ORIGINAL ARTICLE

Journal of Research in Dental and Maxillofacial Sciences
DOI: 10.52547/jrdms.8.1.18

In vitro Antimicrobial Effect of *Punica granatum* Extract versus Chlorhexidine on *Streptococcus sobrinus*, Streptococcus sanguinis, and *Candida albicans*

Elnaz Tavazo Zadeh [1] , Parissa Aref [2] , Nahid Askarizadeh [2] , Fatemeh Emadi [3]

[1] Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[2] Department of Pediatric Dentistry, Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[3] Traditional Persian Medicine, Traditional Medicine Clinical Trial Research Center, Faculty of Medicine, Shahed University, Tehran, Iran

Corresponding author:
Parissa Aref, Department of Pediatric Dentistry, Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

parissa234@yahoo.com

Article History
Received: May 17, 2022
Accepted: July 12, 2022

Abstract

Background and Aim: Considering the search for an effective antimicrobial agent comparable to chlorhexidine (CHX), this study aimed to assess the antimicrobial effect of *Punica granatum (P. granatum)* hydroalcoholic extract on *Streptococcus sobrinus (S. sobrinus), Streptococcus sanguinis (S. sanguinis)* and *Candida albicans (C. albicans)*, in comparison with CHX.

Materials and Methods: In this in vitro study, the disc diffusion test was used to assess the antimicrobial activity of the extract by measuring the growth inhibition zones; while, the microdilution and macrodilution broth tests were applied to find the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against the tested microorganisms. The MBC was measured using the blood agar or Mueller Hinton agar culture medium. The Sabouraud dextrose agar culture medium was used for *C. albicans*. Each test was repeated in triplicate, and data were analyzed by independent samples t-test and Mann-Whitney U test.

Results: None of the tested microorganisms showed any resistance to the extract. CHX had the highest antimicrobial effect against all tested microorganisms. The MIC of the hydroalcoholic extract of *P. granatum* was 2.5 mg/mL for *S. sobrinus* and *S. sanguinis*, and 5 mg/mL for *C. albicans*. Its MBC was 5 mg/mL for *S. sobrinus* and *S. sanguinis*, and 10 mg/mL for *C. albicans*. The mean diameter of the growth inhibition zone for *S. sobrinus* caused by CHX was significantly greater than that caused by *P. granatum* extract (Mann-Whitney U test, P=0.043). The same result was obtained for *S. sanguinis* (Student sample t-test, P=0.002), and *C. albicans* (Mann-Whitney U test, P=0.046).

Conclusion: The hydroalcoholic extract of *P. granatum* has bacteriostatic and bactericidal effects on *S. sanguinis* and *S. sobrinus* and antifungal effect on *C. albicans* comparable to CHX.

Key Words: Pomegranate; Microbial Sensitivity Tests; Antibacterial Agents; Disk Diffusion Antimicrobial Tests; Biological Products

Cite this article as: Tavazo Zadeh E, Aref P, Askarizadeh N, Emadi F. In vitro Antimicrobial Effect of *Punica granatum* Extract versus Chlorhexidine on *Streptococcus sobrinus, Streptococcus sanguinis*, and *Candida albicans*.
J Res Dent Maxillofac Sci. 2023; 8(1):18-27.

Introduction

Dental clinicians are increasingly interested in using natural organic products for inhibition of dental caries. Effective regular mechanical

plaque removal is the basis for almost all preventive and therapeutic approaches for dental caries and chronic periodontal disease. At present, toothbrushing with toothpaste is still the most effective and most practical method to control supragingival plaque in most patients [1,2]. Nonetheless, plaque removal may be ineffective, difficult, or even impossible in children and adults with sensory and/or motor disabilities [3]. If plaque accumulation cannot be prevented, it often leads to the development of dental caries and subsequent destruction of periodontal tissue, bone loss, and eventual tooth loss [4]. Therefore, it is logical to find safe and effective alternatives or adjuncts for plaque control [5,6].

Mouthwashes are among the most effective antimicrobial agents used as an adjunct for plaque control [7]. Chlorhexidine (CHX) has long been the gold-standard antimicrobial mouthwash [8]. Its role in reducing microbial plaque and oral microorganism count and improvement of gingival health status has been previously documented [9-11]. However, it has side effects such as staining, alteration of taste, oral burning sensation, and interference with normal microbial oral flora [12]. Thus, attempts are ongoing to find equally effective alternatives with fewer side effects. Evidence shows that pomegranate fruit or *Punica granatum (P. granatum)* has antimicrobial activity attributed to its peel, internal white membranes and seeds, and may be able to serve as a natural antimicrobial agent.

