

# Evaluation of Efficacy of Green Tea Neem Extract, Frezyderm and Rexitidine Mouthwash on Plaque Induced Gingivitis

## *Running Title: Green Tea Neem Extract, Frezyderm And Rexitidine Mouthwash On Plaque Induced Gingivitis*

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**Abstract**

*Background:* Herbal mouthwashes carry a wide array of advantages such as antimicrobial properties, antioxidant properties and more. They are affordable and have minimal or no side effects. They are more efficient than commercially available mouthwashes in the market.

*Objectives:* The aim of this study was to compare and to analyze the antimicrobial efficacy of green tea mouthwash, neem mouthwash, homeopathic (Frezyderm) mouthwash, rexidine mouthwash on dental plaque.

*Materials and Methods:* This is a double-blinded, random controlled research study conducted in the Department of Periodontics. A total of 200 patients were selected randomly, comprising of four groups (Group-I:green tea, group-II:-neem sticks, Group-III: homeopathic (Frezyderm) mouthwash and group-IV: rexidine mouthwash) with gingival index score II. Saliva samples were collected on at baseline and on day 10 and 20 and microbial colony count was done. The data obtained was statistically analyzed using SPSS version 26 with ANOVA test.

*Results:* Microbial colonies were reduced better in Group IV on the day 10, whereas on day 20, greater reduction was observed in Group II with the difference was statistically significant.

Conclusion: Neem mouthwash formulation had better effects as compared to other three mouthwashes and it could overcome the disadvantages of other mouthwashes as well.

### **Keywords**

Green tea, Neem, Rovidine, Mouthwash, Saliva

### **Introduction**

Plaque is known to be the primary causative factor of gingivitis, dental caries and halitosis-causing microorganisms.<sup>1</sup> There are other means by which plaque can be controlled include chemical therapeutic agents such as chewing gums, sprays, mouthwashes and varnishes.<sup>2</sup> From all these means, mouthwashes are the simplest and easiest mode of oral hygiene.<sup>3</sup> A caries prevention program primarily should be aimed at reducing the cariogenic bacteria. A mouth rinse is a chemotherapeutic means, which is used as an efficient home remedy to improve oral hygiene and to reduce dental caries by targeting the cariogenic bacteria. A variation of synthetic antimicrobial mouthwashes is existing to reduce dental caries. It was observed to reduce plaque formation, inhibit gingival inflammation and prevent dental caries. Chlorhexidine, the most commonly used mouth rinse, shown effective reduction of the oral *S. mutans* levels. However, chlorhexidine as a mouth rinse has reported to be inconsistencies, produces staining of teeth, changes in taste perception and inability to ablate *S. mutans* in the oral cavity. Hence alternative herbal mouth rinses have been studied. The usage of antimicrobial herbal products in dentistry has been well documented in prevention of plaque.<sup>4</sup>

Few mouthwashes are so strong that they can burn the oral tissues and cause ulcers. Clinical studies on herbal and homeopathic mouth washes are very scarce hence the present study was conducted to compare four mouthwashes to analyze whether herbal and homeopathic mouthwashes are better than chemical ones by reducing microbial colony count.

## Materials and Methods

This study is a randomized, controlled, double-blinded study conducted in department of preventive dentistry. Ethical clearance was obtained from the Institutional ethical committee. Informed consent was taken from each patient included in the study.

The inclusion criteria for the study included patients diagnosed with plaque induced gingivitis or periodontitis. The exclusion criteria were patients diagnosed with non-plaque induced gingivitis or periodontitis, patients who were taking antibiotics or have been already using mouthwash for the past 3 months, patients with any systemic diseases, or having a habit of smoking or using smokeless tobacco.

The sample size was calculated with the assumption at a confidence interval of 4 and confidence level of 95% and standard deviation of 0.5 with a sample size of 200 would be sufficient. Hence, for each group 50 patients were taken. Sample size = 200 (Confidence level 95%; Confidence interval 4; Population 300)

200 participants aged 18-22 years were equally divided into 4 mouth wash groups; Group-I:green tea, group-II:-neem sticks, Group-III: homeopathic mouthwash and group-IV: rexidine mouthwash. For evaluation of mouth wash effect, the plaque scoring criteria was taken based on the criteria given by Loe and Silness in 1963.<sup>5</sup>

Preparation of green tea mouthwash: 50 ml of water was warmed till temperature 80 degree celsius with the addition of two tulsi leaves followed by dipping of a single green tea bag. The tea bag used was of commercially available organic tulsi. The water was cooled down and used for mouthwash. The tulsi acted as a preservative.

