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Antibacterial Properties of *Citrus limon* and Pineapple Extracts on Oral Pathogenic Bacteria (*Streptococcus mutans* and *Streptococcus sanguis*)

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Abstract

Background: Micro-organisms resistant to most of the commercial antibiotics are rapidly expanding and there is an urgent need for detection of novel antimicrobial compounds. Tooth decay is a dental infection with bacterial sources such as *Streptococcus mutans* and *Streptococcus sanguis*.

Objectives: The present study aimed to evaluate the in vitro antibacterial effects of different concentrations of *Citrus limon* peel, pineapple fruit, and pineapple peel extracts on oral pathogens such as *S. mutants* and *S. sanguis*.

Materials and Methods: In this experimental study, the hydroethanolic extracts of the selected plants were prepared by maceration method and their antibacterial effects were evaluated by agar well diffusion method.

Results: Two-fold dilutions of plant extract solutions were tested to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against each selected microorganism. The results of the current study revealed that pineapple peel extracts had the highest antibacterial effect on *S. sanguis* (MIC: 1.56 mg/mL and MBC: 3.12 mg/mL). Pineapple fruit had the lowest antibacterial activity against *S. mutans* (MIC: 25 mg/mL and MBC: 100 mg/mL). *C. limon* peel had significant antibacterial activity against *S. mutans* and *S. sanguis*.

Conclusion: The peel of *C. limon* and pineapple had significant antibacterial activity against cariogenic microorganisms such as *S. mutans* and *S. sanguinis*.

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Background

Tooth decay is an infectious progressive disease that disrupts the normal molecular interactions between the tooth surface and microbial biofilm. If not treated early, tooth decay can result in tooth cavity and subsequent dentin loss and pulp injury.[1,2] *Streptococcus mutans* and *Streptococcus sanguis*, belonging to the viridans streptococci, comprise a large group of commensal streptococcal bacteria species which are the most commonly known members of the normal flora of the oral cavity. These bacteria produce large polysaccharides such as levan and dextran from sucrose and are responsible for the development of tooth decay. In addition, after injury or damage to the mucosa, they may enter the bloodstream and cause endocarditis.[3,4] *Citrus limon* is an important medicinal plant which belongs to the Rutaceae family. Different parts of *Citrus* fruit (leaves, stem, root, juice, peel, and flower) have a broad spectrum of biological activity including antifungal, antibacterial, antiviral, antidiabetic and anticancer activities due to the presence of alkaloids.[5] The peel of *Citrus* fruit is a rich source of coumarins, β and α-sitosterol, glycosides, flavonoid, glycosides and volatile oils.[6] Many *Citrus* species are known for their antimicrobial activity and phytochemical compounds.[7-10] In addition, Pandey et al. reported antifungal activity and phytochemical compounds of the hydroalcoholic extracts of *C. limon* peel. Some studies have shown the presence of tannins, reducing sugar and flavonoids in the *C. limon* peels, whereas glycosides and saponins were absent.[11] Pineapple (*Ananas comosus* Linn.) belongs to the Bromeliaceae family and is a tropical fruit that has been used for several years for various medicinal purposes. This fruit has not only been valued for its sweet taste but also has been used for centuries to treat digestion problems and inflammation because of the presence of Bromelain in this plant.[12] Bromelain belongs to the group of protein digesting enzymes obtained from the fruit and stem of the pineapple plant. Previous studies have shown that bromelain, found in pineapples, is currently being used to treat and reduce swelling, bruising, inflammation from tendinitis, sprains, strains and muscle injuries and swelling related to trauma or ear, nose and throat surgeries.[12] It is also used to remove dead and damaged tissue after burn, to reduce allergic fever, and to prevent the collection of water in the lungs.[12] López-García et al carried out a study the purification of bromelain from pineapple stems and reported that the ability of bromelain to inhibit fungal plant pathogens such as *Fusarium verticillioides*, *F. oxysporum* and *F. proliferatum* is because of its proteolytic activity.[13] Bromelain, obtained from pineapple, is a strong antibacterial agent that showed most efficacy against *E. coli* and *Proteus* spp.[14] Liliany et al carried out a study on the enzymatic and antimicrobial activity of Bromelain isolated from pineapple (*A. comosus*) against *Enterococcus faecalis*.[15] Moreover, Zharfan et al reported the antimicrobial efficacy of pineapple *(A. comosus* L. Merr*)* extract against multidrug-resistant *Pseudomonas aeruginosa.*[16] According to the previous literature on the strong antimicrobial properties of *C. limon* and Pineapple fruits and regarding the increased prevalence of drug resistance due to their overuse and side effects of commercial antibiotics, researchers have become interested in herbal medicine to find new sources of antibacterial drugs. This study aimed to assess the antibacterial properties of *C. limon* peel, pineapple peel, and pineapple fruit extracts on *S. mutans* and *S. sanguinis*.