Streptococcus sobrinus (*S. sobrinus*) is a Gram-positive microorganism present in dental plaque, which can cause dental caries and abscess in children [13]. It is the most important cariogenic microorganism responsible for caries development on smooth surfaces [14]. *Streptococcus sanguinis* (*S. sanguinis*) is a Gram-positive microorganism that belongs to the family of viridans streptococci and is among the most critical constituents of primary dental plaque [15]. *Candida albicans* (*C. albicans*) is another culprit found in the oral cavity and gastrointestinal system of 40-60% of adults. It can serve as a pathogenic microorganism in immunocompromised patients and can cause oral candidiasis. It is also involved in development of dental caries [16].

Studies regarding the antimicrobial activity of the hydroalcoholic extract of the whole pomegranate fruit against *S. sobrinus, S. sanguinis,* and *C. albicans* are limited [17-19]. Thus, this study aimed to assess the effect of hydroalcoholic extract of *P. granatum* on *S. sobrinus, S. sanguinis,* and *C. albicans*.

Materials and Methods

In this in vitro experimental study, standard-strain *S. sobrinus* (PTCC 1601), *S. sanguinis* (PTCC 1449), and *C. albicans* (ATCC 10231) were obtained from the Iranian Collection of Industrial Microorganisms. The Ethics Committee of the Faculty of Dentistry at Islamic Azad University provided ethical approval for this study (IR.IAU.DENTAL.REC.1399.126). The sample size was calculated to be 18 according to a previous study [20-22], assuming 99% confidence interval, 99% study power, beta=0.00, and alpha=0.01 by PASS 11 soft-ware; 96-well plates with three repetitions for each concentration of extract were used.

Preparation of hydroalcoholic extract of P. granatum:

Three medium-size pomegranates were purchased, and the entire fruit, including its peel, white membranes, and seeds, were diced into small pieces and crushed in a blender. The water-ethanol solvent in 50:50 ratio was added to the mixture for maceration, and it was filtered with Whatman No.1 filter paper after 24 hours of extraction. The hydroalcoholic extract was then heated at 60°C to concentrate and acquire a honey-like consistency. The concentrated extract was then used to prepare different concentrations [23].

Preparation of microorganisms:

Blood agar, Mueller Hinton agar, and brain heart infusion (BHI) broth were purchased and autoclave-sterilized according to the manufacturers' instructions. The viable bacteria were then cultured on blood agar nutrient under anaerobic conditions in a Gas-Pak jar at 37°C for 24 hours to proliferate and reach maximum confluence. Subculture of all microorganisms was performed three times a week to use fresh microorganisms for each test.

Disc diffusion test:

The disc diffusion test was first performed according to the Clinical & Laboratory Standards Institute recommendations to assess the extract's antibacterial potential [24]. After 24 hours of incubation of microorganisms on blood agar, a certain amount of each microorganism was transferred from the nutrient culture medium to saline to prepare a microbial suspension with 0.5 McFarland standard concentration containing 1.5 x 10^8 colony forming units (CFUs)/mL. Using a sterile swab, the microbial suspensions were then streak-cultured on Mueller Hinton agar. A disc containing 0.12% CHX (as the positive control group), a disc containing *P. granatum* extract (test group), and a blank disc (as negative control) were all placed on the culture medium. The Petri dish was capped and incubated at 37°C for 24 hours. After 24 hours the diameter of the growth inhibition zones was measured by a millimeter ruler. Values < 1 mm were considered zero. To ensure accuracy of the results, each test was repeated under aseptic conditions three times. Each material's antibacterial activity was determined by measuring the diameter of growth inhibition zone around each disc and reported in millimeters (mm) [25].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract:

The microdilution and macrodilution broth tests, recommended by the Clinical & Laboratory Standards Institute, were performed to determine the MIC and MBC of the extract using BHI broth. For this purpose, a certain amount of bacteria was transferred from the Mueller Hinton agar to saline to obtain a microbial suspension with 0.5 McFarland standard concentration containing 1.5 x 10^8 CFUs/mL. To determine the MIC by the microdilution broth test, a 96-well plate was used; 100 µL of the BHI broth was added to all wells by a multi-channel sampler. Next, 100 µL of the pure extract was added to the first well, and after optimal mixing, 100 µL of the contents of this well was transferred to the second well, and so on, until the 10^{th} well. Finally, 100 µL of the contents of the 10^{th} well was discarded. Next, 100 µL of saline containing *S. sobrinus, S. sanguinis*, or *C. albicans* (0.5 McFarland standard concentration) was added to the wells. The same was performed for 0.12% CHX (positive control).

