COMPARISON OF THE EFFICACY OF HOMEMADE HERBAL DISINFECTANTS WITH CHLORHEXIDINE FOR DECONTAMINATION OF TOOTHBRUSH: A RANDOMIZED CONTROLLED TRIAL

DISSERTATION

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In the partial fulfillment of the requirement for the degree

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MASTER OF DENTAL SURGERY

In

PUBLIC HEALTH DENTISTRY

By

DR. ANKITA SINGH

Under the guidance of

DR. ANURADHA P.

Professor & Head

Department of Public Health Dentistry

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

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Submitter email	anuradhap1963@bbdu.ac.in
Similarity	4%
Analysis address	anuradhap1963.bbduni@analysis.urkund.com

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Date: 1/7/ 2021

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Ankita Singh

Dr. Ankita Singh

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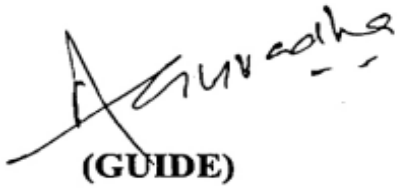

(GUIDE)

Dr. Anuradha P

Professor & Head

Public Health Dentistry

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(CO-GUIDE)

Dr. Tanushri Chatterji

Reader

Department of Microbiology

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Dr. Anuradha P.

Professor & Head

Public Health Dentistry

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Dr. B. Rajkumar

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Babu Banarasi Das College of Dental Sciences,

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Ankita Singh

Dr. Ankita Singh

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Dr. Ankita Singh

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LIST OF ABBREVIATIONS

S. No.	Abbreviations	Full forms
1.	n	Number of subjects
2.	SD	Standard Deviations
3.	±	Plus-Minus
4.	≥	Greater than or equal to
5.	≤	Less than or equal to
6.	S. mutans	Streptococcus Mutans
7.	CHX	Chlorhexidine Gluconate
8.	Group I	Distilled water group
9.	Group II	Neem group
10.	Group III	Garlic group
11.	Group IV	Chlorhexidine group
12.	CFU	Colony Forming Unit
13.	ml	Millilitre
14.	ADA	American Dental Association
15.	DMFT	Decayed Missing Filled Teeth
16.	WHO	World Health Organization
17.	%	Percentage
18.	Sq.km	Square kilometer

19.	ft	Feet
20.	e.g.	Example
21.	hr	Hour
22.	^{0}C	Celsius
23.	SPSS	Statistical Package For Social Sciences
24.	ANOVA	Analysis of Variance
25.	T test	Student t test
26.	IEC	Institutional Ethical Committee

Abstract:

Introduction: Toothbrush is the most common oral hygiene aid used, but maintaining and storing the toothbrush hygienically is commonly neglected. In India people are not aware about the contamination of toothbrushes. Contaminated toothbrushes can act as a vector for the transmission or reinfection of various bacteria, viruses, and fungi. The herbs, which have pharmaceutical properties can be used as an alternative. The aim of the study was to compare antimicrobial efficacy of 3% Neem and 3 % Garlic on *Streptococcus mutans* and to compare with 0.2% Chlorhexidine mouthwash as toothbrush decontaminants.

Materials & Methods: A triple blind randomized controlled trial was done on 120 subjects. The subjects were divided into 4 groups- Group I (Distilled water) i.e. control and 3 study groups having 3% Neem (Group II), 3% Garlic extract (Group III) and 0.2% Chlorhexidine Gluconate (Group IV). Subjects were provided toothbrushes and toothpastes for both baseline and intervention phases. The toothbrushes were collected after 14 & 28 days for microbial analysis in both phases. The data were analysed and compared using appropriate t test and ANOVA. The level of significance was set at $p < 0.05$.

Results: Garlic (Group III) was most effective 98% followed by Chlorhexidine (Group IV) 96% and Neem (Group II) 88% in reducing the level of Streptococcus Mutans. Distilled water (Group I) showed only 3% reduction.

Conclusion: Neem and Garlic were proved to be effective as Chlorhexidine and these herbal products can be used as an alternative to Chlorhexidine as disinfectants for toothbrushes. These are the common ingredients which can be

easily found in Indian households. It is herbal, cost effective and has no side-effects so it can be easily used by every individual as toothbrush decontaminant.

Keywords: Chlorhexidine, Garlic, Neem, Toothbrush, Streptococcus Mutans, Blood agar.

Introduction:

Oral health is an integral part of general health. It directly and indirectly reflects the overall well-being of an individual. A poor oral health can affect the course and pathogenesis of several of systemic disease like cardiovascular disease, bacterial pneumonia, diabetes mellitus, and low birth weight. Thus, maintaining good oral hygiene becomes very important.[1]

Even though there are different oral hygiene aids available in the market, none of them gives complete protection. In chemo-mechanical plaque control a toothbrush plays a vital role for maintaining personal oral hygiene and it is effective against plaque removal.

Most commonly used aid for maintaining oral hygiene is Toothbrush, but maintaining and storing the toothbrushes hygienically is commonly neglected.[2] In India people are not aware about the contamination of toothbrushes.

A newly manufactured toothbrush is free of microorganisms. But after a single use, they may become contaminated by a wide array of microorganisms present both in the oral cavity and in the external environment.[3] Microorganisms can remain viable on toothbrush bristles for periods ranging from 24 hours to seven days.[4] Contaminated toothbrushes can act as a vector for the transmission or reinfection of various bacteria, viruses, and fungi.[5]

In majority of Indian households, toothbrushes of family members are stored in the same container. The routine use of contaminated toothbrushes might lead to disseminate microorganisms within the oral cavity of the same person or between different individuals since.

A method to reduce contamination of toothbrushes might be a helpful means to avoid re–infection and cross–infection risks among individuals. So there is a need for disinfection methods, which are rapidly effective, cost-effective, nontoxic, and can be easily implemented.[6]

With times there is an increase in incidence of drug resistance in the prevalent pathogens and an associated risk with chemotherapeutic agents, it is essential to find an alternative to existing drugs. The herbs, which have known pharmaceuticals properties can be the best source of these alternative drugs.[7]

In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.[8]

Garlic (Allium sativum) is an essential part of cuisine and is used in many parts of the country. The "garlic" word is derived from the old English word *gārlēac* i.e spear leek. The origin of garlic dates back from around 5000 to 6000 years and it was native of central Asia.[9] It has been used in native medicine from ancient times for the treatment of various diseases. It is widely known for having an array of medical benefits, with antiviral, anti-fungal and antibacterial properties. There are a few reports on antimicrobial property of garlic activity against oral microorganisms in which 2.5 and 3% concentrations showed good antimicrobial activity against S. mutans.[10,11]

Neem (*Azadirachta indica*) is a tree that belongs to the mahogany family 'Maliaceae'. Neem is empolyed in traditional medicine as a source of therapeutic agents in the Indian culture and grows well in tropical countries.[12] Every part of the tree has been used as traditional medicine for household remedy against various diseases in India. This tree is also regarded as "Village dispensary" in India. This

tree was proven to have antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer and antifungal properties.[13]

Chlorhexidine Gluconate (CHX) is the biguanide compound having topical antibacterial activity. It is the positively charged molecule and it reacts with the negatively charged microbial cell surface, thereby destroying the integrity of the cell membrane. Subsequently, it penetrates into the cell and causes leakage of intracellular components leading to cell death.[14] Properties of Chlorhexidine gluconate (CHX) has been studied extensively for many years and it is currently one of the safest and most efficient chemotherapeutic agents against S. mutans.[15] It is considered as a "gold standard" but it is associated with few side effects such as discoloration of teeth, irritation of mucosa, altered taste sensation, swelling of parotid, and increased supragingival calculus formation.[15]

There is limited information available comparing the potency of garlic, neem and chlorhexidine gluconate as toothbrush disinfectants. Hence, the present investigation was designed to study the antimicrobial efficacy of 3% Neem and 3 % of Garlic on *Streptococcus mutans* and to compare with 0.2% chlorhexidine as toothbrush disinfectants.

Aim:

To compare the efficacy of 3% Neem, and 3% of Garlic with 0.2% Chlorhexidine mouthwash as toothbrush disinfectants.

Objectives:

1. To compare the efficacy of Neem (3% conc.) with 0.2 % Chlorhexidine mouthwash as toothbrush disinfectants.
2. To compare the efficacy of Garlic (3% conc.) with 0.2 % Chlorhexidine mouthwash as toothbrush disinfectants.
3. To compare the efficacy of Neem (3% conc.) with efficacy of Garlic (3% conc.)
4. To conduct awareness programmes and educate people regarding toothbrush contamination and their decontaminants.

Hypothesis of the study:

Null hypothesis:

There is no difference in the antibacterial effect of Neem, Garlic and Chlorhexidine as toothbrush disinfectants.

Alternate hypothesis:

There is difference in the antibacterial effect of Neem, Garlic and Chlorhexidine as toothbrush disinfectants.

