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Antimicrobial Activities Of Punica granatum Extracts Against Oral Microorganisms

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Abstract: Methanolic and aqueous extract of Pomegranate rind, seed and pith was evaluated for its antimicrobial activity against selected oral microorganisms like *Staphylococcus aureus, Staphylococcus epidermis, Klebsiella pneumonia, Pseudomonas aeuriginosa* and *Candida albicans*. The oral pathogens were cultured in Mueller-hinton media and then paper disks containing the extracts at concentration of 4µg/ml, 8µg/ml, 12µg/ml and 100µg/mlwere inserted on the media. The antimicrobial activity was evaluated using disk inhibition method. The effects of the four different concentrations of different parts of pomegranate against oral microorganisms were compared with the positive control using ANOVA. Methanolic Rind Extract (MRE) and Aqueous Rind Extract (ARE) at the concentration of 100mg/ml were found to be effective against the selected oral microorganisms compared to diluted extracts. The study suggests that pomegranate rind extract can be used as an effect treatment against oral diseases.
Keywords: Punica granatum, oral microorganisms, *Staphylococcus aureus, Staphylococcus epidermis, Klebsiella pneumonia, Pseudomonas aeuriginosa* and *Candida albicans.*

INTRODUCTION

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system[1]. For thousands of years, the practice of ayurvedic medicine has alleviated illness and attributed over all positive health[2]. The Indian subcontinent has a rich flora of various plants used in traditional medical treatments[3]. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopoeia as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens[4].

Plants containing phytochemicals such as alkanoids, tannins, essential oils and flavanoids have pronounced defensive and curative activity. There are many species of medicinal plants belonging to various families which are being used, traditionally, to control and cure a variety of dental problems by the Indian population[5].

Dental diseases are a multifactorial disease and have affected many populations throughout the world. Dental diseases include dental caries, developmental defects of enamel, dental erosion and periodontal disease. Dental diseases are a costly burden to health care services, accounting for between 5% and 10% of total health care expenditures and exceeding the cost of treating cardiovascular disease, cancer and osteoporosis in industrialized countries. Although not life-threatening, dental diseases have a detrimental effect on quality of life in childhood through to old age, having an impact on self-esteem, eating ability, nutrition and health[6,7]. The use of medicinal plants to treat dental problems has been discussed from time to time by many researchers [8, 9] and one of which is Punica granatum[10].

Punica granatum commonly known as pomegranate is native to the region from northern India to Iran. It is a fruit- bearing deciduous shrub or small tree which belongs to the family Lythraceae[11]. The pomegranate tree typically grows 12-16 feet, has many spiny branches. The ripe Pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade-shaped, and crowned by the pointed calyx. The fruit contains many seeds (Arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red juice. The leaves are shiny and about 7.6 cm long[12].

Pomegranate is an amazing source of cyaniding, delphinidin (both are anthocyanidins), caffeic acid, chlorogenic acid (both are phenolic acids), gallic acid, ellagic acid (tannic acids), luteolin, quercetin (flavones), kaempferol (a flavonol), naringenin (a flavanone) as well as 17-alpha-estradiol, estrone, estriol, testosterone, beta-sistosterol, coumesterol, gamma-tocopherol, punicie acid, campesterol and stigmasterol in its juice, peels and seed oil that are chemopreventive and therapeutic potentials of this plant [13, 14].

Since the prevalence of dental disease in India is on rise and the antibiotic resistance exhibited by microbes have paved way for alternative therapy and one such is phytotherapy. The present study was carried out to investigate the anticariogenic properties seed extracts of pomegranate against different oral microorganisms.

MATERIALS AND METHODS

Collection of Fruits

The pomegranate was purchased from the local market. It was cut by making a shallow slit at the top of the pomegranate where the knob/stem is (this part is known as the crown). The shallow circle was created by cutting all the way around the rind. The inner seeds were revealed by pulling the crown of pomegranate. Three shallow slits were made by cutting the outer rind, followed by three of the white pith lines, from the top to the bottom of the fruit. Three large sections were created by pulling the fruit. These sections were merged in a large bowl of cold water. The seeds were separated from the rind and pith. The seeds were drained in a colander to remove any additional pith that is mixed with the seeds.

