Antibacterial Effect of Two Herbal Extracts on the Level of Salivary *Streptococcus mutans* in Children

Fatemeh Sadat Sajadi[1], Sogand Farrokhi[2], Maryam Sharifi[3], Fereshteh Saffari[4], Gholamreza Sepehri[5]

[1, 2] Oral and Dental Diseases Research Center, Kerman University of Medical Sciences, Kerman, Iran. [3]Department of Paediatric Dentistry, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran. [4]Department of Microbiology, Virology and Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran. [5]Department of Physiology and Pharmacology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.

ABSTRACT

BACKGROUND

Streptococcus mutans is the first bacterial pathogen seen in early childhood caries (ECC). The use of plant-derived compounds has seen an increase as anti-microbial agents. The purpose of this study was to compare the antibacterial effect of thyme and chamomile extracts with chlorhexidine on salivary *S. mutans* in children with ECC.

METHODS

In this clinical trial, 90 children aged 4 - 6 years were randomly assigned to three groups of 30. Each of the groups received one of the compounds of 2 % chlorhexidine gel and 5 % methanolic extracts of chamomile and thyme. Saliva samples were collected before intervention, 30 minutes and one week after intervention, and transferred to the laboratory for counting of *S. mutans* using quantitative real time polymerase chain reaction (PCR). Data was analysed by Statistical Package for Social Sciences (SPSS) 22 software using Shapiro-Wilk, Friedman, chi-square, paired-sample t-test, repeated measures ANOVA and Mann-Whitney tests at a significance level of 0.05.

RESULTS

The results demonstrated that all three compounds of thyme, chamomile and chlorhexidine significantly reduced the *S. mutans* level at 30 minutes after administration compared to baseline (P-value < 0.05). In all three compounds, the count of *S. mutans* had an increasing trend after one week, although its count was still lower than the baseline (P-value > 0.05). The lowest rate of elevation in the *S. mutans* level after one week was related to chamomile, thyme and chlorhexidine, respectively.

CONCLUSIONS

The 5 % methanolic extracts of thyme and chamomile, like chlorhexidine, significantly decreased salivary *S. mutans* count.

KEY WORDS

Chlorhexidine, Chamomile, Thyme, *Streptococcus mutans*, Quantitative Real Time PCR, Early Childhood Caries (ECC)

Corresponding Author:
Fatemeh Sadat Sajadi,
Department of Paediatric Dentistry,
Faculty of Dentistry,
Kerman University of Medical Sciences,
Kerman, Iran.
E-mail: fsajadi1234@gmail.com

DOI: 10.14260/jemds/2021/66

How to Cite This Article:
Sajadi FS, Farrokhi S, Sharifi M, et al. Antibacterial effect of two herbal extracts on the level of salivary streptococcus mutans in children. J Evolution Med Dent Sci 2021;10(05):299-304, DOI: 10.14260/jemds/2021/66

Submission 31-08-2020,
Peer Review 01-12-2020,
Acceptance 08-12-2020,
Published 01-02-2021.

BACKGROUND

Dental caries is a multifactorial infectious disease in which nutrition, microbial agents and host responses play an important role and are the most common orodental disease around the world.[1,2] Pain caused by untreated tooth decay in children can affect school activity, nutrition, speech and subsequent growth and development.[3]

Streptococcus mutans is an anaerobic gram-positive bacterium belonging to the normal flora of human oral cavity. This bacterium is the most important cause of dental caries.[4] *S. mutans* is known to be the primary initiator of dental caries due to the ability to adhere to the tooth surface, producing high amounts of acid and the ability to survive and metabolism in acidic pH.[1,2]

Since dental caries is a localized condition, the use of preventive factors in dental caries is more effective than systemic factors.[5] The localized effect means the contact of the active ingredient with dental surfaces in a concentration above the drinking water fluoride, which has a localized effect and should not be swallowed.[6] Antibacterial compounds reduce the dental plaque by inhibiting the colonization, growth and metabolism of bacteria, and prevent the development of dental caries.[7,8]