Review of literature:

1. **Paulo Nelson Filho et al in 2000** [16] conducted a study to determine the level of contamination of toothbrushes by mutans streptococci using microbiological identification, to access the bacterial contamination using scanning electron microscopy (SEM) and to evaluate the efficacy of two toothbrush disinfectants. Nineteen children used their toothbrushes once a day, for five consecutive days. The toothbrushes were then immersed into disinfectant solutions for 20 h: Group I - 0.12% chlorhexidine gluconate; Group II - 1% sodium hypochlorite; Group III - sterile tap water. They were then placed into test tubes containing CaSa B, for 3 to 4 days at 37°C. The number of MS cfu was counted and the toothbrushes were submitted to SEM analysis. They found that was no bacterial growth in Groups I and II; Group III showed MS growth (range, 21 to 120 cfu). Scanning electron microscopy showed biofilm formation on toothbrush bristles.

2. **Suma H P Sogi et al in 2002** [17] conducted a study to evaluate the level of contamination of toothbrush after brushing and at the same time, to know the efficacy of various disinfecting solution in reducing their contamination. Thirty two children in the age group of 12-14, residing in Government Hostel were selected. They were divided into four groups of 8 each, and were supplied with toothbrushes. Toothbrushes were cultured to assess the contamination at different time intervals. Control group had shown the highest percentage of contamination. It was concluded that cleaning of the oral cavity is not the only procedure in maintaining the oral hygiene, the oral hygiene devices should also be kept clean.

3. **Bhat S S , Hegde K S and George R M in 2003** [18] conducted a study to evaluate the presence of microorganisms in the toothbrushes and the effect of disinfectant such as chlorhexidine gluconate or sodium hypochlorite to decontaminate them.

Twenty one children were asked to brush their teeth for five days. The brushes were put in Robertson's Cooked Meat broth and cultured and growth of Streptococcus Mutans were seen. These brushes were then placed in disinfectants such as 0.2% chlorhexidine gluconate (Gp I), 1% sodium hypochlorite (Gp II) and water (Gp III) for 24hrs and then cultured. Disappearance of growth of microorganisms was seen in Gp I and Gp II and remnants of growth seen in Gp III. It can be concluded that the use of disinfectant is a must for every individual at regular intervals.

4. **Sandra Sato et al in 2004** [19] conducted a study to evaluate bacterial survival rate on toothbrushes after brushing and the efficacy of their decontamination by spraying antimicrobial solutions. Thirty subjects were instructed to spray the solutions on toothbrush bristles after brushing. Each volunteer tested three sprays, one solution per week; the sprays were labeled spray 1 (cetylpyridinium chloride - CPC - and basic formulation), 2 (basic formulation only) and 3 (control - sterile tap water). At the end of each week, the brushes were collected and sonicated in Letheen Broth®; the suspensions were ten-fold diluted and the dilutions were plated on various culture media. Anaerobic bacteria, evaluated by colony count of black pigment producing organisms on Ask medium, were recovered from 83.3% of the samples, Streptococci from 80% and aerobic Gram-negative bacilli from 46.7% of them in the control tests. There was a significant decrease in toothbrush contamination with antimicrobial sprays 1 and 2, the first showing the greatest decrease on bacterial counts.

5. **Sandra Sato et al in 2005** [20] conducted a study to evaluate the efficacy of a spray containing an antimicrobial solution for toothbrush disinfection. Three different solutions were sprayed on toothbrush bristles among 30 adults after they had

brushed: (1) basic formulation (base) plus chlorhexidine; (2) base only, and (3) sterile tap water (control). Each solution was tested for 1 week. After that, the toothbrushes were collected and sonicated in Letheen Broth, diluted in 10-fold series, and plated on selective and nonselective media for detection of anaerobes, aerobes, streptococci, and gram-negative bacilli. After incubation, the colonies of those microorganisms were counted. Presence of mutans streptococci on the bristles was also confirmed. Spray 1 produced a significant reduction in the microbial contamination of toothbrushes for all the microorganisms, spray 2 provided some reduction of contaminants, and spray 3 demonstrated the least antimicrobial effect.

6. **MM Fani, J Kohanteb and M Dayaghiin in 2007** [21] conducted a study to investigate in vitro inhibitory activity of garlic extract on multidrug-resistant (MDR) strains of Streptococcus mutans isolated from human carious teeth. Filter sterilized aqueous extract of garlic was prepared and used in the present study. For isolation of S. mutans, extracted human carious teeth were cultured in Todd-Hewit broth and Mitis-Salivarius-Bacitracin agar. S. mutans was characterized by colony morphology, biochemical tests and other conventional bacteriological procedures. Disk sensitivity tests and broth dilution methods were used to determine antibiotic sensitivity profile and inhibitory activity of garlic extract on S. mutans isolated from carious teeth. Of 105 carious teeth tested, 92 (87.6%) isolates of S. mutans were recovered, among which 28 (30.4%) were MDR since they were resistant to four or more antibiotics. The highest rate of resistance was observed for tetracycline (30.4%) and least resistance (0%) to teichoplanin and vancomycin while 22.8% and 23.9% of the isolates were resistant to penicillin and amoxicillin, respectively. Chlorhexidine minimum inhibitory concentration (MIC) for MDR and non-MDR S. mutans varied from 2 to 16 microg ml(-1) and from 0.25 to 1

microg ml(-1), respectively (P<0.05). All isolates, MDR and non-MDR of S. mutans were sensitive to garlic extract with the MIC ranging from 4 to 32 microg ml(-1). Considering in vitro data obtained in the present study, mouthwashes or toothpaste containing optimum concentration of garlic extract could be used for prevention of dental caries.

7. **Saravia ME et al in 2008** [3] conducted a study to assess in vitro the viability of *Streptococcus mutans* on toothbrush bristles relative to the drying time. Forty-five toothbrushes were soaked in a suspension containing *S mutans* (ATCC 25175) in a 1,720.000 cfu/mL concentration (0.5 McFarland scale) for 4 minutes, rinsed in sterile tap water, and assigned to 9 groups. Group 1 toothbrushes were immediately incubated in CaSaB CaSaB (bacitracin sucrose broth—selective enrichment broth) culture medium for 4 days. Toothbrushes from groups 2 to 9 were kept at room temperature for 4, 8, 12, 24, 36, 48, 60, and 72 hours, respectively, and subsequently incubated in CaSaB culture medium. It was observed that micro-organisms were present on toothbrushes of groups 1 to 3, ranging from 50 to 100+ cfu. From the 12-hour drying period on, there was no growth of *S mutans*. Regarding the *S mutans* cfu, the results were expressed in scores and submitted to the Kruskal Wallis statistical test. It was observed that groups 1 to 3 were similar to each other (*P*>.05) and differed significantly (*P*<.001) from other groups, which, in turn, behaved similarly (*P*>0.05). From the 12-hour drying period on, there was a statistically significant decrease in the number of *S mutans* cfu (*P*<.01).

8. **Rafi A Al–Talib, Rayia J Alnaimi and Eman A Mustafa in 2008** [22] conducted a study to evaluate the bacterial survival rate on toothbrushes after brushing and the efficacy of their decontamination by immersing them in different antimicrobial solutions when toothbrushes were not in use. Twenty healthy dental students

participated in this study, they were all supplied with a new toothbrush of the same brand and type together with identical tubes of fluoridated toothpastes and were asked to brush twice daily for a period of 4 weeks during which they were asked to follow their oral hygiene practices. The students were divided into 3 groups, the first group kept their toothbrushes in a ventilated area exposed to air after brushing; the second group kept their toothbrushes soaked in 1% Sodium hypochlorite solution (1 part bleach, 4 parts of distilled water); the third group immersed their toothbrushes in 0.2% chlorhexidine gluconate solution. After one month the toothbrushes were collected, decapitated and examined in the laboratory by making bacteriological cultures to identify the aerobic and anaerobic microorganisms in each toothbrush, also the total live bacterial counts per toothbrush were obtained. The toothbrushes that were not immersed in any antimicrobial solution were heavily contaminated and the immersing of toothbrushes in 0. 2 % chlorhexidine gluconate solution was a very effective method in reducing the mean number of aerobic and anaerobic microorganisms.

9. **Aswini Y Balappanavar et al in 2009** [23] conducted a study to test the efficacy of 3% neem, 2% triclosan, 0.2% chlorhexidine gluconate and 1% sodium hypochlorite as toothbrush disinfectants against Streptococcus mutans. This was a double-blind, linear crossover, within-group comparative experimental trial conducted among 40 children aged 12 to 15 years. The study was divided into five phases: (1) distilled water (control); (2) 3% neem; (3) 2% triclosan; (4) 0.2% chlorhexidine gluconate; and (5) 1% sodium hypochlorite. The toothbrushes were collected after 5 days of brushing and were soaked for 12 h in antimicrobial solutions of separate phases, after which the toothbrushes were submitted for microbial analysis to check for the presence of S. mutans. The mean colony-forming units (CFUs) of S. mutans at phase 1 when compared with baseline was

not significant (P = 0.17). The other phases had a significant drop in mean CFUs of S. mutans from baseline (P < 0.001). The percentage reduction of mean CFUs of S. mutans from baseline was the highest in phase 2 (86%).

