Original Article

Comparative Evaluation of Efficacy of 4% Tulsi Extract (*Ocimum sanctum*), Fluoridated and Placebo Dentifrices against Gingivitis and Plaque among 14–15 years School Children in Davangere City, India – A Triple Blinded Randomized Clinical Trial

Bhuvaneshwari Gangadharamurthy Nadar, G. V. Usha, Nagesh Lakshminarayan[1]

Department of Public Health Dentistry, Bapuji Dental College and Hospital, [1]Department of Public Health Dentistry, Dayanand Sagar Dental College and Hospital, Bangalore, Karnataka, India

Abstract

Context: Gingivitis is the most prevalent periodontal disease among adolescents. The most important factor associated with gingivitis is plaque accumulation. Mechanical plaque control through tooth brushing and mouth rinsing are the most commonly used preventive methods. **Aims:** This study aims to assess and compare the antiplaque and antigingivitis effect of 4% Tulsi leaf extract dentifrice, fluoridated and placebo dentifrice (PD) among 14–15-year-old school children in Davangere city. **Materials and Methods:** A triple blinded concurrent parallel trial. A sample of 84 participants with a baseline mean gingival index score of at least 1.0 and mean plaque index score of at least 1.5 were randomly selected. Participants were divided into three groups by block randomization and concealed random allocation method was used to distribute dentifrices. Postassessment of plaque and gingivitis was done on the 21st day. **Statistical Analysis Used:** Wilcoxon signed rank test for within group comparison and Kruskal–Wallis ANOVA for intergroup comparison was used. **Results:** Significant reduction in the plaque and gingival scores between the groups was observed on the 21st day ($P = 0.001$). Maximum reduction in gingivitis ($P = 0.001$) and dental plaque ($P = 0.01$) was seen in 4% tulsi dentifrice group compared to PD. **Conclusion:** Antiplaque and antigingivitis efficacy of 4% tulsi and commercially available fluoridated dentifrice remained the same after 21 days.

Keywords: *Clinical trial, dental biofilm, dental plaque, dentifrices, fluorides, gingivitis*

Introduction

Children and adolescents are affected by various forms of periodontal diseases, including gingivitis, localized or generalized aggressive periodontitis, and periodontal disease associated with systemic disorders.[1] Gingivitis is the reversible dental plaque-induced inflammation of the gingiva without detectable bone loss or clinical attachment loss. The etiology of gingivitis is multifactorial and the result of more than one factor acting together. A wide range of factors has been identified as significantly associated with gingivitis including the presence of bacteria biofilm, genetic, socioeconomic, demographic, iatrogenic, and behavioral factors. These factors seem to influence the process, making it difficult to identify the risk factors.[1] The most important factor that has been associated with gingivitis is plaque accumulation on the dental surface, resulting in an inflammatory reaction.

Dental plaque, a microbial biofilm, is commonly recovered from oral surfaces and comprises a diverse array of organisms.[2] Plaque reaches the mature stage after 7–14 days and becomes relatively stable around the 21st day.[3] The role of plaque accumulated at the gingival margin in the initiation and progression of gingivitis and periodontitis is well documented.[4] Location and rate of plaque formation present a broad variability between individuals as these factors are influenced by the oral hygiene habits, diet, saliva composition, and the flow rate of each person.[3] Epidemiological studies have shown that gingivitis of varying severity is a universal finding among adolescents and children.[5] Furthermore, it is generally accepted that periodontal disease in children, adolescents, and adults begins as gingivitis, which progresses to periodontitis only in few individuals.

How to cite this article: Nadar BG, Usha GV, Lakshminarayan N. Comparative evaluation of efficacy of 4% tulsi extract (Ocimum sanctum), fluoridated and placebo dentifrices against gingivitis and plaque among 14–15 years school children in Davangere City, India – A triple blinded randomized clinical trial. Contemp Clin Dent 2020;11:67-75.

