Original Article

# Evaluation of the Effect of Four Herbal Extracts on Growth of Streptococcus mutans and Lactobacillus


## N Moezzi ghadim[1], M Taghibakhsh[1*], H Godarzi[2], N Liravinezhad hoseini[3], S Alirezaei[1]

[2]Professor medical sciences , department of microbiology, shahid beheshti university of medical sciences, tehran, iran
[3]Dentist


ARTICLE INFO


*Article History*
*Received: Jan 2018*
*Accepted: Feb 2018*
*ePublished: Mar 2018*



**Corresponding author:**
M Taghibakhsh
Professor medical sciences , department of microbiology, shahid beheshti university of medical sciences, tehran, iran
mehrdad.taghibakhsh@yahoo.com



ABSTRACT

**Background and aim:** Reduction of oral pathogens is very important in the healing of oral ulcers and infections. The side effects of chemical drugs have attracted more attention to medicinal plants. The present study assessed the effect of clove, thyme, garlic, and cinnamon ethanolic extracts on the growth of Streptococcus mutans (S. mutans) and Lactobacillus by measuring the inhibition zone diameter.

**Materials and methods:** In this in-vitro experimental study, suspensions of standard strains of S. mutans (PTCC5027) and Lactobacillus (PTCC1608) were inoculated on Müller-Hinton agar using a sterile swab. 10 g of crushed and dried plants was dissolved in 100 ml of ethanol, and an ethanolic extract was obtained after passing through a filter and evaporation. 10 µl of each extract was poured onto blank discs which were placed on plates containing bacteria. A disc containing the control groups, including 0.2% chlorhexidine and a blank disc, was also placed on the plates. The plates were placed in a jar for 24 hours at 37°C and then the inhibition zone diameter around each disc was measured. Ten replicates were performed for each herbal extract. Data were analyzed by analysis of variance (ANOVA) and post hoc tests.

**Results:** All four ethanolic herbal extracts were able to inhibit the growth of S. mutans and Lactobacilli. For both bacteria, the largest inhibition zone was observed with chlorhexidine, followed by clove, thyme, garlic, and cinnamon, respectively. There was a significant difference between the inhibition zone diameters formed by the extracts ($P<0.01$).

**Conclusion:** Ethanolic extract of clove showed more prominent antibacterial effects than ethanolic extracts of thyme, garlic, and cinnamon.



**Keywords:**
*Clove Oil, Thyme, Garlic, Cinnamon, Chlorhexidine, Streptococcus mutans, Lactobacillus*

**J Res Dent Maxillofac Sci .(2018;Vol2,No.3)**


Original Article

## Introduction:

There are about 500 microbial species in the mouth, some of which are responsible for the development of oral infectious diseases. Reduction of oral pathogenic microorganisms is very important in the healing of oral ulcers and infections.[1,2] Dental caries is the most common chronic disease in the world, and its main etiologic agents are Streptococcus mutans (S. mutans) and Lactobacilli. Symptomatic and regenerative treatments will fail if the cause of the disease is disregarded. [3] Mutans streptococci also play a role in the development of other diseases, such as angular cheilitis and parotid glandular inflammation.[4] Also, Lactobacilli are effective in the development of gingival diseases.[5]

Daily use of mouthwashes in combination with tooth brushing and regular flossing effectively reduces the microbial population of the mouth and ultimately prevents gingival diseases and dental caries and accelerates wound healing.[6] Chlorhexidine is the most effective chemical antimicrobial mouthwash approved by the Food and Drug Administration (FDA) and the American Dental Association (ADA), which is used as a caries inhibitor and to control gingivitis and is recognized as a golden standard for controlling the microbial plaque activity.[6-8] However, this mouthwash has several side effects such as dental discoloration, dysgeusia, burning of oral mucosa, allergies, xerostomia, and adverse systemic effects when swallowed.[7,9]

Recent studies have clearly shown that chemical drugs have adverse effects in addition to their beneficial effects.[10-12] The side effects of chemical drugs, increased drug resistance, high drug costs, environmental contamination by the pharmaceutical industry, and the human disability in the manufacture of drugs from plants have attracted more attention to medicinal herbs. [10-12] The Iranian traditional medicine is one of the old fundaments of medicine and contains valuable information on the use of herbs in the treatment of diseases. Maintenance of oral and dental health has also been addressed in the Iranian traditional medicine, and a chapter has been devoted to oral and dental diseases in the related books.[13] Most previous studies have been conducted on specific pathogens that affect the cutaneous, respiratory, digestive, and urinary systems, and few studies have been done on oral mucosal pathogens.[11] Therefore, in this study, the effect of ethanolic extracts of thyme, garlic, clove, and cinnamon on the growth rate of S. mutans and Lactobacilli was assessed, in comparison with 0.2% chlorhexidine, by measuring the inhibition zone diameter at the microbiology laboratory of Shahid Beheshti University of Medical Sciences in 2017.

