http://www.jrdms.dentaliau.ac.ir e(ISSN): 2383 -2754

JRDMS Journal of Research in Dental and Maxillofacial Sciences

In Vitro Comparative Evaluation of the Antibacterial Efficacy of Licorice Aqueous Root Extract (Glycyrrhiza Glabra) and Chlorhexidine against Lactobacillus Acidophilus(An in-vitro study)

Moini P[1] , Eslami G[2], Taheri S[3], Valaei N[4], Naji Rad MA[5]

[1]*Assistant Professor, Pedodontics Dept, Dental Branch of Tehran, Islamic Azad Univesity, Tehran, Iran.*
[2] *Professor, Microbiology Dept, Shahid Beheshti University, Tehran, Iran.*
[3]*Tutor, Microbiology Dept, Shahid Beheshti University, Tehran, Iran.*
[4] *Faculty Member of Thalassemia Research Center, Mazandaran, Iran.*
[5] *Dentist*

ARTICLE INFO

Article Type
Orginal Article

Article History
Received: Oct 2015
Accepted: Dec 2016
ePublished: March 2016

Keywords:
Lactobacillus Acidophilus;
Glycyrrhiza Glabra;
Chlorhexidine;
Disk Diffusion
Antimicrobial Susceptibility Tests.

ABSTRACT

Background and Aim: Oral infections and dental caries are considered as two serious public health problems, which inflict a costly burden on health care services worldwide, especially in the developing countries. The aim of this study was to evaluate the antibacterial activity of Iranian Licorice aqueous root extract on Lactobacillus Acidophilus in comparison with Chlorhexidine.

Methods and Materials: In this in-vitro experimental study, we evaluated the antibacterial activity of Licorice aqueous root extract and Chlorhexidine against Lactobacillus Acidophilus using Disk Diffusion Method, determining the Minimum Inhibitory Concentration (MIC) by Broth Micro & Macro Dilution Methods and the Minimum Bactericidal Concentration (MBC) by Agar Dilution Method. Research was repeated 3 times and data were analyzed by ANOVA test. The P value of ≤ 0.01 was considered as the level of significance.

Results: Chlorhexidine showed significantly higher levels of antibacterial activity against Lactobacillus Acidophilus in comparison with Licorice aqueous root extract ($P < 0.01$).

Conclusion: Although Licorice aqueous root extract is beneficial, Chlorhexidine is more efficient in the prevention of dental caries and oral infections.

Orginal Article

Please cite this paper as:
Moini P, Eslami G, Taheri S, Valaei N, Naji Rad MA. Comparative evaluation of antibacterial activity of Glycyrrhiza glabra (Licorice) aqueous root extract and Chlorhexidine against Lactobacillus Acidophilus. J Res Dent Maxillofac Sci. 2016;1(2):7-14

***Corresponding author:**
Naji Rad M A , Tel: +98 912 159 8363,
Email: najirad67@yahoo.com

Introduction:

Lactobacillus species are amongst the bacteria responsible for the development of demineralization processes in dental grooves. [1] Nowadays, different methods are being used to prevent dental caries including methods for reducing the number of microorganisms such as Lactobacillus Acidophilus.[2] This includes antibacterial materials such as Chlorhexidine and Xylitol. These substances disrupt bacterial metabolism and block the adhesion of microorganisms to dental surfaces, etc. [3]

Chlorhexidine mouthwashes have some disadvantages such as tooth discoloration, unpleasant taste and interference with the normal oral microbial flora. Other alcohol-containing mouthwashes could also promote the incidence of oral cancer. Therefore, herbal mouthwashes are increasingly used nowadays.[4]

The Licorice root is one of the most prominent medicinal herbs in traditional medicine, which grows in different areas [5] and has many antibacterial properties. [6] It is effective in the treatment of peptic ulcer, gastric cancer, and Staphylococcal and Streptococcal infections.[7] It also prevents dental caries and the growth of harmful bacteria as well as the incidence of oral cancer. [4] Although different studies have examined the effects of this herb on oral microorganisms such as Streptococcus mutans, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Candida albicans [1, 5, 6], but Lactobacilli have been limitedly researched.[8] So far, there has been only one research available which has assessed the effects of Iranian type of Licorice root on oral microorganisms but has not assessed Lactobacilli.[9] In the present in vitro study, we have evaluated the antibacterial activity of Iranian Licorice aqueous root extract against Lactobacillus Acidophilus.