Preparation of neem mouthwash: Neem leaves were boiled in 50 ml water with the addition of turmeric and triphala. The solution was filtered through double-layered muslin cloth. The remnant solution was used as a mouthwash.

All of the patients were advised to use their mouthwash daily twice for 30 seconds for 20 days along with their normal toothbrush and paste activity.

The samples of saliva were collected on day zero, followed by day 10 and 20. The dilution and spread method was used to assess the salivary microbes where 1:1000 dilution of saliva samples was made and further streaked on blood agar containing gel plates which were then incubated at 35 degree celsius for 48 hours.

An automated microbial colony counter was used to assess the growth of microorganisms by counting the microbial colonies. These counts were done for day zero, 10 and 20.

Statistical analyses were performed using SPSS Version 26.0. ANOVA was applied to compare the means among the four groups. To assess the changes from the day of start to day 10 to day 20, turkey's post hoc test was applied. The statistical significance level was set at  $p < 0.05$ .

## **Results**

Comparison among the four groups showed that from day zero to day 10 and day 20, the baseline microbial colony count was reduced. At baseline group-I had microbial count of 141 and on day 20<sup>th</sup> it reduced to 52 cfu, similarly in group-II, III, IV it was 121, 164, 191 at baseline and 58.27, 63.23, 78.71 on day 20<sup>th</sup>, respectively (Table-1, graph-1). It indicates the reduction of microbial count from baseline to 10<sup>th</sup> day to 20<sup>th</sup> day in all groups. It was found

to be ( $P>0.05$ ). Table-II indicates that statistical difference between groups I vs III, I vs IV and III vs IV.

## Discussion

The present study evaluates effect of different mouth rinse on microbial reduction. We found that Chlorhedine is effective in reduction of microorganisms followed by green tea and Frezyderm mouthwash and it was least in neem mouth was.

A study conducted by Baratakke *et al*, Pradeep *et al* found no significant difference on comparing triphala combination mouthwash to CHX in reducing plaque and gingival scores. There was a reduction in plaque, gingival, and oral hygiene index-simplified values.<sup>6,7</sup>

A study conducted by Sushma *et al* stated that on comparing triphala churna and chlorhexidine gluconate, triphala churna was found to be more efficient in reducing the count of *Candida albicans* in patients wearing dentures.<sup>8</sup> Mahajan *et al* reported that as compared to chlorhexidine, tulsi and neem have less antimicrobial properties. The advantage of adding tulsi is that it is active against candida species and can be added in herbal mouthwash while chlorhexidine acts against normal oral microflora as well which in the long term further increases the chances of candidiasis.<sup>9</sup>

A study conducted by Mallikarjun *et al* stated that on using ethanolic extracts of tulsi on periodontal pathogens as compared to doxycycline, tulsi showed an inhibition zone on the agar gel specifically against *Aggregatibacter actinomycetemcomitans* but the inhibition was less against *Porphyromonas gingivalis* and *Prevotella intermedia*. They summarized that tulsi

could be used as an effective adjunct in the management of periodontal diseases along with standard care.<sup>10</sup>

Anand et al reported 3% neem as an effective antimicrobial solution as it reduced the streptococcal mutans on the bristles of the toothbrush.<sup>11</sup> Datta et al used neem extract as an endodontic irrigating agent and reported that 0.94% and 1.88% of the neem extract was effective against *Enterococcus faecalis* and *candida albicans* when compared to 2% chlorhexidine and 3% sodium hypochlorite.<sup>12</sup>

From various studies, it can be concluded that triphala has very beneficial properties such as antioxidant, antimutagenic, antineoplastic, antibacterial, anti-dental caries, chemo and radioprotective ones.<sup>13</sup> It is said that the phenolic and non-phenolic components i.e. the tannic acid, chebulic acid, and flavonoids present in the triphala provides antibacterial action and has a therapeutic potential particularly against streptococci and *candida albicans*.<sup>10</sup>

Neem is said to contain azadarachitin which is the chief active component and is an effective antimicrobial agent. Other major component in neem include trimethylamine, nimbidin, nimbin, nimbolide, lectin, chlorides and fluorides while minor components include sulfur, silica, tannins, Vitamin C, flavonoids, saponins and sterols. These components are said to provide antiseptic and anti-inflammatory effect.<sup>14</sup> Neem contains polyphenolic tannins which have the ability to bind to the bacterial surface proteins effectively which causes bacterial aggregation and loss of glycosyltransferase activity.<sup>11</sup> The polyphenols in the neem help to adhere it to oral mucosa and acts as a synergistic antioxidant in periodontal diseases.