Preparation of Methanolic Extracts

Methonolic rind extract (MRE) was prepared by cutting rind into small squares (approximately 5 mm^2) which was dried at 55°C for 24 hours, and stored in an air tight container in the dark until further use, 10g each of the dried material of rind is taken in a timple. It is then extracted with methanol using soxhlet apparatus for 8hrs. Extract is then is then distilled to remove solvent and used for testing antimicrobial activity.

Methanolic seed extract (MSE) was prepared by drying seed at 55°C for 24 hours. About 10g of the dried material of seed was taken in a timple. It is then extracted with methanol using soxhlet apparatus for 8hours. Extract is then is then distilled to remove solvent.

Methanolic pith extract (MPE) was extracted from Pith which was separated from the seed and it is soaked in 250ml of methanol overnight. It is then filtered and the filtrate is distilled to remove solvent and used for testing the antimicrobial activity.

Preparation of Aqueous Extracts

Aqueous rind extract (ARE) was prepared from 10g of the powdered rind is soaked in 100ml of distilled water for 24 hours and filtered. The filtrate is concentrated and used for testing antimicrobial activity.

Aqueous seed extract (ASE) was prepared from 10g of the powdered seed is soaked in 100ml of distilled water for 24 hours and filtered. The filtrate was concentrated.

Aqueous pith extract (APE) was prepared from pith which was separated from the seed and socked in 100ml of distilled water overnight. It is then filtered and the filtrate concentrated and used for testing the antimicrobial activity

Crude pomegranate extracts (CPE) was prepared from 10g of powdered whole pomegranate soaked in 100 ml of distilled water for 24 hours and filtered. The filtrate was concentrated.

Microorganisms

Type strains were obtained from American Type Culture Collection (ATCC) and National Collection of Industrial Microorganisms (NCIM) as follows: Staphylococcus aureus (ATCC 11105), Staphylococcus epidermis (ATCC 25619), Klebsiella pneumonia (ATCC 9621), Pseudomonas aeuriginosa (ATCC 25619) and

Candida albicans (NCIM 3100) which were all obtained from the microbiology laboratory of Hubert Enviro Care Systems Pvt Ltd. Each of the bacterial specimens was incubated in liquid culture dilutions and incubated at 37°C for 20 minutes to reach the logarithmic stage, then measured to a 0.5 Mc Farland dilution which delivered a final concentration of approximately 105 CFU per ml. Then the agar plates with Methanolic and Aqueous extract of pomegranate were incubated over night at 37°C.

Antimicrobial activity

The antimicrobial potency of Methanolic and Aqueous extract of three different parts such as pith, rind and seed of pomegranate on oral microorganisms was studied using disk inhibition method[15]. In disk inhibition zone method, the Mueller-Hinton agar medium was inoculated with freshly prepared cells of each bacteria and fungi to yield a lawn of growth. After solidification of the agar, a number of sterilized disks were dipped into the solvents (negative controls) and extract solutions of different concentrations (4µg/ml, 8µg/ml, 12µg/ml and 100µg/ml) and placed on the plates. After incubation at 37°C for 24 h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using commercial disks, streptomycin (100µg/ml) and Amphotericin-B (100µg/ml).

Interpretation of inhibition zones of test cultures was adopted from Johnson and Case [16]. Diameter of zone of inhibition of 10 or less indicates test product being resistant to test organism, diameter zone of inhibition of 11 to 15 indicates test product being intermediate resistance to test organism, diameter zone of inhibition of 16 or more indicates test product being susceptible resistance to test organism.

RESULTS

After evaluating the antimicrobial effects of four different concentrations of methanolic and aqueous pomegranate extracts, the positive control produced significantly large inhibition zones for all microorganisms except for crude pomegranate extract (CRE) of Klebsiella pneumonia and Candida albicans. The highest antimicrobial activity against *Staphylococcus aureus* was exhibited by ARE in the concentration of 100mg/ml. The diameter of zone of inhibition exhibits susceptible resistance of 22mm in comparison with the positive control (24mm). The highest antimicrobial activity against *Staphylococcus epidermis* was exhibited by MRE in the concentration of 100 mg/ml. The diameter of zone of inhibition exhibits susceptible resistance of 14mm in comparison with the positive control (21mm). The highest antimicrobial activity against *Klebsiella pneumoniae* was exhibited by CPE in the concentration of 100 mg/ml. The diameter of zone of inhibition exhibits susceptible resistance of 30 mm in comparison with the positive control (25mm). The highest antimicrobial activity against *Pseudomonas aeurginosa* was exhibited by ARE in the concentration of 12mg/ml. The diameter of zone of inhibition exhibits intermediate resistance of 14 mm in comparison with the positive control (15mm). The highest antimicrobial activity against *Candida albicans* was exhibited by MRE in the concentration of 100mg/ml. The diameter of zone of inhibition exhibits susceptible resistance of 22 mm in comparison with the positive control (8mm). Detailed antimicrobial effects of pomegranate extracts against five selected oral microorganisms are presented in Table 1.

