mm in diameter) were made and the plates were incubated at 28°C for two days after and observed for zone of inhibition. As a positive reference, the standard antibiotic Ampicillin was used.

Minimum inhibitory Concentration

To test the *Minimum Inhibitory Concentration levels* (MIC) of the bacterial strains, various concentrations of all extraction samples were prepared separately and the method described by Nuamsetti et al. was adopted to determine MIC.[5]

RESULTS

The antimicrobial activity of different parts of pomegranate has been studied by using agar well-diffusion method (Fig. 1a, b) and the zones of inhibitions were measured (Table 1).

Table 1
Zone of inhibitions in mm showed by the extracts of exocarp (E), mesocarp (M) and seeds (S) of *Aarakta*, *Bhagwa*, *Ganesha* and *Mridula*

	***Aarakta*(A)**			***Bhagwa*(B)**			***Ganesha*(C)**			***Mridula*(D)**			***Ampicillin***
M. organisms	E	M	S	E	M	S	E	M	S	E	M	S	
E. coli	17	20	16	13	24	17	11	27	18	7	17	19	25

P. aeruginosa	15	22	16	14	24	11	9	20	13	13	23	11	19
P. vulgaris	13	22	14	7	24	10	9	23	15	11	21	16	21
A. niger	12	29	11	14	32	12	11	34	14	12	29	11	19
F. oxysporum	12	23	12	12	28	12	10	25	10	13	24	13	20

The exocarp and seed extractions showed fewer zones of inhibitions when compared with the standard antibiotic. In all the cases, mesocarp extract of each variety was found to be highly potent against different microorganisms. The same has shown similar results with the standard antibiotic *i.e.* ampicillin. The treatment of samples and the standard antibiotic against *E. coli* showed that the mesocarp of Ganesha has showed the maximum inhibition zone (27 mm), where the standard showed a zone of 25 mm. The treatment of the same with *P. aeruginosa* showed that the mesocarp of all the varieties (A-22 mm, B-24 mm, C-20 mm, D-23 mm) have broad antibiotic activities compared to *Ampicillin* (19 mm). In the case of *P. vulgaris* the standard antibiotic (21 mm) and all the varieties have shown almost similar effect (A-22 mm, B-24 mm, C-23 mm, D-21 mm). The mesocarp of four varieties selected showed maximum zone of inhibitions against both the fungi when compared to *Ampicillin*. The maximum zone of inhibition was observed with *Ganesha* mesocarp (34 mm). While considering the zone of inhibitions, the antimicrobial agents used in this study can be ordered as: Ganesha > Bhagwa > Ampicillin > Aarakta > Mridula. The parts of pomegranate with potent antimicrobial activity of four varieties can be summarized as follows: *Aarakta* – Exocarp (*E. coli* – 17 mm), Mesocarp (*A. niger*-29 mm), and Seeds – (*E. coli* and *P. aeruginosa*-16 mm); *Bhagwa* – Exocarp (*P. aeruginosa* and *A. niger*-14 mm), Mesocarp (*A. niger*-32 mm), and Seeds – (*E. coli*-17 mm); *Ganesha* – Exocarp (*E. coli* and *A. niger*-11 mm), Mesocarp (*A. niger*-34 mm), and Seeds – (*E. coli*-18 mm); *Mridula* – Exocarp (*P. aeruginosa* and *F. oxysporum* – 13 mm), Mesocarp (*A. niger*-29 mm), and Seeds – (*E. coli* -19 mm).

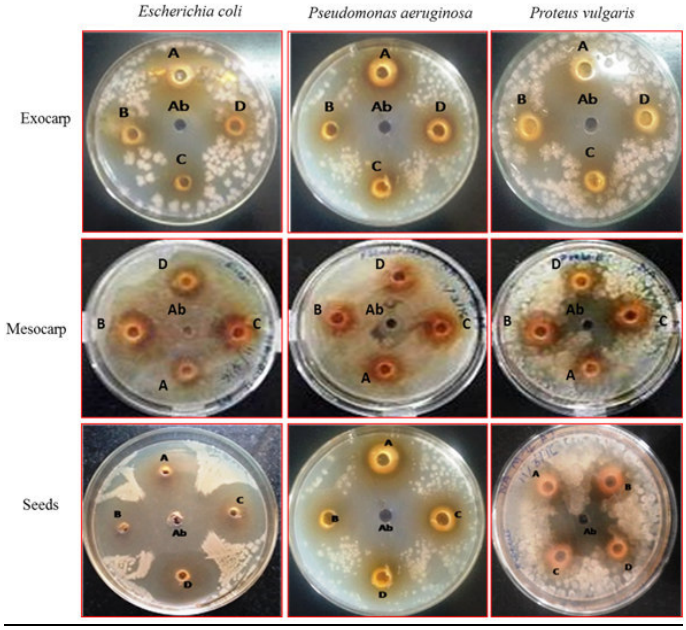

Figure 1 (a)
Antibacterial activity of exocarp, mesocarp and seed extractions of *Aarakta* (A), *Bhagwa* (B), *Ganesha* (C), *Mridula* (M) and Standard Antibiotic (Ab)

