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An Investigation of the Antibiofilm and Antibacterial Effects of Ganoderma Lucidum and Nisin Compared with Chlorhexidine Mouthwash on Streptococcus Mutans

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ABSTRACT

Background and Objective: Various antimicrobial compounds and solutions are used to control oral bacterial infections, especially Streptococcus mutans. Due to their increasing side effects, there is a great deal of interest in using natural substances and antimicrobial peptides to replace chemical drugs. Therefore, the present study was conducted to investigate the antibiofilm and antibacterial properties of Ganoderma lucidum and nisin in comparison with chlorhexidine mouthwash on Streptococcus mutans.

Methods: Streptococcus mutans strain PTCC 1683 was used in this laboratory experiment. Ganoderma lucidum extract and nisin solution were prepared after resuscitation of the standard strain in Brain Heart Infusion (BHI) Broth. Broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MCC) of Ganoderma lucidum extract, nisin and chlorhexidine mouthwash. The antibacterial and antibiofilm effect of the combination of Ganoderma lucidum extract and nisin was determined using the checkerboard assay.

Findings: The MIC of Ganoderma lucidum extract and nisin on the standard strain of Streptococcus mutans were reported to be 2.5 mg/ml and 25 µg/ml, respectively, and the MBC of Ganoderma lucidum extract and nisin on the standard strain were reported to be 2.5 mg/ml and 50 µg/ml, respectively. Moreover, this bacterium was sensitive to all dilutions of chlorhexidine mouthwash. The fractional inhibitory concentration index (FICI) did not show a synergistic effect due to the combined use of Ganoderma lucidum extract and nisin (FICI=2). After reducing the concentration of Ganoderma lucidum extract, nisin, and chlorhexidine mouthwash, the ability to inhibit biofilm formation on the studied strain was significantly reduced ($p<0.05$).

Conclusion: The results of this study indicated the antibiofilm and antibacterial effects of Ganoderma lucidum extract and nisin on Streptococcus mutans, but they are less effective compared with chlorhexidine mouthwash.

Keywords: *Ganoderma Lucidum, Nisin, Chlorhexidine, Streptococcus Mutans*.

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Introduction

Dental caries is a complex issue caused by multiple factors and is one of the most common infectious disorders worldwide (1). High sugar intake in our diet promotes the proliferation of acidogenic bacteria, including the oral cariogenic pathogen Streptococcus mutans, in the microbial composition of dental biofilms (2). Since this bacterium encodes various enzymes, including glycosyltransferases, that are secreted into the extracellular environment and use sucrose in food as a substrate for the synthesis of glucans, it is known as a major producer of exopolysaccharides (3). Consequently, inhibition of biofilm formation by Streptococcus mutans is essential for the effective prevention and management of dental caries (4).

Ganoderma lucidum, a well-known fungus of the Ganodermataceae family, is a valuable source for the extraction of several essential medicinal compounds used in traditional medicine (5). Ganoderma species are classified in the phylum Basidiomycete, Homobasidiomycetes class, and Polyporaceae family (6). Research has shown that Ganoderma lucidum contains diverse chemical constituents such as polysaccharides, triterpenoids, meroterpenoids, alkaloids, and ergosterol (7). Ganoderma lucidum extract contains hydroxybenzoic and cinnamic acids, which have antibacterial activity (8).

Nisin is a polypeptide bacteriocin that has recently attracted attention as a promising antibiotic (9). Nisin is the only antimicrobial peptide widely used in commercial food preservation. Nisin is the most commonly used bacteriocin because of its nontoxicity, tastelessness, heat stability, and low pH tolerance (10). Nisin exhibits antimicrobial activity against most Gram-positive bacteria, especially the spore-forming ones (11). The antimicrobial properties of nisin are attributed to its ability to create pores in the cytoplasmic membrane and inhibit peptidoglycan synthesis, which is critical for bacterial cell wall integrity. This disruption of the membrane potential and pH gradient leads to cell death (12, 13).

Increasing resistance among pathogens to conventional antibiotics and adverse side effects of current treatments have led to a growing interest in exploring traditional medicinal plants and probiotics as potential sources for antimicrobial drug discovery. This study aims to determine the antibiofilm and antibacterial activity of Ganoderma lucidum and nisin compared to chlorhexidine mouthwash on Streptococcus mutans under in vitro conditions.

