Original Article

Comparative Evaluation of Antimicrobial Efficacies of 0.2% Chlorhexidine and 4% Tulsi Extract in the Decontamination of Child Toothbrushes: An Observational Analytical Study

Lekshmy S. R. Nair[1], Anandaraj Soman[1], Sageena George[1], Deepak Jose[1], Shaniya Sain[1], Shiad Salim[2]

[1]Departments of Pediatric and Preventive Dentistry and [2]Oral and Maxillofacial Surgery, PMS College of Dental Science and Research, Trivandrum, Kerala, India

Received : 23-08-21
Revised : 07-11-21
Accepted : 09-11-21
Published : 29-01-22

ABSTRACT

Aims: The aim of this study was to evaluate the efficacy of 0.2% chlorhexidine and 4% tulsi extract as toothbrush decontaminants. **Materials and Methods:** Of 100 children, who attended the outpatient unit of Department of Pediatric dentistry, 81 children, who satisfied all the inclusion criteria were subjected to systematic sampling, after arranging them in the alphabetical order and were grouped into three. The first child came under Group I, second under Group II, third under Group III, fourth one again under Group I, and so on till the 81st child. In the baseline phase, the children were provided precoded toothbrushes and toothpastes and instructed to place those brushes to be put after use, in single-use glasses. After obtaining the baseline value of *Streptococcus mutans* colony count, the participants of Group I was given 0.2% chlorhexidine, Group II was given 4% tulsi extract, and distilled water for Group III, to be used as toothbrush decontaminants for 5 days. The toothbrushes were then collected back, and were subjected to microbial analysis. Statistical analysis was performed by using Kruskal–Wallis one-way analysis of variance (ANOVA) and Mann–Whitney *U* test with $P < 0.05$. **Results:** All test solutions, 0.2% chlorhexidine and 4% tulsi extract, except for distilled water, showed a statistically significant reduction of *S. mutans* count. There was no statistical difference between the efficacies of 4% tulsi extract and 0.2% chlorhexidine, although the latter showed a better reduction. **Conclusion:** Tulsi extract may well be a perfect replacement to chlorhexidine for reducing the *S. mutans* count in the child toothbrushes.

Keywords: *Chlorhexidine, decontamination, distilled water, toothbrush, tulsi extract*

Introduction

Contaminated toothbrushes may play an important role in many oral and systemic diseases.[1] Persistence of microbes on toothbrush could be a viable cause of re-contamination of the mouth.[2] A great many studies have shown that extended use of the toothbrush favors bacterial contamination.[2-4] The chances for these toothbrushes to be related to transferal of serious health problems such as infective endocarditis, arthritis, and stroke have also been registered[5,6] Often, toothbrushes are just rinsed in the plain water after use that forms an ideal niche for microbial agents.[3,4,7] There is a complete lack of awareness among the public regarding the increased chances for cross-infection from toothbrushes kept together in close contact.[8,9] Therefore, it is of prime importance to have proper knowledge about disinfection of toothbrushes.

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_245_21

Address for correspondence: Dr. Lekshmy S. R. Nair, Department of Pediatric and Preventive Dentistry, PMS College of Dental Science and Research, Trivandrum 695028, Kerala, India.
E-mail: lekshmysrnair@gmail.com

How to cite this article: Nair LS, Soman A, George S, Jose D, Sain S, Salim S. Comparative evaluation of antimicrobial efficacies of 0.2% chlorhexidine and 4% tulsi extract in the decontamination of child toothbrushes: An observational analytical study. J Int Soc Prevent Communit Dent 2022;12:85-92.

A number of procedures have been described to reduce the microbiological load of toothbrushes, such as continuous replacement of toothbrushes, submerging the brush into microbicidal solutions such as chlorhexidine, sodium hypochlorite, Listerine, and Dettol or spraying antiseptic solutions using ozone, ultraviolet (UV) radiation rays, and microwaves as well.[10] Only a few number of studies have been carried out with volunteers.[11]

In a meta-analysis with eight randomized controlled trials, natural agents were found to be effective in decreasing the microbial colonization of toothbrush bristles.[7,9,10,12-17] Polyphenolic compounds in herbal products are accountable for their anti-*Streptococcus mutans* property, which inhibits the glucosyltransferase enzyme activity of *S. mutans*.[12,18,19]

