Future Dental Journal of Egypt

Volume 5 | Issue 2 Article 2

2019

Comparison of the efficacy of mouth rinses camellia sinensis extract, guava leaves extract and sodium fluoride solution, on Streptococcus mutans and Lactobacillus in children (an in vivo study)

Sara Abdelkhalek Hassana
sarayounes87@gmail.com

Nadia Ezz Eldin Metwalli

Gehan Gaber Ibrahim

Moustafa Abdelnasser Aly

Future Dental Journal

Comparison of the efficacy of mouth rinses camellia sinensis extract, guava leaves extract and sodium fluoride solution, on Streptococcus mutans and Lactobacillus in children (an in vivo study)

Sara Abdelkhalek Hassan[a,*], Nadia EzzEldin Metwalli[b], Gehan Gaber Ibrahim[b], Moustafa Abdelnasser Aly[c]

[a] *Misr International University and Master Candidate in Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry Ain Shams University, Egypt*
[b] *Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry Ain Shams University, Egypt*
[c] *Microbiology and Immunology Department, Faculty of Medicine, Al Azhar University, Egypt*

A B S T R A C T

Aim: The aim of the present study is to assess and compare intraorally the effectiveness of 0.5% Camellia sinensis extract, 0.5% guava leafs extract, 0.2% sodium fluoride solution on the number of Streptococcus mutans and Lactobacilli spp. in the oral cavity.

Materials and methods: Forty four healthy children of age group 7–12 years was carried out. The subjects were randomly assigned to four groups: (A) 0.5% *C. sinensis* extract, (B) 0.5% guava leafs extract, (C) 0.2% sodium fluoride, and (D) Saline mouth wash, with 11 subjects per group. Plaque samples were taken and streptococcus mutans bacterial count was assessed before and after using the mouth rinses. Salivary samples were taken and lactobacilli bacterial count was assessed before and after using the mouth rinses.

Results: In the in vivo analysis Wilcoxon matched pair test and Mann-Whitney *U* test showed that fluoride, green tea, and guava extract significantly reduced *Streptococcus mutans* colony counts in plaque compared to saline. While in vivo analysis of lactobacilli in saliva showed that there was a significant difference between the reduction percentage changes of all tested groups but there was no significant difference in the bacterial counts between all the tested groups before and after rinsing.

Conclusion: In this in vivo study, green tea extract more effective than guava extract on *streptococcus mutans* in plaque in comparison to Fluoride while guava extract was more effective than green tea extract on *lactobacilli* in saliva in comparison to fluoride.

1. Introduction

Most of mouth rinses are generally used for their analgesic, antimicrobial, anti-inflammatory, and anti-cariogenic activities. Nowadays, a wide range of mouth rinses such as chlorhexidine, sodium fluoride, and essential oils are available in market. The American Dental Association recommends that mouth rinses must be effective at modifying the micro-biota by selectively eliminating pathogens without negatively affecting the normal commensals of oral cavity [1].

The most common plaque-mediated disease in children is dental caries, it is one of the most common chronic diseases among children. It is a preventable, localized infectious, multi-factorial disease resulting from the interaction among host, diet, and microflora on the tooth surface over a period of time, resulting in localized de-mineralization of hard tissues [2].

The main bacterial agents in caries development are *Streptococcus mutans* for its initiation and *Lactobacillus* spp. for its progression. So decreasing these micro-organisms causes a significant decrease in dental caries [3].

Local use of antimicrobial agents is more efficient than their systemic use, because plaque induced caries is local disease [4].

Most of the studies demonstrated that using mouth washes in children provided a significant decrease in the decayed extracted filled surface (defs) index [5].

Periodontal disease has been recognized as a major health problem worldwide. Periodontal diseases are infectious diseases caused by bacteria present in dental plaque [6]. There is a direct relationship present between the presence of dental plaque and development of gingivitis [7].

The goal of this complex periodontal treatment is to cure the inflamed tissues, by reducing the number of periodontal pathogens and alter the host response [8].

Peer review under responsibility of Faculty of Oral & Dental Medicine, Future University.
* Corresponding author.
E-mail address: sarayounes87@gmail.com (S.A. Hassan).