## Materials and Methods:

The research was carried out experimentally. In this study, standard strains of S. mutans (PTCC5027) and Lactobacillus (PTCC1608) were obtained from the Iranian Research Organization for Science and Technology. To confirm the identity of microorganisms, diagnostic tests were used. The bacteria were then stored in Müller-Hinton agar culture medium at -70°C. To prepare bacterial suspensions, 0.5 McFarland concentration was used. Using a sterile swab, we inoculated some of the bacterial suspensions on Müller-Hinton agar culture medium.

The herbs were obtained and then extraction was performed by soaking the herbs. We dried thyme, clove, garlic, and cinnamon plants in the shade at room temperature (25°C) and powdered or chopped the plants using a mill. 100 ml of ethanol was mixed with 10 g of each dried plant such that the whole powder was covered and soaked. After 48 to 72 hours, we filtered the solution using a Whatman no.1 filter paper. The filtered solutions were sterilized using a 0.45-μm membrane filter. These solutions were then evaporated in a vacuum evaporator under pressure reduction conditions. The dried extracts were stored at -20°C.[14]

After preparing the herbal extracts, we dissolved 100 mg of the extract in 1 cc of 2% dimethyl sulfoxide (DMSO) to obtain the stock solution. The amount of 10 μl of each extract was poured onto the prepared blank discs. We waited a few minutes so that the extract on the

**Table 1.Diameter (mm) of inhibition zone according to the type of microorganisms and categorized by groups**

| Group \ Inhibition zone | Streptococcus mutans | | Lactobacillus | |
|---|---|---|---|---|
| | Diameter (mm) | Variation coefficient | Diameter (mm) | Variation coefficient |
| 0.2% chlorhexidine | 22±1 | 4.5 | 19.6±2 | 10.2 |
| Clove ethanolic extract | 17.3±1.3 | 7.5 | 16±1.2 | 7.5 |
| Thyme ethanolic extract | 15.4±1 | 6.5 | 14.7±0.6 | 4.1 |
| Garlic ethanolic extract | 13.1±0.7 | 5.3 | 13.8±0.9 | 6.5 |
| Cinnamon ethanolic extract | 12.2±0.75 | 6 | 12.7±0.5 | 3.9 |
| Test result | P<0.0001 | | P<0.0001 | |

disc was absorbed and then placed the discs on plates containing S. mutans and Lactobacillus bacteria. A disc containing the control groups, including 0.2% chlorhexidine (Iran Daru Pharmaceutical Co., Tehran, Iran) and a blank disc, was also placed on the plates. The plates were placed in a jar for 24 hours at 37°C and then the diameter of the inhibition zone around each disc was measured in millimeters (mm) using a ruler.

In previous studies, the number of samples in each group was at least 1 to a maximum of 10 repetitions. In this research, based on the maximum, 10 replicates and according to 6 groups for each bacterium, 120 samples were examined. In this study, intervening variables such as the concentration of ethanolic extracts, amount of ethanolic extracts, contact time of herbal extracts and microorganisms, microorganism strain, and work environment temperature were matched. Data were analyzed by analysis of variance (ANOVA) and post hoc tests.

## Result:

In the present study, the effect of ethanolic extracts of thyme, cinnamon, clove, and garlic on the growth rate of S. mutans and Lactobacillus microorganisms, in comparison with 0.2% chlorhexidine, was assessed by measuring the inhibition zone diameter with 10 samples per group and a total of 120 samples. The diameters of the inhibition zones according to the type of microorganisms and categorized by medicinal plants are presented in Table 1.

A) S. mutans:

The data showed that the largest inhibition zone diameter was observed with chlorhexidine (22±1 mm), followed by clove (17.3±1.3 mm), thyme (15.4±1 mm), garlic (13.1±0.7 mm), and finally cinnamon (12.2±0.75 mm; Figure 1).

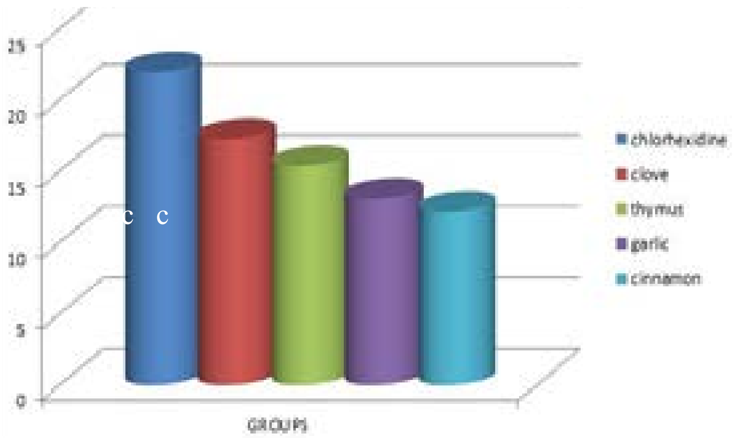


**Figure 1:Inhibition zone diameter (mm) of Streptococcus mutans (S. mutans) divided by the studied groups**

ANOVA showed that the difference in the inhibition zone diameter in the five groups is statistically significant (P<0.0001).

Pairwise comparisons by post hoc tests also showed significant differences (P<0.001).

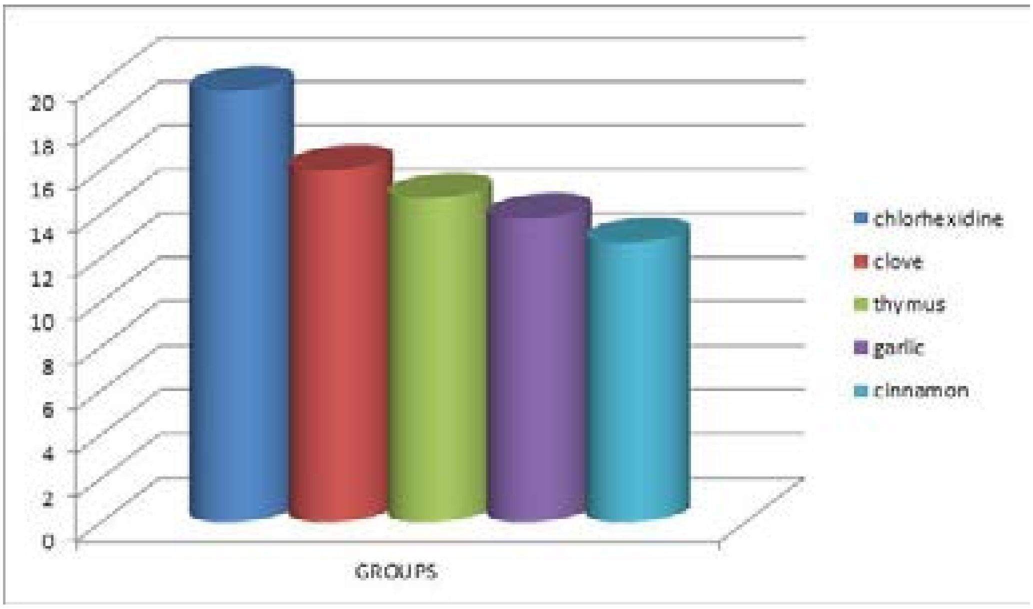


**Figure 2: Inhibition zone diameter (mm) of Lactobacillus divided by the studied groups**

B) Lactobacillus:

The data showed that the largest inhibition zone diameter was observed with chlorhexidine (19.6±2 mm), followed by clove (16±1.2 mm), thyme (14.7±0.6 mm), garlic (13.8±0.9 mm), and finally cinnamon (12.7±0.5 mm; Figure 2). ANOVA showed that the difference in the inhibition zone diameter in the five groups is statistically significant (P<0.0001).

Pairwise comparisons by post hoc tests also showed significant differences (P<0.001).

## Discussion:

In the present study, the effect of ethanolic extracts of clove, thyme, garlic, and cinnamon on the growth rate of S. mutans (PTCC5027) and Lactobacillus (PTCC1608) microorganisms was assessed, in comparison with 0.2% chlorhexidine, by measuring the inhibition zone around discs containing the herbal extracts; we tested 10 samples per group and a total of 120 specimens. The data showed that all four ethanolic herbal extracts were able to inhibit the growth of S. mutans and Lactobacillus. For both bacteria, the largest inhibition zone diameter was observed with chlorhexidine, followed by clove, thyme, garlic, and finally cinnamon. ANOVA and post hoc tests also showed that there is a significant difference in the diameter of the inhibition zone formed by these extracts (P<0.001).

In a study by Hiregoudar et al, the antibacterial activity of ethanolic extracts of clove, turmeric, and ginger was evaluated using zone of inhibition (Z.O.I) and minimum inhibitory concentration (MIC) measurements against S. mutans under laboratory conditions.[15] Their results showed that all the extracts exhibited antimicrobial activity against this bacterium. In both methods, ethanolic extract of clove exhibited the most prominent antibacterial property, followed by turmeric and ginger (P<0.001). (15) These results were similar to those of the present study.

In a study by Mirpour et al, the antibacterial effects of ethanolic and methanolic extracts of clove and gallnut on S. mutans and S. salivarius were assessed.[16] The antimicrobial activity of the extracts was evaluated by measuring the diameter of the Z.O.I and by measuring MIC and minimum bactericidal concentration (MBC). In the Z.O.I method, methanolic and ethanolic extracts of clove showed more significant antibacterial effects than ethanolic and methanolic extracts of gallnut;[16] these results are similar to ours. In the MIC and MBC methods, the antibacterial activity of methanolic extract of clove was higher than that of its ethanolic extract, and the antibacterial activity of ethanolic extract of gallnut was higher than that of its methanolic extract. Methanolic extract of clove showed the best rates of MIC and MBC against both bacteria.[16]

In a study by Soltan Dallal et al, the antimicrobial effect of Shirazi thyme (Zataria multiflora) essential oil on antibiotic-resistant Staphylococcus aureus (S. aureus) strains was evaluated using the MIC and MBC methods.[17] The results showed that the essential oil of Shirazi thyme has good effects on tetracycline-, erythromycin-, trimethoprim-sulfamethoxazole-, and methicillin-resistant S. aureus (MRSA).[17]

Fani et al assessed the inhibitory effect of aqueous garlic extract on multidrug-resistant S. mutans species using disc sensitivity and broth dilution methods. (18) Of the 92 groups of S. mutans, 28 groups (30.4%) showed multidrug resistance, that is, they were resistant to four antibiotics or more. The MIC of chlorhexidine for multidrug-resistant and drug-sensitive S. mutans was 2-16 µg/ml and 0.5-1 µg/ml, respectively. All S. mutans groups showed sensitivity to garlic extract with a MIC of 4-32 µg/ml.[18]

Soleimani et al evaluated the antibacterial effect of Cinnamomum verum and Ferula gummosa essential oils on some gram-positive and gram-negative bacteria.[19] To evaluate the antimicrobial activity of the essential oils, the well-diffusion method was used and the MIC was determined on several standard bacterial strains. In both methods, cinnamon essential oil showed higher antibacterial effects than Ferula gummosa. Based on the results of agar well-diffusion method, the largest inhibition zone diameter by cinnamon was observed against Staphylococcus saprophyticus (47 mm), and the largest inhibition zone diameter by Ferula gummosa was detected against Staphylococcus epidermidis (33 mm). The MIC results showed that cinnamon essential oil has the highest inhibitory effect against Staphylococcus saprophyticus.[19]

In studies by Soltan Dallal et al[17], Fani et al [18], and Soleimani et al [19], the antibacterial effects of thyme, garlic, and cinnamon have been shown, which have also been confirmed by the present study, but more plant extracts were used in the present study, and the antibacterial strength of these extracts was evaluated in comparison with each other, indicating that clove has a higher antibacterial activity than thyme, thyme has a higher antibacterial activity compared to garlic, and garlic has a higher antibacterial activity compared to cinnamon, which is one of the strengths of the current study.

In a study by Haghighati et al, the antimicrobial effects of methanolic extracts of ten medicinal plants (Thymus vulgaris, Syzygium aromaticum, Lavandula angustifolia, Punica granatum, Rosmarinus officinalis, Herate umpersicum, Punica granatum (flower), Quercus infectoria, Terminalia chebula, and Melissa officinalis) on Actinobacillus actinomycetemcomitans (Aa), S. mutans, and Candida albicans (C. albicans) were examined in comparison with chlorhexidine using the well-diffusion method and Z.O.I measurement method.[20] Microbial specimens were obtained from patients with advanced periodontitis and oral candidiasis. Their results showed that Thymus vulgaris, Syzygium aromaticum, Quercus infectoria, Punica granatum, and Terminalia chebula have significant antibacterial and antifungal effects. Other extracts showed no antimicrobial activity. Syzygium extract showed the highest effect on C. albicans (21.7 mm) and Aa (22.5 mm). Terminalia chebula and Thymus vulgaris showed the highest effect on S. mutans (15 and 13.5 mm; $P<0.01$).[20] The results of the cited study are in contradiction with the present study, which can be due to differences in the type of extract, the methodology, and S. mutans strain.

Clove, thyme, garlic, and cinnamon ethanolic extracts were selected in the current study and their antibacterial effects were compared since their superior antibacterial and antifungal effects have been shown by previous studies. [15-20]

From the positive aspects of our study, acceptable microbiologic methodology, a relatively high sample size, use of four herbal extracts (clove, thyme, garlic, and cinnamon), use of chlorhexidine as the control group, and assessment of the antibacterial effect of the extracts on Lactobacillus, which has been limitedly taken into consideration by other studies and is one of the most important cariogenic bacteria, can be mentioned.

In this study, standard strains of bacteria were used in vitro; nevertheless, these herbal extracts may show different antibacterial effects in the oral cavity due to the association of S. mutans and Lactobacillus with other microorganisms. Therefore, it is suggested that future studies examine the antimicrobial effect of these herbal extracts in the oral environment.

Research on clove suggests that its chemical compounds include eugenol and eugenol acetate, flavonoids, saponins, tannins, triterpenoids, and steroids such as phytosterol. This plant has antinociceptive, antifungal, and antibacterial properties; eugenol is its effective ingredient. Aqueous and ethanolic extracts of clove have antimicrobial effects against S. mutans and S. salivarius and antifungal effects against C. albicans.(14,20) Thyme also has antibacterial properties due to its phenolic compounds. These phenolic substances include carvacrol, thymol, and eugenol. Thymol, which has a fragrant smell and spicy taste, is an antibacterial and antifungal agent. The analgesic, anti-inflammatory, antibacterial, and antifungal properties of these two herbs have been of practical value.(21)

## Conclusion:

Ethanolic extracts of clove, thyme, garlic, and cinnamon showed antibacterial effects against standard strains of S. mutans (PTCC5027) and Lactobacillus (PTCC1608). Ethanolic extract of clove has more prominent antibacterial effects than ethanolic extracts of thyme, garlic, and cinnamon.

## Aknowledgement:

This article is based on general dentistry thesis No.2313 registered at the dental branch of Islamic Azad University of Tehran.

## References:

1. Rajabalian S, Mohammadi M, Mozaffari B. Cytotoxicity evaluation of Persica mouthwash on cultured human and mouse cell lines in the presence and absence of fetal calf serum. Indian J Dent Res. 2009 Apr-Jun;20(2):169-73.
2. Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A. Antifungal properties of Salvadorapersica and Juglans regiaL .extracts against oral Candida Strains. Eur J Clin Microbiol Infect Dis. 2010 Jan;29(1):81-8.
3. Slayton RL. Clinical Decision-making for Caries Management in Children: An Update. Pediatr Dent 2015;37(2):106-10.
4. Smith AJ, Jackson MS, Bagg J. The ecology of Staphylococcus species in the oral cavity. J Med Microbiol. 2001 Nov;50(11):940-6.
5. Dal Bello F, hertel C. Oral cavity as natural reservoir for intestinal lactobacilli. Syst Appl Microbiol. 2006 Jan;29(1):69-76.
6. Haffajee AD, Yaskell T, Socransky SS. Antimicrobial effectiveness of an herbal mouthrinse compared with an essential oil and a chlorhexidine mouthrinse. J Am Dent Assoc. 2008 May;139(5):606-11.
7. Fallahzadeh H, Moeintaghavi A, Foruzanmehr M. Clinical comparison of Persica and Chlorhexidine mouthrinses using meta – analysis technique. The J Islamic Dent Assoc. 2006 Spring;18(1):62-72.
8. Santos A. Evidence-based control of plaque and gingivitis. J Clin Periodontol. 2003;30 Suppl 5:13-6.
9. Paknejad M, Jaffarzadeh Kashi TAS, Shamlou AM. Comparison of the efficacy of Matrica and % 0.2 Chlorhexidine mouthwashes in patients with choronic periodontitis. J Islamic Dent Assoc 2006;18(3):92-7.
10. Khosravinia S, Ziaratnia SM, Bagheri A, Marashi SH. Investigation of antibacterial effects of Cell suspension culture and comparison by essential oils and seed extract in Buniumericum. J RIFST.2013;2(1):79-92.
11. Ruddock PS, Liao M, Foster BC, Lawson L, Arnason JT, Dillon JA. Garlic natural Health Products Exhibit Variable Constituent Levels And Antimicrobial Activity Against Neisseria Gonorrhoeae, Staphilococcus Aureus and Enterococcus Faecalis. Phytother Res. 2005 Apr;19(4):327-34.
12. Sakhanda IV, Kosyachenko KL. Assortment of herbal medicines of the treatment of cardiovascular diseases. Wiad Lek. 2018;71(5):1104-1108.
13. Safari MR, Azizi O, Heidary SS, Kheiripour N, Ravan AP. Antiglycation and antioxidant activity of four Iranian medical plant extracts. J

Pharmacopuncture. 2018 Jun;21(2):82-89

14. Borijian Borjeni S, Baba heydari KE, Mortezaei S, Karimian M, Shirzad M, Validi M. The Antibacterial Effects of the Hydroalcoholic Extracts of Aloe Vera and Glycyrrhiza Glabra against Cariogenic Bacteria In vitro. JBUMS. 2016; 18 (4) :14-20.

15. Hiregoudar M, Subramaniam R, Mittal S, Muralikrishna KS, Sakeenabi B, Prashant GM, et al. Antibacterial activity of some spice extracts on Streptococcous mutans: An invitro study. J Indian Assoc Public Health Dent. 2011;9(5):347-51.

16. MirpourM, Gholizadeh Siahmazgi Z, Sharifi kiasaraie M. Antibacterial activity of clove, gall nut methanolic and ethanolic extracts on Streptococcus mutans PTCC 1683 and Streptococcus salivarius PTCC 1448. J Oral Biol Craniofac Res. 2015 Jan-Apr;5(1):7-10.

17.Soltan Dallal MM, Bayat M, Yazdi MH, Aghaamiri S, Ghorbanzade Meshkani M, Abedi Mohtaseb TP, et al. Antimicrobial effect of Zataria multiflora on antibiotic-resistant Staphylococcus aureus strains isolated from food. SJKU. 2012;17(2):21-9.

18.Fani MM, Kohanteb J, Meshki R, Shahine E, Sobhanamayan F, Dayaghi M. Inhibitory effect of aqueous garlic (Allium sativum) extract on multidrug-resistant Streptococcus mutans species: an in vitro study. J Isfahan Dent Sch. 2011 Winter;6(4):348-56.

19.Soleimani N, Ebraze N. Evaluate anti-bacterial effects of Cinnamomum verum and Ferula gummosa essential oil on some pathogen gram positive and negative bacteria. NCMBJ. 2016 Summer;6(23):87-93.

20.Haghighati F, Jafari S, Beitollahi J. Comparison of antimicrobial effects of ten herbal extracts with chlorhexidine on three different oral pathogens; an in vitro study. Hakim Res J. 2003 Fall;6(3):71-6.

21.Nouri F, Beitollahi J, Mansorian A. Comparing antifungal effects of Zatariamultiflora and Punicagranatum extract with Nystatin on Candida Albicans. J Qazvin Univ Med Sci. 2016;20(5):19-25.

**Please cite this paper as: Moezzi ghadim N, Taghibakhsh M, Godarzi H, Liravinezhad hoseini N, Alirezaei S. Evaluation of the Effect of Four Herbal Extracts on Growth of Streptococcus mutans and Lactobacillus. J Res Dentomaxillofac Sci. 2018;3(2):7-13.**