To determine the MIC by the macrodilution test, the abovementioned steps were repeated but with a larger volume (> 1 cc) in mini-tubes along with the positive and negative control groups. The positive control tube contained BHI broth and the microbial suspension (which had to become turbid); while, the negative control tube only contained BHI broth (which had to remain clear). Next, both microplates and macroplates were placed in an incubator and incubated at 37°C for 24 hours under anaerobic conditions. The turbidity of the tubes was visually evaluated to determine the MIC in milligrams per milliliter (mg/mL). In each series of tests, some tubes remained clear after 24 hours of incubation, indicating no bacterial growth in presence of the extract's respective concentration. The first well/tube that remained clear indicated the MIC of the extract. To determine the MBC, samples of three macro-tubes, irrespective of their turbidity or clarity, were streak-cultured on blood agar or Mueller Hinton agar culture medium

with 5% sheep blood (for *streptococci*) or Sabouraud dextrose agar (for *C. albicans*). After 24 hours of incubation at 37°C under anaerobic conditions, the first Petri dish with no bacterial or fungal growth indicated the MBC and minimum fungicidal concentration (MFC) of the extract [26-29].

The Shapiro-Wilk test was applied to assess the normal distribution of data. The independent samples t-test and ANOVA were used to analyze the normally distributed data; while, the Mann-Whitney U and Kruskal-Wallis tests were applied for non-normally distributed data. All statistical analyses were performed using SPSS version 25 at 0.05 level of significance.

Results

Table 1 presents the MIC and MBC of *P. granatum* extract and CHX against the microorganisms. Table 2 presents the mean diameter of the growth inhibition zones. As shown, CHX created the maximum growth inhibition zone for all microorganisms. After CHX, *P. granatum* extract created the maximum growth inhibition zone in *S. sanguinis* culture.

Table 3 and Figure 1compare the diameter of the growth inhibition zones of all three microorganisms around the *P. granatum* extract and CHX discs. As shown, the mean diameter of the growth inhibition zone of *S. sobrinus* caused by CHX was significantly greater than that caused by *P. granatum* extract (non-normal distribution, Mann-Whitney U test, P=0.043). The same result was obtained for *S. sanguinis* (normal distribution of data, Student sample t-test, P=0.002) and *C. albicans* (non-normal distribution, Mann-Whitney U test, P=0.046).

Table 1. MIC and MBC of P. granatum extract and CHX against the microorganisms

Microorganism	Variable	*S. sobrinus*	*S. sanguinis*	*C. albicans*
Extract	**MIC**	2.5 mg/mL	2.5 mg/mL	5 mg/mL
	MBC	5 mg/mL	5 mg/mL	10 mg/mL
Chlorhexidine	**MIC**	1.64 (chx0.12%)	1.64 (chx0.12%)	1.32 (chx0.12%)
	MBC	1.32 (chx0.12%)	1.32 (chx0.12%)	1.16 (chx0.12%)

Table 2. Mean diameter of growth inhibition zone

Material	***S. sobrinus* growth inhibition zone (mm)**			***S. sanguinis* growth inhibition zone (mm)**			***C. albicans* growth inhibition zone (mm)**		
Extract	15	15.5	15	16.5	15	16	11	11	11.5
CHX	22	23	23	20	19	19.5	16	15.5	15

Table 3. Comparison of the diameter of growth inhibition zones of all three microorganisms around the P. granatum extract and CHX discs

Microorganism	Group	Minimum	Maximum	Mean	Std. deviation	Median	IQR	P-value
S. sobrinus	CHX	22.00	23.00	22.67	0.58	23.00	1.00	Mann-Whitney U=-2.02 P-Value=0.043*
	Extract	15.00	15.50	15.17	0.29	15.00	0.00	
S. sanguinis	CHX	19.00	20.00	19.50	0.50	19.50	1.00	t=6.96 P-Value=0.002**
	Extract	15.00	16.50	15.83	0.76	16.00	1.00	
C. albicans	CHX	15.00	16.00	15.50	0.50	15.50	1.00	Mann-Whitney U=-1.99 P-Value=0.046*
	Extract	11.00	11.50	11.17	0.29	11.00	0.00	