With due consideration to available evidence pertaining to side effects and emergence of uncommon infections with the usage of synthetic antimicrobial agents and the fact that resistance to currently used chemotherapeutics is the major factor that necessitates the search for alternative safe, efficacious, and cost-effective treatment options, particularly in developing countries. This study was conducted in the quest of identifying tulsi as a possible alternative or an adjunct in the decontamination of toothbrushes. Several plant products such as garlic, neem, lemon, tea tree oil, and others have been tested for their antimicrobial properties in the past with considerable success.[12] Tulsi is a medicinal herb and is well known for its healing powers. Chlorhexidine is used as a gold standard and is currently the most potent chemotherapeutic agent against *S. mutans* and dental caries.[20] Therefore, an attempt is made to compare the antimicrobial activity of 4% tulsi with 0.2% chlorhexidine against *S. mutans*.

Materials and Methods

A pilot study was conducted initially using and the sample size for this study has been calculated using the following formula:

$n = 2\sigma^2[Z\alpha+Z\beta]^2 \div \delta^2$

where n = sample size per group,

$\sigma = 1.23 \times 10^3$ (pooled standard deviation),

α =5% (type I error),

$Z\alpha = 1.96$,

β = 20% (type II error),

$Z\beta = 0.84$,

δ = (difference in mean) 1×10^3,

Thus, $n = 23.722 \approx 24$.

Considering 10% lost follow up, $n = 24+2.4=26.4\approx 27$.

So, total sample size = $27 \times 3 = 81$(for three groups).

A total of 100 children who attended the outpatient unit of the Department of Pediatric dentistry formed the study population. Institutional Ethical Committee Certification was obtained (Protocol no. PMS/IEC/2015/20). The study had the following inclusion and exclusion criteria:

(a) Inclusion criteria:
Children of age group 10–12 years.
Children under high-risk caries group (with ≥ 1 interproximal lesion).

(b) Exclusion criteria:
Those who were undergoing any kind of dental treatment
Those who have been using any kind of antimicrobial mouth rinses

There was a dropout of 19 children. A total of 81 children who satisfied all the inclusion criteria were subjected to systematic sampling after arranging them in alphabetical order and were grouped into three. The first child came under Group I, second under Group II, third under Group III, fourth one again under Group I, and so on till the 81st child. Each group was assigned different toothbrush decontaminant solutions such as Group I—0.2% chlorhexidine gluconate, Group II—4% tulsi extract, and Group III—distilled water, of which Group III was taken as the control group [Figure 1].

Initially, the children were provided color-coded toothbrushes (Group I—blue, Group II—green, Group III—white) and toothpastes. They were taught the horizontal scrub tooth brushing technique and thereafter subjected to supervised brushing at home, using the same technique for 3 min— in the morning before breakfast and in the night after dinner, for five consecutive days. After brushing, they had to wash the toothbrushes under tap water for 30 s. They were also instructed to keep their toothbrushes in the separately provided disposable glass containers in such a manner that the head of the brush with the bristles should face outside and be left open for drying.

Parents of the children were periodically reminded. The used color-coded toothbrushes were taken back from the participants after 5 days. These toothbrushes were then stored in separate disposable sterile sealed plastic pouches and subjected to baseline microbial analysis.

On the day of collecting the first set of toothbrushes, another set of new color-coded toothbrushes along with the test solutions (Group I—0.2% chlorhexidine, Group II—4% tulsi extract, and Group III—distilled water) was provided to each group. This was done by