The chlorhexidine, an antibacterial compound, possesses cationic and effective factors on *S. mutans*. This compound is available in various types of mouthwashes, gels and varnishes in the market. Although chlorhexidine has been shown to significantly decrease the count of salivary *S. mutans*, there is still not enough evidence to prevent caries.[9,10]

The main clinical problem with the use of chlorhexidine is that its effect on inhibiting the growth of the *S. mutans* bacteria does not exist for a long time and the bacteria are re-colonized on the dental surfaces after a short time and they reach the initial count before the application. The most common side effect of chlorhexidine is the yellow-brown discoloration of the teeth, which occurs with prolonged use of mouthwash. The chlorhexidine triggers a taste sensation that persists for several hours and also has a very unpleasant taste.[9,10]

In addition to chlorhexidine, other antibacterial compounds have been introduced to control oral bacteria and among them the plant-derived compounds are more popular due to availability.

The thyme is an aromatic and medicinal plant, in which two species of wild thyme (*T. serpyllum*) and a typical thyme (*T. vulgaris*) are interested in trading versus other species.[11,12]

Thyme belongs to the family Lamiaceae, one of the most famous genus of aromatic plants.[13] This plant is one of the most important medicinal plants in Iran, which has anti-bacterial and anti-flatulence properties due to the presence of thymol and carvacrol.[14]

There are over 30 types of *T. vulgaris* essential oils, due to diverse monoterpenes, which lead to the preparation of various chemical compounds of a plant species that allows the extraction of various types of essential oil.[15] According to Yamazaki et al., the thyme contains a significant amount of essential oils, and has antibacterial effects due to the presence of phenolic groups, carvacrol and thymol.[16] Gonçalves et al. investigated the effect of thyme essential oil on *S. mutans* growth and found that the thyme had the greatest effect on *S. mutans* compared to triclosan and chlorhexidine.[17] Herman and colleague also concluded that the essential oil and ethanol extract of thyme had the strongest inhibitory effect on all microorganisms isolated from the oral cavity.[18]

Chamomile from a family Asteraceae is an annual plant with a fragrance. This plant grows wildly around the fields and gardens of the central and southern regions of Iran.[19] The chamomile contains various compounds, including chamazulene, flavonoids and mucilage, which develop anti-inflammatory, antispasmodic, antimicrobial, antifungal, soothing, anti-peptic ulcers and wound healing activities.[20] Pourabbas et al. indicated that chamomile mouthwash after two and four weeks can significantly reduce bacterial plaque.[21] Adil Ahmed et al. also found that chamomile tea is effective in reducing plaque and saliva pH, and leads to a decrease in the count of salivary *S. mutans* in short time.[22]

There are different methods for identifying salivary *S. mutans*, and one of the most commonly used methods is the culture of this bacterium in a blood agar medium, so that bacteria are counted and expressed as CFU / ml. This technique is time-consuming, and has low sensitivity in identification. PCR is a method used to identify the oral bacteria due to high sensitivity and specificity.[23] For more accurate and further use of the PCR technique in identification, there have been some changes in the method, one of which is the quantitative real time PCR (qPCR), which is based on fluorescence. Some of the benefits of qPCR are sensitivity, accuracy and speed in obtaining results.[24,25]

Although the chlorhexidine is a known antibacterial compound, its side effects prevent it from being used extensively in the prevention of caries, especially in children. On the other hand, many people around the world have turned to plant products for the treatment and prophylaxis of many diseases. Therefore, this study was conducted to investigate the antibacterial effect of thyme and chamomile on salivary *S. mutans* count in children with ECC compared to chlorhexidine.