DISCUSSION

Results of the present study showed that Methanolic Rind Extract and Aqueous Rind Extract at the concentration of 100mg/ml were found to be effective against the selected oral microorganisms compared to diluted extracts.

Several investigations have been carried out to elicit the antimicrobial activity of Pomegranate against oral pathogens. A hydroalcoholic extract of Punica granatum fruit (HAEP) was investigated for antibacterial effect on dental plaque microorganisms and was found to be effective against Staphylococcus, Streptococcus, Klebsiella, and Proteus species, as well as E. coli. The ellagitannin, punicalagin, is thought to be the fraction responsible for pomegranate's antibacterial activity [10]. McCarrell and coworkers[17] found that aqueous macerated extract of pomegranate rind inhibits growth of *Staphylococcus aureus* and *Pseudomonas aeurginosa*.

Singh and coworkers[18] reported that extracts of *Punica granatum* peel in different concentrations were effective against *S. epidermidis*, *S. aureus*, *S. mutans*, *S. sanguinis* and *S. salivarius*. It is demonstrated that this antibacterial activity may be related to the presence of hydrolysable tannins and polyphenolics in the pomegranate extract specifically punicalagin and gallagic acid. Reddy et al., [19] and Naz et al., [20] demonstrated

that gallic acid (a tannic acid) has the highest anti-bacterial effect against tested sensitive strain seven at low concentrations. In the present study, the extract of *Punica granatum* rind had an effect on *C. albicans* in all concentrations, while the pith and seed did not have any effect. This finding is in line with Singh's investigation [18] who found that Punica granatum peel does not have any effect on C.albicans.

In vitro study conducted by Abdollahzadeh et al., [21] showed that pomegranate-methanolic extract might be used in control of common oral pathogens responsible for caries stomatitis and periodontal diseases. The antibacterial activity of Punica granatum may be related to polyphenol structures because polyphenols may affect the bacterial cell wall, inhibit enzymes by oxidized agents, interact with proteins and disturb co-aggregation of microorganism.

Table 1: Antimicrobial properties of Methanolic and aqueous extracts of Punica granatum at four different concentrations against oral microorganisms

Pathogens	Extracts	Antimicrobial Activity *				Positive Control**
		Conc of 4mg/ml	Conc of 8mg/ml	Conc of 12mg/ml	Conc of 100mg/ml	Conc of 100mg/ml
Staphylococcus aureus	MSE	8	12	14	10	24
	ASE	8	12	14	10	24
	MPE	8	11	13	8	24
	APE	8	12	14	10	24
	MRE	8	10	12	8	24
	ARE	12	14	16	**22**	24
	CPE	7	8	11	13	24
Staphylococcus epidermis	MSE	6	6	6	10	21
	ASE	6	6	6	10	21
	MPE	6	6	6	9	21
	APE	6	6	6	10	21
	MRE	9	11	14	**20**	21
	ARE	12	15	16	8	21
	CPE	6	6	6	8	21
Klebsiella pneumoniae	MSE	6	6	6	27	25
	ASE	6	6	6	10	21
	MPE	6	6	6	22	25
	APE	6	6	6	27	25
	MRE	6	6	6	6	25
	ARE	6	6	6	8	25
	CPE	6	6	6	**30**	25
Pseudomonas aeurginosa	MSE	6	6	6	6	15
	ASE	6	6	6	6	15
	MPE	6	6	6	6	15
	APE	6	6	6	6	15
	MRE	6	6	10	9	15
	ARE	6	6	**14**	6	15
	CPE	6	6	6	6	15
Candida albicans	MSE	6	6	6	6	8
	ASE	6	6	6	6	8
	MPE	6	6	6	6	8
	APE	6	6	6	6	8
	MRE	6	6	10	**22**	8
	ARE	6	6	14	6	8
	CPE	6	6	6	6	8

*measured by the diameter of zone of inhibition in mm, Conc= Concentration,
**Streptomycin and Amphotericin-B are the positive control group
ANOVA indicated significant difference between all the Pomegranate extracts their Control

Thus pomegranate fruit as a whole has superior bioactivity against oral pathogens compared to its purified polyphenols, which illustrates the chemical synergy of the whole fruit's multiple compounds compared to single, purified, active ingredients.