Materials and Methods

Preparation of Extracts

The fresh fruits of *C. limon* and pineapple were purchased from a local market of Ahvaz, Iran. The peel of fruits and the fruit cubes of pineapple were dried in shade and crushed to a fine powder. For the purpose of extraction, an aliquot (10 g) of the powder of peel was soaked in 100 mL of ethanol (85%)[17] in a glass beaker for maceration and then kept on a rotary shaker for 72 hours. Then, the filtration of suspension was carried out using Whatman filter paper No.1. The filtrated ethanolic extracts were dried at room temperature for alcohol evaporation. The dried extracts were stored in sterile air-tight bottles at -20˚C until future use.

Preparation of Bacterial Inoculum

For the antibacterial study, a total of two facultative anaerobic and gram-positive cocci including *S. sanguis* and *S. mutans* were prepared from the frozen stock cultures obtained from the Department of Medical Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran. These samples were stored at -80°C in Trypticase Soy Broth (TSB) (Difco Laboratories, Detroit, Mich., USA) supplemented with 15% glycerol.[18] Then, these samples were subcultured in blood agar, incubated at 37ºC, and complemented with 5% CO2 for 48 hours before assay. The freshly grown stock cultures were diluted in sterile normal saline solution (0.9%) to obtain $1/5 \times 10^8$ CFU/mL adjusted to the turbidity of 0.5 McFarland standard. Then the cultures were diluted again (0.01), to get a turbidity of $1/5 \times 10^6$ CFU/mL.[19]

Screening Plant Extracts for Antibacterial Activity

The minimum inhibitory concentrations (MICs) of selected hydroethanolic extracts were assayed using the agar well diffusion method (AWDM) according to the procedure adopted by previous studies with some modifications.[17] The equal volume (100 µL) of bacterium inoculation was uniformly spread on agar plates with the help of a glass spreader and the plates were dried for 15 minutes. Then, aliquots (1000 mg) of dried plant extracts were dissolved in 2.5 mL of 100% dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA), and the final concentration of each plant extract was adjusted to 400 mg/mL and used within 24 hours. The serial two-fold dilutions of plant extracts were prepared with sterile distilled water at a concentration range of 1.56-100 mg/mL. Five wells were punched in each blood agar and were filled with 100 µL of serial fold dilutions of plant extracts. The plates were incubated in an anaerobic chamber prepared with CO2 at 37°C for 24 hours and the zone of inhibition was observed. Subsequently, to determine MBC, 10 µL of the content of test wells that showed no visible growth were cultured on a new Muller-Hinton agar medium and incubated in an anaerobic chamber prepared with CO2 at 37°C for 24 hours. The lowest concentration of the extract with no growth was recorded as MBC.[20] The zone of inhibition of growth was measured in millimeter.

Statistical Analysis

Differences between the mean values were determined using two-tailed Student's *t* test in SPSS version 20.0. The significance of difference was measured by analysis of variance (ANOVA) and with a confidence interval of 95%. A *P* value less than 0.05 was considered statistically significant.