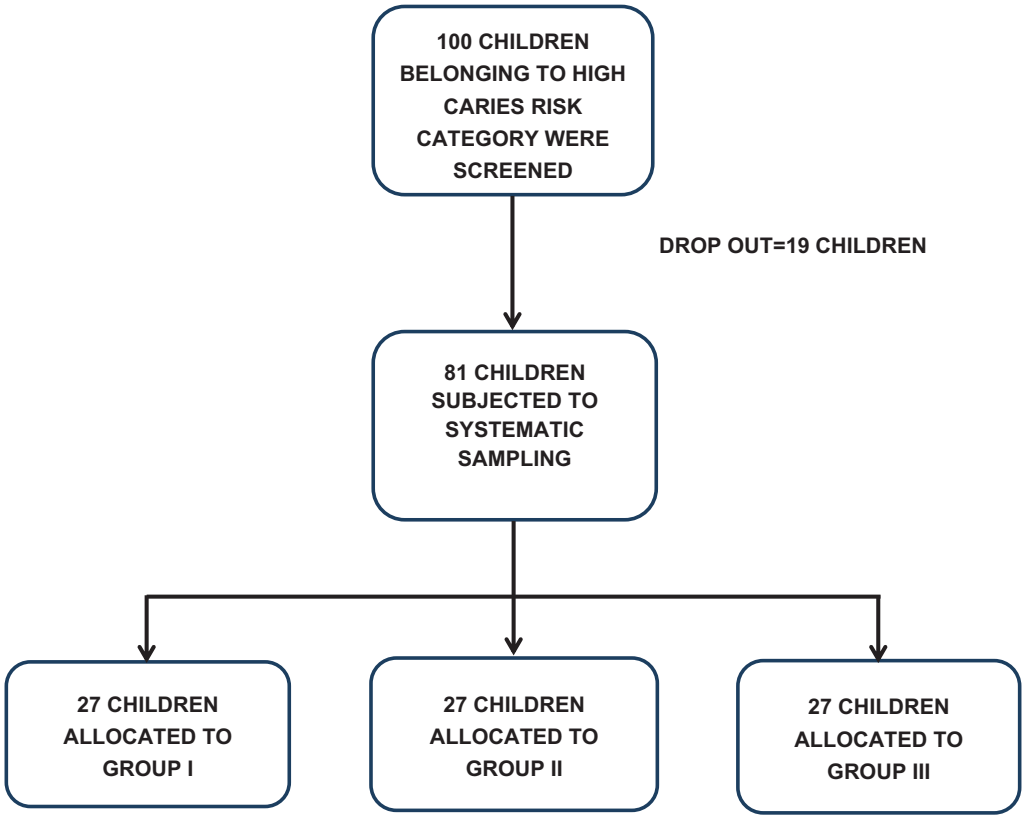


Figure 1: Diagram showing participant flow

a third party, to keep the investigator blinded to the assignment. The participants were given the same instructions for handling the used toothbrushes, but this time, they were to be kept in separate disposable glasses containing the disinfectant agent provided to them. They were supposed to keep their toothbrushes such that the toothbrush head was completely immersed into 10 mL of the solutions taken in the disposable glasses given to them, for a time period of 2 h, following brushing. After rinsing the toothbrushes under running tap water for 30 s, their toothbrushes were to be kept in disposable glasses and leave them for drying.

Care was taken to change the solutions after each use. After 5 days, the brushes were collected as before and subjected to microbial analysis.

Microbiological procedure

To recover *S. mutans* colonies from the used toothbrushes, 100 mg of detached bristles were resuspended in sterile phosphate buffered saline (PBS) and vortexed vigorously in a cyclomixer for 10 min. The solution was then centrifuged at 1200 revolutions per minute for 10 min in a microcentrifuge at 4°C to settle down the bristles. 20 µL of the supernatant was then swabbed onto the Mitis Salivarius Agar uniformly and incubated at 37°C in microaerophilic conditions for 4 days. After incubation, the colonies were counted using Digital Colony Counter, and colony-forming units per mL were calculated [Figure 2].

Statistical analysis

The Kruskal–Wallis one-way analysis of variance (ANOVA) method was used to compare the differences between three groups together. The Mann–Whitney *U* test was used to compare the differences between two groups at a time taking $P < 0.05$ as significant.

Results

Data were entered in Microsoft Excel and the analysis was performed using the SPSS software program. The Kruskal–Wallis one-way ANOVA method was used for the comparison of three groups together. The Mann–Whitney *U* test was used to compare the differences between two groups at a time. In all the above tests, the value of $P < 0.05$ was accepted as indicating significance.

All 81 subjects were present from the beginning to the end of the study. The initial colony count of *S. mutans* ranged from 0.2 to 12.24 $\times 10^3$. The differences in the colony count of *S. mutans* in toothbrushes before and after interventions (0.2% chlorhexidine and 4% tulsi extract) within their own group are given in Table 1.