Methods

The present laboratory experimental research was approved by the Vice-Chancellor of Research and Technology of Hamadan University of Medical Sciences with the ethics approval code IR.UMSHA.REC.1401.643. The standard strain of Streptococcus mutans PTCC 1683 obtained from the Iranian Biological Resources Center (Tehran, Iran) was used in this study. The bacterial strain was cultured in Brain Heart Infusion (BHI) Broth (Ibresco, Iran) at 37 °C. To prepare Ganoderma lucidum extract, this fungus was first purchased from Iran Ganoderma Company and extracted by soaking. First, 50 g of the fungus was powdered by an electric grinder and mixed with 750 ml of Alcohol 99% and 250 ml of distilled water in an Erlenmeyer flask and placed in a shaker for 72 hours. Then, it was extracted from a Buchner funnel using filter paper and a vacuum was created. Its alcohol was distilled, concentrated and extracted by rotary vacuum distillation and was ready for use (14). Nisin N5764 (Sigma-Aldrich, USA) was used in this study. Nisin stock solution was prepared by dissolving 50 mg of nisin in 5 ml of Hydrochloric Acid 0.02N and sterilizing by filtration (0.22 μm pore size filter).

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Broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MCC) of Ganoderma lucidum extract, nisin, and chlorhexidine mouthwash (15). Briefly, 10 µL of Streptococcus mutans culture (approximately 10^9 cfu/ml), 140 µL of BHI (Brain Heart Infusion) medium, and 50 µL of different concentrations of antimicrobial agents were combined in 96-well microplates. The resulting concentrations of the three antimicrobial agents were as follows: nisin concentrations were 100, 50, 25, 12.5, 6.25, 3.125, and 1.562 µg/ml. The concentrations of Ganoderma lucidum extract were 10, 5, 2.5, 1.25, 0.625, 0.132, 0.156, 0.078, 0.039, 0.019, and 0.009 mg/mL. The concentrations of chlorhexidine were 0.2%, 0.1%, 0.05%, 0.025%, 0.00625%, and 0.003125%. The optical density of each well was measured using a spectrophotometer at a wavelength of 570 nm, before and after placing the microplates in an incubator at 37°C for 24 hours. MIC is the lowest concentration of an antimicrobial agent at which bacterial growth is inhibited. MIC was calculated by measuring the changes in OD of each well after a 24-hour incubation period. MBC was determined by inoculating 10 µl of bacterial suspension from the MIC well and two wells above onto Mueller Hinton agar medium. Bacterial colonies were counted after 24 hours at 37 °C. MBC is the lowest concentration of antibiotic that is capable of killing 99.9% of bacteria.

The antibacterial effect of the combination of Ganoderma lucidum extract and nisin was investigated by the checkerboard assay (16). As can be seen in Figure 1, columns 1 to 11 contain two-fold serial dilutions of Ganoderma lucidum extract and rows A to G contain two-fold serial dilutions of nisin. Column 12 contains a serial dilution of nisin alone, while row H contains a serial dilution of Ganoderma lucidum extract alone. To determine the interaction effect of Ganoderma lucidum extract and nisin, an index called the fractional inhibitory concentration index was used, which is calculated according to the following formula.

FICA= MICA+B/MICA

FICB= MICB+A/MICB

FIC Index= FICA+FICB

In this method, if FICI≤ 0.5, it indicates a synergistic effect, FICI= 0.4-3.9 indicates a cumulative or indifferent effect, and FICI≤ 4 indicates a conflicting effect.

