Original Article

Antibacterial and Antifungal Activities of Punica Granatum Peel Extracts Against Oral Pathogens

Sh. Abdollahzadeh [1, 2], RY. Mashouf [3], H. Mortazavi [1, 2], MH. Moghaddam [4], N. Roozbahani [5], M. Vahedi [1, 2]

[1] Assistant Professor, Department of Oral Medicine, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
[2] Assistant Professor, Dental Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[3] Professor, Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[4] Associate Professor, Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5] Dentist, Private Practice

Abstract:

Objective: *Punica granatum* has been used for many years in folk medicine due to several purposes. The aim of the present study was to evaluate the effect of methanolic extract of *Punica granatum* peel (MEPGP) against *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Staphylococcus epidermidis*, *Actynomyces viscosus*, *Lactobacillus acidophilus* and *Candida albicans*.

Materials and Methods: In this *in vitro* study, the mentioned oral organisms were cultured in blood agar and mueller-hinton media and then paper disks containing MEPGP at concentrations of 4 mg/ml, 8 mg/ml and 12 mg/ml were inserted on medias. The antimicrobial activity was evaluated by agar disk diffusion method. The effects of three different concentrations of MEPGP against microorganisms were compared using one-way ANOVA and Tukey tests.

Results: All concentrations of MEPGP had antibacterial activity against *S. aureus* and *S. epidermidis*. Only at concentration of 8 mg/ml and 12 mg/ml MEPGP was effective against *L. acidophilus*, *S. mutans* and *S. salivarius*. Furthermore; no concentrations of MEPGP inhibited *A. viscosus* and *C. albicans*.

Conclusion: This study suggests that MEPGP might be used as an antibacterial agent in controlling oral infections.

Key Words: Punicaceae; Anti-Bacterial Agents; Antifungal Agents; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Lactobacillus acidophilus*; *Streptococcus mutans*; Streptococcus; *Candida albicans*; *Actinomyces viscosus*

Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (2011; Vol. 8, No.1)

Corresponding author:
M. Vahedi, Department of Oral Medicine, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran.
vahedi_md@yahoo.com

Received: 16 July 2010
Accepted: 10 November 2010

INTRODUCTION

In the recent decades, the interest in evaluating therapeutic effects of plants has increased dramatically [1,2] as 80% of the world's people rely on complementary and alternative medicine for their health care needs [3,4]. Phytoplants have been shown to be good alternatives to synthetic chemical antimicrobial agents and antibiotics because of the serious side effects, antimicrobial resistance and the emergence of previously uncommon infections that have been reported to be on the increase due to inappropriate or widespread overuse of antimicrobials [5,6]. On the other hand, clinicians should remind the potential risk of urticaria, alteration in taste, increase of calculus

formation, staining of teeth and mucous membranes and more rarely, oral mucosa desquamation and parotid swelling before prescribing chlorhexidine mouth wash as an antimicrobial agent [7,8]. However, from 250,000-500,000 natures found on earth, only one percent of them have been assessed for their pharmaceutical potential [9]. There are manuscripts proving the effects of Iranian native herbal extracts [10-13]. *Punica granatum* (pomegranate) is native to the region from northern India to Iran. But it is also widely cultivated now in parts of Southwest America, California, Mexico, Arizona and Africa [14,15]. Pharmacological effects of pomegranate represent a long history and have been mentioned in the Greek and Egyptian documents [16,17]. Recently, studies have shown that pomegranate has many potential effects including: bacteriocidal, antifungal, antiviral, immune modulation, vermifuge, stimulant, refrigerant, astringent, stomachic, styptic, laxative, diuretic and anthelmintic. Moreover, it serves to decrease the adverse effects of cardiovascular diseases, diabetes, diarrhea, dysentery, asthma, bronchitis, cough, bleeding disorders, fever, inflammation, acquired immune deficiency syndrome, dyspepsia, ulcers, bruises, sores, mouth lesions, skin lesions, malaria, prostate cancer, atherosclerosis, hypertension, periodontal diseases, hyper lipidemia, denture stomatitis, male infertility, vaginitis, erectile dysfunction, alzheimer, obesity and infant brain ischemia [4,9,14-17]. Furthermore, 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 [14,18]. Review of the literature from 1999 to the present showed that scientific papers relating to the therapeutic effects of pomegranate are increasing compared to only 25 publications from 1950 to 1999 [14,15]. Since pomegranate has varied medical effects, it should be considered by researchers in different parts of medical sciences. Therefore, this study has been based on the antibacterial and antifungal properties of *Punica granatum* on different oral pathogens.