Methods and Materials:

In this experimental study, 500g of Licorice roots were procured from Neyshabur province and were dried in the pharmacognosy laboratory of Ferdowsi University of Mashhad (FUM), (The Faculty of Pharmaceutical Sciences and Nutrition), at room temperature of 25°c . Afterwards, the roots were turned to powder by use of a mechanical cutting machine. Extraction process was performed using distilled water for 72 hours without the use of any heating procedures. The final attained solution was first filtered through a special piece of fabric (muslin cloth) to leave out any unwanted residues and was filtered again using filter-paper grade 1 (Whatman, Little Chalfont, UK). The filtrated solution was thickened in vacuum condition at 40°c using the rotary evaporator instrument (Rotavapor, WBECO, Heidolph Co), and was later frizzed-dried. The licorice extraction was kept at 4°c. Later, 100mg of the extracted powder was weighed on a digital scale (AND GF-300, Tokyo, Japan) and was dissolved in 1cc of distilled water. In this way, a solution with the concentration of 100 mg/ ml was made. [10] With the use of an autoclave device (Hirayama MFG Corporation, Japan) we initiated the preparation of laboratory culture media for accumulating the bacterial samples of the study. The live cultivated bacterial samples comprising of L.Acidophilus PTCC (Persian Type Culture Collection) 1643 and E. coli PTCC 1399 (manufactured at Iranian Scientific and Industrial Research Organization) were added to Blood Agar medium (Q-Lab, Quebec, Canada) in GasPak jars and were cultured for 24 hours to reach their maximum growth. In this study, we produced subcultures of the bacterial samples, 3 times a week and each time we used the regrown fresh bacteria samples for further experimentation. After 24 hours of incubation, a certain amount of bacteria from the Blood Agar medium was added to normal saline in a way that the turbidity of the suspension in the test tube would reach 0.5 McFarland's standard. In other words, the number of bacteria in normal saline equaled 1.5×108 CFU/mL. This standard dilution was used through all the steps of this research. 0.5 McFarland's standard tube contains barium sulfate and was prepared by using spectrophotometer CECIL (CE 2021, Cambridge, UK) at 625 nm (OD625). [9]

Inhibitory Zone Assessment

Disk Diffusion Method was used to investigate the antibacterial activity of the extract in Muller

Hinton Agar medium (MHA, Q-Lab, Quebec, Canada) by following the recommendations of Clinical & Laboratory Standards Institute (CLSI). [11] The bacteria were taken from the 0.5 McFarland's standard suspension with the use of a sterile swab and were lawn cultured on MHA medium. In total, 5 disks were located on MHA medium containing Chlorhexidine 0.12% and Chlorhexidine 0.2% (Hexodine, Tehran, Iran) as the witness group [12], Licorice aqueous extract (made in the pharmacognosy laboratory of the faculty of Pharmaceutical Sciences and Nutrition, Ferdowsi University of Mashhad) as the case group, Licorice alcoholic extract (Iran Dineh, Pharmaceutical Industries Complex) and a blank disk as the control group. The culture medium was then placed inside a GasPak jar anaerobically and was incubated in N-Biotek incubator (NB-203L, Gyeonggi-do, South Korea) at 37°c for 24 hours.[13]After 24 hours, bacterial growth inhibition zone was measured with a millimeter ruler in each of the 5 disks while changes less than 1mm were considered as zero (Fig. 1).