METHODS

In this clinical trial study, 90 pre-schoolers (4 - 6 years old) were selected as research units. This project was initially approved by the ethics committee of the Kerman University of Medical Sciences with the code of ethics of IR.KMU.REC.1395.553, and was then registered in Iranian Registry of Clinical Trials (IRCT) with the code of IRCT20171020036896N4. Inclusion criteria, children with good physical health were considered. Children with no history of any systemic disease, no history of taking antibiotic, xylitol-containing chewing gum and any fluoride-containing mouthwash or gel for at least 3 - 4 weeks before the study, and the absence of any prosthetic and orthodontics appliances, soft tissue lesions and active and severe periodontal diseases were included.[9,10]

Each of the three ingredients thyme, chamomile, and chlorhexidine was prepared in the same manner as the gel. To prepare 5 % methanolic extracts of thyme and chamomile, the fresh leaves and flowers of these two plants (cultivated in Iran) were purchased and their physical and plant properties were confirmed by pharmacognosist. The dried leaves and flowers of the two plants were first crushed to make smaller pieces. Then 100 g of each plant species was soaked in 500 ml of methanol for 48 hours. The resulting solution was passed through the filter, and placed inside the plates at room

temperature for 3 to 4 days. Then, the crystalline powder was scratched from the plate and added to the Carbopol 934 based gel, which has an oral administration. The 5 % methanolic gel was then obtained and kept at a temperature between 0 and 4° C.[26] After preparation, the gel was put in the same tube and the tube was named as A (thyme), B (chamomile) and C (chlorhexidine). Neither the participants nor the executive team members knew who was receiving a particular gel.

At first, informed consent was obtained from parents or legally authorized representative of participants, orodental examinations were performed for each child, and index decayed, missing and filled teeth (DMFT) was recorded. Oral hygiene and brushing techniques were taught to all parents, and they were asked to follow their child's oral hygiene with fluoride-free toothpaste for one week, as a washout period,[27] 0.5 - 1 cc of unstimulated saliva sample was collected from each child and transferred to the laboratory for counting *S. mutans*. In the next step, their mouth was washed with water, and dried with air. Then, the teeth were isolated by cotton roll, and 1 cc from one of the three studied compounds was randomly swabbed onto all teeth of the child. After 5 minutes, the child was asked to completely drain the saliva. Under the supervision of the researcher, for half an hour, the child was asked not to eat and drink. After 30 minutes, other unstimulated saliva sample was prepared again according to previous manner and re-sent to the laboratory.

At second step, a block randomized sequence was prepared by Minitab software version 19. The block length was considered as 9 and the participants were allocated to the groups. This process was done by a third person who didn't belong to the research group. In the third step, the parents were asked to observe their child's oral hygiene by trained methods for one week. A week later, an unstimulated saliva sample was re-collected from all the children. In the laboratory, *S. mutans*. level was determined by qPCR method. First, the frozen samples were melted by placing at 37° C, and 300 µl was taken from each sample of saliva, and the DNA was extracted using a DNA extraction kit (RIBO-prep) according to the manufacturer's protocol. In this study, standard strain of *S. mutans* (ATCC 35668) was prepared from the centre for the collection of industrial bacteria and fungi of Iranian research organization for science and technology (IROST). After extracting DNA from saliva and standard strain samples, the bacteria were identified and counted using qPCR and the Applied Biosystem StepOnePlus Real-Time PCR. In order to count bacteria, serial dilutions of 10 were made from genomic DNA obtained from standard strain (at a concentration of 9 ng / µL), which were used as standard for the preparation of a standard curve. The glucosyltransferase (gtfB) gene coding in *S. mutans* was used to identify this bacterium in saliva samples.

The sample size was calculated by G*power software for repeated measure test. The effect size, power correlation between repeated measures and type one error was considered 0.35, 0.8, 0.8 and 0.05, respectively. The sample size for each group was calculated at 30.

Data were analysed by SPSS 22 software using Shapiro-Wilks, Friedman, Mann–Whitney, paired and independent t test and repeated measure analysis at a significance level of 0.05. Data was described using descriptive statistics of mean and standard deviation.

RESULTS

Compounds	Before Administration	Half an Hour after Administration	A Week after Administration
Chlorhexidine median (IQR)	35.02 (13.04)	11.91 (17.12)	27.01 (8.14)
Thyme mean (SD)	38.43 (7.34)	22.28 (8.31)	30.93 (11.80)
Chamomile median (IQR)	30.15 (9.12)	28.20 (12.13)	38.01 (8.18)

Table 1. Mean Salivary S. mutans Count after Applying Three Compounds in Terms of Time Interval

Compounds	Statistics	P-Value
Chlorhexidine[1]	26.54	< 0.001*
Thyme[2]	19.83	*< 0.001
Chamomile[1]	9.61	*0.008

Table 2. Difference in Salivary S. mutans Count after Administration of Compounds at Three Different Times

[1] calculated by Friedman test
[2]calculated by repeated measure analysis.