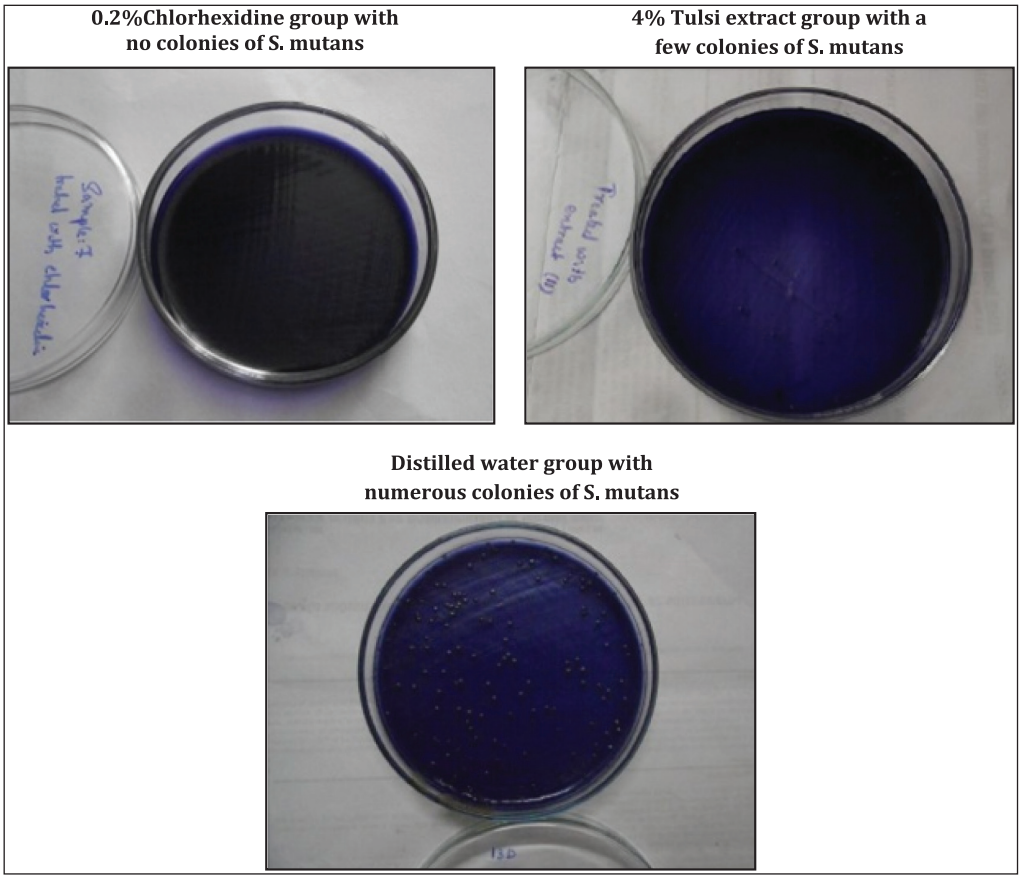


Figure 2: Postintervention microbial colony count in the three groups

The comparison of the potencies of 0.2% chlorhexidine and 4% tulsi extract as toothbrush disinfectants is as shown in Table 2 [Graph 1]. The maximum average reduction was seen in the chlorhexidine group, which was 5935.19 CFU/mL. In the case of tulsi extract, it was 5011.11 CFU/mL and finally the least reduction or rather an increase, in the distilled water group, which was –874.59 CFU/mL. Here, the greater reduction in the mean values was found in the chlorhexidine group, followed by the tulsi extract group and almost no reduction in the mean values were found in the distilled water group.

Multiple comparisons taking two groups at a time were done by using Mann–Whitney *U* test, as shown in Tables 3–5 [Graphs 2-4]. When we compared chlorhexidine and tulsi extract, even though there was a clinically significant difference regarding the average bacterial count; it was not statistically significant. This indicated that chlorhexidine was superior to tulsi extract. When both chlorhexidine and tulsi extract were compared with distilled water, there were highly significant differences. In relation to the distilled water, there was an increase in the bacterial count, whereas in the other groups there was a significant reduction.