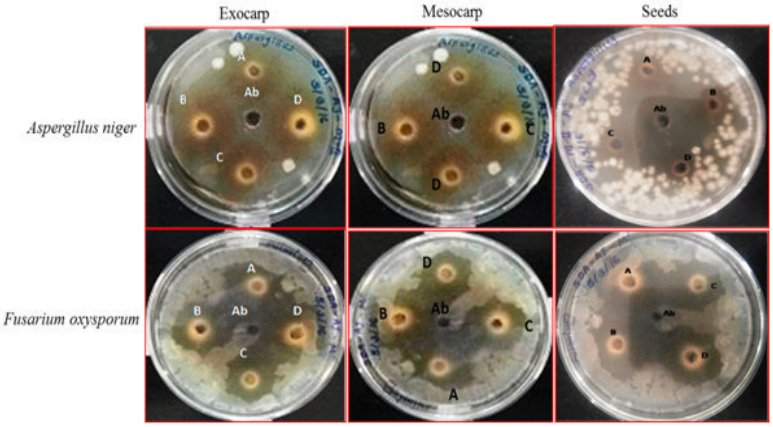

Figure 1 (b)
Antifungal activity of exocarp, mesocarp and seed extractions of *Aarakta* (A), *Bhagwa* (B), *Ganesha* (C), *Mridula* (M) and Standard Antibiotic (Ab)

The *Minimum Inhibitory Concentrations* (MIC) of exocarp, mesocarp and seeds extraction samples was performed against bacteria viz. *E. coli*, *P. aeruginosa* and *P. vulgaris* by using spectrophotometric assay and results were studied. The MIC for *E. coli* was found to be 80% with *Mridula* seeds, 70% with *Ganesha* mesocarp, 60% with *Aarakta* exocarp, *Bhagwa* mesocarp, *Ganesha* seeds, 50% with *Mridula* mesocarp, *Bhagwa* seeds, 40% with the exocarps of *Bhagwa*, *Ganesha*, *Mridula* and the mesocarp of *Aarakta*. *Aarakta* seed extractions showed a MIC of 30% on *E. coli*. The MIC for *P. aeruginosa* was found to be 60% for *Aarakta* mesocarp, *Ganesha* seeds, 50% for all the parts of *Bhagwa* and the seeds of *Aarakta*, 40% for the exocarps of *Aarakta*, *Ganesha*, *Mridula*, mesocarp of *Ganesha*, *Mridula* and the seeds of *Mridula*. MIC for *P. vulgaris* was found to be 70% with *Ganesha* exocarp. *Ganesha* mesocarp and seeds showed a 60% of MIC on *P. vulgaris*. The same has MIC of 50% with *Mridula* exocarp and seeds. There was a 40% MIC observed on *P. vulgaris* from the exocarp and mesocarp of *Aarakta*, *Bhagwa* and seeds of *Aarakta*, *Bhagwa* and *Mridula*.

DISCUSSION

The current study and obtained results were discussed with the previous studies. Nuamsetti *et al*. [5] while studying with different solvent extractions showed that the hot-water extracted peels are highly potent against *E. coli*. The results showed by these authors coincide with the current study. However, the hot-water extractions can be used as replaced with methanol extractions, as the methanol extractions of seeds in the current study showed better zone of inhibition (19 mm) than the previous study (14 mm). Moorthy *et al*. [6] through the ethanolic extracts of pericarp showed a significant inhibitory activity against the bacteria and reported that these extracts are more potent against bacteria than fungi. On contrary, in the current study we found that, the mesocarp of all the varieties studied were more potent against the fungi and also against bacteria. The mesocarp of *Ganesha* showed the maximum zone of inhibition against *Aspergillus niger* (34 mm). Chebaibi *et al*. [7] obtained the bactericidal effect on *P. aeruginosa* with the zone of inhibition (12.5 mm), which is comparatively less than the zone of inhibitions obtained in the current study. While working with pomegranate rind, Ahirrao and Suryawanshi [8] showed the maximum zone of inhibition for *E. coli* in methanolic extract as 15.8 mm, whereas the current study showed a maximum zone of inhibition for *E. coli* as 27 mm with *Ganesha* mesocarp. Bagade *et al*. [11] showed the maximum zone of inhibition for *P. aeruginosa* (38 mm) at a concentration of 3% preparation of gel formulation. In the current study, the maximum zone of inhibition for *P. aeruginosa* was found to be 24 mm *Bhagwa* mesocarp extract. However, these results are similar with various concentrations of marketed silver sulfadazine and marketed Licorice preparations.