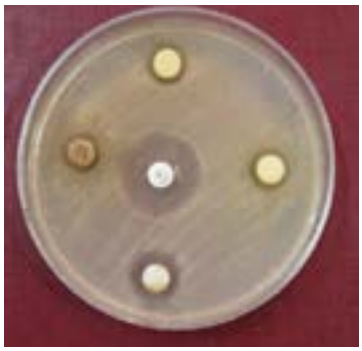

Figure 1- Inhibitory zone assessment

To ensure a precise result, research was conducted under protected aseptic conditions and was repeated 3 times.

MIC (Minimum Inhibitory Concentration) & MBC (Minimum Bactericidal Concentration) Assessment

In the second part of the study, Broth Dilution Method was adopted to evaluate the MIC and MBC. At first, we prepared 1/20 concentration of 0.5 McFarland's standard suspension according to the guidelines of the National Committee on Clinical Laboratory Standards (NCCLS). [14]

For MIC evaluation, we used both Micro-dilution Broth and Macro-dilution Broth techniques in Brain Heart Infusion medium (BHI, Q-Lab, Quebec, Canada) and for MBC evaluation, we cultured bacterial proprietary medium of MRS (de Man, Rogosa and Sharpe, Q-Lab, Quebec, Canada) with Cysteine from each diluted cryovials using Macro-dilution Broth technique.

For evaluation of the MIC with Micro-dilution Broth technique, a 96-well micro-plate was prepared and 100µl of BHI was added to each well with a sampler and then 100µl of pure aqueous root extract was placed in the first well. After mixing the suspension, it was then taken out from the first well and poured into the second well using the sampler and the same procedure was repeated up to the 10th well. Afterwards, 100µl of the 10th well's suspension was discarded and consequently, in each well, 100µl of liquid with different concentrations of Licorice root extract was obtained. The concentrations were as follows: 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39%, 0.19%, and 0.09%.

Later, 10µl of normal saline containing L.Acidophilus with 1/20 concentration of 0.5 McFarland's standard was added to the wells using a sampler. At the same time, to assess the MIC with the use of Macro-dilution Broth technique, same procedure was repeated but with higher volumes (1cc) inside the cryovials along with monitoring the negative and positive tests of both experimentations. (Positive control: a turbid tube that contains BHI broth and bacteria species; Negative control: a clear tube that contains BHI broth only). Afterwards, micro-plates and cryovials were placed inside the incubator to grow anaerobically at 37°c. After 24 hours of incubation, the MIC was evaluated through turbidity observation (Fig 2). In every series of experimentation and after 24 hours of incubation, some of the tubes remained clear, which indicated no growth of bacteria. The first tube that remained clear was considered as the MIC. Since the evaluation of the exact MIC could not be made through observation, the MBC evaluation was carried out.[14]

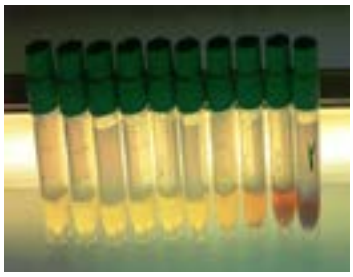

Figure 2- MIC (Minimum Inhibitory Concentration) assessment, using Broth Dilution Method (Turbidity evaluation by observation)

We lawn cultured the contents of each cryovial on its specific Lactobacilli solid culture media of MRS with Cysteine and kept them at 37°c anaerobically to evaluate the MBC. After 24 hours of incubation, the first Petri-dish that showed no bacterial growth was considered as the MBC and a level thinner, was considered as the MIC. [8, 9] (Fig. 3) The same procedure was repeated for Chlorhexidine 0.12% and 0.2% (the witness group). For evaluation of the effect of Licorice root extract, we correspondingly conducted MIC and MBC experiments on E. coli gram-negative bacteria, which lack peptidoglycan cell walls and therefore are more sensitive compared to gram-positive bacteria such as L. Acidophilus. Each experiment was repeated 3 times by the same examiner at different days of the week. Finally, the data were entered into SPSS statistical software (version 20) and ANOVA statistical test was used for further analysis. The P value of <0.01 was considered as the level of significance. Scheffe post hoc test was used to evaluate the significant differences between the four experimental groups.