MATERIALS AND METHODS

Preparation of the Plant Extract

Firstly fresh pomegranates (500 gr) were obtained (in order to prepare fresh extraction) from a public market in the city of Hamadan, Iran. The peels of pomegranate were separated and oven dried at 33°C for 7 days. The dried peels were powdered in an electric grinder and stored in plastic bags for the next step. A 100 gm sample of powder was extracted using 200 ml methanol (99.9%) in an electric blender for 30 min. This suspension was filtered three times per day for 30 days. New methanol dissolvent was used each time. Then methanol was removed in a rotary evaporator to produce a dry powder. The final material was dissolved in methanol for obtaining concentrations of 4mg/ml, 8mg/ml and 12 mg/ml of dry plant powder [9,16,19]. Then specimens were sent to the institute of biological science for assessment of their antibacterial and antifungal effects.

Microorganisms

Type strains were obtained from American Type Culture Collection (ATCC) and Persian Type Culture Collection (PTCC) as follows:
Streptococcus mutans (PTCC 1683), *Streptococcus sanguinis* (PTCC 1449), *Streptococcus salivarius* (PTCC 1448), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (PTCC 1114), *Actinomyces viscosus* (PTCC 1202), *Lactobacillus acidophilus*

(PTCC 1643) and *Candida albicans* (PTCC 5027), which were all obtained from the microbiology laboratory of Hamadan University of Medical Sciences, Hamadan, Iran. Each of the bacterial specimens was incubated in liquid culture dilutions (Tryptone Soy Broth, Oxoid, British) and incubated at 37°C for 20 min to reach the logarithmic stage, then measured to a 0.5 Mc Farland dilution (standard concentrations) which delivered a final concentration of approximately 105 CFU per ml. Then the agar plates with methanolic extract of *Punica granatum* peel (MEPGP) were incubated over night at 37°C [20].

Antibacterial Tests

We tested MEPG at different concentrations using a standard diffusion technique [4,9,21-24]. The MEPGP samples were inserted in 6 mm sterile filter disks (Blank paper, Padtan Teb, Iran) and incubated for 20 min at 37°C and collected in sterile containers. The disks were then placed on the surface of blood agar and meuller-hinton agar (Merk, Germany), in which the microorganisms were cultured. Ciprofloxacin and nystatin disks served as the positive control and diluted methanol was used as the negative control. Finally, we measured the diameter of inhibition zones in millimeter after 24 hours. We placed four disks for each concentration in an 8 cm plate and calculated the mean of inhibition zones. One expert microbiologist conducted all the procedures. We analyzed the data using one-way ANOVA and Tukey test. P value less than 0.05 was considered statistically significant.

RESULTS

After evaluating the antibacterial and antifungal effects of three different concentrations of MEPGP, the positive control produced significantly large inhibition zones for all microorganisms and the negative control showed no markable inhibitory effect. It was found that all concentrations of MEPGP (4 mg/ml, 8 mg/ml and 12 mg/ml) inhibited *S. aureus* and *S. epidermidis*. In addition, the concentration of 12 mg/ml was the most effective extract against *S. aureus* compared with the others. Only two MEPGP concentrations of 8 mg/ml, 12 mg/ml were effective against *S. sanguinis*, *L. acidophilus*, *S. mutans* and *S. salivarius* and there was no significant difference between these concentrations (P=1, P=1, P=0.064). No concentrations of MEPGP inhibited *A. viscosus* and *C. albicans*. Detailed antibacterial and antifungal effects of MEPGP against eight certain oral pathogens are demonstrated in Table 1.

DISCUSSION

In the recent years, the use of plants with preventive and therapeutic effects contributes to health care needs [25]. There are three main reasons to be interested in the treating and healing power of plant extract. First, pharma-

Table 1. Antibacterial and antifungal properties of methanolic extract of punica granatum at three different concentrations.