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Tulsi has antimicrobial activity which is due to the presence of essential oils in it which includes eugenol, caryophyllene, germacrene-A, caryophyllene oxide and clemene. These essential oils are active against a wide variety of organisms which include staphylococci, streptococci, salmonella, and shigella. The phenolic nature of the essential oils

presents in the tulsi exert destabilizing effect on membranes in the microbial strains and stimulate leakage of cellular potassium which kills the bacteria.<sup>16</sup>

Turmeric has anti-inflammatory, antioxidant, antimicrobial, antiseptic and immunostimulant properties. The anti-inflammatory action of turmeric is said to be due to its selective inhibitory action of prostaglandin E2 synthesis and thromboxane and arachidonic acid metabolism inflammatory mediators which can induce change in the structure of the bacterial cells.<sup>17</sup>

Parwani et al reported that 0.2% CHX mouthwash performance was better than herbal mouthwash.<sup>18</sup> Chatterjee et al found out that the effectiveness of herbal oral rinse and chlorhexidine was equal in reducing the periodontal index.<sup>19</sup>

It could be said that herebal extracts have wide variety of benefits in reducing the pathogenic microorganisms of the oral cavity. Also, there are no side effects for the herbal formulations, so they are safe to use and are affordable as well and give better results than the commercially formulated mouthwashes.

However, further research is required to analyses the efficacy of herbal formulations over commercially available mouthwashes over a greater span of duration. These results are helpful in identifying the beneficial effect of herbal mouth rinse in prevention of plaque and gingivitis in practice of primary care.

## **Conclusion**

From the present study, it was concluded that herbal mouth washes are effective in reduction plaque and oral bacterial count and gingivitis. Which can be advised alternative to chlorhexidine.

## Reference

1. Perry DA. Plaque control for the periodontal patients. In: Newmann MG, Takei HH, Klollevoid PR, Carranza FA, editors. Carranza's Clinical Periodontology. 10<sup>th</sup> ed. St. Louis, Missouri: Saunders; 2006. p. 728.
2. Addy M. The use of antiseptics in periodontal therapy. In: Lindhe J, Karring T, Lang NP, editors. Clinical Periodontology and Implant Dentistry. 4<sup>th</sup> ed. New Delhi: Jaypee Brothers Medical Publications (P) Ltd.; 2003. p. 464.
3. Vinod K S, Sunil K S, Sethi P, Bandla RC, Singh S, Patel D. A novel herbal formulation versus chlorhexidine mouthwash in efficacy against oral microflora. J Int Soc Prevent Communit Dent 2018;8:184-90. doi: [10.4103/jispcd.JISPCD\\_59\\_18](https://doi.org/10.4103/jispcd.JISPCD_59_18)
4. Velumurugan A, Madhubala MM, Bhavani S, Satheesh Kumar KS, Satyanarayana SS, Gurucharan N. An *in-vitro* comparative evaluation of two herbal extracts *Emblica*

*officinalis* and *Terminalia chebula* with chlorhexidine as an anticancer agent: A preliminary study. J Conserv Dent 2013;16:546-9. doi: [10.4103/0972-0707.120958](https://doi.org/10.4103/0972-0707.120958)