Results

According to the results of the present study, antibacterial activities of the tested extracts were found at 1.56-100 mg/mL concentration range. *C. limon* peel, pineapple fruit, and pineapple peel extracts showed antibacterial efficacy against both tested facultative anaerobic and gram-positive cocci. Pineapple peel extract revealed the strongest antibacterial agent in our study with highest inhibition zones (30 mm and 28 mm) and the lowest concentration (1.56 mg/L and 6.25 mg/mL) in our research was observed against *S. sanguis* and *S. mutans*, respectively (Figures 1A and 1B; Tables 1 and 2). Pineapple fruit extract showed moderate antibacterial efficacy against *S. sanguis* and *S. mutans* with inhibition zones of 28 mm and 20 mm, MICs

Table 1. The In Vitro Antimicrobial Activity (Inhibition Zone, mm) of Pineapple Fruit, Pineapple Peel, and *Citrus Limon* Peel Extracts on *Streptococcus mutans* and *Streptococcus sanguis*[a]

Extracts	Concentration													
	100 mg/mL		50 mg/mL		25 mg/mL		12.5 mg/mL		6.25 mg/mL		3.12 mg/mL		1.56 mg/mL	
	Sm	Ss	Sm	Ss	Sm	Ss	Sm	Ss	Sm	Ss	Sm	Ss	Sm	Ss
Citrus limon peel	28	25	27	23	20	18	15	15	10	10	NI	NI	NI	NI
Pineapple peel	28	30	26	28	24	25	20	23	15	21	NI	15	NI	10
Pineapple fruit	20	28	10	25	7.0	15	NI	10	NI	NI	NI	NI	NI	NI

Sm: *Streptococcus mutans,* Ss*: Streptococcus sanguis,* NI: No inhibition.
[a]Values represent the mean of 3 replicates.

Table 2. MICs (mg/mL)[a] and MBCs of Pineapple Fruit, Pineapple Peel, and *Citrus Limon* Peel Extracts on *Streptococcus mutans* and *Streptococcus sanguis*

Microorganisms	Plant Extracts					
	Citrus Lemon Peel		Pineapple Fruit		Pineapple Peel	
	MIC	MBC	MIC	MBC	MIC	MBC
Streptococcus mutans	6.25	25	25	100	6.26	12.5
Streptococcus sanguis	6.26	12.5	12.5	25	1.56	3.12

Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.
[a] Values represent the mean of 3 replicates.

of 12.5 mg/mL and 25 mg/mL, respectively. Moreover, the lowest activity (7.0 mm) was observed in pineapple fruit extract against *S. mutans* while the highest activity of this extract was demonstrated against *S. sanguis* with a zone of inhibition of 28 mm. The lowest MIC (1.56 mg/mL) and MBC (3.12 mg/mL) values of pineapple peel extract were observed against *S. sanguis*. *C. limon* peel extract had strong antibacterial efficacy against *S. sanguis* and *S. mutans* with inhibition zones of 25 mm and 28 mm, respectively and MIC of 6.25 mg mL against both *S. sanguis* and *S. mutans* (Figures 1C and 1D; Tables 1 and 2). The *P* values for each plant against tested organisms were presented in Table 3. Table 3 demonstrates the results of the paired comparison of the plants tested.

Discussion

The cause of tooth decay and biofilm formation is microbial flora that adheres to the surfaces of the dental cavity. The dominant organisms in plaque formation and tooth decay are the genus of *Streptococcus.*[21] This experimental study was carried out to assess the antimicrobial properties of *C. limon* peel, pineapple fruit, and pineapple peel extracts against *S. mutans* and *S. sanguis*. These microorganisms are shown to enter the bloodstream and settle in arteries and valves and are known as important causes of bacterial endocarditis.[22] In this study, we used ethanol as a solvent for extraction to get the best results and not to damage the effective compounds of the extract. Moreover, a previous study reported that for the extraction of *C. limon* peel, various solvents like ethanol, methanol, and acetone have been used, however, this extract in ethanol showed higher antimicrobial activity against tested microorganisms in comparison with other solvents such as methanol and acetone.[7] The peel of *Citrus* fruit is a rich source of coumarins, β and α-sitosterol, glycosides, flavonoid, glycosides, and volatile oils.[6] In addition, another study reported that antimicrobial properties of *C. limon* peel are due to the presence of bioactive substances such as