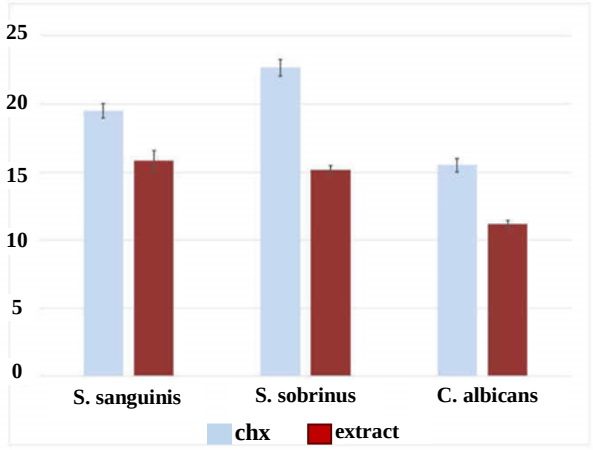

Figure 1. Comparison of the diameter of growth inhibition zones of all three microorganisms around the P. granatum extract and CHX discs

Discussion

This study assessed the effect of 50%-50% hydroalcoholic extract of *P. granatum* on *S. sobrinus, S. sanguinis,* and *C. albicans* compared with 0.12% CHX. The MIC and MBC of the extract against all three microorganisms were also determined. The results revealed that none of the tested microorganisms showed any resistance to the extract. CHX had a significantly higher antimicrobial effect than the extract against all tested microorganisms. The MIC of the hydroalcoholic extract of *P. granatum* was 2.5 mg/mL for *S. sobrinus* and *S. sanguinis*, and 5 mg/mL for *C. albicans*. Its MBC was 5 mg/mL for *S. sobrinus* and *S. sanguinis*, and its MFC was 10 mg/mL for *C. albicans*.

Pomegranate is a natural source of phenolic compounds and antioxidants such as tannin, ellagitannin, punicic acid, polyphenol, flavonoids, and vitamin C. It also contains tocopherol and anthocyanins [30]. Tannin-rich ellagitannins and phenolic acids have antibacterial properties, probably due to their secondary metabolites [31]. Phenolic compounds, particularly gallic acid, have significant antibacterial activity [32]. Tannins and polyphenolic compounds are the main phytochemical agents in pomegranate [33]. Tannins can pass through the cell wall of microorganisms and degrade the membrane proteins, destroying the microorganisms. They also inhibit enzymes such as glycosyltransferase that play a key role in the adhesion of bacteria to tooth surfaces and prevent bacterial adhesion [34]. Tannins have different mechanisms of action against Candida species. They exert their antifungal effect by inhibition of extracellular microbial

enzymes and depletion of nutrients and iron. Also, they directly affect microbial metabolism by inhibiting oxidative phosphorylation [35]. Evidence shows that *P. granatum* extract boosts the activity of several antibiotics such as chloramphenicol, gentamycin, ampicillin, tetracycline, and oxacillin against 30 *S. aureus* strains [36]. The pomegranate peel extract has maximum antimicrobial activity compared with other parts of this fruit due to the presence of polyphenolic compounds [34,37]. The antibacterial activity of the white membranes and internal parts of the fruit is due to the presence of phytocompounds such as hydrolyzing tannins, polyphenols, and flavonoids, including punicalagin, gallic acid, and catechins [38,39]. The phenolic compounds in pomegranate peel extract can react with microorganisms' cellular proteins, change their cell wall structure, and denature some microbial enzymes.

Also, phenolic compounds form complexes with carbohydrates, proteins, vitamins, and minerals and cause their depletion. By doing so, they adversely affect the metalloenzymes, degrade the cell wall, and prevent coaggregation of microorganisms [40]. Polyphenols also significantly decrease the bacterial load by reducing the environmental pH [41]. Evidence shows that punicalagin is the most effective antibacterial compound in a pomegranate; however, the pomegranate fruit's synergistic effects are mainly responsible for its cariostatic properties [42].