Compounds	Comparison of Administration Time by Paired t-Test and Mann-Whitney (P-Value)		
	Before Administration - Half an Hour after Administration	Before Administration - a Week after Administration	Half an Hour after Administration - a Week after Administration
Chlorhexidine[1]	< 0.001*	0.10	0.008*
Thyme[2]	< 0.001*	0.07	0.005*
Chamomile[1]	0.01*	0.67	0.26

Table 3. Comparison of Salivary S. mutans Count after Administration of Three Compounds at Three Different Times

[1] Mann–Whitney,
[2] paired t-test

Time Intervals	Compounds Comparison	P–Value
Half an Hour after Administration	Chlorhexidine-Chamomile[1]	0.02*
	Chlorhexidine-Thyme[1]	0.06
	Chamomile-Thyme[1]	0.21
A Week after Administration	Chlorhexidine-Chamomile[1]	0.58
	Chlorhexidine-Thyme[1]	0.56
	Chamomile-Thyme[1]	0.45

Table 4. Comparison of Salivary S. mutans Count after Administration of Three Compounds in Terms of Different Compounds

[1] Mann - Whitney test

Table 1 shows the mean salivary *S. mutans* count after applying three compounds in terms of study time intervals.

The Shapiro-Wilk test showed that the data from chlorhexidine and chamomile did not follow the normal distribution at three different times. Therefore, the mean difference in three different times was evaluated by the Friedman test as a nonparametric version of repeated measure. The results revealed a significant difference in the mean salivary *S. mutans* count after administration of chlorhexidine (P–value = 0.001) and chamomile (P–value = 0.008) between three different times (Table 2).

The Shapiro-Wilk test showed that the data from thyme follow the normal distribution at three different times. To do this, the mean difference in three different times was evaluated by repeated measures of ANOVA. The results indicated a significant difference in the mean salivary *S. mutans* count after applying thyme (P-value < 0.001) between three different times (Table 2).

The paired comparison (parametric and non-parametric) was used to compare salivary *S. mutans* count after

administration of chlorhexidine, thyme and chamomile at three different times (Table 3). In table 4, the Mann-Whitney test was used to compare the salivary *S. mutans* count after applying three different compounds as paired comparison at different times. The results indicated that there was a significant difference only between the mean salivary *S. mutans* count after administration of chlorhexidine and chamomile and at half an hour after administration at 0.05 level.

DISCUSSION

The ECC is the most common chronic infectious disease in childhood today. Acidogenic and acid-tolerant *S. mutans* species have been identified as the first pathogen in the ECC.[28] Clarke JK et al. in 1924 reported for the first time the role of *S. mutans* in the aetiology of dental caries.[29]

The results of the present study showed that the administration of three compounds of 2 % chlorhexidine gel and 5 % methanolic extracts of thyme and chamomile resulted in a significant reduction in the mean salivary *S. mutans* count in 30 minutes after administration. Although this reduction in bacterial count for chlorhexidine was higher than the rest of the agents, it was observed with a lower but acceptable degree in the thyme and then in the chamomile.

The results also showed that salivary *S. mutans* count exhibited a decrease in the duration of one week after administration of three compounds compared to the time before administration, but there was no significant difference. Also, the rate of increase in salivary *S. mutans* count at the end of one week was much higher in chlorhexidine than the other two compounds, and the chamomile had the lowest rate of increase, which could be due to the sustainability or long-term effect of chamomile.

In comparing the effect of three different compounds on salivary *S. mutans* count, the results showed that there was a significant difference in mean salivary *S. mutans* count after administration of chlorhexidine and chamomile 30 minutes after administration. In other cases, there was no significant difference between chlorhexidine, chamomile and thyme in reducing the salivary *S. mutans* count, which indicates that the two herbal compounds of thyme and chamomile had the same effect as chlorhexidine in reducing the count of bacteria.

The chlorhexidine is a broad-spectrum antimicrobial mouthwash that has been widely considered recently. The side effects, such as dental discoloration, mucosal stimulation, taste changes, parotid swelling and increased formation of supra-gingival calculus, were reported for the chlorhexidine. Since the chlorhexidine is known to be a strong compound against dental plaque,[30] this study considered this substance as a control group for comparing the effect of thyme and chamomile on the salivary *S. mutans*.

The herbal compounds have been successfully used to clean teeth and also as antimicrobial compounds in dentistry.[31] The herbal mouthwashes have also been given special attention in recent years because of their chemical and industrial properties. The chamomile is known to be a plant aspirin that plays a role in reducing the count of *S. mutans* and has been shown to be effective in reducing oral malodour and improving oral health.[22]

The chamomile contains various bio phytochemicals, which can provide therapeutic effects. The chamomile can also help to improve cardiovascular conditions, stimulate the immune system and develop cancer protection.[32] Adil Ahmed et al. showed that the count of *S. mutans* and salivary pH decreased significantly 15 and 30 minutes after administration of chamomile tea, and this decrease was due to the inhibitory effect of bactericidal catechins found in chamomile.[22] Another study also found that chamomile mouthwash could significantly reduce bacterial plaque.[21] Natural herbal extracts of chamomile have grown in importance, due to phenolic compounds with anti-microbial and antioxidant properties.[22] In the present study, these compounds also led to a decrease in salivary *S. mutans* count. The dried flower of chamomile contains many terpenoids and flavonoids, which helps its medicinal properties. The chamomile medicines are commonly used for many human diseases such as hay fever, inflammation, muscle spasm, menstrual disorders, insomnia, ulcers, digestive disorders, rheumatic pain and haemorrhoids. Different products of chamomile have been produced, of which the most popular is herbal tea with an administration of more than one million cups a day.[20]

A relatively small percentage of people are sensitive to chamomile and have allergic reactions. People allergic to melon, chrysanthemum and other members of the family are more susceptible to mucosal allergy to chamomile. The chamomile is listed in the generally recognized as safe (GRAS) Food and Drug Administration (FDA) list (commonly known as Safe).[32]

The thyme is a species of evergreen plant in the family Lamiaceae, which is obtained from Mediterranean regions and is compatible with many different climates around the world. *Thymus vulgaris* is the most important species that is traditionally used for treating cough, bronchitis, gastritis, laryngitis, airway obstruction and diarrhoea. Extract of this plant has been considered as antiseptic, antibacterial, antioxidant and anti-cancer agent. The main components of thymus leaves consist of phenolic compounds, thymol and its structural isomers and carvacrol. The antimicrobial activity of thyme often seems related to thymol and carvacrol content.[33]

Moezzi Ghadim et al. showed that the thyme extract after administration of chlorhexidine and clove can be effective in inhibiting *S. mutans* growth.[34] Soltan Dallal et al. also suggested that the thyme had antibacterial effects.[32] Haghighati et al. showed that the thyme had antibacterial and antifungal effects. This study emphasises that plant extracts had satisfactory effects against microorganisms compared to chlorhexidine.[35] Hammad confirmed the inhibitory effect of thyme on adhesion and growth of *S. mutans* on buccal mucosa cells.[36] Gonçalves et al. investigated the effect of thyme on the growth of *S. mutans* and found that the thyme essential oil in the minimum concentration had the highest effect against *S. mutans* compared to triclosan and chlorhexidine.[17] Herman and colleague showed that the essential oil and ethanolic extract of *T. vulgaris* had the strongest inhibitory effect on all microorganisms isolated from the oral cavity. Therefore, they stated that the essential oil and extract of this plant might be useful in the prevention and treatment of oral diseases caused by oral microorganisms.[18]

Although the results of the mentioned studies are similar to those of the present study, the studies were different from the present study in terms of sample size, consistency and type

of compounds used, administration method and follow-up duration, and none of them used the qPCR method, which can be the most accurate method for counting the bacteria.