Received 13 November 2018; Accepted 16 December 2018

Several studies have indicated that green tea is able to decrease the process of caries formation through several different mechanisms [9,10].

Because Green tea is reported to be very rich in fluoride and catechin, a bioactive component, which have an anti-cariogenic efficacy by decreasing the proliferation of the streptococcal agent, interfering with the process of adhesion of the bacteria to tooth surface enamel and also act as inhibitors of glucosyltransferase and amylase [11].

Green tea mouth wash proved to be equally effective compared to chlorhexidine which is considered as gold standard. This may also be a valuable public health intervention as it is economical and has multiple health benefits [12].

Guava is also shown high antibacterial activity against Gram-negative and Gram-positive bacteria [13].

Guava extract has demonstrated *in vitro* antiplaque actions by inhibiting growth, adherence and co-aggregation of dental plaque bacteria. Guava extracts may inhibit plaque development without disrupting homeostasis of the oral cavity. Thus, guava is an excellent antibacterial and antiplaque agent which may be a good adjunct to the mainstream periodontal treatment [14].

Fluoride is an established antimicrobial agent. Because of its anticariogenic and remineralization properties, it is extensively used in the prevention of dental caries. However, due to risk of ingestion and fluoride toxicity, in addition, a high amount of systemic fluoride application is toxic [15]. So it is not recommended in small children. Sodium fluoride is regarded as a gold standard of caries prevention.

The aim of the present study is to assess and compare intraorally the effectiveness of 0.5% Camellia sinensis extract, 0.5% guava leafs extract, 0.2% sodium fluoride solution on the number of *Streptococcus mutans* and *Lactobacilli* spp. in the oral cavity. This study was conducted to come up with novel and cost-effective mouth washes that can be used by people for reducing the oral diseases.

2. Materials and methods

This study was composed of in vivo study and it was conducted using three experimental groups and a control group. Randomized selection was used when groups were allocated. Totally, forty four children from the outpatient's clinic of the Pediatric Dentistry Department at the Faculty of Dentistry, Ain Shams University, were included in this study. Their age ranged from 7 to 12 years old. Ethical approval was obtained from the research and ethics committee of the Faculty of Dentistry of Ain Shams University. The study was explained to all the parents' participants and they signed informed consent included their agreement to participate in this study. Also verbal assent from the children was obtained. The children were selected according the inclusion criteria: 1- Children with no regular use of chewing xylitol gum. 2- Children without systemic disease. 3- Children without orthodontic appliance. 4- Children with healthy periodontium. 4- Children with no history of topical fluoride application. [16], and its exclusion criteria: 1- Children with fixed or removable orthodontic appliances. 2- Children with prescribed antibiotics in the last month. 3- Children with gingival diseases.

Children were divided into four groups consisting of 11 child in each group. Group A: 0.5% Green tea (*C. sinensis*) extract. Group B: 0.5% guava leafs extract. Group C: 0.2% sodium fluoride. Group D (control group): Saline mouth wash.

2.1. Preparation of mouth washes

Green tea mouth wash was extracted from the plant leaves of *Camellia Sinensis* in the laboratory of faculty of pharmacy in the pharmaceutical Department of AL-Azhar University in Cairo by an expert pharmacologist. The dried Leaves of the plant were broken into small pieces, chopped, fragmented and grounded into powdered form. The powdered net weight of the tea leaves were 2.050 kg. Then soaked in 6 L of 70% ethanol and were stirred 24 h for 3 days with electrical stirrer. Preparation of the extract have been done. Thereafter, the solution was transferred into rotary flask. The rotary flask were maintained in 60°c temperature by the rotary evaporator (Rotavapor) in the laboratory. Then the residue of extract was scraped from the rotary flask and put in the refrigerator at 4°c to be used. Finally green tea mouthwash 0.5% was prepared (0.5 g of extract in 100 ml distilled water) and poured into bottles each contains 240 ml [17].