Evidence shows that ethanolic extract (65-85%) maintains the tannins, polyphenols, and procyanidines and has inhibitory effects on *S. aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* (*P. aeruginosa*), and *C. albicans* [43]. Also, it has been reported that the whole fruit extract of pomegranate has higher antioxidant activity than the extract of each component alone [44]. Thus, the whole fruit extract was evaluated in this study. Gosset-Erard et al. [39] identified punicalagin as the main antibacterial agent present in pomegranate peel. Among the evaluated ex-tracts in their study, ethanolic extract had the greatest antimicrobial activity with a growth inhibition diameter of 20 mm, which was close to the present findings. This effect was attributed to active antimicrobial agents in pomegranate peel. The MIC of a and b forms of punicalagin was 0.3 and 11.2 µg/mL, respectively. Veloso et al. [45] evaluated the antibacterial activity of medicinal herbs including pomegranate against oral bacteria. The diameter of growth inhibition zones ranged from 1.6 to 10.3 mm. Pomegranate ethanolic (80%) extract was the only extract that inhibited all the tested microorganisms and had high level of polyphenols. It had a MIC of 50 to 400 µg/mL. Their results were in agreement with the present findings. Ferrazzano et al. [46] confirmed the optimal antibacterial activity of hydroalcoholic extract of pomegranate peel and pomegranate juice against cariogenic bacteria. They reported that pomegranate peel extract with MIC and MBC of 10 and 15 µg/µL, respectively, inhibited the proliferation of cariogenic bacteria; while pomegranate juice had inhibitory effects on *Streptococcus mutans (S. mutans)* with MIC and MBC of 25 and 40 µg/µL, respectively. The concentration of polyphenols in the pomegranate peel is higher than that in the pomegranate juice. Thus, the peel extract has higher antimicrobial activity than the pomegranate juice. The MIC and MBC values reported in their study were higher than those in our study, which may be due to the fact that we used the whole fruit extract, which is more potent. Although they did not measure the

growth inhibition zones, the optimal efficacy of the hydroalcoholic extract of *P. granatum* in their study was in line with our findings. Nozohour et al. [47] evaluated the antibacterial activity of alcoholic extract of pomegranate seed and peel against *S. aureus* and *P. aeruginosa* and reported that ethanolic extract of pomegranate had a greater inhibitory effect than tetracycline and chloramphenicol against the above mentioned bacteria. The MIC and MBC of the pomegranate seed extract (25 and 50 mg/mL, respectively) were significantly higher than those of the pomegranate peel extract (12.5 and 25 mg/mL, respectively). However, this difference was not significant for *P. aeruginosa*. The diameter of the growth inhibition zone caused by the pomegranate peel extract ranged from 22.5 to 27.5 mm (9 mg/disc) while this range was 18.7 to 22 mm (9 mg/disc) for the pomegranate seed extract; these values were close to the values obtained in the present study (11.17 to 15.83 mm). Such results indicate higher antibacterial activity of pomegranate peel extract than seed extract. Their results were in agreement with our findings. Benslimane et al. [31] evaluated the antioxidant and anti-biofilm activity of pomegranate peel extract against the bacteria isolated from carious lesions and subgingival plaque. They prepared the pomegranate peel extract by using 70% acetone, 70% ethanol, and 70% methanol. All tested bacteria were sensitive to the extracts with a MIC of 0.0125 to 100 mg/mL. Gram-positive bacteria were more sensitive. The ethanolic extract showed higher antimicrobial activity. Their results were in line with ours. Millo et al. [48] evaluated the antibacterial effect of a formulated gel of pomegranate fruit against *S. mutans, S. sanguinis*, and *Lactobacillus casei*. They performed high-performance liquid chromatography to identify and quantify punicalagin and determine its MBC. They showed that the formulated pomegranate gel had an optimal inhibitory effect on *S. mutans*, although its effect was smaller than that of CHX. They also reported that the amount of punicalagin was 2033.58±25.29 pg/mL in aqueous extract of P. granatum and 0.234 pg/mL (w/w) in the formulated gel. The MBC of the extract was 500, 250, and 125 mg/mL for *Lactobacillus casei, S. mutans*, and *S. sanguinis*, respectively. The antibacterial activity of the formulated gel in their study was lower than the hydroalcoholic extract activity in our study, which can be due to the lower amount of punicalagin in the formulated gel. Aravindraj et al. [49] evaluated the antimicrobial effect of ethanolic, methanolic, acetone, ethyl acetate, chloroform, hexane, and dimethyl sulfide extracts of pomegranate against *S. aureus, S. mutans, Lactobacillus acidophilus, Enterococcus faecalis*, and *C. albicans.* They reported a significant difference between different extracts. They reported a 9-12 mm diameter of growth inhibition zone for *C. albicans*, close to the value in the present study (11.17 mm). Anibal et al. [17] evaluated the antifungal activity of 70% ethanolic extract of aril, seed, pericarp, peel, and the whole fruit of pomegranate and the resultant morphological changes in the structure of *C. albicans*. The pure ethanolic extract was analyzed by mass spectrometry, and a number of ingredients such as punicalagin were isolated. The pericarp and peel extracts with 125 µg/mL MIC had inhibitory effects on C. albicans. The effect of pericarp and seed extract on the morphology and structure of *C. albicans* was evaluated by transmission electron microscopy. The results confirmed the antimicrobial potential of ethanolic extract of *P. granatum* against Candida genus and the ability of its bioactive compound to change this microorganism's structure and morphology. The obtained MIC value was lower than the

value in our study, which can be due to the use of pure ethanolic extract in their study. However, their results were generally in agreement with our findings.