Address for correspondence:
Dr. Bhuvaneshwari Gangadharamurthy Nadar, Department of Public Health Dentistry, Bapuji Dental College and Hospital, Davangere, Karnataka, India. E-mail: bhuvana15689@gmail.com

Submitted : 09-Feb-2019
Accepted : 09-Feb-2019
Published : 13-Jul-2020

Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_109_19

Quick Response Code:

According to data from National Health and Nutrition Examination Survey III, gingival bleeding is most prevalent in the 13–17-year-old age group (63%) and declined gradually through the 35–44-year-old group.[6] The prevalence percentage of gingivitis among 15-year-old Indian adolescents is 67.7%.[7] The cornerstone of prevention of periodontal disease is mechanical plaque control of supragingival biofilm. However, mechanical plaque removal is not always performed to an adequate standard as many surfaces on the teeth and gingiva are relatively inaccessible. It also requires adequate patient compliance. As a result, chemotherapeutics have been introduced as an adjunct to mechanical removal of plaque. The supplementation of these chemotherapeutic agents was found to have a beneficial effect on gingiva health as the chemicals used could prevent the growth of dental plaque on human teeth. Tooth brushing is the most frequently used means of performing oral hygiene. To improve the antiplaque action during tooth brushing, chemical control methods are utilized.

Chemotherapeutic agents commonly used are chlorhexidine, triclosan, and various other antimicrobials. Triclosan, a chlorinated bisphenol, is low toxic and nonionic in nature.[8] It is compatible with toothpaste components, such as fluoride and surfactants, and it exhibits its anti-inflammatory effects by promoting the inhibition of cyclooxygenase/lipoxygenase pathways.[8] Fluoride is another antimicrobial agent, which is incorporated in toothpaste and mouth rinses to prevent the plaque accumulation on the tooth surface. The excess use of the fluoride can cause dental fluorosis, so only the recommended amount of the fluoride should be used in the toothpaste.

Interest in alternative toothpaste based on the plant extracts has increased recently. Among the plants known for medicinal value, *Ocimum sanctum* Linn. (also known as *Ocimum tenuiflorum,* tulsi) belonging to genus *Ocimum* and family Labiatae are very important for their therapeutic potentials.[9] Tulsi was recognized by ancient Rishis and has been used for thousands of years in Ayurveda for its diverse healing properties.[10] *O. sanctum* is also globally known for more than 2000 years as one of the most versatile medicinal plants, having a wide spectrum of biological activities. Basils are native to tropical Asia and some varieties of tulsi readily grow wild in many areas of Asia and Africa. Tulsi has been considered as the Queen of herbs and the legendary "Incomparable one" of India.[10] It is well known for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East.[10] Its strong aroma and astringent taste is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity.[10] Even today, the shrub (tulsi) is planted in most homes in India because of its continuing importance in healing, religion, spirituality sanctity, culture, and decorative esthetics.[9]

In developing countries like India, oral health is given least importance. The factors such as affordability, accessibility, and acceptability play major importance as a majority of the population (69%) resides in the rural area.[11] Inculcating better oral hygiene aids during childhood may improve the health of oral cavity in the life course. Literature search revealed only one study by Gupta *et al*.,[12] evaluated the plaque and gingivitis of *O. sanctum* as mouthrinse. Therefore, oral health of adolescents can be improved through better oral health measure by herbal dentifrice which is traditional medicine, readily available at backyard, and cost-effective. As mouthrinses are considered as adjunct and tooth brushing are the routine behaviors, therefore delivery of herbal chemotherapeutic agent as a dentifrice is more attractive, acceptable, and readily adaptable behavior. Literature search revealed no studies evaluating the antiplaque and antigingivitis effect of tulsi leaf extract when incorporated in the form of a dentifrice. Therefore, the present study is formulated with the aim to assess the antiplaque and antigingivitis efficacy of 4% tulsi leaf extract dentifrice as compared to commercially available fluoridated dentifrice among 14–15-year-old school children in Davangere city. We hypothesize that there is a difference in the antiplaque and antigingivitis effect of 4% tulsi leaf extract dentifrice and commercially available fluoridated dentifrice.

Materials and Methods

The trial was designed, analyzed, and interpreted according to the extension of CONSORT for herbal intervention.