Guava leaves extract, Guava leaves were cleaned and dried in the air for three days away from any sun rays to prevent over dryness of the leaves. The dried leaves were grounded to powdered form. The powdered net weight of the guava leaves were 2.050 kg. Then 6 L of 70% ethanol were added to the powder and were stirred 24 h for 3 days with electrical stirrer. Preparation of the extract have been done. The extracts were filtered by using Whatman No. 4 filter paper and then dried in a rotary evaporator at 60 °C in the laboratory. The dried extract was converted into powder form which was utilized for the preparation of desired concentrations of the extracts. The required concentrations of 5% of ethanolic extract was prepared by adding 0.5% of powder in 10 ml of distilled water. The extracts was stored at 4 °C in dark sterile bottles [18].

Sodium fluoride mouth wash is commercially available, Fluoride mouth wash is made by using sodium fluoride powder of Sigma Aldrich Company and adding distilled water with mannitol as a flavor. Fluoride mouth rinse is a concentrated solution for daily or weekly use. The fluoride form of mouth rinse solutions of 0.2% sodium fluoride (920 ppm fluoride) were used under supervision for weekly use [19].

Saline mouth wash (sodium chloride 0.9 gm/100 ml) was the control group and commercially available.

2.2. Plaque samples

Plaque samples were taken before and after using the mouth rinse from the outpatient clinic of the Pediatric Dentistry Department at the Faculty of Dentistry and Oral Medicine, Ain Shams University. Plaque samples were collected from each child on each experimental group using sterile buds from lingual side of lower molars, then diluted with sterile saline in a ratio of 1:100 and added to 10 ml Thioglycolate broth to reach 1:100 [20].The samples were transported to the laboratory of Microbiology Department of the Faculty of Medicine Al-Azhar University in ice box within 6 h of collection. In the microbiology lab, 0.1 ml from each dilution was spread on its selective media:

- Mitis salivarius agar (Difco Co. USA) was used as selective media for *Streptococcus mutans* counts.
- Tomato Juice (Rogosa) agar was used as selective media for *Lactobacilli* bacterial counts [18].

Plates were incubated aerobically and anaerobically at 37 °C for 48 h using a gaspak jar then allowed to develop at room temperature for 24 h to enhance colony development. The number of bacterial colonies before and after rinsing the mouth washes in the four groups were counted.

After collection of plaque samples from each experimental group the children were asked to rinse their mouth for 1 min with 40 ml of each mouth wash, then after 2 min from rinsing with each mouth wash according to the study group the plaque samples were collected again and transported to microbiological laboratory within 1 h in order to calculate the density (CFU/ml) of *streptococcus mutans* and *lactobacilli* for each subject before and after using the mouth wash. All children were reinforced weekly and had oral hygiene instructions.

2.3. Saliva samples

Saliva samples were taken before and after using the mouth rinse

from the outpatient clinic of the Pediatric Dentistry Department at the Faculty of Dentistry and Oral Medicine, Ain Shams University. Samples were collected from each child on each experimental group by asking the child to spit in a sterile plastic container until a suitable amount of saliva was collected (in the morning before tooth brushing and breakfast). After collection of the saliva samples the subjects were instructed to rinse with the mouth rinse according to the study group for 1 min. Saliva samples were collected again from each child after 2 min from rinsing their mouth. The samples were transported in ice box to the laboratory within 6 h of collection. In the lab 0.1 ml from each sample was spread on Tomato Juice (Rogosa) agar were used as selective media for *Lactobacilli* bacterial counts.Plates were incubated anaerobically at 37 °C for 48–96 h using a gaspak jar then allowed to develop at room temperature for 24 h to enhance colony development. The number of bacterial colonies before and after rinsing the mouth washes in the four groups were counted.

2.4. Microbial evaluation

The selective medium Mitis salivarius bacitracin "MSB" (Difco Co. USA) was used as for *Streptococcus mutans* counts and the selective medium Tomato Juice (Rogosa) agar was used for *Lactobacilli* bacterial counts as following:

1% potassium tellurite was added and then poured on sterile petri plates. After cooling to around 50%, they were allowed to set for 24 h and finally, the plaque samples were taken in serial dilutions. Then plates were incubated at around 37 °C in the biological incubator for 24–48 h and clear colonies were seen on the plates, indicating the growth. The plates were incubated aerobically at 37 °C for 48–96 h for *Streptococcus mutans* and anaerobically *Lactobacilli* for at 37 °C for 96 h. The number of bacterial colonies before and after rinsing the mouth washes in the four groups were counted.