The limitations of this study were the problem of the possibility of satisfying parents for their children in the study. Parental consent was drawn with explanations on the objectives and importance of the project and that the solutions would not be detrimental to their child. Preparation of saliva samples from children was accompanied by many problems. Encouraging children to collect and drain saliva into special containers was possible by playing games and entertainment and giving gifts.

CONCLUSIONS

Considering the acceptable effect of chamomile and thyme extracts on reducing the salivary *S. mutans* count and their lower complications compared to long-term use of chlorhexidine, as well as the availability and affordability of these substances, these two compounds can be suggested as antibacterial agents for controlling the ECC and substitution to chlorhexidine in children.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
Financial or other competing interests: This study was supported by Kerman University of Medical Sciences.
Disclosure forms provided by the authors are available with the full text of this article at jemds.com.

REFERENCES

[1] Colak H, Dülgergil CT, Dalli M, et al. Early childhood caries update: a review of causes, diagnoses and treatments. J Nat Sci Biol Med 2013;4(1):29-38.
[2] Anil S, Anand PS. Early childhood caries: prevalence, risk factors and prevention. Front Pediatr 2017;5:157.
[3] Moezizadeh M. Anticariogenic effect of tea: a review of literature. J Dent Oral Hyg 2013;5(9):89-91.
[4] Damle SG, Loomba A, Dhindsa A, et al. Correlation between dental caries experience and mutans streptococci counts by microbial and molecular (polymerase chain reaction) assay using saliva as microbial risk indicator. Dent Res J (Isfahan) 2016;13(6):552-9.
[5] Tehrani MH, Asghari G, Hajiahmadi M. Comparing streptococcus mutans and lactobacillus colony count changing following green tea mouth rinse or sodium fluoride mouth rinse use in children (Randomized double-blind controlled clinical trial). Dent Res J (Isfahan) 2011;8(Suppl 1):S58-63.
[6] Patricia R. Topical fluoride therapy. Chap 15. In: Harris O, Garcia-Godoy F, eds. Primary Preventive Dentistry. 8th edn. Pearsin Prentice Hall 2013:274-5.
[7] Karami M, Mazaheri R, Pour MM. Comparing the effectiveness of two fluoride mouthrinses on streptococcus mutans. J Mash Dent Sch 2011;35(2):115-22.
[8] Jeevarathan J, Deepti A, Muthu MS, et al. Effect of fluoride varnish on streptococcus mutans counts in plaque of caries - free children using dentocult SM strip mutans test: A randomized controlled triple blind study. J Indian Soc Pedod Prev Dent 2007;25(4):157-63.
[9] Autio-Gold J. The role of chlorhexidine in caries prevention. Oper Dent 2008;33(6):710-6.
[10] Rath SK, Singh M. Comparative clinical and microbiological efficacy of mouthwashes containing 0.2 % and 0.12 % chlorhexidine. Dent Res J (Isfahan) 2013;10(3):364-9.
[11] Nabavi SM, Marchese A, Izadi M, et al. Plants belonging to the genous thymus as antibacterial agents: from farm to pharmacy. Food Chem 2015:173:339-47.
[12] Badi HN, Yazdani D, Ali SM, et al. Effects of spacing and harvesting time on herbage yield and quality / quantity of oil in thyme, *Thymus vulgaris* L. Industrial Crops and Products 2004:19(3):231-6.
[13] Naghibi F, Mosaddegh M, Motamed MM, et al. Labiateae family in folk Medicine in Iran from ethnobotany to pharmacology. Iranian Journal of pharmaceutical Research 2005;4(2):63-79.
[14] Kalvandi R, Atri M, Jamzad Z, et al. Taxonomic study of Thymus eriocalyx (Ronniger) Jalas in Iran with emphasis on Floristic marker and using special station method. Taxonomy and Biosystematics 2012;4(1):63-76.
[15] Guillen MD, Manzanos MJ. Study of the composition of the different parts of a Spanish thymus vulgaris L. plant. Food Chem 1998;63(3):373-83.
[16] Yamazaki K, Yamamoto T, Kawai Y, et al. Enhancement of antilisterial activity of essential oil constituents by nisin and diglycerol fatty acid ester. Food Microbiol 2004;21(3):283-9.
[17] Gonçalves GMS, Bottaro M, Nilson AC. Effect of the thymus vulgaris essential oil on the growth of streptococcus mutans. Rev Ciênc Farm Básica Apl 2011;32(3):375-80.
[18] Herman A, Młynarczyk A. Essential oils and plant extracts with activity against oral microorganisms: prevention and treatment of oral diseases. Israel Journal of Plant Sciences 2015;62(4):250-7.
[19] Salamon I, Ghanavati M, Abrahimpour F. Potential of medicinal plant production in Iran and variability of chamomile (Matricaria recutita L.) essential oil quality. Journal of Essential Oil Bearing Plants 2010;13(5):638-43.
[20] Srivastava JK, Shankar E, Gupta S. Chamomile: a herbal medicine of the past with bright future. Mol Med Rep 2010;3(6):895-901.
[21] Pourabbas R, Delazar A, Chitsaz MT. The effect of german chamomile mouthwash on dental plaque and gingival inflammation. Iranian Journal of Pharmaceutical Research 2005;2:105-9.
[22] Ahmed MAA, Pavani B, Tanzila TL, et al. Effects of green tea and chamomile tea on plaque pH, salivary pH, streptococcusmutans count. Indian J Pharm Biol Res 2017;5(4):1-3.
[23] Childers NK, Osgood RC, Hsu KL, et al. Real-time quantitative polymerase chain reaction for enumeration of streptococcus mutans from oral samples. Eur J Oral Sci 2011;119(6):447-54.
[24] Valasek MA, Repa JJ. The power of real-time PCR. Adv Physiol Educ 2005;29(3):151-9.