Study design and population (participants)

The present study was a single-center, block randomized, triple-blinded, placebo-controlled parallel group study. It was conducted among selected school children aged 14–15 years in Davangere city, India for a period of 21 days from January to February 2018. The study protocol was approved by the Ethical Review Board of Bapuji Dental College and Hospital, Davangere. The trial was registered in Clinical Trial Registry – India with trial registry number CTRI/2017/12/010749. Permission to conduct the study was obtained from the Deputy Director of Public Interest and school authorities. To select schools, Davangere was arbitrarily divided into four zones (Northeast, Northwest, Southeast, and Southwest). From each zone, two schools were selected randomly by employing lottery method. From the selected eight schools, three schools were selected randomly by lottery method. List of all the students of 14–15 years age group from each of the selected schools was obtained from school records. Voluntary written informed assent and consent was obtained from selected children and from their parents. The students were examined in the school premises under natural light using sterilized mouth mirror and Community Periodontal Index – probe. Plaque was assessed using Turesky modification of the Quigley-Hein Plaque Index including

all the teeth. Modified plaque scoring system by Turesky *et al*. was used as the index provides a clear objective definition of each score included in the index.[13] Gingivitis was assessed using gingival index (Loe and Sillness 1964) as the index is entirely confined to qualitative changes in gingival soft tissues.[13]

Individuals with a baseline mean gingival index score of at least 1.0 as determined by the use of the Loe-Silness Gingival Index, and a mean plaque index score of at least 1.5 as determined by the use of the Turesky modification of the Quigley-Hein Plaque Index, were included.[14,15] Individuals with presence of more than 20 scoreable teeth in the oral cavity were permitted to participate in the present study. Individuals with the history of hypersensitivity to any products used in the study, having a history of antibiotic therapy in the previous 1 month till the start of the study, individuals undergoing orthodontic treatments, and those who were unable to comply with the study appointment schedules were excluded from the study.

Preparation of tulsi extract

Dried black tulsi leaves were subjected to cold maceration technique to prepare a 4% ethanolic extract [Figures 1 and 2]. Moreover, 4% concentration of tulsi extract was prepared because maximum inhibition zone of 22 mm against *Streptococcus mutans* was observed at the same concentration in a study conducted by Agarwal and Nagesh.[16]

Preparation of toothpaste

Formulation of the dentifrices was done at Department of Pharmacognocy, Bapuji Pharmacy College, and Davangere. The only difference in the content of the test dentifrice was the 4% tulsi extract. All the three formulations were filled in identical tubes [Figure 3].

Intervention

All the participants, along with the toothpaste received a soft bristle toothbrush. They were instructed to refrain from any other oral hygiene aids for the next 21 days. Demonstration for brushing technique was given and they were instructed to brush by modified bass technique for a period of 4–5 min twice daily using a pea-sized toothpaste. After a period of 21 days, the plaque levels and gingival status were assessed by indices as mentioned.

Outcomes

All the outcome measures were assessed at baseline and after 21 days of treatment at the follow up visit. The primary outcome measures were a reduction in the mean gingival and plaque scores as determined by Loe and Silness gingival index and Turesky Gilmore Glickman modification of the Quigley Hein Plaque index. Secondary outcome measures included the assessment of the patient compliance, acceptance and any adverse effect through checklist and questionnaire [Annexure 1 and 2].

Figure 1: Preparation of 4% tulsi extract by cold maceration method

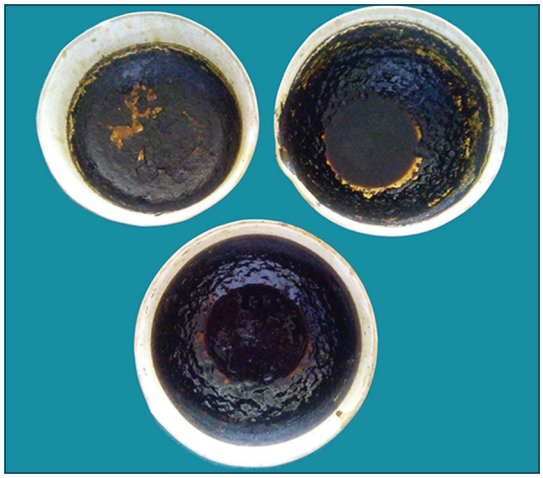

Figure 2: 4% Tulsi extract

Figure 3: Masking of the dentifrices using identical tubes

Sample size

The sample size was calculated using G*Power software for windows version 3.1.9.2 (Universität Düsseldorf, Düsseldorf, Germany). We assumed *a priori* that

20% of individuals would be lost to follow-up by 21 days (α – 0.05, β – 0.20). A sample of 84 individuals were enrolled in the study. Each group consisted of 28 individuals.