10. **Yu-Ying Den; Hsien-Chung Chiu and Yi-Bing Wang in 2009** [24] conducted a study to examine the effects of filtered garlic extracts and diallyl sulfide on the acid production and the growth of Streptococcus mutans 10449 and BHT. Results showed that both garlic extracts and diallyl sulfide could enhance the rate of acid production and inhibit the growth of S. mutans. Strain BHT was highly susceptible, and strain 10449 less sensitive. These data indicate that garlic extracts stimulates the acid production and may thus be harmful to the teeth if eaten with glucose-containing foods, but may inhibit the growth of S. mutans and subsequently reduce the caries incidence.

11. **Nanjunda Swamy KV et al in 2011** [25] conducted a study to determine the level of contamination of tooth brushes by Streptococcus mutans using microbiological identification, to assess the bacterial contamination using SEM and to evaluate the efficacy of different disinfectant solutions in reducing the contamination of tooth brushes. 24 children used their tooth brushes once a day, for five consecutive days. The tooth brushes were then immersed into disinfectant solution for 20 hours. Group I -0.12% chlorhexidine gluconate, Group II- 1% sodium hypochlorite, Group III - 3% hydrogen peroxide, Group IV- Tap water. They were then placed into test tubes containing bacitracin sucrose solution for 3 to 4 days at 370 C. Streptococcus mutans colonies were assessed and the tooth brushes were submitted for SEM analysis. There was no bacterial growth observed in Group I, II and III, Group IV showed Streptococcus mutans growth. SEM showed biofilm formation on tooth brush bristles.

12. **Aarati Nayak et al in 2011** [26] conducted a study to determine the inhibitory effect of Azadirachta indica (aqueous and alcoholic extract of neem) on Streptococcus mutans, Enterococcus faecalis and Candida albicans. The activity of Azadirachta indica against Candida albicans, Streptococcus mutans and Enterococcus faecalis was tested by serial broth dilution method and was expressed by minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) of the aqueous neem extract to all the organisms was 7.5%. The MIC of the alcoholic neem extract for E. faecalis, S.mutans, C. albicans were 1.88%, 7.5%, and 3.75% respectively.

13. **Padma K Bhat et al in 2012** [2] conducted a study to determine the effectiveness of antimicrobial solutions on Streptococcus mutans in used tooth brushes. 60 children used their toothbrushes twice a day for seven consecutive days. The toothbrush bristles were then immersed into antimicrobial solutions for 12 hours: Group I-3% neem ; group II – 5% turmeric; group III- 0.5 % cetylpyridinum chloride, group IV- 0.2% chlorhexidine glucoate. They were then placed into test tubes containing the resultant suspensions were three-folds diluted. Aliquots of 0.1 ml were plated in Mitis Salivarius Agar using dilution and plating method. Incubation was done in an anaerobic jar for 72 hours at 37^0C. The results obtained showed that there was 88% reduction in the Streptococcus mutans in the 3% neem group, 86% reduction in the streptococcus mutans in the 0.5% cetylpyridinium chloride group.78% reduction in the streptococcus mutans in the 5% turmeric group, 65% reduction in the streptococcus mutans in the 0.2% chlorhexidine gluconate group. The difference between them was found to be statistically significant ($p<0.001$).

14. **Amin Mansour et al in 2012** [27] conducted a study to compare the effect that garlic juice and Chlorhexidine mouthwash has on oral pathogens. Fresh garlic

bulbs were used to extract the antibacterial juice. The yellow juice was separated from the pulp with Whatman filter paper. After filtration the liquid was freeze dried and stored at 4 C until required. The bacteria that were tested were: Streptococcus mu tans, S. sanguis, S. salivarius and Lactobacillus casei. After cultivation of the bacteria, the Minimal Inhibitory Concentration (MIC) of the garlic juice and Chlorhexidine were measured using the E-test method, then the Minimal Bactericidal Concentration (MBC) of the Chlorhexidine and garlic juice were measured by tube test. The lowest MIC of garlic juice was for S. mutans 0.25 mu g/mL and the highest was for L. casei 2.5 mu g/mL. The MIC of Chlorhexidine for these two bacteria was 0.62 mu g/mL ands mu g/mL respectively. The MBC of Chlorhexidine and garlic for S. mutans had the lowest concentration compared with the other tested bacteria. The MBC of Chlorhexidine and garlic for S. mutans was 0.35 mu g/mL and 0.3 mu g/mL respectively. The highest MBC of Chlorhexidine was for S. salivarius 10 mu g/mL. The MBC of garlic for S. sanguis was similar at 10.4 mu g/mL.

15. **Dithi Chandrdas et al in 2014** [28] conducted a study to assess and compare the efficacy of 3% garlic extract, 0.2% tea tree oil, 0.2% chlorhexidine, 0.05% cetylpyridinium chloride, and ultravoilet (UV) toothbrush sanitizing device as toothbrush disinfectants against Streptococcus mutans. It was a double blind randomized controlled parallel study was done on 210 dental students. The subjects were divided into one control group using distilled water and five study groups representing 0.2% tea tree oil, 3% garlic extract, 0.2% chlorhexidine gluconate, 0.05% cetylpyridinium chloride and UV toothbrush sanitizing device. Participants were provided with new toothbrushes and toothpastes for both baseline and intervention phases. The toothbrushes were collected after two weeks for microbial analysis in both phases. The data were analysed and compared using

appropriate statistical analysis. On comparing pre- and post-intervention, S. mutans colony counts, a highly significant ($P < 0.001$) difference was observed in all the groups. Differences of 77.74 colony forming units (CFU) in tea tree oil group, 102.87 CFU in garlic group, 68.13 CFU in chlorhexidine group, 82.47 CFU in cetylpyridinium group and 42.67 CFU in UV toothbrush sanitizer group were observed. Garlic group showed the highest reduction (100%) whereas UV toothbrush sanitizer group showed the least reduction (47.4%) in S. mutans colonies.

16. **Selvamani Manickam et al in 2014** [29] conducted a study to determine the presence of microorganism in the toothpaste and used tooth bristles at 15 min and 24 h after brushing. 25 subjects were involved in the study. They were requested to follow their normal oral hygiene practices for a period of 2 weeks and to store their toothbrushes and pastes in the usual storage places. At the end of 2nd week, sample from the bristle were collected from each brush 15 min and 24 h after brushing. Samples of toothpaste left at the opening of toothpaste tube were also collected with the help of sterile straight probe. Samples were placed in Brain Heart Infusion (BHI) broth and incubated for 24 h at 37°C. Twenty-eight percent of the toothbrush showed contamination 15 min after brushing with a mean colony forming unit (CFU) count of 1.20 ± 2.12 and almost all (96%) the toothbrushes showed contamination after a period of 24 h with mean CFU count of 71.68 ± 54.96. Thirty-two percent of the toothpastes showed contamination 15 min after brushing with a mean CFU count of 5.40 ± 14.65.

17. **P J Swathy Anand et al in 2016** [6] conducted a study to compare the efficacy of 3% neem, garlic of concentration 4.15 mg/mL and green tea of concentration 40 mg/mL with 0.2% chlorhexidine mouthwash as toothbrush disinfectants. The study

was a parallel in vitro comparative experimental trial conducted among 75 randomly selected boys aged between 18 years and 21 years. The subjects were divided into five groups, namely, Group I, Group II, Group III, Group IV, and Group V. They were provided with a new set of precoded toothbrushes and nonfluoridated tooth pastes. After 14 days of tooth brushing, the toothbrushes were immersed in antimicrobial solution for 12 h [Group I—distilled water (control), Group II—3% neem, Group III—garlic of concentration 4.15 mg/mL, Group IV—green tea of concentration 40 mg/mL, and Group V—0.2% chlorhexidine] and then subjected to microbial analysis to check the presence of Streptococcus mutans. The t-test and analysis of variance (ANOVA) were done using Statistical Package for the Social Sciences (SPSS) software version 16. All test solutions showed a statistically significant reduction of Streptococcus mutans count ($P < 0.001$). There was no statistical difference between the efficacies of neem, garlic, and green tea when compared with chlorhexidine mouthwash ($P > 0.05$).

18. **Vethakkan Bijivin Raj et al in 2017** [30] conducted a study to evaluate the effectiveness of vinegar, lime, and salt water as potential household decontaminants for toothbrushes. It is an in vitro study, 120 used toothbrushes were collected and divided into four groups comprising 30 samples each. Group I was treated with plain water alone after use. Group II was treated with salt water. Group III was treated with a solution which contained lime juice. Group IV was treated with vinegar. Treatment duration for the groups was set to be 12 h. After the treatment, the brush heads of the four groups were incubated in brain-heart infusion agar at 37°C for 24 h. Statistical analysis was performed using the Statistical Package for the Social Sciences version 20.0 software. One-way analysis of variance and Bonferroni post hoc analysis were performed for multiple

comparisons. Vinegar group showed statistically significant result for decontamination of toothbrushes when compared to other test and control agents.