REFERENCES

1. Yineger H., Yewhalaw D. Traditional medicinal plant knowledge and use by local healers in Sekoru District, Jimma Zone, Southwestern Ethiopia. J Ethnobiol Ethnomed. 2007, Jun 4;3:24.
2. Samy R.P., Pushparaj P.N. and Gopalakrishnakone P.A., Compilation of bioactive compounds from Ayurveda, Bioinformation, 2008, 3, 100–110 2008
3. Ballabh B., and Chaurasia O.P., Traditional medicinal plants of cold desert Ladakh--used in treatment of cold, cough and fever, J. Ethnopharmacol., 2007, 112(2), 341-349
4. Devi A, Singh V, and Bhatt A.B., Comparative antibacterial study of different extract of pomegranate and its wild variety IJPSR, 2011, 2(10): 2647-2650
5. Bhardwaj A. and Bhardwaj, S. V., Ethno-dentistry: popular medicinal plants used for dental diseases in India. Journal of Intercultural Ethnopharmacology, 2012, 1 (1), 62-65.
6. Sheiham A. Dietary effects on dental diseases. Public Health Nutrition, 2001, 4:569--591.
7. Kelly M. Adult dental health survey: oral health in the United Kingdom 1998. London, The Stationery Office, 2000.
8. Arya K.R., and Prakash V. Ethnomedicinal study of a remote tribal area of Almora district: A survey reportPart I. In: Ethnobotany and Medicinal plants of Indian Subcontinent (J. E. Maheshwari Ed.) Scientific Publishers(India), Jodhpur, 2003, 247-252.
9. Acharya D., Sancheti G., Shrivastava A., and Pawar S. Traditional medicines of Gonds and Bharias-26-Herbal medicines for Dental Diseases 2006.
10. Menezes S.M., Cordeiro L.N., Viana G.S., Punica granatum (pomegranate) extract is active against dental plaque. J Herb Pharmacother 2006, 6:79-92
11. Adler L., Modin C., Friskopp J., Jansson L. Relationship between smoking and periodontal probing pocket depth profile. Swed Dent J. 2008, 32(4):157-63.
12. Qnais E.Y., Elokda A.S., Abu Ghalyun Y.Y. and Abdulla F.A., Antidiarrheal activity of the aqueous extract of *Punica granatum* (Pomegranate) peels, *Pharm. Biol*., 2007, 45(9), 715–720
13. Lansky E.P., Newman R.A., Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol. 2007, Jan 19; 109(2):177–206.
14. Kim N.D, Mehta R., Yu W., Neeman I., Livney T., Amichay A., Chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. Breast Cancer Res Treat. 2002 Feb; 71(3):203–17.
15. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med. 2006 Oct;6:35.
16. Johnson T., and Case C., "Chemical Methods of Control," adapted from *Laboratory Experiments in Microbiology, Brief Edition, 4th ed.* Redwood City, CA: Benjamin/Cummings Publishing Co., 1995.
17. McCarrell E.M., Gould S.W.J., Fielder M.D., Kelly A.F., Sankary W.E., Naughton D.P. Antimicrobial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C. BMC Complement Altern Med, 2008, 8:64.
18. Singh R.P., Chidambara Murthy K.N., Jayaprakasha G.K. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. J Agric Food Chem 2002, Jan 2; 50(1):81-6.
19. Reddy M.K., Gupta S.K., Jacob M.R., Khan S.I., Ferreira D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punica granatum L. Planta Med 2007, May;73(5):461-7.
20. Naz S., Siddiqi R., Ahmad S., Rasool S.A., Sayeed S.A. Antibacterial activity directed isolation of compounds from Punica granatum. J Food Sci 2007, Nov; 72(9):M341-5.
21. Abdollahzadeh S.H., Mashouf R.Y., Mogahaddam M.H., Roozbahani N, Vahedi M. Antibacterial and antifungal activities of Punica granatum extract against oral pathogens. J Dent Tehran Unversity Med Sci 2011, 8(1): 1-6.

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