Random allocation

The three dentifrices, namely 4% tulsi extract, Colgate Total, and placebo dentifrices (PDs) constituted the three interventions. Each school was considered as one block or group. Random allocation of the dentifrice was done by a person not involved in the present study.

Blinding and calibration

A single investigator, who was trained and calibrated to record the plaque and gingival scores, recorded the findings. All the participants, investigator and statistician were blinded to the allocation of dentifrice in the intervention arms. The examiner was calibrated by experts for plaque and gingivitis assessment using indices. Using kappa statistics, interexaminer reliability was 0.80 for the gingival index and 0.84 for the plaque index.

Statistical analysis

Data analysis was done using SPSS software version 20.0 (IBM Corp., Armonk, N.Y., USA). The statistical significance was fixed at $P < 0.05$. Comparison of baseline plaque scores was done using one-way ANOVA. Comparison of baseline and posttest gingivitis and posttest plaque scores for three dentifrices groups were done using Kruskal–Wallis ANOVA followed by *post hoc* Mann–Whitney U-test, as the data were not normally distributed.

Results

A sample of 84 participants were included, of which 41 were males and 43 were females; 59 participants belonged to 14 years and 25 were in 15 years of age [Figure 4]. Each group consisted of 28 participants with tulsi extract, Colgate Total and PDs as Group 1, 2, and 3, respectively. Baseline gingivitis and plaque scores were assessed between the groups, and there was no statistically significant difference between the groups [Tables 1 and 2]. It was done to ensure baseline comparability. At the end of the study, five participants from the placebo group dropped out of the study accounting for 6% dropout [Figure 5]. Dropouts were less than expected; hence, dropout analysis was not done. Reasons for dropouts were loss of interest in the middle of the trial by 2 (2.5%) participants and 3 (3.7%) participants were absent on the day of assessment. The test dentifrice had a good acceptance and did not show any adverse effects. Majority of participants had reported slight bitter taste of the tulsi dentifrice (TD), but none of the participants dropped out of the study from the test group.

Gingival index showed a significant decrease from baseline to the 3rd week in tulsi and fluoride dentifrice (FD) group that is from mean rank 40.54 to 30.66 in tulsi group and from 42.05 to 34.57 in FD group. At the 21st day, there was a significant reduction in gingivitis score between all the three groups with $P = 0.001$. *Post hoc* analysis showed a statistically significant difference between the tulsi and PD and between Fluoride and PD group. Between the tulsi and FD group, no statistical significance was found [Table 3].

Comparison of postthird week assessment of plaque scores showed a statistically significant difference between the groups ($P = 0.001$). *Post hoc* analysis showed a statistically significant difference between the tulsi and PD and between fluoride and PD group. Between the tulsi and FD group, no statistical significance was observed [Table 4]. To find out within group difference, Wilcoxon signed rank test was applied. There was a statistically significant difference within the groups TD and FD from baseline to 21 days' posttest period. No significant difference was seen within the PD group from baseline to posttest. The test group (TD) showed a clinical effect size of 0.39 and 0.59 for antiplaque and antigingivitis action respectively. This can be considered a moderate degree of effect size. [Tables 3 and 4].