Table 1: Differences in the colony count of *Streptococcus mutans* in toothbrushes before and after interventions (0.2% chlorhexidine, 4% tulsi extract, and distilled water) within their own group

	Difference in bacterial counts		
Groups	**Mean**	**Median**	**Standard deviation**
Chlorhexidine	5935.19 (increase)	5720.00	3154.697
Tulsi extract	5011.11 (increase)	4500.00	2767.411
Distilled water	–874.59 (d ecrease)	–80.00	2847.560

Discussion

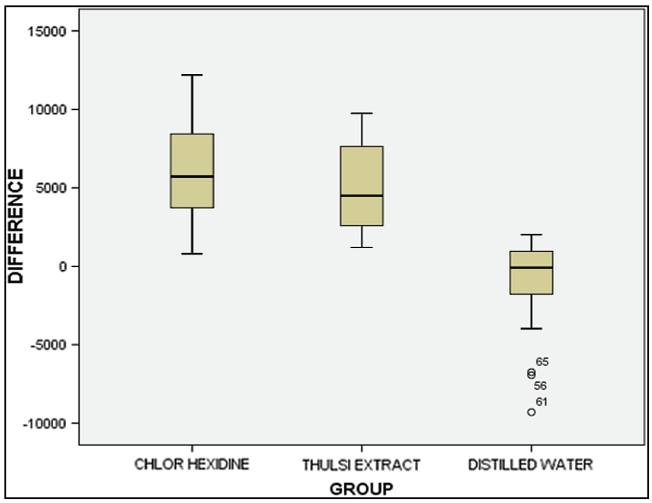
In order to have sound oral hygiene, pertinent toothbrush care and maintenance are required and an individual should replace his/her toothbrush every 3–4 months.[21] Nevertheless, periodic change of toothbrush raises the maintenance cost, giving more economical advantage to the use of toothbrush disinfectants.[15]

In this research, an attempt had been made to evaluate and compare the efficacies of chlorhexidine and tulsi extract as toothbrush disinfectants against *S. mutans*. Study participants, of 10–12 years, having high-risk of caries, with≥ 1 interproximal lesion were considered.[22] This age group was chosen, for in this age; they are

Table 2: Comparison of the potencies of 0.2% chlorhexidine and 4% tulsi extract as toothbrush disinfectants

Comparisons	Mean	Median	Standard deviation	Test used	*P* Value
Chlorhexidine	5935.19	5720.00	3154.697	Kruskal–Wallis test	
Tulsi extract	5011.11	4500.00	2767.411	Chi-square = 48.941	0.0001 HS
Distilled water	–874.59	–80.00	2847.560		

HS = highly significant



Graph 1: Box–Whisker plot of data comparing the potencies of 0.2% chlorhexidine, 4% tulsi extract, and distilled water

through the mixed dentition period, which is the time for transitional changes in the oral microbiota, and also it is of uttermost importance to make the school children aware about oral health as they would continue these habits all through their life.

In this study, the initial number of colony-forming units of *S. mutans* ranged 0.2–12.24 $\times 10^3$ per mL. In another study by Anand *et al.*,[12] the initial number of *S. mutans* ranged 5×10^3 to 8.8×10^3 CFU/mL. The disparity in CFU could be due to the dissimilar age groups and varying time intervals for toothbrush collection used in these studies.

In this study, 4% tulsi extract and distilled water were compared with chlorhexidine as the antimicrobial agent to know the efficacy in reducing contamination of the toothbrush.

Routine identification of mutans streptococci is generally based on growth on selective media based on Mitis Salivarius Agar, colony morphology, and biochemical characteristics.[23] Thus, in this study, Mitis Salivarius Agar was used for the growth of *S. mutans.*

The toothbrushes from the participants were collected after 5 days, after the prescribed method of decontamination, unlike in previous studies.[12,24] The time interval of 5 days was selected because specimen collection toward weekends was expected to reduce the dropouts.

The toothbrushes were dipped in disinfecting solutions for 2 h only. It has been reported that 0.12% chlorhexidine successfully eliminated microbial species such as *S. mutans, Candida albicans, Staphylococcus aureus*, and *S. pyogenes* within 10 min.[25] As tulsi is a natural product and applicable for molecular deterioration, the disinfection period was limited to 2 h to evaluate the rapid antimicrobial activity of the extract.