19. **Prasad Jathar, Amey Panse and Aniket R. Desai in 2018** [31] conducted a study to evaluate the effectiveness of different disinfectant agents in decontaminating the toothbrushes and educate the children, parents, and the community about toothbrush decontamination. Seventeen children were asked to brush their teeth for 1 month. After 1 month, toothbrushes were collected. The bristles from these brushes were then placed in disinfectants such as 0.2% chlorhexidine (Group I), water (Group II), hydrogen peroxide (Group III), 5% sodium hypochlorite (Group IV), and Group V as a control for 12 hrs and then cultured. Hydrogen peroxide (Group III solution) considerably reduced the bacterial colonies.

20. **Rohan Sachdev et al in 2019** [32] conducted a study to evaluate the effectiveness and compare the negative oral microbial flora of dental plaque after the use of a self-contaminated multiple use toothbrush and that of a single-use toothbrush. The study conducted with 60 participants who were free from any systemic or oral disease and without any adverse habits. In these participants, plaque samples were collected after 1 month use of a self-contaminated multiple use toothbrush. Each participant was given a set of 30 new toothbrushes and a toothpaste tube and instructed to use one toothbrush everyday and discard it after use. The plaque samples were collected on seven days interval and cultured on Mitis Salivarius agar. The colonies were identified and their count was recorded. Student *t* test was applied. *Streptococcus mutans*, *S. sanguis*, *S. milleri*, and *Candida* were identified from the samples. A highly marked decrease in their numbers was found after the use of a single-use toothbrush over multiple use toothbrushes.

Materials & Methods:

The present study was done to compare the efficacy of 3% Neem, and 3% Garlic with 0.2% Chlorhexidine mouthwash as toothbrush disinfectants.

Study area:

The study was performed at Department of Public health dentistry, Babu Banarasi Das College of Dental Sciences in collaboration with Department of microbiology Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow for the microbial analyses.

Lucknow is the capital city of the Indian state of Uttar Pradesh.

The city stands at an elevation of approximately 123 metres (404 ft) above sea level. Lucknow district covers an area of 2,528 square kilometres (976 sq m).

Bounded on the east by Barabanki, on the west by Unnao, on the south by Raebareli and in the north by Sitapur and Hardoi, Lucknow sits on the northwestern shore of the Gomti River.

According to the provisional report of 2011 Census of India, Lucknow city had a population of 2,815,601, of which 1,470,133 were men and 1,345,468 women.

The city has a total literacy level in 2011 of 84.72% compared to 67.68% for Uttar Pradesh as a whole.

Study population:

The study population consisted of 18-25 years old students of Babu Banarasi Das University.

Study Design:

A parallel, multiple arm, in vitro comparative experimental trial was conducted to compare the efficacy of 3% Neem, and 3% Garlic with 0.2% Chlorhexidine mouthwash as toothbrush disinfectants.

Sampling technique:

Simple random sampling was done.

Eligibility Criteria:

Inclusion criteria

- The subjects aged between 18 - 25 years
- Atleast three single-rooted and two multi rooted functional teeth per quadrant (third molars excluded)
- Decayed, missing, and filled teeth (DMFT) score of <3 were included in the study.

Exclusion Criteria

- Subjects using antibiotics or antiseptic mouthwashes for at least 3 months prior to the study,
- Undergoing any dental treatment, with orthodontic or with extensive intraoral prosthesis,
- Medically compromised patients (e.g., in diabetic patients)

- Subjects using neem, garlic and chlorhexidine in any form as an oral hygiene aid were excluded from the study.
- Subjects not willing to participate in the study.

Pilot study:

Pilot study was conducted on 20 participants to check for the feasibility of the study and they were not included in the final study.

A pilot study saves precious time, identify potential difficulties and prompt modifications that may be necessary before the actual start of the study.

Sample size estimation:

Sample size was calculated with the help of G-power analysis software, keeping the power of the study at 80%, alpha error at 5%, effect size at 0.25,[6] beta error 20% and confidence interval at 95% a sample size of 28 was obtained for each group. The final sample size was decided to be 30 in each group. There are 4 groups in the study so a total of 120 healthy volunteers were involved in the study.

Calibration and Training

Prior to the study, the investigator (1) was trained and calibrated in the department of Public Health Dentistry.

Selection of study participants:

Clinical examination:

Type III clinical examination was done.

The examination was carried in the BBD university premises with consistent lighting and by wearing surgical gown & face shield.

Subjects were examined seated in a chair with a head supported by a wall with the examiner standing by the side of the chair.

A study specific proforma was used to record demographic details, medical and dental history of each participant.

The caries experience was calculated using DMFT index by Klein, Palmer and Knutson (WHO modified 1997) and participants who had a DMFT score of <3 were included in the study.

Out of the 325 students screened, 152 students fulfilled the inclusion criteria. From the eligible 152 study participants, the final required 120 subjects were randomly selected.

Recording clerk:

Recording clerk was assigned to record the data. The person was made familiar with the terms and codes of the survey before hand to avoid any discrepancy of information.

Survey forms :

Appropriate number of designed proforma was used during the study.

Trial design:

Allocation ratio: 1:1:1:1

Allocation sequence: participants were allocated to the group by envelope method

Type of randomization: Simple random

Unit of randomization: DMFT score <3

Blinding:

It was a triple blinded clinical trial. The study subjects remained blind to further procedures after collection of toothbrushes. The subjects, microbiologist and statistician remained blind regarding the disinfectant solutions the toothbrush was kept in.

Implementation:

Participants were assigned to the group and generation of sequence was done by the organizing clerk.

Ethical Issues:

Ethical clearance was obtained by the Institutional Ethical committee of Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Consent:

The purpose of the study was explained and the informed written consent was obtained from all the study participants.

Patients informed consent is a legal regulation and a moral principle. It represents the patient's rights to take part in the clinical decision.

Schedule of the survey:

The study was done from October 2020- December 2020.

Intervention:

None

All the participants reverted back after 14 and 28 days.

Materials and Equipments:-

For clinical examination:

Mouth mirror

Explorer

Tweezer

Kidney tray

Mouth mask

Face shield

Gloves

Headcap

Surgical gown

Hand sanitizer

Surface Disinfectant

Cotton & cotton holder

Toothbrush

Tooth paste

Sterilized pouches

For microbiological study:

3% Neem extract

3 % Garlic concentrate

Chlorhexidine mouthrinse (0.2%)

Whatman's No. 1 filter paper

Muslin cloth

Weighing scale

Examination gloves

Sterile test tube

Sterile micropipettes with disposable tips

Autoclave

Culture Media Plate

Inoculating loop

Incubator

Vortex

Diagnostic instrument

Kidney tray

Cotton & cotton holder

Mouth mask

Gloves

Face shield

Headcap

Surgical gown

Hand sanitizer &
Surface Disinfectant

Figure 1: Materials and Equipments

Neem

Garlic

Water

Chlorhexidine mouthrinse

Muslin cloth

Filter paper

Sterilized pouches

Toothpaste

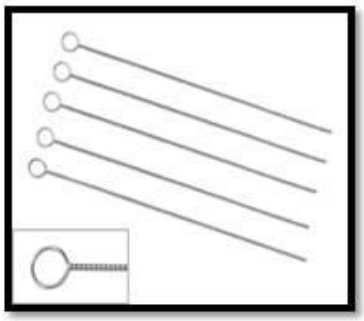
Toothbrush

Figure 2: Materials and Equipments

Weighing scale

Mixer grinder

Sterile Micropipettes



Inoculating loop

Autoclave

Incubator

Culture media plate

Vortex

Sterile test tube

Figure 3: Materials and Equipments

Preparation of solutions:

3 % Neem

100 grams of Neem leaves were blended in a mixing jar and the extract was filtered first through a muslin cloth and then it was filtered into a jar using a Whatman's No. 1 filter paper. To make it 3%, 3 ml of Neem leaves extract was mixed with 100 ml of distilled water. Solution was prepared freshly on the day of microbial examination.

3 % Garlic

For preparing 3% Garlic extract, 100 grams of fresh garlic obtained from the local market was peeled and blended in a mixer and the extract was first filtered by using a muslin cloth and then into another jar using a Whatman's No. 1 filter paper. To make it 3%, 3 ml of garlic extract was mixed with 100 ml of distilled water. Garlic solution was prepared freshly on the day of microbial examination.

0.2% Chlorhexidine

Chlorhexidine 0.2% (Hexidine, ICPA Health Products Ltd, India) was used, which was readily available in the market.