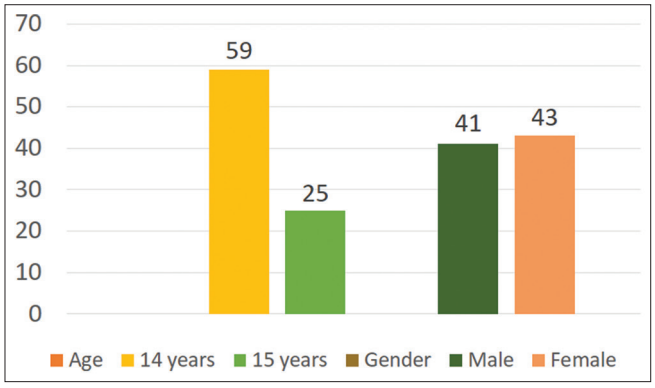

Figure 4: The agewise and genderwise distribution of the study participants

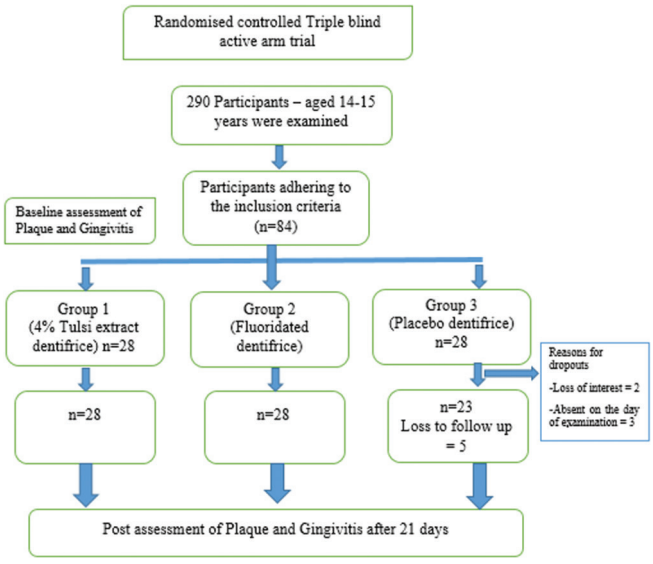

Figure 5: Flowchart of the study

Discussion

The present study assessed and compared the antiplaque and antigingivitis effect of 4% TD. It is first of its kind to evaluate the efficacy of 4% tulsi extract when incorporated in the form of toothpaste. The study results showed that antiplaque and antigingivitis effect of 4% tulsi extract dentifrice was comparable to that of Colgate Total dentifrice. The results demonstrated that there was a significant reduction in the plaque and gingival scores after 21 days in both tulsi and FD group. Placebo group showed a slight increase in the plaque and gingival index score, but the results were not statistically significant.

The results of 4% tulsi extract dentifrice on plaque and gingivitis could not be compared with other studies as the exploration of the available literature revealed that no studies have been reported in the literature which has tried to assess the same effect. A study by Gupta *et al*.[12] showed a significant reduction of plaque and gingival index scores when 4% tulsi mouthrinse was used over a period of 15 and 30 days. The results of the present study are in line with the study by Gupta *et al*.[12]

Table 1: Comparison of mean rank of gingivitis scores between groups at baseline

Groups	Number of participants	Mean rank	χ^2	*P*
TD	28	40.54	0.67	0.71
FD	28	42.05		
PD	28	36.85		

TD: Tulsi dentifrice; FD: Fluoride dentifrice; PD: Placebo dentifrice

Table 2: Comparison of baseline mean dental plaque scores between groups

Groups	Number of participants	Mean	SD	*P*
TD	28	0.85	0.35	0.5
FD	28	0.73	0.36	
PD	28	0.79	0.45	

TD: Tulsi dentifrice; FD: Fluoride dentifrice; PD: Placebo dentifrice; SD: Standard deviation

Tulsi has been considered as a sacred and medicinal plant in ancient Indian literature. In traditional literature, preparations of the leaves of *O. sanctum* are claimed to be effective in a wide spectrum of inflammatory and infectious diseases. The anti-gingivitis effect of *O. sanctum* extract dentifrice can be attributed to compounds isolated from *O. sanctum* extract. Civsilineol, Civsimavatine, Isothymonin, Apigenin, Rosavinic acid, and Eugenol compounds in tulsi were considered to have anti-inflammatory activity or cyclooxygenase inhibitory activity.[17] Singh,[18] Singh and Majumdar[19] in their study reported that linoleic acid present in different species of *O. sanctum* L. can also be attributed for the anti-inflammatory activity, by blocking both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism.