5. Kulkarni P, Singh DK, Jalaluddin M, Mandal A. Comparative evaluation of antiplaque efficacy between essential oils with alcohol-based and chlorhexidine with nonalcohol-based mouthrinses. J Int Soc Prev Community Dent 2017;7:S36-41 doi: 10.4103/jispcd.JISPCD\_131\_17
6. Baratakke SU, Raju R, Kadanakuppe PS, Savanur NR, Gubbihal R, Kousalya PS. Efficacy of triphala extract and chlorhexidine mouth rinse against plaque accumulation and gingival inflammation among female undergraduates: A randomized controlled trial. Indian J Dent Res 2017;28:49-54.
7. Pradeep AR, Suke DK, Martande SS, Singh SP, Nagpal K, Naik SB, *et al.* Triphala, a new herbal mouthwash for the treatment of gingivitis: A randomized controlled clinical trial. J Periodontol 2016;87:1352-9. doi: 10.1902/jop.2016.130406
8. Sushma R, Sathe TT, Farias A, Sanyal PK, Kiran S. Nature cures:” an alternative herbal formulation as a denture cleanser. Ann Afr Med 2017;16:6-12. doi: [10.4103/aam.aam\\_43\\_16](https://doi.org/10.4103/aam.aam_43_16)
9. Mahajan R, Khina PK, Gill AS, Kaur J, Saravan SP, Shewale A, *et al.* Comparison of efficacy of 0.2% chlorhexidine gluconate and herbal mouthrinses on dental plaque: An *in vitro* comparative study. Eur J Med Plan 2016;13:1-11. <https://doi.org/10.9734/EJMP/2016/23318>.
10. Mallikarjun S, Rao A, Rajesh G, Shenoy R, Pai M. Antimicrobial efficacy of tulsi leaf (*Ocimum sanctum*) extract on periodontal pathogens: An *in vitro* study. J Indian Soc Periodontol 2016;20:145-50. doi: [10.4103/0972-124X.175177](https://doi.org/10.4103/0972-124X.175177)

11. Anand PJ, Athira S, Chandramohan S, Ranjith K, Raj VV, Manjula VD, *et al.* Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial. *J Int Soc Prev Community Dent* 2016;6:22-7. doi: 10.4103/2231-0762.175406.
12. Datta PS, Chand GP, Sashidhar RK, Sunil KC, Melpati H, Swamy RR. Evaluation of antimicrobial efficacy of neem and *Aloe vera* leaf extracts in comparison with 3% sodium hypochlorite and 2% chlorhexidine against *E. faecalis* and *C. albicans*. *J NTR Univ Health Sci* 2016;5:104-10. DOI: 10.4103/2277-8632.185436
13. Peterson CT, Denniston K, Chopra D. Therapeutic uses of triphala in ayurvedic medicine. *J Altern Complement Med* 2017;23:607-14. doi: [10.1089/acm.2017.0083](https://doi.org/10.1089/acm.2017.0083)
14. Sharma P, Tomar L, Bachwani M, Bansal V. Review on Neem (*Azadirachta indica*): Thousand problems one solution. *Int Res J Pharm* 2011;2:97-102.
15. Heyman L, Hourri-Haddad Y, Heyman SN, Ginsburg I, Gleitman Y, Feuerstein O, *et al.* Combined antioxidant effects of neem extract, bacteria, red blood cells and lysozyme: Possible relation to periodontal disease. *BMC Complement Altern Med* 2017;17:399. doi: 10.1186/s12906-017-1900-3.
16. Eswar P, Devaraj CG, Agarwal P. Anti-microbial activity of tulsi (*Ocimum sanctum* (Linn.)) extract on a periodontal pathogen in human dental plaque: An *in vitro* study. *J Clin Diagn Res* 2016;10:ZC53-6. DOI: [10.7860/JCDR/2016/16214.7468](https://doi.org/10.7860/JCDR/2016/16214.7468)
17. Suhag A, Dixit J, Dhan P. Role of curcumin as subgingival irrigant: A pilot study. *PERIO* 2007;4:115-21.
18. Parwani SR, Parwani RN, Chitnis PJ, Dadlani HP, Sai Prasad SV. Comparative evaluation of anti-plaque efficacy of herbal and 0.2% chlorhexidine gluconate

mouthwash – A 4 day plaque re-growth study. J Indian Soc Periodontol 2013;17:72-7.  
doi: 10.4103/0972-124X.107478.