Figure 3- MBC (Minimum Bactericidal Concentration) assessment, using Agar Dilution Method

Results:

In this study, we conducted Disk Diffusion Method on Muller Hinton Agar (MHA) standard medium to measure the inhibitory zones. The inhibitory zone diameters are presented in Table 1 which shows that the largest inhibitory zone diameter belongs to Chlorhexidine 0.2% (19 mm), followed by Chlorhexidine 0.12% (15 mm), Licorice alcoholic extract (9 mm) and Licorice aqueous extract (8.3 mm), respectively. ANOVA statistical test showed that the differences between the four inhibitory zone diameters were significant ($p<0.01$). Kolmogorov Smirnov test proved the normal distribution of data. Further experimentation with Scheffe Post hoc test showed that the data were well-distributed and were significantly different.

Table 1- The inhibitory zone diameters divided by study groups (crude disk = 6mm)

Inhibitory Zone Diameter/ Groups	Dimensions/mm	Min	Max
Chlorhexidine 0.2%	19±1.73	18	21
Chlorhexidine 0.12%	15±1.0	12	19
Licorice Alcoholic Extract (Iran Dineh)	9±0	9	9
Licorice Aqueous Extract	8.33±1.88	6	10

The MIC and MBC for each group are presented in Table 2 and the results show that the MIC of Chlorhexidine 0.2% is less than that of Chlorhexidine 0.12% and Licorice aqueous extract. The MBC of Chlorhexidine 0.2% is less than that of Chlorhexidine 0.12%, while Licorice aqueous extract shows the highest level.

The overall results of the disk inhibitory zone diameter in disk plate method revealed that Licorice aqueous root extract (100 mg/ml) has antibacterial effect on L.Acidophilus, but it's significantly less effective compared to Chlorhexidine

Table 2- The MIC and MBC of each group

Criteria/ Groups	MIC ($\frac{mg}{ml}$)	MBC ($\frac{mg}{ml}$)
Chlorhexidine 0.2%	0.0625	0.125
Chlorhexidine 0.12%	0.075	0.15
Licorice Aqueous Extract	100 > MIC > 50	100 > MBC > 50

In order to evaluate the effect of Licorice aqueous root extract on gram-negative bacteria, we conducted the MIC and MBC evaluation procedure on E. coli to control the data. The results show that the MIC of Licorice aqueous extract for L. Acidophilus and E. Coli was measured approximately 100 > X > 50 and 25 mg/ml. The MBC of Licorice aqueous root extract for L. Acidophilus and E. coli was measured approximately 100 > X > 50 and 50 mg/ml.

Table 3- Evaluation of the MIC and MBC of Licorice aqueous root extract

Criteria/ Groups	MIC ($\frac{mg}{ml}$)	MBC ($\frac{mg}{ml}$)
L. Acidophilus	50<	50<
E. Coli	25	50

Discussion:

This experimental study was conducted in laboratory environment and evaluated the effect of Licorice aqueous root extract on microorganisms such as standard L. Acidophilus (PTCC 1643) and E. Coli (PTCC 1399) in comparison with the effect of Chlorhexidine 0.12% and 0.2% and Licorice alcoholic extract (Iran Dineh Pharmaceutical Industries Complex) by using disk inhibitory zone method and evaluating the MIC/ MBC by Broth Dilution technique.0.12% and 0.2%. On the other hand, evaluation of the MIC and MBC with Broth Dilution method showed that the effective concentration of Licorice aqueous root extract that can prevent the growth or fully eradicate the lactobacilli is not in the range of the studied concentrations, but a number between 50-100 mg/ ml.