Sterility control:

5 new toothbrushes (Oral B Shine) was freshly opened from the packets and were subjected to microbial analysis to check for *S.mutans* colonies on bristles. This was done so as to ensure that the new toothbrushes were free from contamination before its use by study participants.

Methodology:

This study was a single center parallel in vitro comparative experimental trial.

After obtaining informed consent from the study participants, eligible participants were assigned into 4 groups (envelope method). The selected 120 subjects were divided into: four groups. (30x4)

Group 1- Distilled water (control)

Group 2- 3% Neem extract

Group 3- 3% of Garlic extract

Group 4- 0.2% Chlorhexidine gluconate

All the subjects were provided with pre-color coded toothbrushes (Oral B Shine) and toothpaste (Colgate) during the entire study procedure. All of them were instructed to brush twice daily using a horizontal scrub method for 2-3 min and to rinse the toothbrushes under running tap water for 20 seconds after brushing.

They were instructed to keep their toothbrushes in such a manner that the head of the brush with the bristles should face outside and be left open for drying. Subjects

were periodically reminded to follow the instructions via mobile phone. The study was conducted in two phases.

First phase:

In the first phase, all participants were provided with a new set of pre-color coded toothbrushes and toothpaste. The toothbrushes were collected back after 14 days.

These toothbrushes were then stored in separate disposable sterile sealed plastic pouches and then transferred to the laboratory for microbial analysis. This was done to obtain the baseline analysis.

Second phase:

In the second stage, the 120 subjects were again provided with a new set of toothbrushes and toothpastes. The toothbrushes were collected back after 28 days. The collected toothbrushes were again transferred to the laboratory.

Microbial analysis:

The bristle from one tuft from each toothbrush was separated aseptically and Group I was aseptically introduced directly into test tubes containing 5 mL of distilled water (control). Similarly, the bristles from the subjects in Group II, Group III and Group IV were immersed in the test tubes containing 3% neem, 3 % of garlic, and 0.2% chlorhexidine mouthwash for 12 hours.

The samples were then vortexed for 15 second to dislodge the bacteria from the bristles to the media using a cyclomixer and the solution was inoculated using a platinum inoculation loop. 10 μL of the vortexed sample was transferred on blood agar medium by spread plate method. The plate was incubated for 24-48 hours at

37°C in an incubator. After 24 hours the colonies were counted through manual method and it was expressed as colony forming units (CFU)/ml. After inoculation & incubation total colony count was calculated. Total colony count was calculated using the formulae: colony forming unit (cfu)/ml=total colony count × 1000. Further, the colony count was subjected to statistical analyses to observe the efficacy best obtained among the three decontaminants. The laboratory procedure was similar for both the stages.

Data analysis:

Data was entered in Microsoft Excel and the analysis was performed using SPSS software version 20 (IBM Corporation). The t-test was used for comparing the baseline values and test values; analysis of variance (ANOVA) was used for multiple group comparison. In all the above tests, $P < 0.05$ was considered as statistically significant.