This study did not separately assess the antimicrobial activity of the constituents of the extract. Thus, future studies are required to assess the antimicrobial activity of the isolated fractions of pomegranate extract. Also, *P. granatum* extract's antibacterial activity should be investigated against a broader spectrum of pathogenic microorganisms, particularly Gram-negative bacteria. Moreover, the cytotoxicity of *P. granatum* extract against human oral fibroblasts should be evaluated in vitro. Given that its safety is confirmed, clinical trials can be carried out to assess its use in the clinical setting and its mass production in the form of mouthwash, chewing gums, or lollypops.

Conclusion

The hydroalcoholic extract of *P. granatum* has bacteriostatic and bactericidal effects on *S. sanguinis and S. sobrinus and antifungal* effect on *C. albicans*.

References

1.Löe H, Theilade E, Jensen SB, Schiott CR. Experimental gingivitis in man. J Periodontol (1930). 1965 May-Jun; 36: 177-87.

2.Suhasini.SJ, Valiathan M. Brushing Techniques. Eur J Mol Clin Med. 2020;7(2):6601-11.

3.Waldron C, Nunn J, Mac Giolla Phadraig C, Comiskey C, Guerin S, van Harten MT, et al. Oral hygiene interventions for people with intellectual disabilities. Cochrane Database Syst Rev. 2019 May 31;5(5): CD012628.

4.Newman MG, Takei HH, Klokkevold PR, Carranza FA. Newman and Carranza's Clinical Periodontology. 13th ed. Philadelphia: Elsevier Saunders; 2019. 944 p.

5.Addy M. Chlorhexidine compared with other locally delivered antimicrobials. A short review. J Clin Periodontol. 1986 Nov;13(10):957-64.

6.Akula S, Nagarathna J, Srinath K. Anti-Plaque and Anti-Gingivitis Efficacy of 0.25% Lemongrass Oil and 0.2% Chlorhexidine Mouthwash in Children. Front Dent. 2021 Sep 6;18:32.

7.Vyas T, Bhatt G, Gaur A, Sharma C, Sharma A, Nagi R. Chemical plaque control - A brief review. J Family Med Prim Care. 2021 Apr;10(4):1562-8.

8.Flötra L, Gjermo P, Rölla G, Waerhaug J. Side effects of chlorhexidine mouth washes. Scand J Dent Res. 1971; 79(2): 119-25.

9.Yarahmadi N, Hashemian F, Hosseini Doust R. Clinical Effects of Chlorhexidine 0.2% and Cetylpyridinium 0.05% Combination in Comparison with Chlorhexidine, Cetylpyridinium and Persica in Reducing Oral Bacteria in Healthy Individuals. J Pharm Care. 2020;8(3):116-22.

10.Pandiyan I, Rathinavelu PK, Arumugham MI, D S, Balasubramaniam A. Efficacy of Chitosan and Chlorhexidine Mouthwash on Dental Plaque and Gingival Inflammation: A Systematic Review. Cureus. 2022 Mar 19;14(3):e23318.

11.Sreenivasan PK, Haraszthy VI. Chlorhexidine Improves Hygiene Reducing Oral Polymorphonuclear Leukocytes with Antimicrobial Effects at Distinct Microenvironments amongst Subjects Stratified by Health Status. Antibiotics (Basel). 2022 Apr 29;11(5):603.

12.Gazi MI, Lambourne A, Chagla AH. The antiplaque effect of toothpaste containing salvadora persica compared with chlorhexidine gluconate. Clin Prev Dent. 1987 Nov-Dec; 9 (6):3-8.

13.Zhao Y, Zhong WJ, Xun Z, Zhang Q, Song YQ, Liu YS, Chen F. Differences in carbon source usage by dental plaque in children with and without early childhood caries. Int J Oral Sci. 2017 Dec;9(12):e6.

14.Mungi S, Perona G. Genotypic concordance of streptocccus sobrinus ATCC® 33402TM in mother and child pairs detecting by molecular AP-PCR assays. J Dent Health Oral Disord Ther. 2019;10(6):310-5.