3. Results

3.1. A-In vivo analysis for Streptococcus mutans in plaque

Intergroup comparisons of bacterial counts (10^3 CFU/ml):

Mean, Standard deviation (SD) values for intergroup comparisons of bacterial counts (10^3 CFU/ml) before and after rinsing (see Table 1 and Fig. 1)

Before rinsing: (Saline) group had the highest mean value followed by **(Guava extract)**, then **(Fluoride)**, while the lowest value was scored by **(Green tea extract)** group. There was no significant difference in the bacterial counts between all the tested groups.

After rinsing: (Saline) group had the highest mean value followed by **(Guava extract)**, then **(Green tea extract)**, while the lowest value was scored by **(Fluoride)** group. There was a significant difference between the bacterial counts of all tested groups. Pairwise comparisons showed that, there was no significant difference between **(Saline)**, **(Green tea extract)** and **(Guava extract)** groups. However, (**Fluoride)** group was significantly different from both **(Saline)** and **(Guava extract)** groups, it showed no significant difference from (**Green tea extract)** group.

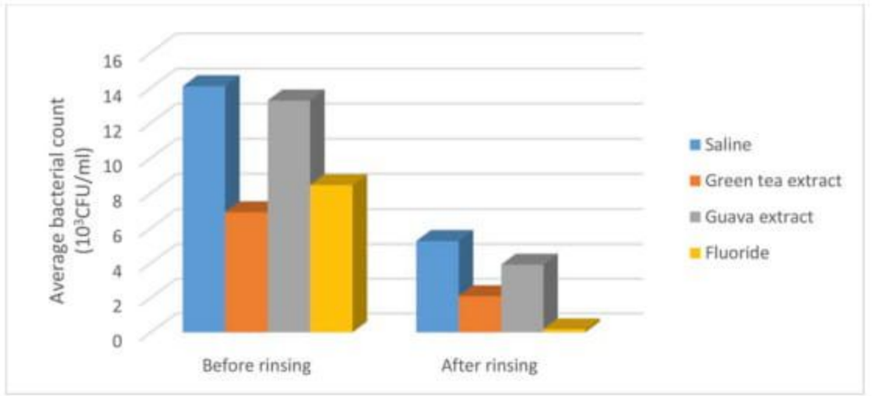

Fig. 1. Bar chart showing average intergroup bacterial count (10^3 CFU/ml) before and after rinsing.

3.2. B—in vivo analysis for Lactobacillus in saliva

Intergroup comparisons of bacterial counts (10^3 CFU/ml):

Mean, Standard deviation (SD) values for intergroup comparisons of bacterial counts (10^3 CFU/ml) before and after rinsing. Were presented in table [9] and figure [21]. (see Table 2 and Fig. 2)

Different superscript letters within the same row indicates a statistically significant difference *; significant ($p \leq 0.05$) ns; non-significant ($p > 0.05$).

Before rinsing: (Guava extract) group had the highest mean value followed by (Fluoride), then (Saline), while the lowest value was scored by (Green tea extract) group. There was no significant difference in the bacterial counts between all the tested groups.

After rinsing: (Saline) group had the highest mean value followed by (Guava extract), then (Fluoride), while the lowest value was scored by (Green tea extract) group. There was no significant difference in the bacterial counts between all the tested groups before and after rinsing.

4. Discussion

Dental caries is one of the most common chronic diseases in the world. It is an infectious disease caused by the colonization of bacteria [21].

Although great efforts have been made to decrease dental caries, its prevalence is still high. As a supplementary tool alongside mechanical methods such as brushing and flossing, mouth rinses have an important role in the reduction of bacterial counts in the mouth, including Streptococcus mutans in decreasing dental caries [22,23].