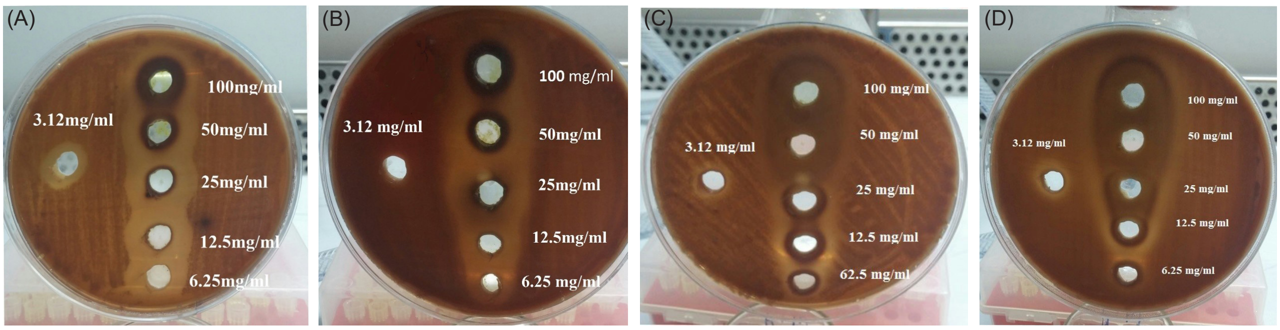

Figure 1. (A) Inhibition Zones Increased by Pineapple (Peel Extract on *S. sanguis* on Blood Agar). **(B)** Inhibition Zones Increased by Pineapple (Peel Extract on *S. mutans* on Blood Agar). **(C)** Inhibition Zones Increased by Citrus Lemon (Peel Extract on *S. sanguis* on Blood Agar). **(D)** Inhibition Zones Increased by Citrus Lemon (Peel Extract on *S. mutans* on Blood Agar).

Table 3. Paired Samples *T* Test for Comparison of Means

Medicinal Plants	Mean ± SD	Significant (2-tailed)	
Pair 1. Lemon peel-pineapple peel	-4.21429 ± 3.68320	0.001	*P*<0.05
Pair 2. Lemon peel-pineapple fruit	4.71429 ± 6.53309	0.018	*P*<0.05
Pair 3. Pineapple peel-pineapple fruit	8.92857 ± 8.02366	0.001	*P*<0.05