Microbial Strains	Antimicrobial Activity at *							P.Value	F
	Conc. of 4		Conc. of 8		Conc. of 12		Positive		
	Mean	SD	Mean	SD	Mean	SD	Control**		
Staphylococcus aureus	7.5	0.57	11.5	0.56	12.5	0.58	30	0.000	155.66
Staphylococcus epidermis	11.5	0.57	13.5	0.59	13.5	0.58	29	0.000	20.00
Lactobacillus acidophilus	6.5	0.57	10.0	0.00	10.0	0	14	0.000	227.00
Actinomyces viscosus	6.0	0.00	6.5	0.57	6.5	0.57	25	0.168	2.00
Streptococcus mutans	6.0	0.00	9.5	0.57	9.5	0.57	24	0.000	98.00
Streptococcus sanguinis	6.5	0.57	10.0	0.00	11.5	0.58	25	0.000	172.00
Streptococcus salivarius	6.5	0.58	8.5	0.59	9.5	0.60	26	0.000	43.66
Candida albicans	6.0	0.00	6.5	0.57	6.5	0.57	40	0.168	2.00

* measured by the diameter of zone of inhibition in mm, Conc= Concentration, **Ciprofloxacin and nystatin are the positive control group.

cological studies have demonstrated that many of plants are known to possess antimicrobial agents; second, people are becoming aware of the side effects associated with the over prescription of traditional antibiotics; third, time to time resistant microorganisms against antibiotics are increasing [9,22,25]. Among these plants, punica granate has an important role in folk medicine. Pomegranate is known as a rich source of pharmacological properties which have been evaluated due to antiparasitic, antibacterial, antifungal, antiproliferative, apoptotic and anti-cancer effects as well as protection against herpes virus, inhibition of LDL oxidation and decrease in atheromatous plaque formation and reduction of systolic blood pressure [17,18,22]. Results of the present study showed that MEPGP was effective against some common oral pathogens such as *S. epidermidis*, *S. aureus*, *L. acidophilus*, *S. mutans*, *S. sanguinis* and *S. salivarius*, but not effective against *A. viscosus and C. albicans*. Review of literature showed that some researchers such as Naz et al [22], Vasconcelos et al [26] and Singh et al [27] also 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 [17,19]. It means that the antimicrobial effect of tannins is related to its toxicity and molecular structure. Tannins may act on the cell wall and across the cell membrane because they can precipitate proteins [19,26]. They may also suppress many enzymes such as gycosyltransferases [26]. Reddy et al [17] and Naz et al [22] demonstrated that gallic acid (a tannic acid) has the highest antibacterial effect against tested sensitive strains even at low concentrations. Hence, 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 microorganisms [22,26]. In the present study, the extract of *Punica granatum* peel did not have an effect on *C. albicans* in all concentrations. This finding is in agreement with Singh's study [27], but is in disagreement with the report of Vasconcelos et al [26] and Duraipandiyan et al [4]. Vasconcelos et al showed that *Punica granatum* may be used as a topical antifungal drug against *C. albicans* in two reports [19,26]. The real mechanism of the antifungal effect of tannins (the major components of *Punica granatum* extract) against Candida albicans is not clear; however, it may be related to their toxicity, astringent, molecular structure or other ways [19,26]. Furthermore, in the present study *Punica granatum* extract had no effect on the growth of A. viscosus. It may be related to the fact that gram positive bacteria such as Actynomyces, Aspergillus flavus and Aspergillus parasiticus are more sensitive against antibacterial agents compared to gram negative bacteria because of the difference in their cell wall structures [27]. In the present study, we have used the disk diffusion method for the antimicrobial evaluation of MEPGP; however, the MIC method (Minimum Inhibitory Concentration) applied along with disk diffusion may be recommended in future studies.

CONCLUSION

Many herbs have preventive or therapeutic potentials. Therefore, further studies are required to find these effects in order to replace synthetic medications with natural remedies. According to the results of this study, the extract of *Punica granatum* might be used in the control of common oral pathogens responsible for caries, stomatitis and periodontal diseases. On the other hand, further photochemical studies are required to determine the type of compounds responsible for the antibacterial effect of pomegranate.

ACKNOWLEDGMENTS

This work was based on an undergraduate thesis of the Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran. We are also grateful to Mr. Mani Kashani for his valuable help in statistical analysis.

REFERENCES

1-Thuille N, Fille M, Nagl M. Bactericidal activity of herbal extracts. Int J Hyg Environ Health 2003 Jun;206(3):217-21.

2-Alanís AD, Calzada F, Cervantes JA, Torres J, Ceballos GM. Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol 2005 Aug 22;100(1-2):153-7.