CONCLUSION

The current study concluded that the mesocarp of pomegranates contains certain antimicrobial compounds, which can control the microbial action to cause diseases. The exocarp and seeds are also having some important medicinal properties, however, less potent than the mesocarp. With these results, it is clear that, the mesocarp of pomegranate can be used as a potential antimicrobial agent against the microorganisms studied so far. As the non-edible parts of the pomegranates i.e. exocarp and mesocarp, are not consumed by the people, these portions of the fruits can be used in pharmacological purposes. This study can be used in the preparation of novel drugs to cure against the diseases caused by specific organisms.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Dahham SS, Ali MN, Tabassum H, Khan M. Studies on Antibacterial and Antifungal Activity of Pomegranate (*Punica granatum* L.). Amer-Eurasian J of Agri. & Envi. Sci. 2010; 9 (3): 273-281.
2. Khandelwal S, Khurana SMP. Antimicrobial Activity of Leaf Extracts of Three Medicinal Plants. Int. J. of Pharma. And Bio Sci. 2016; 7 (2): 226-233.
3. Swadhini SP. Phytochemical Screening and Antimicrobial Activity of Five Medicinal Plants against *Myrothecium Sp.* Int. J. of Pharma. And Bio Sci. 2011; 2 (1): 272-279.
4. Devi A, Singh V, Bhatt AB. *Invitro* Antibacterial Activity of Pomegranate and Daru (Wild Pomegranate) Against Dental Plaque Bacteria. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3 (4): 182-184.
5. Nuamsetti T, Dechayuenyong P, Tantipaibulvu S. Antibacterial activity of pomegranate fruit peels and arils. Science Asia. 2012; 38: 319-322.
6. Moorthy K, Punitha T, Vinodhini R, Sureshkumar BT , Vijayalakshmi P, Thajuddin N. Antimicrobial activity and qualitative phytochemical analysis of *Punica granatum* Linn. (PERICARP). J. of med. Plants. res. 2013; 7 (9): 474-479.
7. Chebaibi A, Filali FR. Bactericidal activity and phytochemical screening of Moroccan pomegranate (*Punica granatum* Linn.) peel aqueous extracts J. of med. Plants. res. 2013; 7 (14): 887-891.
8. Ahirrao SD, Surywanshi SP. Phytochemical Screening and antimicrobial activities of medicinally important plant *Punica granatum* L.

rind against various microorganisms. Int. J. of Sci. inn. And dis. 2013; 3 (3): 330-335.
9. Nikfallah F, Venugopal A, Tejani H, Lakshmikantha HT. Evaluation of the antibacterial activity in pomegranate peels and arils by using ethanolic extract against *Streptococcus mutans* and *Lactobacillus acidophilus*. Glob. J of Med. Res. 2014; 14 (2): 1-5.
10. Bassiri-Jahromi S, Katiraee F, Hajimahmoodi M, Mostafavi E, Talebi M, Pourshafie MR. Invitro antifungal activity of various Persian cultivars of *Punica granatum* L. extracts against *Candida* Species. Jund. J. of nat. Phar. Pro. 2015; 10 (3): 1-6.
11. Varsha B. Bagade, Varsha M. Jadhav and Vilasrao J. Kadam. Study on Antimicrobial activity of Herbal Formulation. Int. J. of Phar. & Life Sci. 2013; 4 (11): 3099-3104.
12. Growther L, Sukirtha K, Savitha N, Andrew NS. Antibacterial activity of *Punica grantum* peel extracts against Shiga toxin producting E. coli. Int. J. of Life Sci. Bt. & Pharm. Res. 2012; 1 (4): 164-172.
13. Devatkal SK, Jaiswal P, Jha SN, Bharadwaj R, Viswas KN. Antimicrobial activity of aqueous extract of pomegranate peel against *Pseudomonas stutzeri* isolated from poultry meat. J. of food Sci. & Tech. 2013; 50 (3): 555-560.
14. Akkiraju PC, Suryawanshi DD, Jawakekar AJ, Harshad ST, Mamillapalli SL. Phytochemical analysis and HPLC study of vitamin-C from *Punica granatum* L. *Aarakta* variety of India, J. Med. Plants stu.. 2016; 4(6): 09-12.]
15. Akkiraju PC, Harshad ST, Suryawanshi DD, Mamillapalli SL. Jawakekar AJ. Phytochemistry of Three Indian Varieties of *Punica granatum* and Vitamin-C Study by HPLC Technique. Int. J. of Adv. Res. 2017; 5 (2): 512-518.

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