flavonoids, terpenes, and coumarins in it.[6] Moreover, Dhanavade et al reported that *C. limon* peel extract showed strong antimicrobial activity against microorganisms such as *P. aeruginosa*, *Salmonella typhimurium*, and *S. aureus*.[7] Ali et al also reported that the antimicrobial activity of the peel extracts of *C. limon* against *S. aureus*, *E. coli* and *Candida albicans* was due to the presence of alkaloids, saponin, flavonoids, carbohydrates, glycosides, citric acids and tannins.[8] Pandey et al conducted a study on the antimicrobial activity and phytochemical compounds of *C. limon* peel extract. The finding of this study revealed that lemon peel extracts showed maximum zone of inhibition against *P. aeruginosa* and *S. aureus*. The antifungal activity was observed only in the hydroalcoholic extracts of *C. limon* peels against *T. rubrum* whereas no activity was observed against *Aspergillus niger*, *C. albicans*, and *Microsporum canis*. Phytochemical analysis showed the presence of tannins, reducing sugars, and flavonoids. These finding revealed that the compounds in *C. limon* are responsible for its antimicrobial activity.[9] Saeb et al evaluated the antibacterial properties of *C. limon* against pathogenic bacteria such as *S. aureus, E. coli, Bacillus subtilis*, and *S. typhimurium*. The antibacterial activity of the essential oils obtained from *C. limon* leaf was higher against Gram-positive bacteria such as *S. aureus* and *B. subtilis* than against gram-negative bacteria like *E. coli* and *S. typhimurium*.[10] Lawal et al conducted phytochemical analysis and evaluated the antibacterial activities of *C. senensis* peel extract against *Salmonella paratyphi* and *Aeromonas hydrophila*. The results of the phytochemical analysis showed the presence of flavonoids, alkaloids, saponins, tannins, triterpenoids, phytosterols and steroids and found that the bacterial isolates were sensitive to the *C. senensis* peel extract with MIC of 0.25-2.5 mg/mL and MBC of 0.5-5.0 mg/ml.[23] A large number of researchers have focused on the study of leaf and peel of *Citrus* fruits and their antimicrobial activities against the gram-positive and gram-negative aerobic bacteria,[6-11,23] however, no studies have been done on the facultative anaerobic and gram-positive cocci (oral *Streptococcus*) like *S. mutans* and *S. sanguis* so far. Regarding the antimicrobial effect of pineapple, Thanish et al carried out a study on the antimicrobial effect of pineapple extract and reported zones of 26 mm and 22 mm against *S. mutans* and *E. faecalis*, respectively.[12] An inhibition zone of 20 mm was recorded against *S. mutans* in this study which is in agreement with the study done by Thanish et al who reported that pineapple extract was active against *S. mutans*.[12] In addition, Zharfan et al reported antimicrobial efficacy of pineapple *(A. comosus* L. Merr*)* extract against multidrug-resistant *P. aeruginosa*.[16] In a study conducted by Kabir et al, the ethanolic extracts of apple (*Malus pumila*), guava (*Psidium guajava*), and pineapple (*A. comosus)* showed antimicrobial activities against eight bacteria including *S. aureus,* enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), *Enterobacter cloacae, Shigella flexineri, E. faecalis, Klebsiella,* and *P. aeruginosa*. The results of this study revealed that all fruits showed antibacterial activity with the highest activity of the ethanolic extracts of pineapple against EAEC.[24] Praveen et alevaluated the antibacterial effect of pineapple extract (Bromelain) on periodontal pathogens. In this evaluation, *S. mutans* showed the highest sensitivity at the lowest concentration (MIC=2 mg/mL) as compared to *E. faecalis* (MIC=31.25 mg/mL), *Porphyromonas gingivalis* (MIC=4.15 mg/mL) and *Aggregatibacter actinomycetemcomitans* (MIC=16.6 mg/mL).[25] In our study, the hydroethanolic extracts of pineapple peel revealed the highest sensitivity at the lowest concentration (MIC=1.56 mg/mL) against *S. sanguis* as compared to *S. mutans*. Another study reported that pineapple extract alone was inactive against *S. sanguis*, however, the pineapple in combination with vancomycin was active against *S. sanguis*.[26] On the contrary, in our study, the peel extracts of pineapple fruit alone revealed high antibacterial properties against *S. sanguis* with MIC of 1.56 mg/mL. Pineapple contains a protease namely bromelain. Bromelain has revealed antimicrobial properties against *P. aeruginosa, K. pneumonia* and *S. aureus*.[27] The phytochemical analysis of pineapple has revealed numerous compounds such as flavonoids and steroids, which are known antimicrobial agents. Liliany et al carried out a study on the antimicrobial effect of bromelain isolated pineapple (*A. comosus*) against *E. faecalis*. They found that the bromelain extract showed effective inhibitory and bactericidal activity against *E. faecalis*.[15] The chemotherapeutic potential of the pineapple fruit and peel could be due to the presence of flavonoids, steroids, and bromelain which are recognized as antimicrobial agents. A large number of studies have been conducted on the antimicrobial activities of the fruits and peel of pineapple on the Gram-positive and Gram-negative aerobic bacteria,[12,15,16,24,25,27] however, only a few studies have been done on the facultative anaerobic bacteria like *S. mutans* and *S. sanguis*. Therefore, the current study was conducted to evaluate the antibacterial activity of the hydroethanolic extracts of the fruits of pineapple and *C. limon* on oral pathogens like streptococci.