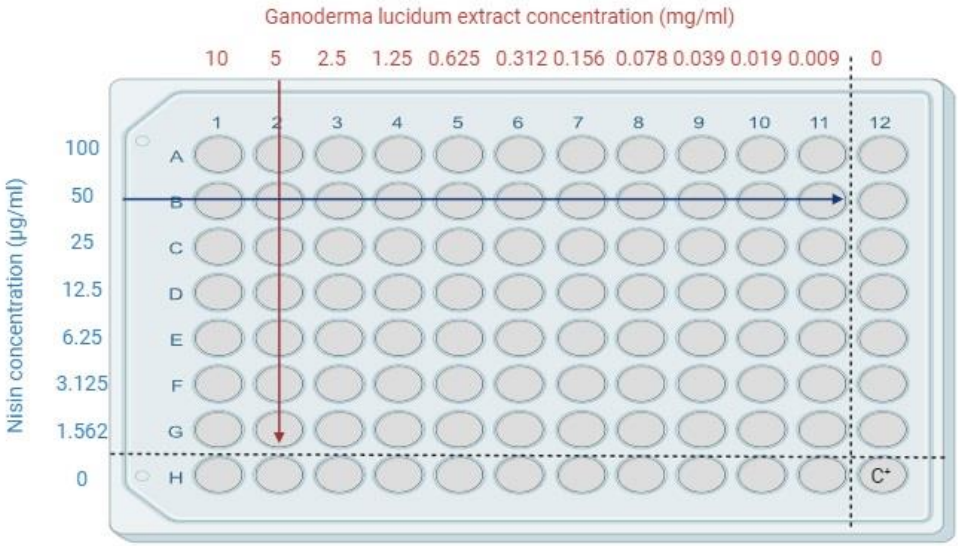

Figure 1. Combined antimicrobial activity of Ganoderma lucidum extract and nisin by checkerboard assay

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The effect of Ganoderma lucidum extract, nisin and chlorhexidine on Streptococcus mutans was evaluated using the microtiter plate method (17). Streptococcus mutans strain was incubated with 1.2 MIC, 1.4 MIC and 1.8 MIC concentrations of Ganoderma lucidum extract, nisin and chlorhexidine in BHI broth medium containing 0.2% sucrose at 37°C for 24 hours. The well containing Streptococcus mutans without the presence of antimicrobial agents was considered as the positive control and the well containing the culture medium without the presence of antimicrobial agents and bacteria was considered as the negative control. After incubation, the supernatant was removed from each well and washed three times with PBS solution. For fixation, 200 µl of 99% methanol was added to each well for 15 minutes. The wells were stained with 200 µl of crystal violet 0.1% for 5 min and excess dye was removed with sterile distilled water. Dyes bound to the biofilm were dissolved using 200 µl of Acetic Acid 33% for 15 min. In the final step, the OD of each well was determined and recorded using an ELISA reader at a wavelength of 570 nm. All experiments were repeated twice.

The checkerboard assay was used to evaluate the antibiofilm activity of Ganoderma lucidum extract in combination with nisin (18). First, the isolate was inoculated in BHI medium containing 0.2% sucrose and incubated for 24 hours at 37 °C. Then, a suspension with a standard turbidity of 0.5 McFarland was prepared from the grown bacteria. To reach a bacterial count of 10^6 CFU/ml in a final volume of 200 µL, a 1:100 dilution was prepared. Then, 100 µL of the above suspension was added to the wells of a 96-well microplate containing 50 µL of different MIC concentrations of Ganoderma lucidum extract and nisin. Then, the plate was incubated at 37 °C for 24 hours. After incubation, the biofilm was stained with crystal violet according to the method mentioned in measuring the effect of substances alone on biofilm inhibition. In the final step, the OD of each well was determined and recorded using an ELISA reader at a wavelength of 570 nm. The test results were obtained as a percentage of biofilm inhibition after treatment with antimicrobial agents according to the following formula (18).

$[(C-B) - (T-B)]/[(C-B)] \times 100$

C: Mean OD of control, B: Mean OD of negative control well, T: Mean OD of treatment well.

Data were entered and analyzed in SPSS version 27 (IBM Corp., Armonk, NY, USA). One-way ANOVA was used for statistical testing, and $p<0.05$ was considered significant.

Results

The MIC of Ganoderma lucidum extract, nisin and chlorhexidine mouthwash were determined using the broth microdilution method (Figure 2). The MIC of Ganoderma lucidum extract and nisin on the standard strain of Streptococcus mutans was reported to be 2.5 mg/ml and 25 µg/ml, respectively, and the MBC of Ganoderma lucidum extract and nisin on the standard strain was reported to be 2.5 mg/ml and 50 µg/ml, respectively. Furthermore, this bacterium was sensitive to all dilutions of chlorhexidine mouthwash. Therefore, chlorhexidine mouthwash has a stronger antimicrobial effect compared to Ganoderma lucidum extract and nisin against Streptococcus mutans. FICI was used to determine the interaction effect of Ganoderma lucidum extract and nisin, which indicated an indifferent effect of these two substances on the inhibitory power of the studied bacteria (FICI= 2).