15.Rodriguez AM, Callahan JE, Fawcett P, Ge X, Xu P, Kitten T. Physiological and molecular characterization of genetic competence in Streptococcus sanguinis. Mol Oral Microbiol. 2011 Apr;26(2):99-116.

16. Blocker T. Analysis of the CYC1 Promoter in Candida Albicans [Thesis]. Langston: McCabe Thesis Collection; 2008. 35 p.

17.Anibal PC, Peixoto IT, Foglio MA, Höfling JF. Antifungal activity of the ethanolic extracts of Punica granatum L. and evaluation of the morphological and structural modifications

of its compounds upon the cells of Candida spp. Braz J Microbiol. 2013 Dec 17;44(3):839-48.

18.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.

19.Lavaee F, Motaghi D, Jassbi AR, Jafarian H, Ghasemi F, Badiee P. Antifungal effect of the bark and root extracts of Punica granatum on oral Candida isolates. Curr Med Mycol. 2018 Dec;4(4):20-4.

20.Jain E, Pandey RK, Khanna R. Liquorice root extracts as potent cariostatic agents in pediatric practice. J Indian Soc Pedod Prev Dent. 2013 Jul-Sep;31(3):146-52.

21.Sedighinia F, Safipour Afshar A, Soleimanpour S, Zarif R, Asili J, Ghazvini K. Antibacterial activity of Glycyrrhiza glabra against oral pathogens: an in vitro study. Avicenna J Phytomed. 2012 Summer;2(3):118-24.

22.Ajagannanavar SL, Battur H, Shamarao S, Sivakumar V, Patil PU, Shanavas P. Effect of aqueous and alcoholic licorice (glycyrrhiza glabra) root extract against streptococcus mutans and lactobacillus acidophilus in comparison to chlorhexidine: an in vitro study. J Int Oral Health. 2014 Jul;6(4):29-34.

23.Caiyun W, Ling-ling SH, Litong F, Youfang D, Shan Z, Yujun L, et al. Optimization of extraction and enrichment of phenolics from pomegranate (Punica granatum L.) leaves. Ind Crops Prod. 2013; 42:587-94.

24.Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, et al. CLSI Methods Development and Standardization Working Group of the Subcommittee on Antimicrobial Susceptibility Testing. CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests. J Clin Microbiol. 2018 Mar 26;56(4):e01934-17.

25.Rahimi VB, Askari VR, Shirazinia R, Soheili-Far S, Askari N, Rahmanian-Devin P, Sanei-Far Z, Mousavi SH, Ghodsi R. Protective effects of hydro-ethanolic extract of Terminalia chebula on primary microglia cells and their polarization (M1/M2 balance). Mult Scler Relat Disord. 2018 Oct;25:5-13.

26.Wikler MA, Cockerill FR, Bush K, Dudley MN, Eliopoulos GM, Hardy DJ, et al. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard M7-A8. 2009.

27.Alexander BD, Procop GW, Dufresne P, Fuller J, Ghannoum MA, Hanson KE, et al. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts-4th ed. Clin Lab Stand Inst. 2017;37(13):M27-A3.

28.Miller RA, Gaunt PS, Burbick CR, Avendaño-Herrera R, Buller N, Chuanchuen R, et al. Methods for Antimicrobial Broth Dilution and Disk Diffusion Susceptibility Testing of Bacteria Isolated From Aquatic Animals. 2nd ed. Clin Lab Stand Inst. 2020;40(3):VET03-A.

29.Petrus EM, Tinakumari S, Chai LC, Ubong A, Tunung R, Elexson N, et al. A study on the minimum inhibitory concentration and minimum bactericidal concentration of Nano Colloidal Silver on food-borne pathogens. Int Food Res J. 2011;18(1):55-66.

30.Shaygannia E, Bahmani M, Zamanzad B, Rafieian-Kopaei M. A Review Study on Punica granatum L. J Evid Based Complementary Altern Med. 2016 Jul;21(3):221-7.

31.Benslimane S, Rebai O, Djibaoui R, Arabi A. Pomegranate Peel Extract Activities as Antioxidant and Antibiofilm against Bacteria Isolated from Caries and Supragingival Plaque. Jordan J Biol Sci. 2020;13(3):403-12.

32.Duman AD, Ozgen M, Dayisoylu KS, Erbil N, Durgac C. Antimicrobial activity of six pomegranate (Punica granatum L.) varieties and their relation to some of their pomological and phytonutrient characteristics. Molecules. 2009 May 13; 14(5):1808-17.