Conclusion

The present study suggested that the *C. limon* and pineapple fruits have great potential antibacterial activity against selected bacteria and they can be used as an alternative medicine in the treatment or control of periodontal disease. Moreover, previous studies reported that Bromelain in pineapple fruit showed antibacterial effects against periodontal pathogens; however, in order to validate this hypothesis, clinical trials are required.

Authors' Contributions

MG and BH designed the bacteriological studies and did study supervision. MMP was the dental consultant in this study. SSN participated in the collection of specimens. BSN contributed to microbiological experiments, statistical analyses, and manuscript preparation.

Ethical Approval

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1395.316).

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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References

1. García-Cortés JO, Medina-Solís CE, Loyola-Rodriguez JP, et al. Dental caries' experience, prevalence and severity in Mexican adolescents and young adults. Rev Salud Publica (Bogota). 2009;11(1):82-91.
2. Jain M, Mathur A, Sawla L, et al. Oral health status of mentally disabled subjects in India. J Oral Sci. 2009;51(3):333-340.
3. Acevedo AM, Montero M, Machado C, Sáez I, Rojas-Sánchez F, Kleinberg I. Dental caries experience in school children and the impact of non-cavitated lesions on the caries index. Acta Odontol Latinoam. 2013;26(1):8-14.
4. Nomura R, Naka S, Nemoto H, et al. Potential involvement of collagen-binding proteins of *Streptococcus mutans* in infective endocarditis. Oral Dis. 2013;19(4):387-393. doi:10.1111/odi.12016
5. Akhilesh K, Raghvendra P, Vikas S, Madhulika G. Antimicrobial property of lemon peel extract. Natl J Universal Pharm Life Sci. 2012;52:382-386.
6. Sultana S, Ali M, Ansari SH, Bagri P. A new sesquiterpene derivative from the fruit peel of *Citrus limon* (Linn.) Burm. f. Sci Pharm. 2007;75(4):165-170. doi:10.3797/scipharm.2007.75.165
7. Dhanavade MJ, Jalkute CB, Ghosh JS, Sonawane KD. Study Antimicrobial Activity of Lemon (*Citrus lemon* L.) Peel Extract. Br J Pharmacol Toxicol. 2011;2(3):119-122.
8. Ali J, Das B, Saikia T. Antimicrobial Activity of Lemon peel (*Citrus limon*) extract. Int J Curr Pharm Res. 2017;9(4):79-82. doi:10.22159/ijcpr.2017v9i4.20962
9. Pandey A, Kaushik A, Kumar Tiwari S. Evaluation of antimicrobial activity and phytochemical analysis of *Citrus limon*. J Pharm Biomed Sci. 2011;13(17):1-7.
10. Saeb S, Amin M, Seyfi Gooybari R, Aghel N. Evaluation of antibacterial activities of *Citrus limon, Citrus reticulata,* and *Citrus grandis* against pathogenic bacteria. Int J Enteric Pathog. 2016;4(4):e37103. doi:10.15171/ijep.2016.13
11. Gunwantrao BB, Kaveri Bhausaheb S, Ramrao BS and Subhash KS. Antimicrobial activity and phytochemical analysis of orange (*Citrus aurantium* L.) and pineapple (*Ananas comosus* (L.) Merr. peel extract. Ann Phytomed. 2016; 5(2): 156-160.
12. Thanish Ahamed S, Vishnu Priya V, Gayathri R, Geetha RV. Evaluation of anti microbial activity of pineapple extract against selected oral pathogen. J Pharm Sci Res. 2016;8(6):491-492.