In the present investigation, chlorhexidine showed a 100% reduction in the *S. mutans* count. The result of this study was concurrent with studies conducted that produced a 100% reduction of the *S. mutans* count by the use of chlorhexidine.[25] Chlorhexidine kills bacteria by disrupting the cell membrane.

Ocimum sanctum L. has specific aromatic odor because of the presence of essential or volatile oil, which are polyphenolic compounds, mainly concentrated in the leaf. An array of extractants have been tried to solubilize antimicrobials from plants like water or alcohol (methanol/ ethanol), which is used for the preparation of large quantity of crude extracts.[23] Ethanolic extracts are more powerful due to the better solubility of active components in organic solvents.[26] In this study, we had used an ethanolic extract of tulsi for its greater antimicrobial effect.

Agarwal *et al.*[13] had conducted a study that concluded that 4% tulsi extract showed an antimicrobial property against *S. mutans.*

In this study, when the differences in the colony count of *S. mutans* in toothbrushes before and after interventions (0.2% chlorhexidine and 4% tulsi extract) within their own group were compared, greater reduction in the mean values was found in chlorhexidine group, followed by tulsi extract group and almost no reduction in the mean values were found in the distilled water group. In comparing the potencies of 0.2% chlorhexidine and 4% tulsi extract as toothbrush disinfectants, maximum average reduction was seen in the chlorhexidine group which was 5935.19 CFU/mL. In the case of tulsi extract, it was 5011.11 CFU/mL and finally the least reduction

Table 3: Comparison between chlorhexidine and tulsi extract

Comparisons	Mean	Median	Standard deviation	Test used	*P* Value
Chlorhexidine	5935.19	5720.00	3154.697	Mann–Whitney *U* test Value = 305.000	0.303 NS
Tulsi extract	5011.11	4500.00	2767.411		

NS = nonsignificant

Table 4: Comparison between chlorhexidine and distilled water

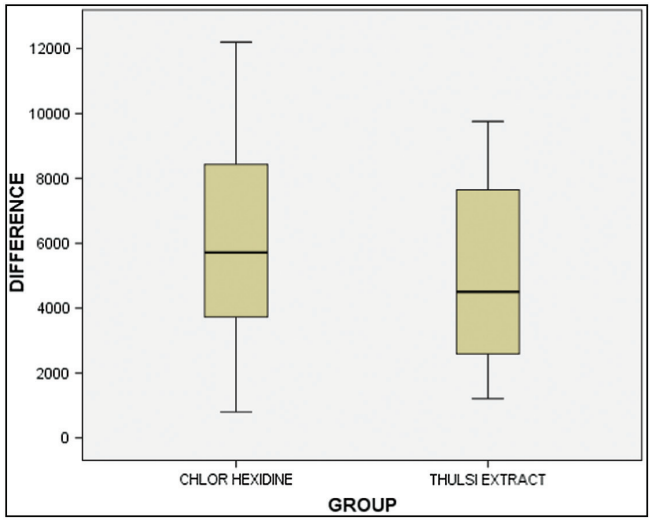
Comparisons	Mean	Median	Standard deviation	Test used	*P* Value
Chlorhexidine	5935.19	5720.00	3154.697	Mann–Whitney *U* test Value = 18.000	0.0001 HS
Distilled water	–874.59	–80.00	2847.560		

HS = highly significant

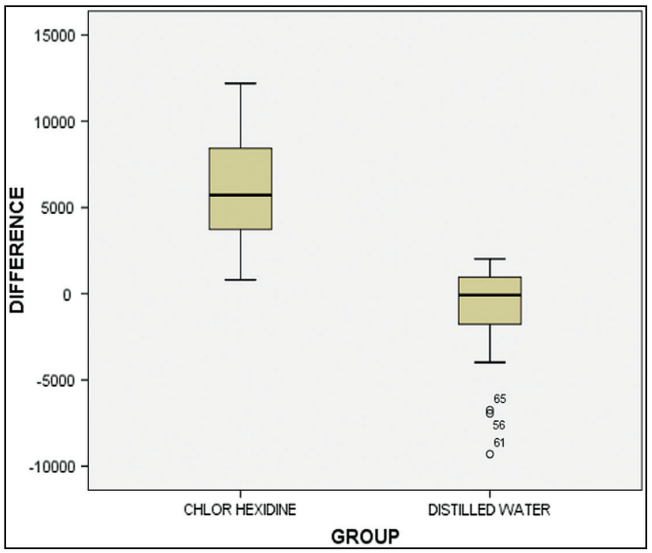
Table 5: Comparison between tulsi extract and distilled water