19. Chatterjee A, Saluja M, Singh N, Kandwal A. To evaluate the antigingivitis and antipalque effect of an *Azadirachta indica* (neem) mouthrinse on plaque induced gingivitis: A double-blind, randomized, controlled trial. J Indian Soc Periodontol 2011;15:398-401. doi: [10.4103/0972-124X.92578](https://doi.org/10.4103/0972-124X.92578)

### **Legends for illustrations**

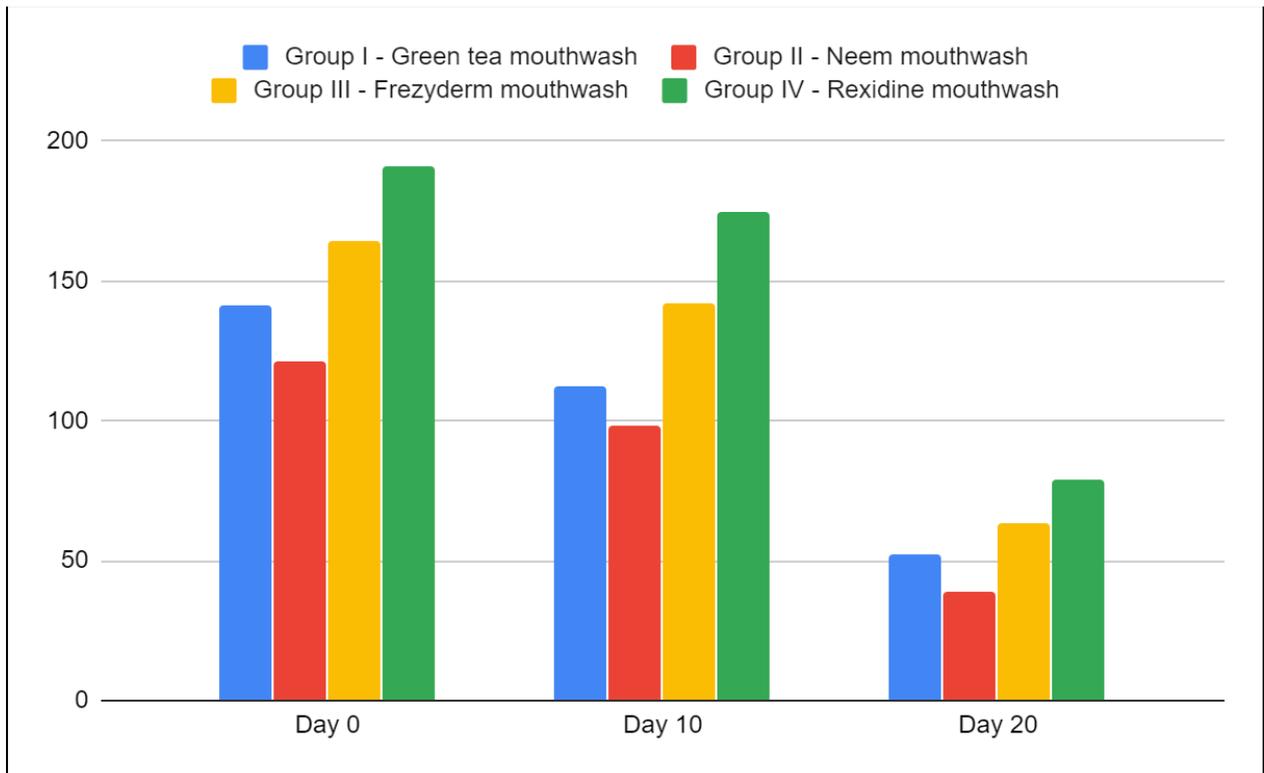
#### **Tables**

Table 1: different mouth wash effect on microbial colony count

Day of microbial colony count	Group I - Green tea mouthwash	Group II - Neem mouthwash	Group III - Frezyderm mouthwash	Group IV - Rexidine mouthwash
Day 0	141.24	121.11	164.21	191.23
Day 10	112.23	98.6	142.08	174.92
Day 20	52.34	58.27	63.23	78.71

P>0.05

Graps;1: comparison of mouth rinses



**Table-2: Comparison of reduction in microbial count among groups**

Comparison	difference of CFU	std. Error of Difference	Critical Rang	Results P value
Group-I vs Group-II	9.245	1.77631052	7.4358	<0.05
Group-I vs Group-III	3.457	1.77631052	7.4358	>0.05
Group-I vs Group-IV	2.799	1.77631052	7.4358	>0.05
Group-II vs Group-III	7.236	1.77631052	7.4358	<0.05
Group-II vs Group-IV	7.769	1.77631052	7.4358	<0.05
Group-III vs Group-IV	4.869	1.77631052	7.4358	>0.05

CFU- colony forming units