3-Magee KA. Herbal therapy: a review of potential health risks and medicinal interactions. Orthod Craniofac Res 2005 May;8(2):60-74.

4-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.

5-Pawar PL, Nabar BM. Effect of Plant Extracts Formulated in Different Ointment Bases on MDR Strains. Indian J Pharm Sci 2010 May;72(3):397-401.

6-Olila D, Olwa-Odyek, Opuda-Asibo J. Antibacterial and antifungal activities of extracts of Zanthoxylum chalybeum and Warburgia ugandensis, Ugandan medicinal plants. Afr Health Sci 2001 Dec;1(2):66-72.

7-Zanatta FB, Antoniazzi RP, Rösing CK. Staining and calculus formation after 0.12% chlorhexidine rinses in plaque-free and plaque covered surfaces: a randomized trial. J Appl Oral Sci 2010 Sep-Oct;18(5):515-21.

8-Amanlou M, Beitollahi JM, Abdollahzadeh S, Tohidast-Ekrad Z. Miconazole gel compared with Zataria multiflora Boiss. gel in the treatment of denture stomatitis. Phytother Res 2006 Nov; 20(11):966-9.

9-Meléndez PA, Capriles VA. Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine 2006 Mar;13(4):272-6.

10-Amanlou M, Beitollahi JM, Abdollahzadeh S, Tohidast-Ekrad Z. Miconazole gel compared with Zataria multiflora Boiss. gel in the treatment of denture stomatitis. Phytother Res 2006 Nov; 20(11):966-9.

11-Mahmoudabadi AZ, Dabbagh MA, Fouladi Z. In Vitro Anti-Candida Activity of Zataria multiflora Boiss. Evid Based Complement Alternat Med 2007 Sep;4(3):351-353.

12-Bonjar S. Evaluation of antibacterial properties of some medicinal plants used in Iran. J Ethnopharmacol 2004 Oct;94(2-3):301-5.

13-Rahimi R, Shams-Ardekani MR, Abdollahi M. A review of the efficacy of traditional Iranian medicine for inflammatory bowel disease. World J Gastroenterol 2010 Sep 28;16(36):4504-14.

14-Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 2007 Jan 19;109(2):177-206.

15-Jurenka JS. Therapeutic applications of pomegranate (Punica granatum L.): a review. Altern Med Rev 2008 Jun;13(2):128-44.

16-Braga LC, Shupp JW, Cummings C, Jett M, Takahashi JA, Carmo LS, et al. Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production. J Ethnopharmacol 2005 Jan;96(1-2):335-9.

17-Reddy MK, Gupta SK, Jacob MR, Khan SI, 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.

18-Kim ND, Mehta R, Yu W, Neeman I, Livney T, Amichay A, et al. Chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. Breast Cancer Res Treat 2002 Feb;71(3):203-17.

19-Vasconcelos LC, Sampaio FC, Sampaio MC, Pereira Mdo S, Higino JS, Peixoto MH. Minimum inhibitory concentration of adherence of Punica granatum Linn (pomegranate) gel against S. mutans, S. mitis and C. albicans. Braz Dent J 2006;17(3):223-7.

20-Koru O, Toksoy F, Acikel CH, Tunca YM,

Baysallar M, Uskudar Guclu A, et al. In vitro antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. Anaerobe 2007 Jun-Aug;13(3-4):140-5.
21-Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices: results of an in vitro diffusion method study. J Am Dent Assoc 2004 Aug;135(8):1133-41.
22-Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA. Antibacterial activity directed isolation of compounds from Punica granatum. J Food Sci 2007 Nov;72(9):M341-5.
23-Gohari AR, Saeidnia S, Mollazadeh Moghaddam K, Yassa N, Malmir M, Shahverdi AR. Isolation of a new quinic acid derivative and its antibacterial modulating activity. DARU 2010;18(1):69-73.
24-Shantiaee Y, Dianat O, Janani A, Kolahi Ahari G. In vitro evaluation of the antibacterial activity of three root canal sealers. IEJ 2010;5(1):1-5.
25-Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 2002 Oct;97(7):1027-31.
26-Vasconcelos LC, Sampaio MC, Sampaio FC, Higino JS. Use of Punica granatum as an antifungal agent against candidosis associated with denture stomatitis. Mycoses 2003 Jun;46(5-6):192-6.
27-Singh RP, Chidambara Murthy KN, Jayaprakasha GK. 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.