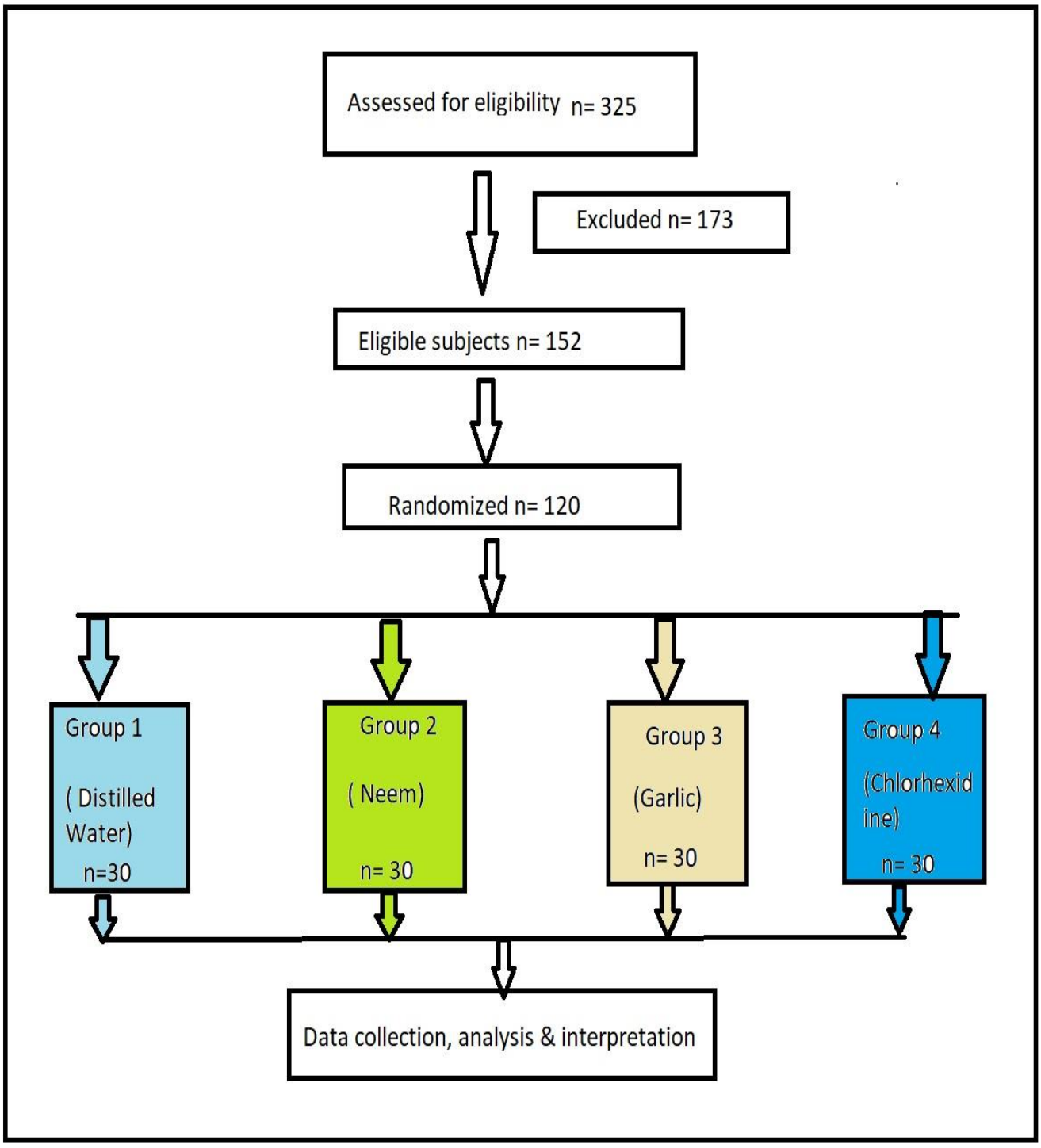


Figure 4: Schematic representation of methodology

Figure 5: Clinical examination

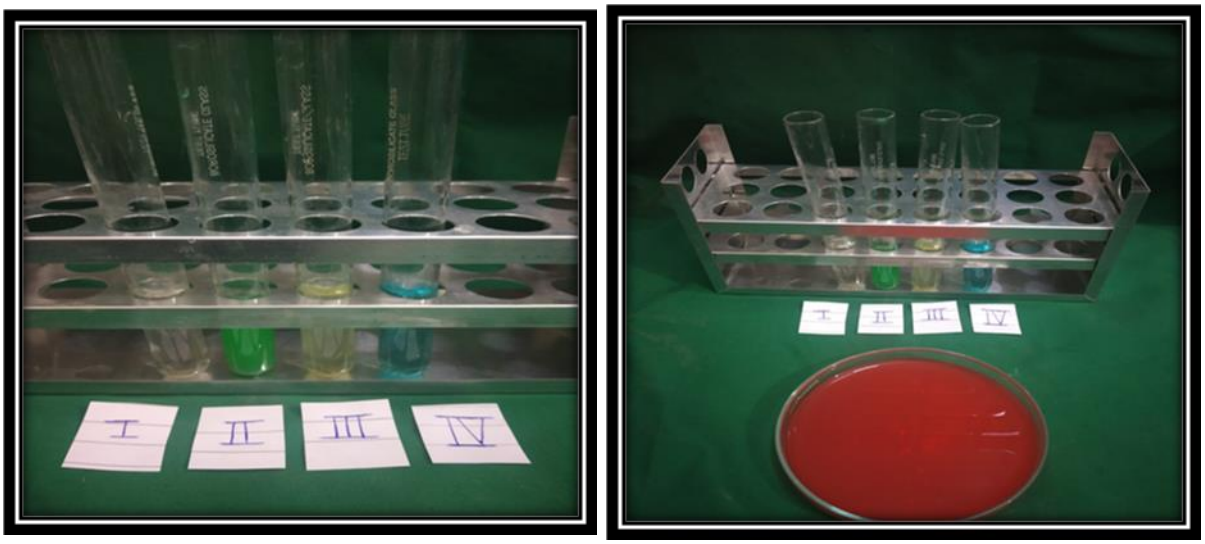


Figure 6: Toothbrush bristles in test tube

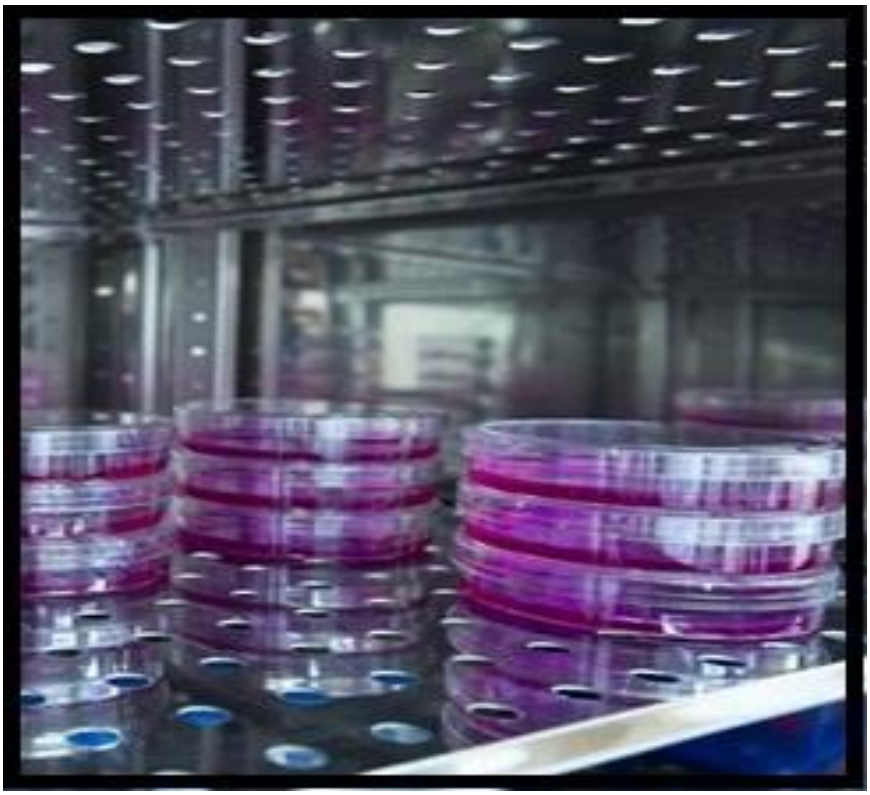


Figure 7: Culture plate in incubator

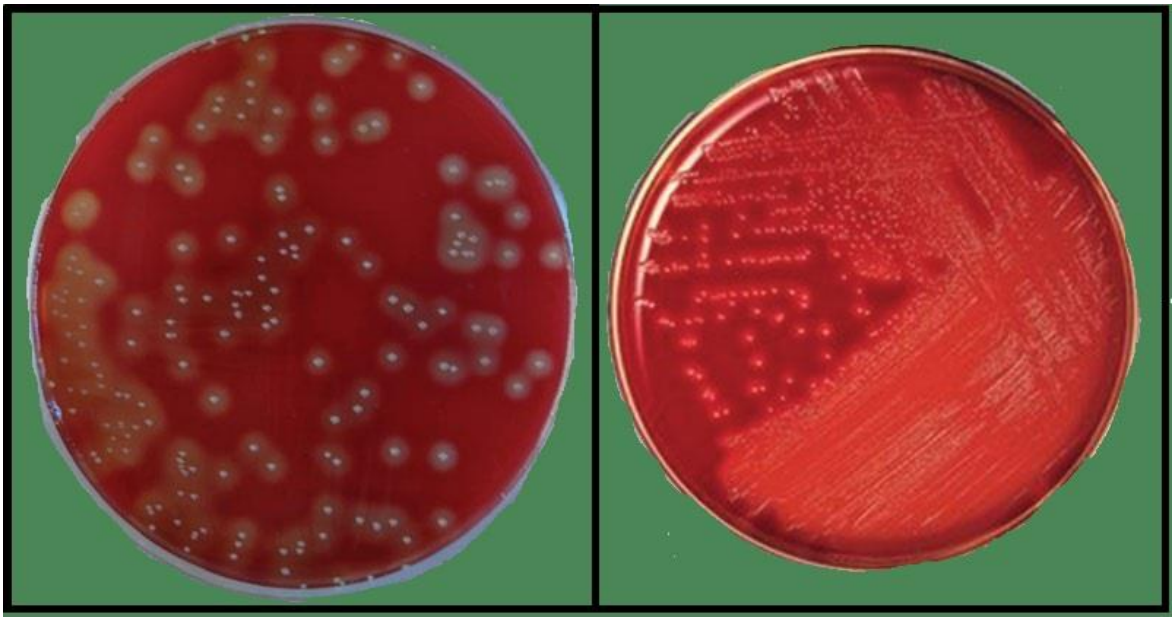


Figure 8: Streptococcus Mutans colony (blood agar)

Results:

There were a total of 120 subjects involved in this study. It consists of 78 male (65%) and 42 females (35%) as shown in **figure 9**. All the participants were present from the beginning to the end of the study and there was no loss to follow-up.

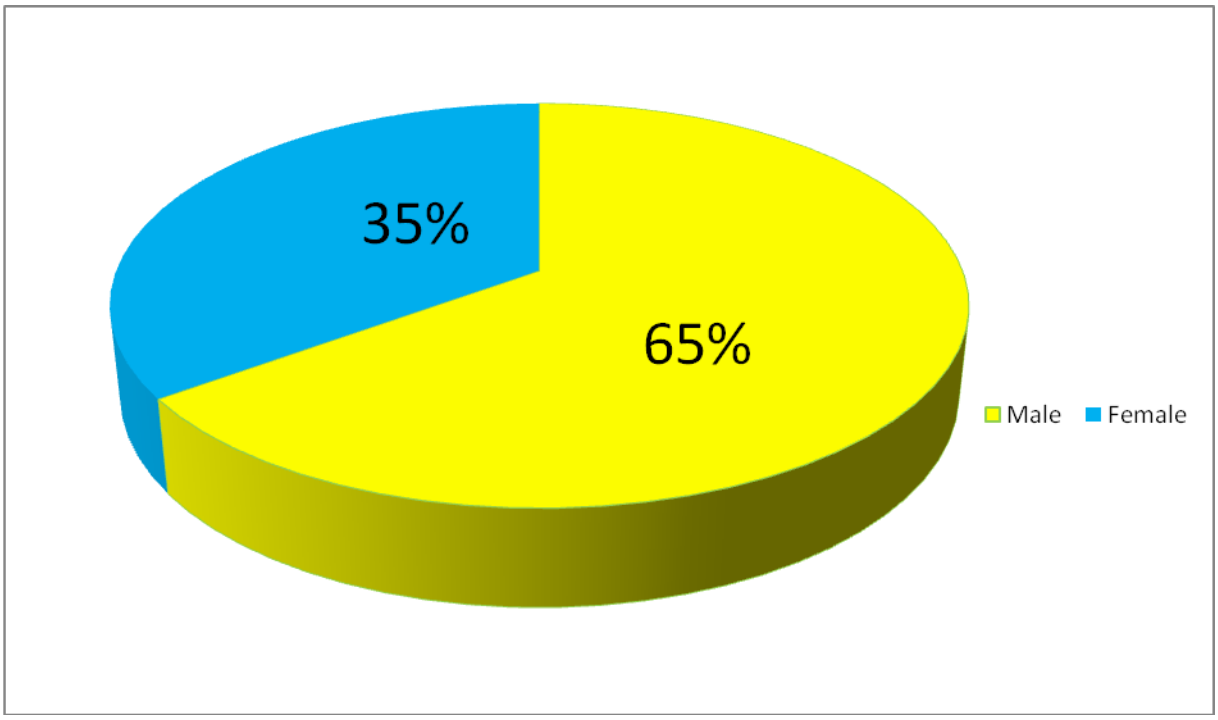


Figure 9: Gender Distribution

The mean level of contamination of toothbrush by Streptococcus mutans (baseline level) in each group are as follows: group I had 14.56 ± 0.76, group 2 had 14.82 ±0.54, group 3 had 15.06 ± 0.46 and group 4 had 14.68 ± 0.36 as shown in **table 1.**

Group	**Baseline*** **(Mean+SD)**
Group I (Distilled water)	14.56 ± 0.76
Group II (Neem)	14.82 ±0.54
Group III (Garlic)	15.06 ± 0.46
Group IV (chlorhexidine)	14.68 ± 0.36

***Level of contamination of toothbrush by S. Mutans ($x10^3$CFU)

Table 1: Mean CFU of Streptococcus mutans in different groups (Baseline)

After decontamination the mean level of contamination of toothbrush by Streptococcus mutans in each group are as follows: group I had 14.12 ± 1.58, group 2 had 1.82 ±2.4, group 3 had 0.34 ± 2.2 and group 4 had 0.58 ± 2.5 as shown in **table 2.**

Group	**After decontamiation * (Mean+SD)**
Group I (Distilled water)	14.12 ± 1.58
Group II (Neem)	1.82 ±2.4
Group III (Garlic)	0.34± 2.2
Group IV (chlorhexidine)	0.58 ± 2.5

*****Level of contamination of toothbrush by S. Mutans ($x10^3$CFU)

Table 2: Mean CFU of Streptococcus mutans in different groups after decontamination

The mean difference in the level of Streptococcus mutans from baseline and intervention groups are shown in **table 3**.