In a study by Sedighinia et al. in 2011, the effect of Licorice alcoholic extract on some microorganisms was evaluated. They concluded that Licorice alcoholic extract was effective on Streptococcus mutans, Streptococcus sanguinis, Actinomyces Viscosus and Enterococcus Faecalis. In the mentioned study, S. aureus and E. coli served as the control group. They also reported that none of the mentioned bacteria could resist against Licorice alcoholic extract and that it was more effective than Chlorhexidine 0.2%. [9] However, in the current study, 100 mg/ml concentration of Licorice aqueous extract was significantly less effective than Chlorhexidine 0.2% and 0.12%. The MIC and MBC for all the species in the study by Sedighinia et al. were about 12.5- 50 mg/ml and the inhibitory zone diameters were between 14- 26mm, which differ significantly from the results of our study. It should be noted that, the bacterial species and the extract types were different in these two studies. On the other hand, same values were obtained for Chlorhexidine in both studies. Although the extract prepared in our research was efficient, but it had less effective components compared to the extract used in the study by Sedighinia et al., which could be due to the different types of solvents used for extraction. It should also be noted that MHA 5% (sheep blood) medium was used in the mentioned study, which is not a standard culture medium. [13, 15] It is worth mentioning that the Licorice roots in the above mentioned study were collected from the same location as ours but the roots had been collected during the summer and used within 6 months. According to a study conducted by Hosseini et al. in 2014, the temperature, harvesting season, species type and the ecologic conditions could all influence the efficacy of the extract's components. [16]

In a study by Jain et al. in 2013, the efficacy of 0.03% to 30% Licorice aqueous and alcoholic root extracts was evaluated in-vivo and in-vitro on S.mutans, separated from children's saliva. The approximate MBC of Licorice aqueous and alcoholic extracts and Chlorhexidine was 0.156, 37.5 and 150mg/ml. The overall result was in line

with our research and the findings confirmed that Chlorhexidine was significantly more effective against S.mutans. In the mentioned study, the Licorice root powder was soaked in distilled water for 24 hours to attain Licorice aqueous extract. They only used Macro Tube Dilution Method to evaluate the MIC. One of the benefits of their research was the assessment of Licorice extract in-vivo by counting the number of S.mutans and assessing the pH level of children's saliva. [15]

In another study by Ajagannanavar et al. in 2014, the effectiveness of 0.09 to 50% Licorice aqueous and alcoholic root extracts against S.mutans and L.acidophilus was evaluated in comparison with Chlorhexidine 0.2% and the obtained results revealed the effectiveness of Licorice on these bacterial species. [8] The inhibitory zone diameter of lactobacilli equaled 14mm, MIC was 12.5 mg/ml and MBC equaled 25 mg/ml . Although these results concur with our findings, the obtained numbers are different. The MIC and MBC of Chlorhexidine were not evaluated and they only relied on evaluation of the inhibitory zone diameter. It should be noted that they had soaked the Licorice extract powder in distilled water or alcohol for one week before the experiment, which could influence the efficacy of the contents of Licorice root extract. Moreover, they used BHI Agar medium for inhibitory zone evaluation which is not a standard culture medium for this method.[13, 15]

In another study by Hu et al. in 2011, Licorice alcoholic extract was included in a sugar-free candy and its inhibitory effect on S.mutans was evaluated. The results revealed the significant inhibitory effect of Licorice alcoholic extract on S.mutans. They soaked the Licorice root powder in ethanol 95% for 72 hours to transform it into the alcoholic form. But the effect of Licorice extract on lactobacilli species was not evaluated. [17] A study conducted by Ahn et al. in 2012, evaluated the influence of deglycyrrhizinated Licorice aqueous and 95% alcoholic extracts on S.mutans in both planktonic and biofilm cultures. [13] Then, they evaluated the cytotoxicity of the extracts on human normal fibroblastic gingival cells. The results of their study certified the antibacterial efficacy of Licorice extract. In the mentioned study, Disk Diffusion Method was not used while the MBC was evaluated in BHI Agar medium, which is not the standard culture medium for this method. [13, 15] It should also be noted that the bacterial species and the method of extraction in the above study are different from those of our study. They used Licorice alcoholic extract and applied heat during the extraction procedure. They mixed distilled water and Licorice root powder by the ratio of 1/20 and heated it in the heating flask for 2 hours till the distilled water was evaporated. Then, they added 95% ethanol and again heated it for another 2 hours, and repeated the same procedure after adding 99% ethanol. This method of extraction was lacking the effective ingredient (glycyrrhizin), however the results do not contradict ours.