Comparisons	Mean	Median	Standard deviation	Test used	*P* Value
Tulsi extract	5011.11	4500.00	2767.411	Mann–Whitney *U* test Value = 16.000	0.0001 HS
Distilled water	–874.59	–80.00	2847.560		

HS = highly significant



Graph 2: Box–Whisker plot of data comparing between chlorhexidine and tulsi extract



Graph 3: Box–Whisker plot comparing between chlorhexidine and distilled water

or rather an increase, in the distilled water group, which was –874.59 CFU/mL.

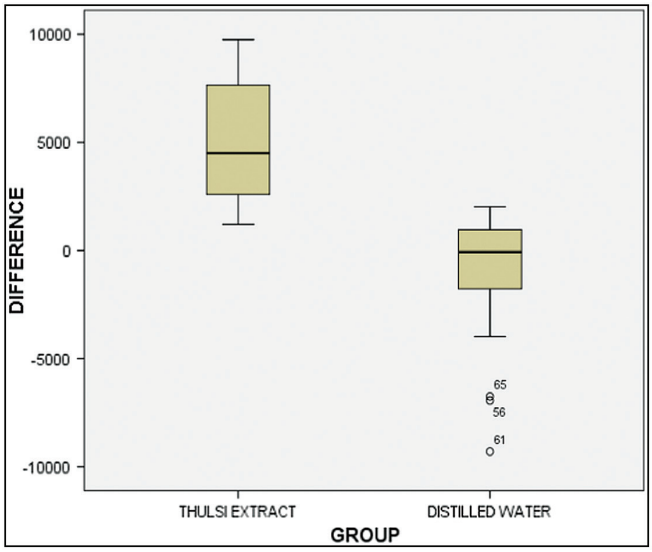
When we compared chlorhexidine and tulsi extract, even though there was a clinically significant difference regarding the average bacterial count, it was not statistically significant. This indicated that chlorhexidine was superior to tulsi extract. Thus, this study suggested that both chlorhexidine and tulsi extract are successful agents that could be used in disinfection of toothbrushes. As there was a notable increase in contamination of toothbrushes dipped in distilled water, it is an inefficient procedure of toothbrush cleaning.

Conclusion

This study showed a clinically significant difference in the colony count of *S. mutans* in toothbrushes before and after interventions (0.2% chlorhexidine and 4% tulsi extract) within their own group. Although 0.2% chlorhexidine had the highest efficacy, 4% tulsi extract, which is a herbal product, can be used as a potent alternative to chlorhexidine as a disinfectant for toothbrushes.

Future scope/clinical significance

To the best of our knowledge, this is the first study comparing 4% tulsi extract with 0.2% chlorhexidine



Graph 4: Box–Whisker plot comparing between tulsi extract and distilled water

in toothbrush decontamination, being performed by volunteers to simulate the natural conditions of daily life. Another advantage is that the tulsi extract that is commercially available has increased shelf life than chlorhexidine by more than 2 years. As this study considered only single immersion period (2 h) and time interval (5 days), studies could be tried with various immersion times and intervals to know more about the decontaminating abilities. Additional clinical research is needed to widen our knowledge of various antimicrobial agents, especially natural products, in the prevention of dental caries.

Ethical policy and institutional review board statement

The study had been approved by Institutional Ethical Committee, PMS College of Dental Science and Research (Protocol no. PMS/IEC/2015/20, dated November 28, 2015). Informed written consent for participation in the study and publication of the data for research and educational purposes was obtained. All the procedures have been performed as per the ethical guidelines laid down by Declaration of Helsinki.

Data availability statement

The data set used in this study is available from the corresponding author Dr. Lekshmy S. R. Nair (email: lekshmysrnair@gmail.com) upon request.

Acknowledgement

I would like to express my special thanks of gratitude to Dr. Swathy Anand PJ, Reader, Department of Public Health Dentistry, PMS College of Dental Science and Research, Trivandrum who helped me in doing a lot of research regarding the current topic and finalizing this project within the limited time frame.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Authors' contribution

Not applicable.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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