33.Yu M, Gouvinhas I, Barros A. Variation of the Polyphenolic Composition and Antioxidant Capacity of Freshly Prepared Pomegranate Leaf Infusions over One-Day Storage. Antioxidants (Basel). 2021 Jul 26;10(8):1187.

34.Dahham SS, Ali MN, Tabassum H, Khan M. Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.). Am-Euras. J. Agric. & Environ. Sci. 2010;9(3): 273-81.

35.Endo EH, Cortez DA, Ueda-Nakamura T, Nakamura CV, Dias Filho BP. Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against Candida albicans. Res Microbiol. 2010 Sep;161(7):534-40.

36.Braga LC, Shupp JW, Cummings C, Jett M, Takahashi JA, Carmo LS, Chartone-Souza E, Nascimento AM. Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production. J Ethnopharmacol. 2005 Jan 4;96(1-2):335-9.

37.Peršurić Ž, Saftić Martinović L, Malenica M, Gobin I, Pedisić S, Dragović-Uzelac V, Kraljević Pavelić S. Assessment of the Biological Activity and Phenolic Composition of Ethanol Extracts of Pomegranate (Punica granatum L.) Peels.

Molecules. 2020 Dec 14;25(24):5916.

38.Rosas-Burgos EC, Burgos-Hernández A, Noguera-Artiaga L, Kačániová M, Hernández-García F, Cárdenas-López JL, Carbonell-Barrachina ÁA. Antimicrobial activity of pomegranate peel extracts as affected by cultivar. J Sci Food Agric. 2017 Feb;97(3):802-10.

39.Gosset-Erard C, Zhao M, Lordel-Madeleine S, Ennahar S. Identification of punicalagin as the bioactive compound behind the antimicrobial activity of pomegranate (Punica granatum L.) peels. Food Chem. 2021 Aug 1;352:129396.

40.Estabraghi E, Sadeghpour M, Mehrabani A. Study of pomegranate hydromethanol extract on Staphylococcus aureus and Escherichia coli by microplate in laboratory conditions. J Payavard Salamat. 2018;12(3):183-92.

41.Ismail T, Sestili P, Akhtar S. Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. J Ethnopharmacol. 2012 Sep 28;143 (2):397-405.

42.Lalwani V, Koneru A, Vanishree M, Vardendra M, Hunasgi S, Surekha R. Anti-microbial activity of Punica granatum on Streptococcus in dental caries patients and healthy individuals: A comparative study. J Adv Clin Res Insights. 2014;1(3):94-8.

43.Mehdipour Biregani Z, Sharifan A. Extraction of bioactive compounds from pomegranate: A review. J Food Bioprocess Eng. 2021;4(1):37-42.

44.Mirjalili A. A review on biochemical constituents and medicinal properties of pomegranate (Punica granatum L.). J. Med. Plants 2015;14(56):1-22.

45.Veloso DJ, Abrão F, Martins CHG, Bronzato JD, Gomes BPFA, Higino JS, Sampaio FC. Potential antibacterial and anti-halitosis activity of medicinal plants against oral bacteria. Arch Oral Biol. 2020 Feb;110:104585.

46.Ferrazzano GF, Scioscia E, Sateriale D, Pastore G, Colicchio R, Pagliuca C, Cantile T, Alcidi B, Coda M, Ingenito A, Scaglione E, Cicatiello AG, Volpe MG, Di Stasio M, Salvatore P, Pagliarulo C. In Vitro Antibacterial Activity of Pomegranate Juice and Peel Extracts on Cariogenic Bacteria. Biomed Res Int. 2017;2017:2152749.

47.Nozohour Y, Golmohammadi R, Mirnejad R, Fartashvand M. Antibacterial activity of pomegranate (Punica granatum L.) seed and peel alcoholic extracts on Staphylococcus aureus and Pseudomonas aeruginosa isolated from health centers. J Appl Biotechnol Rep. 2018;5(1):32-6.

48.Millo G, Juntavee A, Ratanathongkam A, Nualkaew N, Peerapattana J, Chatchiwiwattana S. Antibacterial Inhibitory Effects of Punica Granatum Gel on Cariogenic Bacteria: An in vitro Study. Int J Clin Pediatr Dent. 2017 Apr-Jun;10(2):152-7.

49.Aravindraj S, Preethi M, Sivapathasundharam B. Antimicrobial Effects of Punica granatum Extracts on Staphylococcus aureus, Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis and Candida albicans. Int J Curr Microbiol Appl Sci. 2017;6(9):2762-74.