The aim of this study was to assess and compare intraorally the effectiveness of two different extracts, 0.5% camellia sinesis (green tea), and 0.5% guava leaves extract, with 0.2% sodium fluoride mouth washes on the bacterial count of streptococcus mutans and lactobacilli in plaque, in children ranging from 7 to 12 years old.

This study was conducted on children from 7 to 12 years old to avoid the swallowing of the mouth wash during rinsing and the toxicity of fluoride in children less than 6 years. Because high amount of fluoride ingestion may lead to acute poisoning and its low repeated ingestion causes fluorosis especially in children. So sodium fluoride mouth rinse is not recommended for children younger than 6 years who may swallow it [24].

Children having serious general systemic disease taking antibiotics within the last month, were excluded in this study to avoid any factors that may affect the bacterial count in the oral cavity [25].

Plaque is the main cause of dental caries due to its highly colonization by bacteria for this reason plaque samples were chosen in this

Table 1
Mean, Standard deviation (SD) values for intergroup comparisons of bacterial counts (10^3 CFU/ml) before and after rinsing.

Study Period	Groups	Saline	Green tea extract	Guava extract	Fluoride	*P*-value
Before	(mean ± SD)	(14.09 ± 14.48)	(6.90 ± 11.70)	(13.27 ± 12.23)	(8.45 ± 13.09)	0.157ns
After	(mean ± SD)	$(5.27 \pm 6.00)^{A}$	$(2.09 \pm 4.59)^{AB}$	$(3.90 \pm 5.06)^{A}$	$(0.18 \pm 0.60)^{B}$	0.003*

Different superscript letters within the same row indicates a statistically significant difference *; significant ($p \leq 0.05$) ns; non-significant ($p > 0.05$).

Table 2
Mean, Standard deviation (SD) values for intergroup comparisons of bacterial counts (10^3 CFU/ml) before and after rinsing.

Study Period	Groups	Saline	Green tea extract	Guava extract	Fluoride	*P*-value
Before	(mean ± SD)	(32.00 ± 13.74)	(21.33 ± 9.07)	(50.66 ± 16.25)	(36.33 ± 25.66)	0.313ns
After	(mean ± SD)	(31.66 ± 12.66)	(10.33 ± 5.50)	(24.00 ± 11.53)	(15.33 ± 8.09)	0.225ns

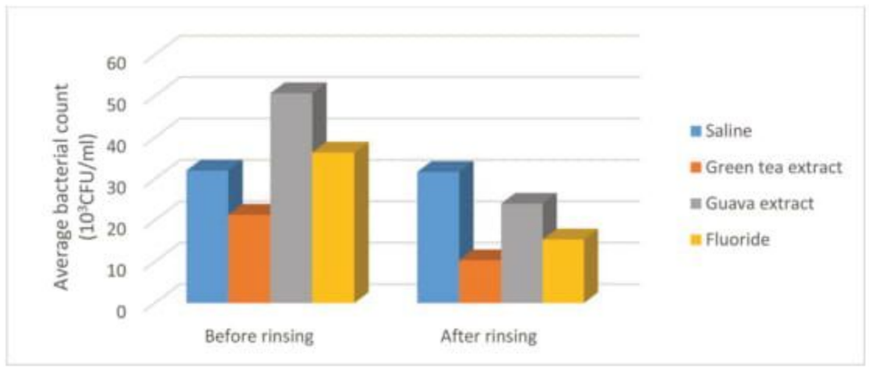

Fig. 2. Bar chart showing average intergroup bacterial count (10^3 CFU/ml) before and after rinsing.

study to determine the level of streptococcus mutans and lactobacilli to eliminate the difficulties associated with collection of saliva in children. Plaque sample were taken by sterile cotton swab from the gingival 1/3 of the lingual part of the 1st molar, where plaque is likely to accumulate the most, and the brushing in this part is misused [26].

After taking the plaque samples there was no growth of lactobacilli in all samples, so saliva samples have been taken to check the effect of mouth rinses on lactobacilli due its presence in saliva samples more than in plaque samples. This was in agreement with **Teanpaisan H.J. et al (2007)** [27] who stated that it is difficult to have an exact idea of the relation between lactobacilli and dental plaque. Lactobacilli have been reported to be less detectable in the dental plaque than in saliva.