In the present study, the *O. sanctum* extract dentifrice reduced the plaque formation during the 21 days of the trial. A possible explanation for this effect could be antibacterial agents present in stem and leaves of *O. sanctum*. They are eugenol, ursolic acid and carvacrol, linalool, limatrol, caryophyllene, estragol, saponins, flavonoids, triterpenoids, and tannins. These constituents form high molecular weight complexes with soluble proteins in saliva. They increase bacterial lysis on the tooth surface and saliva and also interfere with bacterial adherence mechanisms on tooth surfaces.[10,20]

As Colgate Total dentifrice has the added benefit of fluoride and triclosan. Comparative studies have shown that the triclosan containing dentifrice is more efficient in plaque control and improvement of gingival health than conventional FD.[21] Therefore Colgate Total dentifrice was selected as positive control. The use of Colgate Total also showed a significant reduction in the mean plaque scores. The result is in line with the study conducted by Gupta *et al*.,[22] plaque scores were assessed after 4 weeks. Triclosan blocks lipid biosynthesis by specifically inhibiting the enzyme Enoyl-acyl carrier protein reductase (ENR).[23] This

Table 3: Comparison of mean rank gingivitis scores between the groups after 21 days

Groups	Number of participants	Mean rank	χ^2	*P*	Effect size
TD	28	30.66[b]	20.339	0.001[a] (HS)	0.59
FD	28	34.57[c]			
PD	23	57.98[b,c]			

[a]Statistical significance; Kruskal–Wallis ANOVA; [b,c]*P*<0.05 Mann–Whitney U-test. HS: Highly significant; TD: Tulsi dentifrice; FD: Fluoride dentifrice; PD: Placebo dentifrice

Table 4: Comparison of mean rank dental plaque scores between the groups after 21 days

Groups	Number of participants	Mean rank	χ^2	*P*	Effect size
TD	28	39.04[b]	22.366	0.001[a] (HS)	0.39
FD	28	26.79[c]			
PD	23	57.26[b,c]			

[a]Statistical significance; Kruskal–Wallis ANOVA; [b,c]*P*<0.05 Mann–Whitney U-test. HS: Highly significant; TD: Tulsi dentifrice; FD: Fluoride dentifrice; PD: Placebo dentifrice

feature of fluoride toothpaste can be attributed to the antimicrobial efficiency.[23] A study by de Oliveira *et al*. showed a significant reduction in plaque and gingival index scores after the use of fluoridated dentifrice for a period of 1 month.[24] A study by Bruhn *et al*. evaluated the efficacy of triclosan containing dentifrice. It showed marked antigingivitis as well as plaque-inhibitory effects during 28-week maintenance period in periodontitis patients.[25] A study by Barnes *et al*. showed that Colgate Total significantly reduced plaque regrowth as compared to Crest Pro-Health when assessed for 24-h time period ($P \leq 0.05$).[26]

The present study has the advantage of a placebo group, which is the most rigorous test of evaluating treatment efficacy for a medical therapy.[27] Furthermore, in the present study, there was an increase in the plaque and gingival index scores seen in the placebo group. In the present study 14–15 years' children were selected, as this group represents a full complement of permanent dentition (except for third molars). Fifteen years is considered as the global indicator age and represents the end of mixed dentition period. At this age the permanent dentition has been exposed to the oral cavity for 3–9 years, so the assessment of plaque and gingivitis levels found to be more meaningful. A study done by Honkala *et al*. revealed the strong effect of duration of toothbrushing on plaque removal.[28] Therefore the type of toothbrush, dentifrice, frequency and method of brushing are controlled in the present study. A study done by Loe *et al*. showed that clinical signs of gingivitis will be observed within 10–21 days.[4] Therefore the gingival scores were assessed at baseline and at 21 days.