In another study by Ahn et al., the objective was set to evaluate the MIC of deglycyrrhizinated Licorice extract against eleven type strains of S.mutans and three type strains of S.sobrinus, which had been obtained from the saliva of South Korean subjects. [11] The extraction method used in this study was the same as the study which was conducted in 2012 by same researchers but the differences were appointed to evaluation of the MIC using Micro Tube Dilution method and the use of TH Broth medium (Todd Hewitt), which is not the standard culture medium for this method. [13, 15] Of course, the obtained extract was completely different from ours and the lactobacilli were not investigated.

In 2015, Ahn et al. conducted another study to separate and specify the contents of Licorice alcoholic root extract and to evaluate their MIC against S.mutans in comparison with Chlorhexidine 0.2%. [14] They declared that the antibacterial effect of each of the three effective separated substances is 2 to 4 times higher than that of the deglycyrrhizinated whole extract. Further evaluation showed that the bacteriostatic and bactericidal ability of Chlorhexidine's effective content is at least 2 times higher than that of Licorice's effective substances. Due to the side effects of glycyrrhizin including acute hypertension, hypokalemia and also increased mineralocorticoid (cortisol) by inhibiting the transformation of cortisol to cortisone in the urine, the extract used in

Ahn et al's three studies lacked glycyrrhizin.[13] They used a different extraction method and an especial method that allowed them to omit glycyrrhizin and used alcohol as a solvent. Moreover, the inhibitory zone was not measured. Micro Dilution Method was adopted for evaluation of the MIC. The MBC was assessed in BHI Agar medium. Each bacterial type was cultured in TH Broth or TH Agar media and then was sub-cultured.

These are all the differences that are not in line with our study and are not considered as the standards. [13, 15] The bacteria types in our study were also different from that of the mentioned studies.

In our research, the antibacterial activity of Licorice aqueous root extract was evaluated by the microbiology group of Shahid Beheshti University of Medical Sciences according to the standard protocol of CLSI. In addition, the bacteria type (PTCC1643) that was evaluated in this study was locally produced by the Iranian Scientific and Industrial Research Organization and every step of the experiment was repeated 3 times by the same researcher. We also performed the inhibitory zone test on Licorice alcoholic extract, which is a readymade substance in the market, in order to verify the applied extraction method. Likewise, for evaluation of the extract effectiveness on gram-negative bacteria species, which lack peptidoglycan cell walls and are less resistant compared to gram-positive bacteria,[9] we carried out the MIC and MBC tests on E. coli (PTCC1399). We concluded that Licorice aqueous root extract has antibacterial effect against L. acidophilus (gram-positive) bacteria, and even higher antibacterial effect against E. coli (gram-negative) bacteria while overall, it is less effective than Chlorhexidine.

Conducting this research on a greater range of bacterial species and using different solvents such as alcohol, ether and chloroform are highly recommended while it is best to use an extract that lacks glycyrrhizin due to its side effects such as hypertension.[13] We also recommend that separated Licorice fractions be evaluated individually. [14] Moreover, if the safety of Licorice extract can be proved on viable human cells, higher concentrations of this extract could be used to inhibit the growth of oral microorganisms.

Conclusion:

It can be concluded that Licorice aqueous root extract has antimicrobial effect against L. acidophilus, but it is less efficient in comparison with Chlorhexidine.