The most commonly used mouth rinse in children for therapeutic purposes, is 0.05% sodium fluoride for daily use. In this study we prefer to use 0.2% for weekly use to decrease the false result which may happen if the patient used it by himself daily [28].

Fluoride mouth wash act as an antimicrobial agent; because of its anti-cariogenic and remineralization properties, it is extensively used in prevention of dental caries. But due to risk of ingestion and fluoride toxicity, it is not recommended in younger children [29].

Herbal mouth rinses that are receiving special attention nowadays were introduced in this study, because they are non-chemical, non-synthetic, in comparison to fluoride. Moreover, they have been already used in traditional medicine [30].

In this study during preparation of green tea extract and guava extract 70% of ethanol alcohol was used as a solvent because of being safe to use as well as its effectiveness in the extraction of bioactive molecules of plants. Electrical stirrer was used for being accurate and stable [31].

In the current study saline mouth wash was used as control group due to its very limited effect on bacteria and it was used because it's rinsing effect comparing to other groups.

In this study Mitis Salivarius Agar was used as a selective medium for *streptococcus mutans* with 1% potassium tellurite which is highly selective medium, which enable to isolate streptococci from highly contaminated specimens like exudates from body cavities, as it inhibits a wide variety of bacteria. And Tomato juice was included in media for *lactobacilli* and was found to be advantageous for its growth, and for the isolation, cultivation and enumeration of Lactobacilli, from clinical specimens and foodstuffs [32].

In this study there was significantly reduction of bacterial count of *streptococcus mutans* for all groups which includes sodium fluoride mouth wash, green tea extract mouth wash, guava leaves extract mouth wash. In spite of sodium fluoride was highly significant effect, green tea extract has the nearest effect to it in comparable with guava extract.

In the present study there was a significant decrease in mean bacterial counts of *streptococcus mutans* after using 0.2% fluoride mouth wash in comparison with other groups, and this was consistent with other study that reported significant decrease in *streptococcus mutans* bacterial count that was conducted by **Hambire C. U. et al (2015)** [33].

Also the result of this study was in agreement with **Aminabadi et al (2007)** [28] and **Jothika et al (2015)** [34] that there is effect of 0.2% sodium fluoride mouth wash in prevention of dental caries, and fluoride mouthwash also showed a significant reduction in the colony count of *streptococcus mutans*.

According to data from this current study, it was found that there was no statistically significance difference between the mean bacterial counts of streptococcus mutans of the three groups, and the control group of plaque samples before using the mouth rinse, this goes in consistence with the results of a clinical study done by **Neturi R. et al (2014)** [26].

In this study it was shown that green tea inhibit the growth of S. mutans significantly and this may be due to the polyphenolic compounds prevented the attachment of S. mutans to tooth structure through modification of its phenotype. And that catechin present in green tea inhibits glucosyl transferase and leads in significant reduction of the plaque. The result of this study was consistent with the study conducted by **Tehrani et al. (2011)** [29].

Also the result of this study showed that green tea is effective mouth wash against *S. mutans* and having better action in plaque as compared to saliva. So, it can be used as an adjunct to commercially available mouthwashes and this was in agreement with **Goyal et al (2017)** [35].

In this study there is significantly effect of guava extract mouth wash on streptococcus mutans bacterial count that is consistent with study conducted by **Singla et al (2017)** [36], that showed antibacterial efficacy against oral streptococci by guava extract.

According the data of this present study there was no growth of lactobacilli before and after using the mouth rinses that were used in this study in plaque samples and that was consistent with the conducted study done by **Mutisuki et al, (2005)** [37] The most suitable method to detect the *Lactobacillus* spp. level in the oral cavity is the stimulated whole saliva method.

In this current study there was a significant difference between the reduction percentage changes of all tested groups included sodium fluoride mouth wash, green tea extract mouth wash, and guava leaves extract mouth wash on lactobacilli in saliva. In spite of sodium fluoride was highly significant effect, guava extract has the nearest effect to it in comparable with green tea extract.