The strengths of the study include random selection of the participants, block randomization method, concealed random allocation, and blinding of the investigator, participant, and statistician. These methods reduced selection bias, allocation bias, and confounder bias. Furthermore, the oral hygiene technique was standardized for all the participants. The compliance was assessed directly through the checklist and indirectly by assessing the dentifrice tubes of each participant. Limitations of the study are as follows. Participants did not adhere to the protocol of tooth brushing and majority of participants brushed only once daily. Few participants (24) did not return the filled checklist. As dentifrices were distributed to participants for home use, the amount of paste used at each interval and technique of brushing couldn't be evaluated. Majority of participants reported slight bitter taste of TD, but despite the taste, the participants utilized the dentifrice during the study period. The PD became hardened and participants were unable to dispense and use it in the last few days (4–5 days). The study results showed comparable antiplaque and antigingivitis effect of TD to FD. Medicinal properties of TD are globally known; therefore, it can be recommended for regular use among adolescents for maintenance of oral health in areas where fluoride is considered endemic and fluoridated dentifrices are not advocated. Future studies are recommended to evaluate the long term effect of tulsi extract dentifrice and its effect among people of different age groups and in different time intervals in the reduction of dental plaque and gingivitis.

Conclusion

In the present study, there was a significant reduction in the plaque and gingivitis indices scores in tulsi and FD groups. However, there was no significant difference in the antiplaque and antigingivitis efficacy of 4% tulsi and commercially available Fluoridated dentifrice among 14–15 years school children in Davangere city.

Acknowledgement

We duly acknowledge Bapuji Pharmacy College for the preparation of Tulsi extract dentifrice, placebo dentifrice and Principals, teachers, Parents and students of the three schools for their cooperation. I extent my gratitude to Dr. Mahesh (Public Health Dentist) for statistical assistance and Dr. Chandrabhaga (Post graduate - Public Health Dentistry) for conducting concealed randomization and demonstration of brushing technique to participants.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Chrysanthakopoulos NA. Prevalence of gingivitis and associated factors in 13-16-year-old adolescents in Greece. Eur J Gen Dent 2016;5:58-64.
2. Sreenivasan PK, Prasad KV, Javali SB. Oral health practices and prevalence of dental plaque and gingivitis among Indian adults. Clin Exp Dent Res 2016;2:6-17.
3. Mazumdar M, Chatterjee A, Majumdar S, Chandrika M, Patki PS. Evaluation of the safety and efficacy of complete care herbal toothpaste in controlling dental plaque gingival bleeding and periodontal diseases. J Homeop Ayurv Med 2013;2:124.
4. Loe H, Theilade E, Jensen SB. experimental gingivitis in man. J Periodontol 1965;36:177-87.
5. Pari A, Ilango P, Subbareddy V, Katamreddy V, Parthasarthy H. Gingival diseases in childhood – A review. J Clin Diagn Res 2014;8:ZE01-4.
6. Singh DK, Jayanti I. Gingival diseases: World and Indian scenario a background check. J Dent Otolaryngol 2015;15:1-6.
7. Bali RK, Mathur VB, Talwar PP, Chanana HB. National Oral Health Survey and Fluoride Mapping, 2002-2003, India. Delhi: Dental Council of India; 2004.
8. De Rossi A, Ferreira DC, da Silva RA, de Queiroz AM, da Silva LA, Nelson-Filho P. Antimicrobial activity of toothpastes containing natural extracts, chlorhexidine or triclosan. Braz Dent J 2014;25:186-90.
9. Bhateja S, Arora G. Therapeutic benefits of holy basil (tulsi) in general and oral medicine: A review. Int J Res Ayurveda Pharm