On comparing the level of Streptococcus mutans in Group I (Distilled water) before and after decontamination of toothbrush bristles, it was found to be non-significant $p>0.05$.

On comparing the level of Streptococcus mutans in Group II (Neem) before and after decontamination of toothbrush bristles, it was found to be statistically significant $p<0.05$.

On comparing the level of Streptococcus mutans in Group III (Garlic) before and after decontamination of toothbrush bristles, it was found to be statistically significant $p<0.05$.

On comparing the level of Streptococcus mutans in Group IV (Chlorhexidine) before and after decontamination of toothbrush bristles, it was found to be statistically significant $p<0.05$.

Group	**Baseline*** **(Mean+SD)**	**After decontamiation *** **(Mean+SD)**	**Mean Difference**	**p value**
Group I (Distilled water)	14.56 ± 0.76	14.12 ± 1.58	0.44±0.82	>0.05!
Group II (Neem)	14.82 ±0.54	1.82 ±2.4	, 13±1.86	<0.05#
Group III (Garlic)	15.06 ± 0.46	0.34± 2.2	14.72±1.74	<0.05#
Group IV (Chlorhexidine)	14.68 ± 0.36	0.58 ± 2.5	14.1±2.14	<0.05#

***Level of contamination of toothbrush by S. Mutans ($x10^3$CFU)

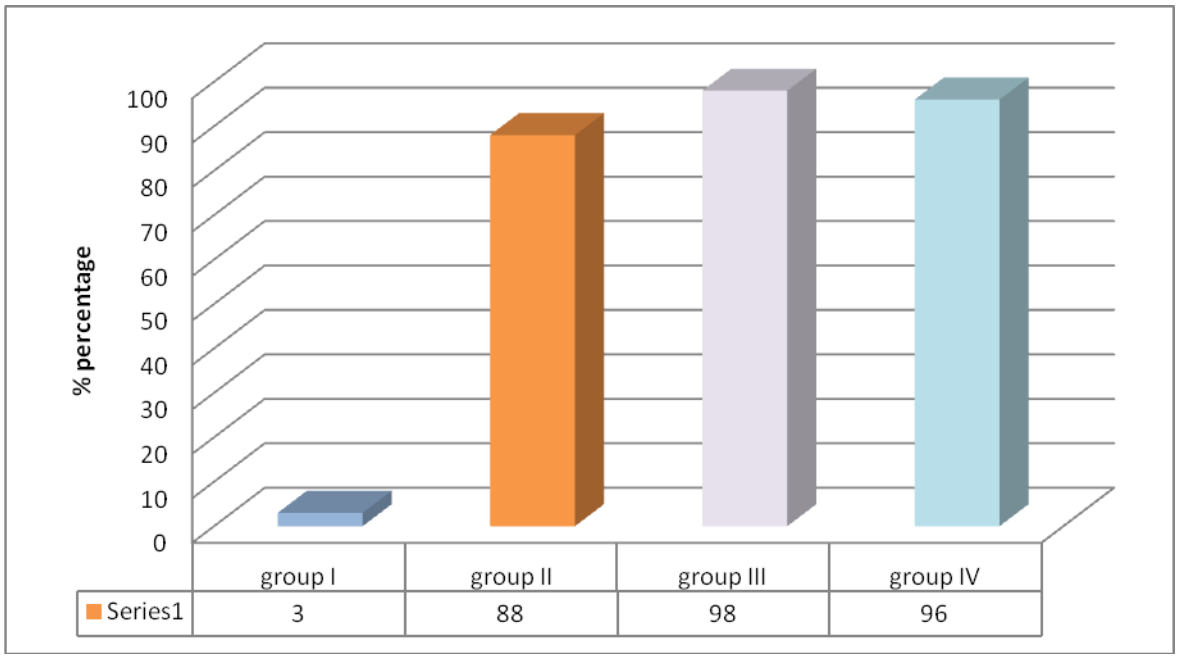
! $p>0.05$ (Non-Significant)

# $p<0.05$ (Significant)

Table 3: Mean difference in the level of Streptococcus mutans from baseline and intervention groups

The percentage of reduction done by different solutions in the level of Streptococcus mutans is shown in **Graph 1**. Group I (Distilled water) showed only 3% reduction, Group II (Neem) showed a reduction of 88%, Group III (Garlic) showed the maximum reduction of 98% and Group IV (Chlorhexidine) showed a reduction of 96% in the level of *Streptococcus mutans*.

The highest reduction in *S. mutans* was seen in the garlic group and distilled water group showed the least reduction.



Graph 1: Mean percentage reduction of Streptococcus mutans colony forming units in four study groups from baseline

When comparing the mean percentage reduction in Streptococcus mutans, CFUs between the control group (I) and each experimental group (II, III, and IV) the differences noted were all statistically significant ($p < 0.05$) as shown in **Table 4.**

When the mean percentage reduction produced by experimental Groups II and III, were compared with experimental Group IV, the differences noted were not statistically significant ($p > 0.05$) as shown in **Table 4.**

When the mean percentage reduction produced by experimental Groups II was compared with experimental Group III, the differences noted was not statistically significant ($p > 0.05$) as shown in **Table 4.**

Group	Mean reduction %	I	II	III	IV
I	3	--	$p<0.05$*	$p<0.05^{\#}$	$p<0.05^{\#}$
II	88	--	--	$p>0.05^{\#}$	$p>0.05^{\#}$
III	98	--	--	--	$p>0.05^{\#}$
IV	96	--	--	--	--

* $p>0.05$ (Non-Significant)

\# $p<0.05$ (Significant)

Table 4: Comparison of the mean percentage reductions in S. mutans count between different groups after decontamination

Discussion:

From the very first day of life, the mouth became contaminated with a wide variety of microbes such as *Streptococcus, Nisseria, Candida*.[33] But the main etiological agent for dental caries is *Streptococcus mutans*. Cobb CM. et al in 1920 stated that toothbrush is a major cause of repeated infection in the mouth.[34] Svanberg M. in his study stated that toothbrushes can become heavily infected with microorganisms especially *Streptococcus mutans* within 24hrs of use.[35]

In routine households, for preventing contamination of toothbrush rinsing and drying is usually adopted. Toothbrushes gets contaminated with microorganisms on toothbrushing, which can act as a source for inoculation or reintroduction of microorganisms from infected to uninfected tissues which can lead to recurrent infections in the mouth.[36] Glass RT in his study states that the air drying method for decontamination of toothbrushes is incomplete.[37] So we need an alternative method that should be effective, economical and doesn't have any side effects.

Toothbrush and toothpaste were used in this study as an oral hygiene aid, since it is the most commonly used aid and it is the most effective, safest device for maintaining oral hygiene. In Indian household toothbrushes of family members are kept in same containers, so there is a further risk of cross-infection, microorganisms can get transmitted from one toothbrush to others if they are kept in close proximity or even on sharing toothbrushes.[38]

American Dental Association (ADA) in 1996 recommended that for every three months everyone should discard their old toothbrush.[39] Glass and Jenson[40] and Denny[41] had advised that patients undergoing chemotherapy should change their toothbrush after every three days; and those patients who had to undergo major

surgeries should change their toothbrush every day. Those who are sick should change their toothbrush at the beginning of disease, when they first feel better and when they are completely well.

Transmission of *Streptococcus mutans* occurs even through dentifrices.[23] Many people however, reported to have psychological, economical, and environmental barriers on changing their toothbrushes frequently.[16,42] Many people change their toothbrushes on the flaring of bristles since there is no awareness regarding decontamination of toothbrushes.

Mutans streptococci is the primary agent for dental caries, it can also be transmitted by toothbrushes, intra- or inter-individual thus, increasing the incidence of dental caries, especially in children.[43] This means of transmission is of great importance in a country such as India, where the frequency of collective toothbrush use is very common, especially in low income families.

Dayoub M.B. et al in his study stated that a wet environment is an ideal factor for the growth of microorganisms and the use of a disinfectant is a must at regular intervals.[44] In spite of millions of toothbrushes sold worldwide, there is very little public awareness that the bristles get contaminated with use.

Each subject was provided with an individual toothbrush and toothpaste to maintain uniformity in the study. For maintaining the sterility control five new toothbrushes that were freshly removed from the packets were subjected to microbial analysis and none of the unused toothbrushes showed any colonies of *Streptococcus mutans*. This suggests that the origin of these microorganisms is from the oral cavity.