In this current study there was considerable inhibitory effect against S. mutans and lactobacilli spp., also there was significant difference found between the mean zones of inhibition of the different mouth rinses for the two microbes, this was agreement with **Thomas et al (2015)** [38].

References

[1] John NR, Gala VC, Sawant CS. Inhibitory effects of plant extracts on multi-species dental biofilm formation in-vitro. Int J Pharm Biol Sci 2013;4:487–95.
[2] Tanner AC, Mathney JM, Kent RL, Chalmers NI, Hughes CV, Loo CY. Cultivable anaerobic microbiota of severe early childhood caries. J Clin Microbiol 2011;49:1464–74.
[3] Zafar S, Harnekar SY, Siddiqi A. Early childhood caries: etiology, clinical considerations, consequences and management. Int Dent Saudi Arabia 2009;11:24–36.
[4] Kocak MM, Ozcan S, Kocak S, Topuz O, Erten H. Comparison of the efficacy of three different mouth rinse solutions in decreasing the level of Streptococcus mutans in

saliva. Eur J Dermatol 2009;3:57–61.
[5] Lobo PLD, de Carvalho CB, Fonseca SG, de Castro RS, Monteiro AJ, Fonteles MC. Sodium fluoride and chlorhexidine effect in the inhibition of Mutans Streptococci in children with dental caries: a randomized, double-blind clinical trial. Oral Microbiol Immunol 2008;23:486–91.
[6] Kornman KS. Mapping the pathogenesis of periodontitis: a new look. J Periodontol 2008;79:1560–8.
[7] Powell RN. The relationship of forming and mature dental plaque to the tooth surface. J Dent Res 1965;44:1171–5.
[8] Kumar P, Ansari SH, Ali J. Herbal remedies for the treatment of periodontal disease: a patent review. Recent Pat Drug Deliv Formulation 2009;3:221–8.
[9] Hamilton-Miller JM. Anti-cariogenic effects of tea (Camellia sinensis). J Med Microbiol 2001;50:299–302.
[10] Hassani AS, Amirmozafari N, Ordouzadeh N, Hamdi K, Nazari R, Ghaemi A. Volatile components of Camellia sinensis inhibit growth and biofilm formation of oral Streptococci in vitro. Pakistan J Biol Sci 2008;11:1336–41.
[11] Si W, Gong J, Tsao R, Kalab M, Yang R, Yin Y. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. J Chromatogr A 2006;1125:204–10.
[12] Rosy SN, Srinivas R, Vikram SB, Sandhya SY, Chandra ST, Siva KP. Effects of green tea on Streptococcus mutans counts- a randomised control trail. J Clin Diagn Res 2014 Nov;8(11):128–30.
[13] Ushimaru PI, Silva MT, Di Stasi LC, Barbosa L, Fernandes A. Antibacterial activity of medicinal plant extracts. Braz J Microbiol 2007;38:717–9.
[14] Kocak MM, Ozcan S, Kocak S, Topuz O, Erten H. Comparison of the efficacy of three different mouth rinse solutions in decreasing the level of Streptococcus mutans in saliva. Eur J Dermatol 2009;3:57–61.
[15] Vargas CM. Fluoride supplements prevent caries but can cause mild to moderate fluorosis. J Evid Base Dent Pract 2011;11:18–20.
[16] Niloofar J, Moghadamnia AA, Elaheh K, Poorsattar BM. The effect of *Camellia Sinensis* (green tea) mouthwash on plaque-induced gingivitis: a single-blinded randomized controlled clinical trial. Daru J Pharmaceutical Med 2012;20(1):39.
[17] Steinmann J, Buer J, Pietschmann T, Steinmann E. Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. Brit J Pharma 2013;168(5):1043–289.
[18] Ramamurthy PH, Swamy HS, Bennete F, Rohini M, Nagarathnamma T. Relationship between severe-early childhood caries, salivary mutans streptococci, and lactobacilli in preschool children of low socioeconomic status in Bengaluru city. J Indian Soc Pedod Prev Dent 2014;32:44–7.