2012;3:761-4.
10. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev 2010;4:95-105.
11. Narayan A, Thomas SJ, Daniel A, Fatima S, Mathew RA. An overview of oral health in India: Current scenario and challenges. Int J Oral Care Res 2016;4:280-3.
12. Gupta D, Bhaskar DJ, Gupta RK, Karim B, Jain A, Singh R, *et al*. A randomized controlled clinical trial of *Ocimum sanctum* and chlorhexidine mouthwash on dental plaque and gingival inflammation. J Ayurveda Integr Med 2014;5:109-16.
13. Damle SG, Patil A, Jain S, Damle D, Chopal N. Effectiveness of supervised toothbrushing and oral health education in improving oral hygiene status and practices of urban and rural school children: A comparative study. J Int Soc Prev Community Dent 2014;4:175-81.
14. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc 1962;65:26-9.15.
15. Loe H, Silness J. Periodontal disease in pregnancy. I. prevalence and severity. Acta Odontol Scand 1963;21:533-51.
16. Agarwal P, Nagesh L. Comparative evaluation of efficacy of 0.2% chlorhexidine, listerine and Tulsi extract mouth rinses on salivary *Streptococcus mutans* count of high school children – RCT. Contemp Clin Trials 2011;32:802-8.
17. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomedicine 2000;7:7-13.
18. Singh S. Comparative evaluation of antiinflammatory potential of fixed oil of different species of *Ocimum* and its possible mechanism of action. Indian J Exp Biol 1998;36:1028-31.
19. Singh S, Majumdar DK. Evaluation of antiinflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. Indian J Exp Biol 1997;35:380-3.
20. Shokeen P, Bala M, Singh M, Tandon V. *In vitro* activity of eugenol, an active component from *Ocimum sanctum*, against multiresistant and susceptible strains of *Neisseria gonorrhoeae*. Int J Antimicrob Agents 2008;32:174-9.
21. Davies RM, Ellwood RP, Davies GM. The effectiveness of a toothpaste containing triclosan and polyvinyl-methyl ether maleic acid copolymer in improving plaque control and gingival health: A systematic review. J Clin Periodontol 2004;31:1029-33.
22. Gupta P, Agarwal N, Anup N, Manujunath BC, Bhalla A. Evaluating the anti-plaque efficacy of meswak (*Salvadora persica*) containing dentifrice: A triple blind controlled trial. J Pharm Bioallied Sci 2012;4:282-5.
23. Kurian M, Geetha RV. Effect of herbal and fluoride toothpaste on *Streptococcus mutans* – A comparative study. J Pharm Sci Res 2015;7:864-5.
24. de Oliveira SM, Torres TC, Pereira SL, Mota OM, Carlos MX. Effect of a dentifrice containing *Aloe vera* on plaque and gingivitis control. A double-blind clinical study in humans. J Appl Oral Sci 2008;16:293-6.
25. Bruhn G, Netuschil L, Richter S, Brecx M, Hoffmann T. Effect of a toothpaste containing triclosan on dental plaque, gingivitis, and bleeding on probing – An investigation in periodontitis patients over 28 weeks. Clin Oral Investig 2002;6:124-7.
26. Barnes VM, Richter R, DeVizio W. Comparison of the short-term antiplaque/antibacterial efficacy of two commercial dentifrices. J Clin Dent 2010;21:101-4.
27. Castro M. Placebo versus best-available-therapy control group in clinical trials for pharmacologic therapies: Which is better? Proc Am Thorac Soc 2007;4:570-3.
28. Honkala E, Nyyssönen V, Knuuttila M, Markkanen H. Effectiveness of children's habitual toothbrushing. J Clin Periodontol 1986;13:81-5.

Annexure 1: Daily brushing checklist

Name:____

Name of School:-

Compliance checklist

Days	Morning brushing	Quantity of toothpaste dispensed ½/1/2 PEA sized	Night brushing	Quantity of toothpaste dispensed ½/1/2 PEA sized
				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				

If you have used the given toothpaste and toothbrush, kindly put (✓) and if not put ×mark. ✓Used the toothpaste, ×Did not use the toothpaste

Annexure 2: Questionnaire

Q1. If you have not used the given toothpaste, give reasons by selecting the options given below

a. I forgot to use
b. The toothpaste and brush was not kept in routine place
c. I forgot to carry the toothpaste and toothbrush to my visitors place
d. If others, please specify

Q2. Did you like the taste of the toothpaste?

a. Yes
b. No

Q3. Did you like the color of the toothpaste?

a. Yes
b. No

Q4. Are you satisfied with the foam, while brushing?

a. Yes
b. No

Q5. Do you recommend any changes required in the toothpaste?

a. Yes
b. No

If yes, Please specify

Thank you