[25] Bustin SA, Benes V, Nolan T, et al. Quantitative real-time RT-PCR--a perspective. J Mol Endocrinol 2005;34(3):597-601.
[26] Jenabian N, Moghadamnia A, Karami E, et al. The effect of camellia sinensis (green tea) mouthwash on plaque-induced gingivitis: a single-blinded randomized controlled clinical trial. Daru 2012;20(1):39-44.
[27] Kulkarni V, Damle SG. Comparative evaluation of efficacy of sodium fluoride, chlorhexidine and triclosan mouth rinses in reduing the mutans streptococci count in saliva: an in vivo study. J Indian Soc Pedod Prev Dent 2003;21(3):98-104.
[28] Tanner ACR, Mathney JMJ, Kent RL, et al. Cultivable anaerobic microbiota of severe early childhood caries. J Clin Microbiol 2011;49(4):1464-74.
[29] Clarke JK. On the bacterial factor in the aetiology of dental caries. Br J Exp Pathol 1924;5(3):141-7.
[30] Menendez A. Comparative analysis of the antibacterial effects of combined mouthrinses on streptococcus mutans. Oral Microbiol Immunol 2005;20(1):31-4.
[31] Kumar G, Jalaluddin M, Rout P, et al. Emerging trends of herbal care in dentistry. J Clin Diagn Res 2013;7(8):1827-9.
[32] Dallal MMS, Yazdi MH, Aghaamiri S, et al. Antimicrobial effect of zataria multiflora and rosemarinus officinalis on antibiotic-resistant staphylococcus aureus strains isolated from food. Journal of Medical Plants 2014;13(52):41-7.
[33] Fani M, Kohanteb J. In vitro antimicrobial activity of thymus vulgaris essential oil against major oral pathogens. J Evid Based Complementary Altern Med 2017;22(4):660-66.
[34] Ghadim NM, Taghibakhsh M, Godarzi H, et al. Evaluation of the effect of four herbal extracts on growth of streptococcus mutans and lactobacillus. Journal of Research in Dental and Maxillofacial Sciences 2018;3(2):7-13.
[35] 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;6(3):71-6.
[36] Hammad M, Sallal AK, Darmani H. Inhibition of streptococcus mutans adhesion to buccal epithelial cells by an aqueous extract of thymus vulgaris. Int J Dent Hyg 2007;5(4):232-5.