[19] Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney: practical medical microbiology. fourteenth ed. Churchill Livingstone; 1996.
[20] Kulp WL. Science 1927;66:512.
[21] Jazaeri M, Rezaei SL, Abdolsamadi H, Rafieian N. Cariostatic effect of green tea in comparison with common anticariogenic agents: an in vitro study. J. D.D.D. 2015;9(1).
[22] Dean JA, Avery DR, Mc Donald RE. Dentistry for the child and adolescent. ninth ed. London: Mosby Co; 2011. p. 177.
[23] Arya V, Thakur NM, Kashyap C. Preliminary phytochemical analysis of the extracts of psidium leaves. J Pharmacogn Phytochem 2012;1:1–5.
[24] Goldstep F. Proactive intervention dentistry: a model for oral care through life. Comp Cont Educ Dent 2012;33:394–6.
[25] Chaturvedi TP. Uses of turmeric in dentistry: an update. Indian J Dent Res 2009;20:107–9.
[26] Neturi R, Srinivas R, Simha B, andhya Y, Chandra T, Siva P. Effects of green tea on *Streptococcus mutans* counts- a randomised control trail. J Clin Diagn Res: J Clin Diagn Res 2014;8:11.
[27] Vargas CM. Fluoride supplements prevent caries but can cause mild to moderate fluorosis. J Evid Base Dent Pract 2011;11:18–20.
[28] Aminabadi NA, Balaei E, Pouralibaba F. The effect of 0.2% sodium fluoride mouthwash in prevention of dental caries according to the DMFT index. J Dent Res Dent Clin Dent Prospects 2007;1(2):71–6.
[29] Tehrani MH, Asghari G, Hajiahmadu M. Comparing Streptococcus mutans and Lactobacillus colony count changes following green tea mouth rinse or sodium fluoride mouth rinse use in children. Dent Res J 2011;8:S58–63.
[30] Cabrera C, Artacho R, Gimenez R. Beneficial effects of green tea – a review. J Am Coll Nutr 2006;25(2):79–99.
[31] Arya V, Thakur NM, Kashyap C. Preliminary phytochemical analysis of the extracts of psidium leaves. J Pharmacogn Phytochem 2012;1:1–5.
[32] MacFaddin JF. Media for isolation-cultivation-identification-maintenance of medical bacteria vol. 1. 1985.
[33] Hambire CU, Jawade R, Patil A, Wani VR, Kulkarni AA, Nehete PB. Comparing the antiplaque efficacy of 0.5% Camellia sinensis extract, 0.05% sodium fluoride, and 0.2% chlorhexidine gluconate mouthwash in children. J Int Soc Prev Community Dent 2015;5(3):218–26.
[34] Jothika M, Vanajassun PP, Someshwar B. Effectiveness of probiotic, chlorhexidine and fluoride mouthwash against Streptococcus mutans – randomized, single-blind, in vivo study. J Int Soc Prev Community Dent 2015;5(1):S44–8.
[35] Goyal AK, Bhat M, Sharma M, Garg M, Khairwa A, Garg R. Effect of green tea mouth rinse on *Streptococcus mutans* in plaque and saliva in children: an *in vivo* study. J Indian Soc Pedod Prev Dent 2017;35:41–6.
[36] Singla S, Malhotra R, Shashikiran ND, Saxena S. Antibacterial efficacy of mouthwash prepared from pomegranate, grapeseed and guava extract against oral streptococci: An in vvo study. J Clin Pediatr Dent 2017.
[37] Motisuki C, Monti LL, Spolidorio PMD, Pinto LS. Influence of sample type and collection method on Streptococcus mutans and Lactobacillus spp. counts in the oral cavity. Oral Biology 2005;50(3):341–5.
[38] Thomas A, Thakur S, Mhambrey S. Comparison of the antimicrobial efficacy of chlorhexidine, sodium fluoride, fluoride with essential oils, alum, green tea, and garlic with lime mouth rinses on cariogenic microbes. J Int Soc Prev Community Dent 2015;5(4):302–8.