The rate of biofilm formation in Streptococcus mutans was reduced after treatment with different concentrations of Ganoderma lucidum extract, nisin, and chlorhexidine mouthwash alone compared to the positive control well. Moreover, the rate of reduction in biofilm production in the studied strain was dependent on the concentration of the antimicrobial agent; significantly less biofilm was formed at higher concentrations ($p<0.05$) (Table 1).

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The checkerboard assay was used to determine the effect of the combination of Ganoderma lucidum extract and nisin on biofilm formation in Streptococcus mutans. The results after treatment of the standard strain of Streptococcus mutans with different concentrations of Ganoderma lucidum extract and nisin can be seen based on the rate of biofilm formation (Table 1). The results showed that during treatment with the combination of the two antimicrobial substances, the inhibition of biofilm formation was greater than when each antimicrobial substance was used alone.

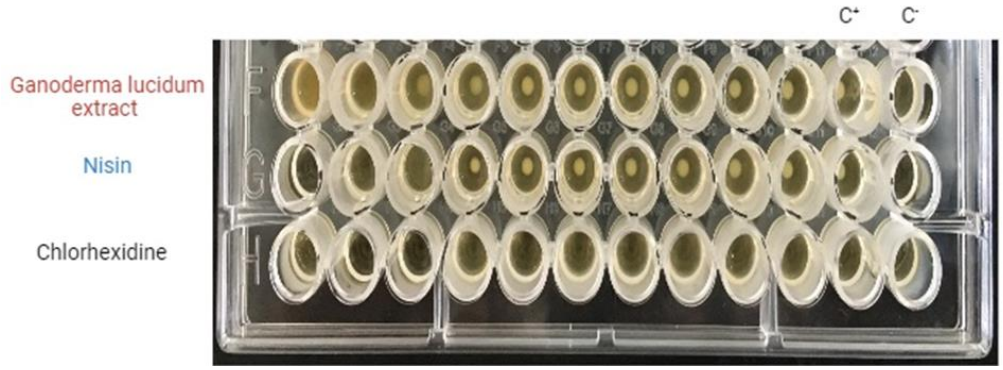

Figure 2. Evaluation of bacterial susceptibility to Ganoderma lucidum extract, nisin, and chlorhexidine mouthwash using the broth microdilution method

Table 1. Effect of different concentrations of Ganoderma lucidum extract, nisin, and chlorhexidine mouthwash alone and in combination on the inhibition of biofilm formation of the standard strain of Streptococcus mutans

Type of antimicrobial agent	Standard strain of Streptococcus mutans					p-value
Ganoderma Lucidum Extract	Concentrations of Ganoderma lucidum extract used in µg/mL	2.5	1.25	0.625	0.312	0.021
	Mean OD at 570 nm	0.323	0.35	0.365	0.386	
	Mean percentage of biofilm inhibition	65	61	59	56	
Nisin	Nisin concentrations used in µg/mL	25	12.5	6.25	3.125	0.044
	Mean OD at 570 nm	0.237	0.315	0.358	0.435	
	Mean percentage of biofilm inhibition	77	66	60	49	
Ganoderma Lucidum Extract + Nisin	Concentrations of Ganoderma lucidum extract used in mg/mL + Concentrations of nisin used in µg/mL	2.5+25	1.25+12.5	0.625+6.25	0.312+3.125	0.012
	Mean OD at 570 nm	0.152	0.273	0.308	0.358	
	Mean percentage of biofilm inhibition	89	72	67	60	
Chlorhexidine mouthwash	Dilutions of chlorhexidine mouthwash in percentage	0.2	0.1	0.05	0.025	0.031
	Mean OD at 570 nm	0.139	0.188	0.237	0.287	
	Mean percentage of biofilm inhibition	91	84	77	70	

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Discussion

Based on the results of this study, Ganoderma lucidum extract and nisin have inhibitory, bactericidal, and anti-biofilm effects on Streptococcus mutans, but they were less effective compared to chlorhexidine mouthwash. In addition, treatment of this bacterium with a combination of Ganoderma lucidum extract and nisin increased biofilm inhibition compared to when each antimicrobial agent was used alone.

In the present study, the MIC and MBC of Ganoderma lucidum extract were 2.5 mg/mL. On the other hand, a study by Erbiai et al. showed that the MIC of Ganoderma lucidum extract against Escherichia coli and Staphylococcus aureus was 8 and 4 mg/mL, respectively. This study also showed that the MBC of this extract against Escherichia coli and Staphylococcus aureus was 8 and 8 mg/mL, respectively (19). Therefore, the extract prepared in this study showed growth inhibitory and apoptotic properties at a lower dilution compared to Streptococcus mutans. Of course, the method of extract preparation and the resistance of different bacteria may be effective in these results.

In the present study, the MIC and MBC of nisin were 25 and 50 μg/mL, respectively. Shin et al. showed that the MIC and MBC of nisin against Streptococcus mutans UA159 were 20 and 100 μg/mL, respectively (20). Jiamboonsri et al. showed that the MIC and MBC of nisin against Streptococcus mutans ATCC 25175 were greater than 4000 international units per milliliter (IU/mL) (21). The difference in the MIC and MBC of our study with other studies may be due to the type of nisin used and the standard strain studied.

A study by Mozaffari et al. showed that chlorhexidine mouthwash at dilutions of 0.2, 0.1, 0.01, and 0.02% inhibited the growth of Streptococcus sanguinis, Streptococcus mutans, and Lactobacillus casei in culture media (22). These results are consistent with the findings of the present study and indicate the relatively strong antimicrobial properties of chlorhexidine on Streptococcus mutans.

A study by Karaca et al. showed that the biofilm formation ability of Enterococcus strains was reduced by treatment with different concentrations of Ganoderma lucidum extract. Therefore, the higher the concentration of extract, the greater the percentage reduction in biofilm production (23). A study by Tong et al. also showed a decrease in the biofilm formation ability of Streptococcus mutans bacteria after treatment with nisin (24). On the other hand, the results of the present study are consistent with these studies and indicate an increase in the rate of inhibition of biofilm formation of the studied bacteria through treatment with different concentrations of Ganoderma lucidum extract and nisin. Moreover, the combination of these two substances reduced the biofilm formation ability of Streptococcus mutans.

The results of this study may suggest that Ganoderma lucidum extract can be used in combination with nisin to enhance the anti-biofilm activity of nisin. However, for the practical application of this combination, it is suggested that their effects be measured using other antimicrobial agents and on other pathogens.

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References

1.Al-Ibraheem J, Zyara Y, Al-Quraine N, Abdulridha WM. Correlation between salivary immunoglobulin A and interleukin-1beta in smokers with dental caries. F1000Res. 2023;12:175.

2.Cai JN, Kim D. Biofilm ecology associated with dental caries: understanding of microbial interactions in oral communities leads to development of therapeutic strategies targeting cariogenic biofilms. Adv Appl Microbiol. 2023;122:27-75.

3.Ribeiro SM, Fratucelli ÉDO, Bueno PCP, de Castro MKV, Francisco AA, Cavalheiro AJ, et al. Antimicrobial and antibiofilm activities of Casearia sylvestris extracts from distinct Brazilian biomes against Streptococcus mutans and Candida albicans. BMC Complement Altern Med. 2019;19(1):308.

4.Yan L, Wu W, Tian S. Antibacterial and antibiofilm activities of Trollius altaicus C. A. Mey. On Streptococcus mutans. Microb Pathog. 2020;149:104265.

5.Du Y, Tian L, Wang Y, Li Z, Xu Z. Chemodiversity, pharmacological activity, and biosynthesis of specialized metabolites from medicinal model fungi Ganoderma lucidum. Chin Med. 2024;19(1):51.

6.Mowna Sundari T, Alwin Prem Anand A, Jenifer P, Shenbagarathai R. Bioprospection of Basidiomycetes and molecular phylogenetic analysis using internal transcribed spacer (ITS) and 5.8S rRNA gene sequence. Sci Rep. 2018;8(1):10720.

7.Gholami A, Karami E, Ebrahimpoorghalehjoghi S, Hajjarzadeh M, Javadi K, Bakhtiari M, et al. The therapeutic effects of Ganoderma lucidum on cancer and immune system such as Anti-Tumor, Anti-Metastatic, Antioxidant, Anti-Angiogenic, Anti-Inflammatory and Immuno-Modulating. Plant Biotechnol Persa. 2023;5(2):68-85.

8.El-Khashab IH. Antiangiogenic and Proapoptotic Activities of Atorvastatin and Ganoderma lucidum in Tumor Mouse Model via VEGF and Caspase-3 Pathways. Asian Pac J Cancer Prev. 2021;22(4):1095-104.