13. López-García B, Hernández M, Segundo BS. Bromelain, a cysteine protease from pineapple (*Ananas comosus*) stem, is an inhibitor of fungal plant pathogens. Lett Appl Microbiol. 2012;55(1):62-67. doi:10.1111/j.1472-765X.2012.03258.x
14. Ali AA, Milala AM, Gulani IA. Antimicrobial effects of crude bromelain extracted from pineapple fruit (*Ananas comosus* (Linn.) Merr.). Adv Biochem. 2015;3(1):1-4. doi:10.11648/j.ab.20150301.11
15. Liliany D, Widyarman AS, Erfan E, Sudiono J, Djamil MS. Enzymatic activity of bromelain isolated pineapple (*Ananas comosus*) hump and its antibacterial effect on *Enterococcus faecalis*. Sci Dent J. 2018;2(2):39-50. doi:10.26912/sdj.v2i2.2540
16. Zharfan RS, Purwono PB, Mustika A. Antimicrobial activity of pineapple (*Ananas comosus* L. Merr) extract against multidrug-resistant of Pseudomonas aeruginosa: an in vitro study. Indonesian J Trop Infect Dis. 2017;6(5):118-123. doi:10.20473/ijtid.v6i5.4159
17. Sadeghi-Nejad B, Moghimipour E, Yusef Naanaie S, Nezarat S. Antifungal and antibacterial activities of polyherbal toothpaste against oral pathogens, in vitro. Curr Med Mycol. 2018;4(2):21-26. doi:10.18502/cmm.4.2.65
18. Baron EJ, Finegold SM. Diagnostic microbiology. 8th ed. St. Louis: Mosby; 2017:171-186.
19. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria That Grow Aerobically; Approved Standard (A07-A10). 10th ed. CLSI; 2015.
20. Bali EB, Acik L, Akca G, et al. Antimicrobial activity against periodontopathogenic bacteria, antioxidant and cytotoxic effects of various extracts from endemic *Thermopsis turcica.* Asian Pac J Trop Biomed. 2014;4(7):505-514. doi:10.12980/apjtb.4.2014apjtb-2013-0010
21. Ebrahimi Saravi M, Vojdani M, Bahrani F. Evaluation of cellular toxicity of three denture base acrylic resins. J Dent (Tehran). 2012;9(4):180-188.
22. Vacca-Smith AM, Jones CA, Levine MJ, Stinson MW. Glucosyltransferase mediates adhesion of *Streptococcus gordonii* to human endothelial cells in vitro. Infect Immun. 1994;62(6):2187-2194.
23. Lawal D, Bala JA, Aliyu SY, Huguma MA. Phytochemical screening and in vitro anti-bacterial studies of the ethanolic extract of *Citrus senensis* (Linn.) peel against some clinical bacterial isolates. Int J Innov Appl Stud. 2013;2(2):138-145.
24. Kabir S, Jahan SM, Hossain MM, Siddique R. Apple, Guava and Pineapple Fruit Extracts as Antimicrobial Agents against Pathogenic Bacteria. Am J Microbiol Res. 2017;5(5):101-106.
25. Praveen NC, Rajesh A, Madan M, Chaurasia VR, Hiremath NV, Sharma AM. In vitro evaluation of antibacterial efficacy of pineapple extract (bromelain) on periodontal pathogens. J Int Oral Health. 2014;6(5):96-98.
26. Khosropanah H, Bazargani A, Ebrahimi H, Eftekhar K, Emami Z, Esmailzadeh S. Assessing the effect of pineapple extract alone and in combination with vancomycin on *Streptococcus sanguis*. Jundishapur J Nat Pharm Prod. 2012;7(4):140-143.
27. Ashik Ahamed A. Vishnu Priya V, Gayathri R, Geetha RV. Evaluation of anti microbial activity of pineapple extract against selected microbes. Int J Pharm Sci Rev Res. 2016;39(1):277-278.