Acknowledgement

We are thankful to those who helped us in this article including research center of Islamic Azad University, Dental Branch of Tehran.

References:

1.Vyas YK, Bhatnagar M, Sharma K. In vitro evaluation of antibacterial activity of an herbal dentifrice against Streptococcus mutans and Lactobacillus acidophilus. Indian J Dent Res 2008;19 (1): 26-8.

2.Malhotra N, Rao SP, Acharya S, Vasudev B. Comparative in vitro evaluation of efficacy of mouthrinses against Streptococcus mutans, Lactobacilli and Candida albicans. Oral Health Prev Dent 2011; 9(3):261-8.

3.Subramaniam P, Nandan N. Effect of xylitol, sodium fluoride and triclosan containing mouth rinse on Streptococcus mutans. Contemp Clin Dent 2011; 2(4):287-90.

4.Messier C, Epifano F, Genovese S, Grenier D. Licorice and its potential beneficial effects in common oro-dental diseases. Oral Dis 2012; 18(1):32-9.

5.Villinski JR , Bergeron C, Cannistra JC, Gloer JB, Coleman CM, Ferreira D, et al. Pyrano -isoflavones from glycyrrhiza uralensis with antibacterial activity against streptococcus mutans and porphyromonas gingivalis. J Nat Prod 2014; 28;77(3):521-6.

6.Hamdon SM , Ghada Y, Rahman A. Glycyrrhiza glabara as antibacterial agent on biofilm and planktonic cell of aggregatibacter actinomycetemcomitans. Int J Dent Sci Res 2014; (2) 42-6.

7.Asl MN, Hosseinzadeh H. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother Res 2008;22(6):709-24.

8.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; 6(4):29-34.

9.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; 2(3):118-24.
10.More G, Tshikalange TE, Lall N, Botha F, Meyer JJ. Antimicrobial activity of medicinal plants against oral microorganisms. J Ethnopharmacol 2008; 28;119(3): 473-7.
11.Ahn S-J, Song YD, Mah SJ, Cho EJ, Kook JK. Determination of optimal concentration of deglycyrrhizinated licorice root extract for preventing dental caries using a bacterial model system. Journal of Dental Sciences 2014;(9):214-20.
12.Jayaprakash R, Sharma A, Moses J. Comparative evaluation of the efficacy of different concentrations of chlorhexidine mouth rinses in reducing the mutants streptococci in saliva: An in vivo study. J Indian Soc Pedod Prev Dent 2010;28(3):162-6.
13.Ahn SJ, Cho EJ, Kim HJ, Park SN, Lim YK, Kook JK. The antimicrobial effects of deglycyrrhizinated licorice root extract on Streptococcus mutans UA159 in both planktonic and biofilm cultures. Anaerobe 2012; 18(6):590-6.
14.Ahn SJ, Park SN, Lee YJ, Cho EJ, Lim YK, Li XM, et al. Invitro antimicrobial activities of 1-Methoxyficifolionol, Licorisoflavan A, and 6,8-Diprenylgenistein against streptococcus mutans. Caries Res 2015; 49(1):78-89.
15.Jain E, Pandey RK, Khanna R. Liquorice root extracts as potent cariostatic agents in pediatric practice. J Indian Soc Pedod Prev Dent 2013; 31(3):146-52.
16.Hosseini A, Shafiee-Nick R, Mousavi SH. Combination of Nigella sativa with Glycyrrhiza glabra and Zingiber officinale augments their protective effects on doxorubicin-induced toxicity in H9c2 cells. Iran J Basic Med Sci 2014; 17(12):993-1000.
17.Hu CH, He J, Eckert R, Wu XY, Li LN, Tian Y, et al. Development and evaluation of a safe and effective sugar-free herbal lollipop that kills cavity-causing bacteria. Int J Oral Sci 2011; 3(1): 13-20.