9.Tavares TD, Ribeiro ARM, Silva C, Antunes JC, Felgueiras HP. Combinatory effect of nisin antimicrobial peptide with bioactive molecules: A review. J Drug Deliv Sci Technol. 2024;91:105246.

10.Roshanak S, Shahidi F, Tabatabaei Yazdi F, Javadmanesh A, Movaffagh J. Evaluation of Antimicrobial Activity of Buforin I and Nisin and Synergistic Effect of the Combination of them as a Novel Antimicrobial Preservative. J Food Prot. 2020;83(11):2018-25.

11.Yu X, Lu N, Wang J, Chen Z, Chen C, Regenstein JM, et al. Effect of N-terminal modification on the antimicrobial activity of nisin. Food Control. 2020;114:107227.

12.Zhou H, Fang J, Tian Y, Lu XY. Mechanisms of nisin resistance in Gram-positive bacteria. Ann Microbiol. 2014;64:413-20.

13.Sun Z, Li P, Liu F, Bian H, Wang D, Wang X, et al. Synergistic antibacterial mechanism of the Lactobacillus crispatus surface layer protein and nisin on Staphylococcus saprophyticus. Sci Rep. 2017;7(1):265.

14.Akbari S, Chabavizadeh J, Abtahi SM, Yegdaneh A, Namdar F, Saberi S. Evaluation of Antileishmanial Effect of Hydroalcoholic Extract of Ganoderma Leucidum on Leishmania Major in Vitro. J Isfahan Med Sch. 2019;36(511):1628-34. [In Persian]

15.Tong Z, Zhou L, Jiang W, Kuang R, Li J, Tao R, et al. An in vitro synergetic evaluation of the use of nisin and sodium fluoride or chlorhexidine against Streptococcus mutans. Peptides. 2011;32(10):2021-6.

16.Tong Z, Zhang Y, Ling J, Ma J, Huang L, Zhang L. An in vitro study on the effects of nisin on the antibacterial activities of 18 antibiotics against Enterococcus faecalis. PLoS One. 2014;9(2):e89209.

17.Mataraci E, Dosler S. In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin-resistant Staphylococcus aureus biofilms. Antimicrob Agents Chemother. 2012;56(12):6366-71.

[DOI: 10.22088/jbums.27.1.37]

18.Rosato A, Sblano S, Salvagno L, Carocci A, Clodoveo ML, Corbo F, et al. Anti-Biofilm Inhibitory Synergistic Effects of Combinations of Essential Oils and Antibiotics. Antibiotics (Basel). 2020;9(10):637.
19.Erbiai EH, Amina B, Kaoutar A, Saidi R, Lamrani Z, Pinto E, et al. Chemical Characterization and Evaluation of Antimicrobial Properties of the Wild Medicinal Mushroom Ganoderma lucidum Growing in Northern Moroccan Forests. Life (Basel). 2023;13(5):1217.
20.Shin JM, Ateia I, Paulus JR, Liu H, Fenno JC, Rickard AH, et al. Antimicrobial nisin acts against saliva derived multi-species biofilms without cytotoxicity to human oral cells. Front Microbiol. 2015;6:617.
21.Jiamboonsri P, Kanchanadumkerng P. Influence of Gallic Acid and Thai Culinary Essential Oils on Antibacterial Activity of Nisin against Streptococcus mutans. Adv Pharmacol Pharm Sci. 2021;2021:5539459.
22.Mozaffari B, Mansouri S, Rajabalian S, Alimardani A, Mohammadi M. In vitro study between antibacterial and cytotoxic effects of chlorhexidine and persica mouthrinses. J Dent Sch-Shahid Beheshti Univ Med Sci. 2005;23(3):494-509. [In Persian]
23.Karaca B, Cihan AÇ, Akata I, Altuner EM. Anti-Biofilm and Antimicrobial Activities of Five Edible and Medicinal Macrofungi Samples on Some Biofilm Producing Multi Drug Resistant Enterococcus Strains. Turk J Agr -Food Sci Technol. 2020;8(1):69-80.
24.Tong Z, Zhang L, Ling J, Jian Y, Huang L, Deng D. An in vitro study on the effect of free amino acids alone or in combination with nisin on biofilms as well as on planktonic bacteria of Streptococcus mutans. PLoS One. 2014;9(6):e99513.

[DOI: 10.22088/jbums.27.1.37]