Hence, establishing an easy and effective method for disinfecting a toothbrush would be an important and economical way to prevent reinfection of oral diseases.

In the present study 120 students were involved and two herbal disinfectants were compared with chlorhexidine (gold standard) antimicrobial [23,24] agent to know the efficacy in reducing contamination of the toothbrush against S. mutans.

All the study subjects involved in this study had a DMFT score of less than 3 since WHO classifies less than 3 DMFT to be ideal for 18-year old individuals.[45]

The participants involved in the study were given oral hygiene instructions for standardization of the study procedure. Storage conditions of toothbrushes are also an important factor for bacterial survival.[42] The number of bacteria on the toothbrushes kept in aerated conditions is less as compared to brushes stored in plastic containers.[16,44]

In most of the households, the daily procedure for toothbrush care is rinsing and drying them. The results of our study revealed that Streptococcus mutans colonies remained on the bristles after usage and drying by the usual method. Hence, disinfecting the toothbrushes after their use is of great importance to prevent reinfection of a person with pathogenic bacteria and the toothbrush becoming a reservoir of environmental microorganisms.[19]

Many studies have found that there was an increased risk of spreading oral and systemic diseases from the contaminated toothbrushes. Fischer state that there was a relationship between pharyngitis and toothbrush contamination.[46]

Brook and Gober [47] found that the contaminated toothbrushes contain group A beta hemolytic streptococci of pharyngotonsillitis. Hence, it can be postulated that load

of microrganisms in a toothbrush might have a significant impact for the risk of systemic diseases.

In our study the toothbrushes were collected from study participants after 14 days. In different study, different study intervals was found such as 5 h, 6 h, 24 h, 48 hour[17], 3 days, 5 days,[16,48] and 7 days.[19,49]

14 days, 15 days, 24 days, 28 days and 3 months[23] In our study, we have taken 14 days because a study conducted by Sogi et al. states that maximum microbial contamination was found in toothbrushes after 14 days and 28 days.[17,48]

The toothbrushes were immersed in disinfecting solutions for 12 hours, which was similar to other studies.[23,49]

Twice-a-day tooth brushing was used in this study so, a soaking period of 12 hours in disinfectant solutions was used. However, other studies have used different soaking times of 20 hours,[16] 24 hours,[18] or 20 minutes[17] and all had employed once-a-day brushing.

Neem (*Azadirachta indica*) possesses various therapeutic properties, and is widely available in most rural and urban areas of our country. In our study, 3% neem was found to be an effective antimicrobial solution against Streptococcus mutans on toothbrush bristles and showed 88% reduction. This was similar to the studies conducted by Anand PJ et al[6] , Balappanavar et al.[23] and Padma K Bhat et al[2] where it shows 88%, 86% and 86% reductions respectively. This reduction could be due to the presence of polyphenolic tannins which are present in the extract. It gets bound to the surface-associated bacterial proteins and results in bacterial aggregation and also loss of glucosyltransferase activity. This bacterial aggregate reduces the count of Streptococcus mutans. Aarati et al. conducted a study and

states that both aqueous and alcoholic extracts of neem have significant antibacterial activity against Streptococcus mutans.[26]

Garlic showed the maximum reduction of 98% in the Streptococcus mutans count in our study and it was similar to the study conducted by Anand PJ et al[6] and Dithi Chandrdas et al[28] where it shows 96% and 100% reductions respectively. This antibacterial activity of garlic is due to the presence of allicin. Allicin possesses strong anti-Streptococcus mutans activity which was reported by Nikolic et al.[50]

On doing the literature search not many studies were found. Fani et al. conducted a study on mouthwash containing garlic and stated that garlic mouthwashes can be used for prevention of dental caries.[21] Garlic extracts of 2.5[10,51] and 10% [52] were used as mouthwash in studies; which also showed significant reduction in salivary Streptococcus mutans counts. Despite having the antimicrobial property, garlic has side effects like unpleasant taste, halitosis, and nausea were reported.[53]

Chlorhexidine is a cationic agent and it exhibits broad spectrum antimicrobial effects. It has both bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria) mechanisms of action, depending on its concentration.[3] It is considered as a benchmark control in various studies. In our study, chlorhexidine showed 96% reduction in the Streptococcus mutans count. It kills bacteria by disrupting the cell membrane.[54]

In studies conducted by Bhat et al,[2] Bhat SS et al,[18] Nanjunda Swamy et al[25] chlorhexidine produced 100% reduction of the Streptococcus mutans count and study conducted by Anand PJ et al[6] showed 92% reduction. Suma Sogi et al. in her study stated that chlorhexidine produces 88 % reduction in S.mutans count after 14

days.[17] But the result of the present study was not in concurrence with the study reported by Balappanavar et al.[23], Padma K Bhat et al.[2] and Dithi Chandrdas et al[28] where the percentages of reduction in Streptococcus mutans count were only 64% ,65% and 78.3 % respectively, which were less than the present study.

As per the present results, neem and garlic have been proved to be as effective disinfectants as chlorhexidine for toothbrushes. Kudva et al. reported that garlic and chlorhexidine are effective against Streptococcus mutans.[55] Fani et al. showed that the inhibitory activity of garlic on Streptococcus mutans was comparable with chlorhexidine.[21]

In the present study garlic showed the maximum reduction in the *S. mutans* count. It produced 98% reduction with garlic followed by chlorhexidine 96% and neem 88%. Distilled water showed only a 3% reduction. When comparing the mean difference and also the mean percentage reduction obtained in Streptococcus mutans colony count between the groups, only the comparison with distilled water showed a statistically significant difference. But when comparing the mean difference or mean percentage reduction in CFUs of *Streptococcus mutans* in garlic and neem, with chlorhexidine, the differences were not statistically significant from each other. So the present investigation showed that immersion of toothbrush for 12 hours in 3% neem and 3% garlic were as efficacious as 0.2% chlorhexidine for disinfection. So neem and garlic, can be used as toothbrush disinfectant as chlorhexidine.

Limitations:

The present study considered only *S. mutans* microorganism for evaluating the efficacy of disinfecting agents and did not take into account all other microorganisms present on the toothbrushes.

It also did not assess the acceptability of toothbrushes dipped in these solutions by the study subjects as antimicrobial agents are known to have unpleasant smell, bitter and altered taste especially with garlic.

It also did not test whether the properties of toothbrush bristles can be affected by the herbal solutions they are dipped in.

Comparison with Green tea extract & Vinegar can also be taken into consideration for further research.

Therefore, further study is required to analyze various antimicrobial agents with different concentrations and different soaking time.

Conclusion:

Based on the results of the study, it can be concluded that neem and garlic are equally efficacious as chlorhexidine and these herbal products can be used as potent alternatives to chlorhexidine as disinfectants for toothbrushes.

It is seen that toothbrushes can act as a carrier of infection in the oral cavity. Thus, it is mandatory for every individual to disinfect his brush at regular intervals so as to maintain good oral hygiene.

Neem and Garlic are the common ingredients that can be easily found in Indian society and it is herbal, cost effective and having no side-effect so it can be easily used by every individual as toothbrush decontaminants.

Among the test agents, garlic was the most effective decontamination agent followed by neem.

Further clinical research is required to broaden our understanding of various antimicrobial agents, especially the natural/herbal agents for the prevention of dental caries.

Recommendation:

To maintain oral hygiene we use toothbrush and it needs to be decontaminated regularly.

The decontaminating solutions available in the market are mostly chemical and may be expensive. This may lead to avoidance of use of decontaminants.

To overcome this problem present study showed efficacy of 3% Neem and 3 % Garlic solution almost equal 0.2% Chlorhexidine but Garlic & Neem has bitter taste & smell.

So pharmaceutical companies can carry out further research to remove the bitter taste & smell and also make it available for common man at economical rates so that each and everyone can afford it.

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Comparison of Efficacy of Homemade Herbal Disinfectants with Chlorhexidine for Decontamination of Toothbrush: A Randomized Control Trial."** submitted by **Dr Ankita Singh** Post graduate student from the **Department of Public Health Dentistry** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **15th October, 2020** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

16/10/2020

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 16 (Revised) **BBDCODS/10/2020**

Title of the Project: Comparison of Efficacy of Homemade Herbal Disinfectants with Chlorhexidine for Decontamination of Toothbrush: A Randomized Control Trial.

Principal Investigator: Dr. Ankita Singh **Department:** Public Health Dentistry

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: Revised, MDS Project Protocol

Dear Dr. Ankita Singh,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 22nd October, 2020.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry & Nutrition, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.

The proposal was reviewed, comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 22/10/20

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

CASE RECORDING PROFORMA

Date:

Name: **Code:**

Age/ Gender:

Education:

Address: **Phone no.:**

Medical history:

Dental history:

DMFT index: