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Effect of Bael, Cinnamon and Neem Extract Mouthwashes on Dental Plaque, Gingival Health and Salivary pH among adults residing in a hostel in Chennai, India – A triple blinded, randomized controlled trial

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Abstract

Aim: To assess and compare the effect of mouthwash containing 0.3% *Aegle marmelos* (Bael) leaves extract with other herbal mouthwashes containing 0.3% *Cinnamomum zeylanicum* (Cinnamon) barks extract and 0.3% *Azadirachta indica* (Neem) leaves extract and with 0. 2% chlorhexidine mouthwash on dental plaque, gingival health and salivary p H among adults.

Materials and Methods: A triple blinded randomized controlled trial with concurrent parallel study design was carried out. Around, 48 young adults were randomly allotted to four groups - Group A (Chlorhexidine), Group B (Cinnamon), Group C (Bael) and Group D (Neem) by simple randomization technique. They were advised to rinse with 10 ml of their respected mouthwashes twice daily for 1 minute for a period of 28 days. The dental plaque score and gingival inflammation score were recorded using Plaque index and Gingival index at the baseline, 15th day, 22nd day and 29th day. Statistical analysis were done using ANOVA and repeated

measure ANOVA for the plaque and gingival scores and Paired sample t test for p H

Results: Mouthwashes containing Bael, Neem and Chlorhexidine showed a stastistically significant reduction in the plaque levels at the end of second week (mean difference from baseline to 2nd week = 0. 158, 0. 100 and 0. 108 respectively with p value < 0. 05). However, at all the time points considered in the study, Bael was more effective in reducing the gingivitis.

Conclusion: Bael extract mouthwash is as effective as the gold standard Chlorhexidine mouthwash in reducing the plaque and gingivitis and opens new perspectives for its use.

Key words*: Bael, Cinnamon, Neem, plaque, gingivitis*

Long before the advent of modern medicine, herbs were the mainstream remedies for nearly all ailments. The worldwide consumption of herbal medicines today is enormous and hence it is essential to identify the risks associated with their use. As the popularity of these herbal products continues to rise, dental professionals are expected to provide information to patients about these products' safety and efficacy. This can be difficult, however, owing to a lack of professional consensus on the subject. Herbal medicine is said to be both promotive and preventive in its approach.[1] The World Health Organization estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health car e needs. [2] Moreover, natural products have a long history of treating oral diseases. [3]
Today' s dentists are practicing in an era where the patients are more concerned about both their oral health and their overall medical wellbeing. Aerobic and anaerobic bacteria in the dental plaque are incompatible with gingival tissues leading to gingivitis, which may progress to periodontitis, so plaque control becomes essential. Nonsurgical periodontal treatment remains the core component of periodontal therapy. Thus, in the midst of growing evidence of the connection between oral health and whole body health, herbal medicines with their 'naturally occurring' active ingredients, offers a gentle and enduring way for restoration of health by a least harmful way. [4]

Bael *(Aegle marmelos)* is known in India since the prehistoric time and has been mentioned in the ancient system of medicine. [4] It is considered as "Mahaphala" (Fruit with all qualities) in Ayurvedic System of medicine. Every part of the plant such as its leaves, fruit, seed, bark and root are an important ingredient of several traditional formulations. [4] Due to its curative properties, i t is one of the most useful medicinal plants of India. *Aegle marmelos* is utilized as an effective ethenomedicine as astringent, antidiarrheal, antidysentric, demulcent, antipyretic, anti - oxidant and anti-inflammatory treatment. [5] Plant also contains compounds useful for treatment of several diseases including cancer, diabetes, and cardiovascular diseases. It has a great mythological significance also. [5]

It is a sacred tree from India, of Rutaceae family, related to citrus. The plant is mostly cultivated for

its aromatic character and medicinal properties. [6] It is a medium size tree (average is 8. 5 m tall), with spines on its branches and very aromatic. Leaves are pale green and trifoliate.[7] It is a good source of vitamin C and protein. It is chiefly composed of Limonene (82.4%) which has an antibacterial property is the main constituent present in the leaves.[8] Leaves extracts are used in Ayurveda as a medicine for fever, diabetes mellitus, peptic ulcers, respiratory infections, jaundice, wound healing, conjunctivitis, anaemia, swollen joints, typhoid, irritable bowel syndrome and also in the treatment of snakebites.[4]

Overview of the constituents present in the leaves of *Aegle marmelos* [9]

COMPOUND	BIOLOGICAL ACTIVITY
Skimmianine (Tannins)	Analgesic, antipyretic, antidiuretic, anticancer, antimalaria anticonvulsive
Aegelin (Alkaloid)	Cardioactive, Antihyperglycemic Antidislipidemic
Limonene (Terpenoid)	Antioxidant and anticancer
Lupeol	Cardioactive, anti-inflammatory
Cineol	Antiulcer
Citral	Antiallergic, antiseptic
Citronellal	Antiseptic
Cuminaldehyde	Antibacterial
Eugenol	Antioxidant, antibacterial, antiulcer
Marmesinin (coumarin)	Antioxidant

The National Medicinal Plants Board of Government of India has placed Bael in the priority list of medicinal plants at 4th position. [10]

Two hundred years old palm leaf manuscript conserved in the TKDL – Traditional Knowledge Digital Library which is in CCRS - Central Council for Research in Siddha Traditional Medicine, India, reveals the medicinal uses of *Aegle marmelos* (Figure 1a – Front view, 1b – Back view). The manuscript reveals the therapeutic uses of Bael and explicit that bael leaves were used to prevent any kind of fever. [12] The 6th century ancient Sanskrit text on medicine and surgery – Sushruta Samhita reveals that the leaves of b ael have been used in curing humoral disorders and internal abscess. [13]

"varun .a –dirgan . o hyes .a kaphamedoniva – ran . ah. vinihanti sfirah . sfu –lagulma – bhyantaravidradhi –n"

The above statement reveals that Bilva falls in Varun. a–di group of plants; and helps in eliminating kapha and medas and cures headache, gulma and internal abscess. [13]

Cinnamon *(Cinnamomum cassia)* is a member of the Lauraceae family. The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. [14] Cinnamon is thought to have many health benefits, so it is used as an herbal medicine. [15] The bark of the cinnamon tree contains an essential oil called cinnamonaldehyde, which give cinnamon its characteristic flavor and aroma. The inner bark of the cinnamon tree has been used as a spice for thousands of years. Apart from these, cinnamon barks has proved to have antibacterial, anti - inflammatory and antifungal properties due to the presence of cinnamaldehyde and eugenol. [16] Two hundred years old palm leaf manuscript conserved in the TKDL – Traditional Knowledge Digital Library which is in CCRS - Central Council for Research in Siddha Traditional Medicine, India, reveals the medicinal uses of *Cinnamomum zeylanicum*[12] (Figure 2)

Neem (*Azadirachta indica*) is a member of the Meliaceae family and its role as health-promoting effect is attributed because it has a rich source of antioxidants. Importance of neem tree has been recognized by US National Academy of Sciences where neem is entitled as "a tree for solving global problems." [17] It has been used in India and South Asia for thousands of years as a perfect tool for maintaining healthy periodontium.[18] Neem has been long considered to have an astringent, antiseptic, insecticidal, antiulcer and medical properties due to the presence of aza dirachtin and nimbidin.[18] The 350 year old Agathiyar Gunavadagam – a palm leaf manuscript, which was written by the great Siddhar Agathiyar in the 6th century B. C reveals the therapeutic uses of neem and explicit that neem flowers will be used to prevent a nd treat bile disorders; neem leaf will be used to prevent and treat ulcers; and neem bark will be used to prevent and treat CNS disorders, paralysis and psychiatric disorders (Figure 3). [19]

As the popularity of the above mentioned herbal products continue s to rise, dental professionals are expected to provide information to patients about these products safety and efficacy. Until today, an insufficient amount of clinical research on herb- based mouth rinses and dentifrices has been reported in Asia, especia l ly in India and other south east Asian countries, where these products are most popular and widely used. [19]

Various commercial mouth rinses are available in the market, amongst which Chlorhexidine is the most popular. It has also been recognized by the pharmaceutical industry as the positive control against which the efficacy of alternative antiplaque agents should be measured, and has earned its eponym of gold standard. [20] But it cannot be used on a long term basis because of various side effects like brow n discoloration, taste perturbation, oral mucosal lesions, parotid swelling, enhanced supragingival plaque formation and sometimes unacceptable taste. [21] An effective alternative to Chlorhexidine is highly desirable and has been long awaited. Many studies have evaluated the effectiveness of mouthwashes containing Cinnamon and Neem. However no study have been conducted to assess the effectiveness of Bael mouthwash. Hence the aim of the present study was to assess and compare the effect of mouthwash containin g 0. 3% *Aegle marmelos* (Bael) leaves extract with other herbal mouthwashes like 0.3% *Cinnamomum zeylanicum* (Cinnamon), 0.3% *Azadirachta indica* (Neem) and with the gold standard 0.2% chlorhexidine mouthwash on dental plaque, gingival health and salivary pH among adult population.

Materials and methodology:

The study was carried out among 20 to 30 years old female young adults residing in a hostel in Chennai for a period of two months (June to August 2018). The ethical clearance was obtained to conduct the study. The trial protocol was registered in Clinical Trials Registry- India with the registration number CTRI/ 2018/10/016097. Subjects aged 20 - 30 years, willing to give written informed consent, those with a minimum of 20 functional teeth and have mild to moderate gingivitis were included in the study. Subjects undergoing orthodontic treatment, with any kind of ulcers/ lesions, present in oral cavity, pregnant women, tobacco users, those undergoing or have taken antibiotic treatments for the past one month, with any systemic diseases and those who are und er regular medications for systemic illness and those using any other mouthwashes or history of hypersensitivity to mouthwash were excluded from the study. Before the commencement of the study, t he investigator was trained and calibrated to ensure uniform recording of plaque and gingival indices. Following the training and calibration exercises, the inter examiner reproducibility between the investigator and the calibrator for the gingival index was recorded for a group of 10 young adults and the inter examiner reproducibility was found to be 90%. The taxonomic identification of the leaves was performed at Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute (CSMRADDI) under the Central Council for Research in Ayurvedic Sciences (CCRAS), Min istry of AYUSH, Government of India, Arumbakkam, Chennai, Tamil Nadu Fresh leaves of all three products were thoroughly washed using distilled water and individually shade dried for about two weeks (Figure 4). After this period, the dried leaves and barks were powdered, individually and transferred to sterile air tight containers (Fig 5. 1, 5.3).

The extraction procedure was done using Cold maceration technique and was carried out at the Central Research Laboratory, Meenakshi Ammal Dental College. About 100 grams each of powdered Bael leaves, Neem leaves and cinnamon barks were individually dissolved in 1 liter of solvent (ethanol and distilled water - 1:1).The bottles were kept in the orbital shaking incubator at 37 ^{o}c for about 3 days with intermittent mixing. On the 4th day, solvents were filtered with the Whatman filter paper. The filtrate obtained was further subjected to distillation process for four days to yield the respective herbal extract. (Fig 5. 6). The concentrated extract was then transferred to a porcelain dish with minimum quantity of ethanol and dried over water bath to evaporate the ethanol completely.

An experimental, in-vitro study was conducted to assess the antimicrobial activity of Bael, Cinnamon and Neem extracts and compare with the gold standard Chlorhexidine mouthwash against six test pathogens namely *Streptococcus mitis, Streptococcus mutans, Enterococcus faecalis, Campylobacter rectus, Porphyromonas gingivalis* and *Candida albicans* in terms of zone of inhibition, palatability and shelf life. About five concentrations were prepared initially, which are as follows: 0.1%, 0. 2%, 0. 3%, 0.4% and 0.5%. (Figure 5) When the palatability was assessed, 0. 4% and 0.5% were rejected due to the unacceptability in taste. Hence, the first three concentrations were subjected to microbial analysis and assessed for shelf life. At the end of 7th day, the maximum zone of inhibition was seen in 0.3 % in all the extracts. The shelf life of the extracts with this concentration was also found to be 7 days since when checked on the 9th day, a mild turbidity was seen in the test tubes containing mouthwash es, representing microbial contamination. The palatability was also acceptable at this concentration. Hence, 0.3% was selected as the concentration of mouthwashes to be used in the in -vivo study.

Mouthwashes each containing 0.3% of the Bael, Cinnamon and Neem were prepared by mixing 300 mg of the extract in 100 ml of distilled water. (Figure 6)

1 person = 20ml/day

 1 week = 20* 7 = 140 ml/week

The extracts were prepared and dispensed in amber colored bottles and dispatched in batches on a weekly basis.

A pilot study was conducted to know the feasibility and to determine the sample size for the main study. A concurrent, parallel study design was followed for which twenty females (5 in each group) fulfilling the inclusion and exclusion criteria were randomly allotted to each of the four treatment groups:

- Group A (Test group): Mouthwash containing 0.3% Bael extract;
- Group B (Positive control 1): 0.3% Cinnamon extract;
- Group C (Positive control 2): 0.3% Neem extract; and
- Group D (Gold standard): 0. 2% Chlorhexidine mouthwash.

The effect of using the allotted mouthwash on dental plaque, gingival inflammation and salivary p H were evaluated. Dental plaque was assessed using Plaque Index described by Silness J and Loe H, 1964 with the modified criteria given by Loe H in 1967 (without the use of a disclosing agent), Gingival inflammation was assessed using Gingival Index (Loe H and Silness J in 1963)[60] and salivary p H using digital p H meter. Baseline data was collected prior to the use of respective mouth washes. Re- evaluation of dental plaque and gingival inflammation were carried out on 15th, 22nd and 29th day post intervention. Salivary pH was evaluated on the baseline and at the 29th day after administration of the mouthwash.

Sample size determination:

Keeping the power of the study at 90% and probability of type I error (α) at 5%, the sample size for the main study was calculated based on the dental plaque score obtained from the pilot study. The final sample size was estimated to be **12** in each group. With 4 groups in the study, the total sample size for the study was 48.

Oral examination was carried out to record the Gingival Index (Loe H and Silness J, 1963) for all the women residing in the hostel who gave informed consent. Forty Eight subjects with mild to moderate gingivitis were identified. The trial commenced in the second week of July 2018. The eligible subjects were randomly allotted to four groups - Group A, Group B, Group C and Group D by simple randomization (lottery method). Although the eligible subjects were identified and enrolled in the study by the investigator, administration of the intervention was carried out by a senior faculty based on the allocation sequence generated by a software called randomizer.org. The allocation sequence was coded, sealed and maintained by the senior faculty. It was decoded only after the data analysis

was completed. In this manner, the allocation sequence was concealed and maintained throughout the trial duration. (Figure 7)

The study subjects, the investigator assessing the clinical outcomes at baseline and during follow up visits and the statistician analyzing the data were blinded to group assignment. Hence, this study was a *triple blinded trial*. Subjects in group A were advised to rinse with 10 ml of 0.2% Chlorhexidine mouthwash (Hexidine) according to the manufacturer's instructions (10 ml of mouthwash to be swished in the mouth for one minute, twice a day). Subjects in group B were advised to rinse with 10 ml of 0.3 % Cinnamon mouthwash for 1 minute, group C to rinse with 10ml of 0.3% Bael mouthwash for 1 minute, subjects in group D was advised to rinse with 0.3 % Neem mouthwash for 1 minute. They were instructed to rinse their mouth with the respective mouthwashes, half an hour after brushing the ir teeth in the morning and once in the night before going to bed. During the study period, the participants were provided with a toothbrush and a fluoridated non-herbal toothpaste and were asked to continue their normal oral hygiene routine.

Oral examination was carried out in the hostel premises. The study subjects were seated upright in an ordinary chair and were examined under natural light. The dental plaque score and gingival inflammation score were recorded using Plaque Index described by Silness J a nd Loe H, 1964 with the modified criteria given by Loe H in 1967 and Gingival Index given by Loe H and Silness J in 1963. Oral examination was carried out prior to the daily tooth brushing on the days of data collection. The pH of the saliva was assessed using a digital pH meter (CyberTech)

Statistical analysis was performed using SPSS software version 19 (IBM Corp. Chicago, IL, USA). Descriptive data was expressed in terms of mean and standard deviation. The intra- group comparison was carried out using repeated measures ANOVA test followed by post hoc test. The inter - group comparison of Plaque Index score and Gingival Index score were carried out using One way ANOVA test followed by post hoc test (Tukey HSD test). Inter- group comparison of percentage difference in the dental plaque and gingival scores between each time point was carried out using ANOVA test. The inter- group comparison of salivary p H was carried out using One way ANOVA test followed by post hoc test (Tukey HSD test). Th e intra- group comparison of salivary pH was carried out using paired sample t test. In all tests, P value of <0.05 was considered as the level of statistical significance.

RESULTS

The quantitative data were described using mean and standard deviation. All continuous variables were tested for normality using Shapiro Wilk test and the data was found to be normally distributed. Hence, within and between comparisons were made using parametric tests of significance.

The mean age of subjects in 0. 2% Chlorhexidine group, 0.3% Cinnamon mouthwash group, 0. 3% Bael mouthwash group and 0.3% Neem mouthwash group were 26.5 + 2. 15 years, 25. 8 + 1.58 years, 26.25 + 2.66 years and 26.08 + 2.16 years respectively. There was no statistically significant difference in the distribution of the study subjects between the four groups based on their age (P=0.89) (Table 1)

INTERGROUP COMPARISON OF PERCENTAGE REDUCTION IN PLAQUE INDEX SCORES

The percentage reduction in mean plaque scores from Baseline to 15 th day in Chlorhexidine group, Cinnamon group, Bael group and Neem group were 6. 79± 4.88, 4.26±4. 80, 12. 33 +8. 28 and 8.12 +8.21 respectively. These differences in the percentage reduction in plaque scores were statistically significant (P=0 . 04) (Table 2A). Post hoc analysis showed that the differences in the percentage reduction in mean plaque scores between Bael and Cinnamon alone was statistically significant (P=0.03) (Table 2B) (Graph 1, 2)

The percentage reduction in mean plaque scores from Baseline to 22nd day in Chlorhexidine group, Cinnamon group, Bael group and Neem group were 19.85±8.71, 15.16±6. 09, 23. 38 +2. 11 and 15.46 +7.15 respectively. These differences in the percentage reduction in plaque scores were statistically highly significant (P=0.009) (Table 2A). Post hoc analysis showed that the differences in the percentage reduction in mean plaque scores between Bael and Cinnamon and between Bael and Neem were statistically significant (P=0. 02) (Table 2B).

The percentage reduction in mean plaque scores in Chlorhexidine group, Cinnamon group, Bael group and Neem group were 30. 22±8. 67, 19.80±5.97,

37.24+5.47 and 23. 89 +8.22 respectively. These differences in the percentage reduction in plaque scores were statistically very highly significant (P<0.001) (Table 2A). Post hoc analysis showed that the differences in the percentage reduction in mean plaque scores between Chlorhexidine and Cinnamon was statistically highly significant (P=0.005) and between Bael and Cinnamon and between Bael and Ne em, were statistically very highly significant (P<0. 001) (Table 2B). (Graph 3)

INTERGROUP COMPARISON OF PERCENTAGE REDUCTION IN GINGIVAL INDEX SCORES

The mean percentage reduction in gingival index scores from baseline to 15th day in Chlorhexidine group, Cinnamon group, Bael group and Neem group were 6.88±4. 51, 5.23±3.47, 13. 26±8.59 and 4.68±3.65 respectively. These differences in the percentage reduction in gingival index scores were statistically very highly significant (P <0. 001) (Table 3A). Post hoc analysis showed that the differences in the percentage reduction in gingival index scores between Bael and Chlorhexidine was statistically significant (P=0.03) and between Bael and Cinnamon and between Bael and Neem was statistically very highly significant (P=0. 004, 0. 002) (Table 3 B).

The percentage reduction of mean gingival index scores from baseline to 22nd day in the Chlorhexidine group,

cinnamon group, Bael group neem group were 14. 31±5.91,13.19±5. 27,25.68±8. 77 and 15.31±5. 41 respectively. These differences in the percentage reduction in gingival index scores were statistically very highly significant (P<0.001) (Table 3 A). Post hoc analysis showed that the difference s in the mean gingival index scores between Bael and Chlorhexidine and between Bael and Cinnamon was statistically very highly significant (P <0. 001) and between Bael and Neem it was statistically highly significant (P=0.002) (T able 3 B).

The percentage reduction in mean gingival index scores from baseline to 29th day in Chlorhexidine group, Cinnamon group, Bael group and Neem group were 20.74±7.22, 23.79± 5.77, 38.55± 9. 13 and 26.46 +9.31 respectively. These differences in the percentage reduction in gingival i ndex scores were statistically very highly significant (P<0.001) (Table 3A). Post hoc analysis showed that the differences in the mean gingival index scores between Bael and Chlorhexidine and between Bael and Cinnamon was statistically very highly significant (P<0. 001) and between Bael and Neem it was statistically highly significant (P=0.003) (Table 3B). (Graph 4)

CLINICAL SIGNIFICANCE

The clinical significance of the difference in the plaque and gingival index scores between the groups was assessed using effect size.

When compared to Chlorhexidine, the effect size was medium (0.40) for Cinnamon, large (0. 84) for Bael and small (0.23) for Neem mouthwash with regard to the plaque index scores. (Table 4 A)

When compared to Chlorhexidine, the effect size was medium (0.58) for Cinnamon, large (1. 67) for Bael and small (0.25) for Neem mouthwash with regard to the gingival index scores (Table 4 B)

DISCUSSION

In the present study, it was observed that with the progression of time, there was a statistically significant decrease in the intra group mean values of plaque scores as well as gingival scores in all the four intervention groups. With regard to Neem, the plaque scores at baseline, 15 th day, 22nd day and 29th day were 1.23 +0.27, 1. 13 +0. 27, 1.05+0.27 and 0.94 +0.24 respectively and the gingival index scores at baseline, 15 th day, 22nd day and 29th day were 1.42±0. 27, 1. 35± 0.26, 1. 20±0.24 and 1.05±0. 23 respectively. For Chlorhexidine, the plaque index scores at baseline, 15th day, 22nd day and 29 th day were 1.42 +0. 35, 1.31 +0.28, 1.14 +0. 29 and 1.00+0.28 respectively and the gingival index scores at baseline, 15th day, 22nd day and 29th day were 1.41±0. 29, 1. 31±0.27, 1.21±0. 27 and 1.10±0.16 respectively.

Similar results were seen in a study conducted by Nitish Abrol et al (2015)[3] where there was a significant difference in the reduction of plaque index scores and gingival index scores in the Neem and Chlorhexidine group {(Neem- Plaque index scores: at baseline, 15th day, 22nd day and 29th day were 0.38 +0. 09, 0.34 +0. 08, 0.27+0.06 and 0.22 +0.05 respectively; Gingival index scores: at baseline, 15th day, 22nd day and 29th day were 0. 46±0.07, 0.42±0. 06, 0.34±0. 06 and 0. 29± 0.06 respectively); (Chlorhexidine - Plaque index scores: at baseline, 15th day, 22nd day and 29th day were 0.40 +0. 08, 0.38+0.08, 0.33 +0.08 and 0.31

+0.07 respectively; Gingival index scores: at baseline, 15th day, 22nd day and 29th day were 0.51±0. 08, 0.49±0.08, 0.42±0. 07 and 0.39± 0.07 respectively)}.

Bael mouthwash was found to have higher percentage reduction in gingival index score when compared to Chlorhexidine at the end of two weeks (13. 26% versus 6. 88%) which is in contrast to the study conducted by Yeturu et al (2016) [23] where the percentage reduction in gingival index score was higher for Chlorhexidine than the herbal mouthwash containing Aloe vera (16.30% versus 9.88%). This difference can be attributed to the high content of tannins in Bael extract which has strong astringent effect thereby reducing the gingival bleeding and thus the gingival index score. In the present study, the antiplaque effect of Bael mouthwash was as eff ective as Chlorhexidine at the end of two weeks in contrast to Yeturu et al (2016) [23] in which Chlorhexidine was more effective than Aloe vera in reducing plaque score.

On the other hand, Bael mouthwash had higher percentage reduction than Chlorhexidine in reducing gingival index scores at the end of three weeks (25. 68% versus 14. 31%) which is in contrast to the results obtained by Mali et al (2012) [24] where no difference in percentage reduction of gingival index scores was seen between Chlorhexidine and Turmeric mouthwash was seen at the end of three weeks (61.15% versus 62.54%). However, no difference existed in the percentage reduction of plaque index between Bael and Chlorhexidine (25.68% and 14.31%) at the end of 3rd week which is in concordance with the findings of Mali et al (2012) [24] in which turmeric mouthwash was compared to Chlorhexidine (62 54% and 61.15%).

Similarly, at the end of 4th week, Gupta et al (2014) [19] did not find any significant difference in plaque scores between Tulsi mouthwash and Chlorhexidine (2. 49 +0. 46 and 2. 10 +0. 57 respectively) which is analogous to the present study where no difference was found between Bael and Chlorhexidine against dental plaque after four weeks of usage (1. 00 +0. 28 and 0. 81 +0.15 respectively). This is also in line with the findings of Pradeep et al (2016) [26] who found no difference between Triphala and Chlorhexidine at the end of four weeks. (2. 82 + 0.29 and 1.90 + 0.21 respectively). As per the above literature, it could be stated that Bael, Tulsi and Triphala can be used in par with Chlorhexidine in reducing plaque scores.

In the present study, Bael was found to be more effective than Chlorhexidine at the end of four weeks, (38. 55 +0.16 and 0.59 +0. 19) in reducing gingival index score which is in contrast to Pradeep et al (2016)[26] who found no difference in the anti - gingivitis effect between Triphala mouthwash and Chlorhexidine at the end of four weeks (0.6 +0. 16 and 0.59+0.19).

In the current study, chlorhexidine was maintained as a gold standard. Hence achieving a large effect size for the plaque and gingival score reflects good clinical significance apart from the statistical significance in favour of mouthwash containing 0.3% Bael (*Aegle marmelos)* when compared to chlorhexidine. Merits can be attributed to the generalizablility of the study to wider population due to the following reasons : In this pragmatic trial, the subjects were randomly selected, sample size estimation was done scientifically based on the inputs from the pilot study which was carried out in a population that was very s imilar to the study population, steps were adopted to reduce the risk of bias and also the trial was preceded by an *in- vitro* study which gave the necessary inputs to arrive at a concentration of the mouthwashes intended to be used in the *in-vivo* study.

However, the results of the study should be interpreted cautiously since this study has certain

limitations. The possibility of Hawthorne effect cannot be ruled out since the subjects had prior knowledge on the regular oral examinations to be carried out by the investigator, which itself would have been a motivation to perform a better oral hygiene and thus bringing a difference in the level of dental plaque and thereby in the gingival inflammation.

This study was conducted only on female subjects with mild to moderate gingivitis. Hence future studies are recommended to assess the effectiveness of *Aegle marmelos* with both the genders included in the study and considering severe forms of gingival inflammation as eligibility criteria. Studies of longer duration are also recommended to strengthen the evidence on the long term effect of *Aegle marmelos* with regard to its antimicrobial and anti-inflammatory properties. This clinical trial carried out to evaluate the effect of 0.3% Bael *(Aegle marmelos)* mouthwash against dental plaque and gingival inflammation advocates new possibilities of using this well-known potential medicinal plant in the pharmaceutical industry to devise novel mouthwash which can be incorporated in dentistry.

CONCLUSION

At the end of the 4 weeks trial, mouthwash containing 0. 3% *Aegle marmelos* (Bael) leaves extract, 0. 3% *Cinnamomum zeylanicum* (Cinnamon) barks extract, 0.3% *Azadirachta indica* (Neem) leaves extract and 0.2% Chlorhexidine showed effectiveness in reducing the dental plaque and gingival inflammation. Mouthwash containing 0.3% Bael extract was found to be as effective as gold standard Chlorhexidine mouthwash in reducing plaque, but was more effective compared to mouthwash containing 0. 3% Neem extract and 0.3% Cinnamon extract.

With regard to the gingival inflammation, 0.3% Bael mouthwash was found to be more effective than 0.3% *Cinnamomum zeylanicum* (Cinnamon) barks extract, 0.3% *Azadirachta indica* (Neem) leaves extract and 0.2% Chlorhexidine mouthwashes. Thus it could be concluded that, use of Bael mouthwash can be a better choice over Chlorhexidine in reducing the plaque accumulation and gingival inflammation as the former is a natural product with no side effects.

Table 1: Mean age of the study subjects allocated to the intervention groups

Groups	Intervention	Number of Study Subjects (n)	Age (in years) (Mean±SD)	P value[a]
Group A	0.2% Chlorhexidine	12	26.5 + 2. 15	0. 895
Group B	0.3% Cinnamon	12	25.8 + 1. 58	
Group C	0.3% Bael	12	26.25 + 2.66	
Group D	0.3% Neem	12	26.08 + 2.16	

**P<0.05 is Statistically significant*
[a]ANOVA – Analysis of Variance

Table 2A: Intergroup comparison of percentage reduction in plaque index scores

PERCENTAGE REDUCTION					**P value[a]**
Time points	**Chlorhexidine**	**Cinnamon**	**Bael**	**Neem**	
	Mean± SD	**Mean± SD**	**Mean± SD**	**Mean± SD**	
Baseline to 15th day	6.79+4.88	4.26+4.80	12.33+8.28	8.12+8.21	**0.041***
Baseline to 22nd day	19.85+8.71	15.16+6.09	23.38+2.11	15.46+7.15	**0.009****
Baseline to 29th day	30.22+8.67	19.80+5.97	37.24+5.47	23.89+8.22	**<0.001*****

**P<0. 05 is Statistically significant*
***P<0.01 is Statistically highly significant*
****P<0.001 is Statistically very highly significant*
[a]ANOVA – Analysis of Variance

Table 2B: Post Hoc analysis for intergroup comparison of percentage reduction in plaque index scores

PERCENTAGE REDUCTION			**P value[a]**
TIME POINTS	**COMPARITIVE GROUPS**	**MEAN DIFFERENCE**	
End of 2weeks	Chlorhexidine vs Cinnamon	2.53	0.79
	Chlorhexidine vs Bael	5.53	0.20
	Chlorhexidine vs Neem	1.33	0.96
	Cinnamon vs Bael	8.07	**0.03***
	Cinnamon vs Neem	3.86	0.50
	Bael vs Neem	4.20	0.43
End of 3 weeks	Chlorhexidine vs Cinnamon	4.68	0.30
	Chlorhexidine vs Bael	3.53	0.54
	Chlorhexidine vs Neem	4.38	0.35
	Cinnamon vs Bael	8.22	**0.02***

	Cinnamon vs Neem	0.29	0.99
	Bael vs Neem	7.92	**0.02***
End of 4 weeks	Chlorhexidine vs Cinnamon	10.41	**0.005****
	Chlorhexidine vs Bael	7.02	0.09
	Chlorhexidine vs Neem	6.32	0.15
	Cinnamon vs Bael	17.44	**<0.001*****
	Cinnamon vs Neem	4.09	0.51
	Bael vs Neem	13.35	**<0.001*****

**P<0. 05 is Statistically significant*
***P<0.01 is Statistically highly significant*
****P<0.001 is Statistically very highly significant*
[a]Tukey's Post hoc test

Table 3A: Intergroup comparison of percentage reduction in gingival index

Time points	PERCENTAGE REDUCTION				P value[a]
	Chlorhexidine	Cinnamon	Bael	Neem	
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Baseline to 15th day	6.88+4.51	5.23+3.47	13.26+8.59	4.68+3.65	**<0.001**
Baseline to 22nd day	14.31+5.91	13.19+5.27	25.68+8.77	15.31+5.41	**<0.001**
Baseline to 29th day	20.74+7.22	23.79+5.77	38.55+9.13	26.46+9.31	**<0.001**

**P<0. 001 is Statistically very highly significant*
[a]ANOVA – Analysis of Variance

Table 3B: Post Hoc analysis of intergroup comparison of percentage reduction in gingival index

TIME POINTS	COMPARITIVE GROUPS	MEAN DIFFERENCE	P value[a]
End of 2 weeks	Chlorhexidine vs Cinnamon	1.66	0.88
	Chlorhexidine vs Bael	6.37	**0.03***
	Chlorhexidine vs Neem	2.19	0.76
	Cinnamon vs Bael	8.02	**0.004****
	Cinnamon vs Neem	0.54	0.99
	Bael vs Neem	8.57	**0.002****

End of 3 weeks	Chlorhexidine vs Cinnamon	1.12	0.97
	Chlorhexidine vs Bael	11.36	**≤0.001*** **
	Chlorhexidine vs Neem	1.00	0.98
	Cinnamon vs Bael	12.48	**<0.001*** **
	Cinnamon vs Neem	2.12	0.85
	Bael vs Neem	10.36	**0.002** **
End of 4 weeks	Chlorhexidine vs Cinnamon	3.04	0.788
	Chlorhexidine vs Bael	17.80	**<0.001*** **
	Chlorhexidine vs Neem	5.71	0.31
	Cinnamon vs Bael	14.76	**<0.001*** **
	Cinnamon vs Neem	2.67	0.84
	Bael vs Neem	12.08	**0.003** **

**P<0. 05 is Statistically significant*
***P<0.01 is Statistically highly significant*
****P<0.001 is Statistically very highly significant*
[a]*Tukey's Post hoc test*

Table 4A: Determination of clinical significance using effect size for plaque index scores

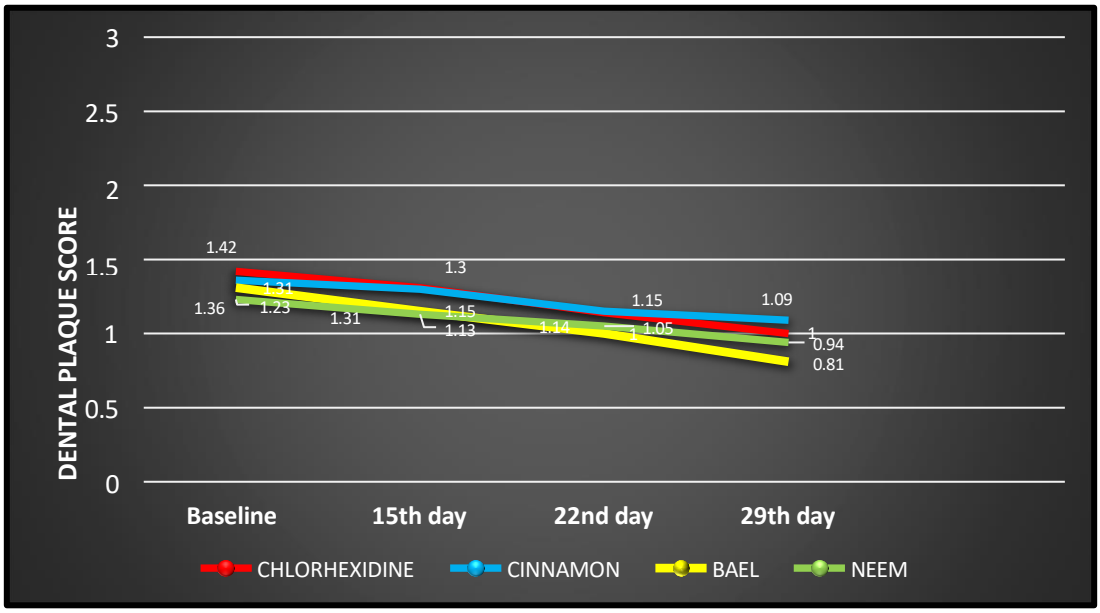
Groups	Mean + SD	Effect size when compared to the Gold standard	Interpretation
Chlorhexidine	1.00 + 0.28	---	---
Cinnamon	1.09 + 0.15	0.400	Medium
Bael	0.81 + 0.15	0.845	Large
Neem	0.94 + 0.24	0.230	Small

Table 4B: Determination of clinical significance using effect size for gingival index scores

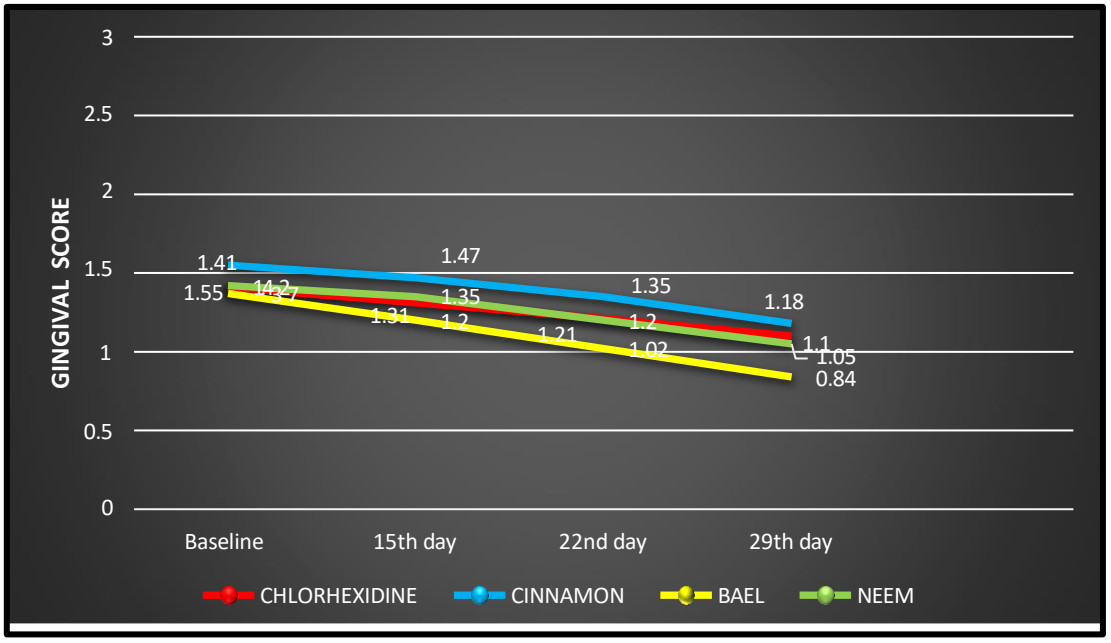
Groups	Mean + SD	Effect size when compared to the Gold standard	Interpretation
Chlorhexidine	1.10 + 0.16	---	---
Cinnamon	1.18 + 0.11	0.582	Medium
Bael	0.84 + 0.15	1.676	Large
Neem	1.05 + 0.23	0.252	Small

GRAPHS

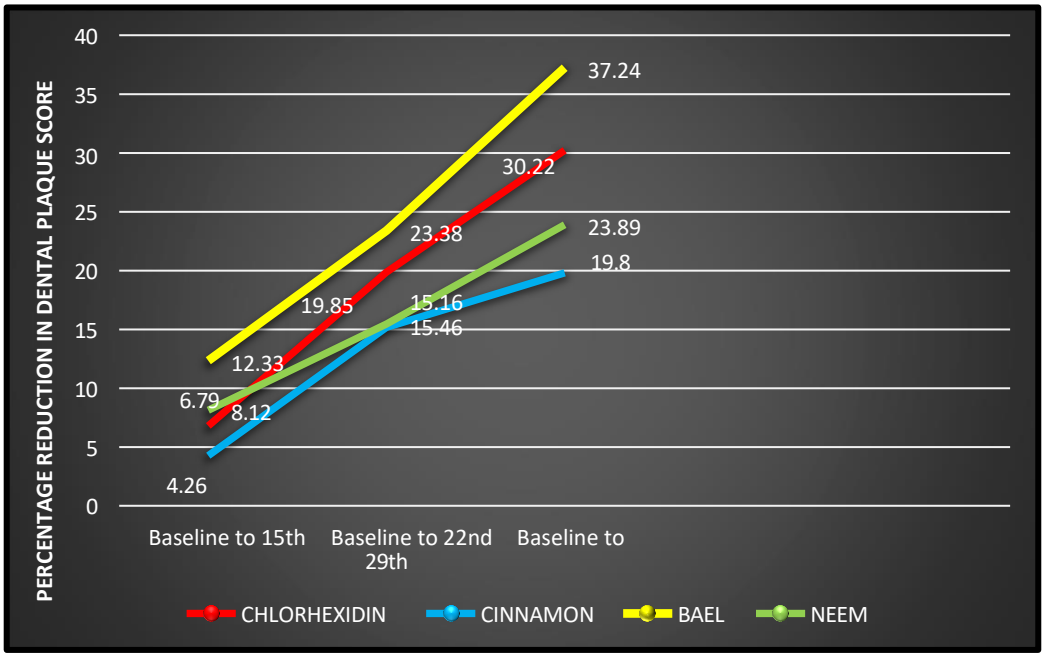
Graph 1 - Mean dental plaque index scores from baseline to 4th week in test groups and control group



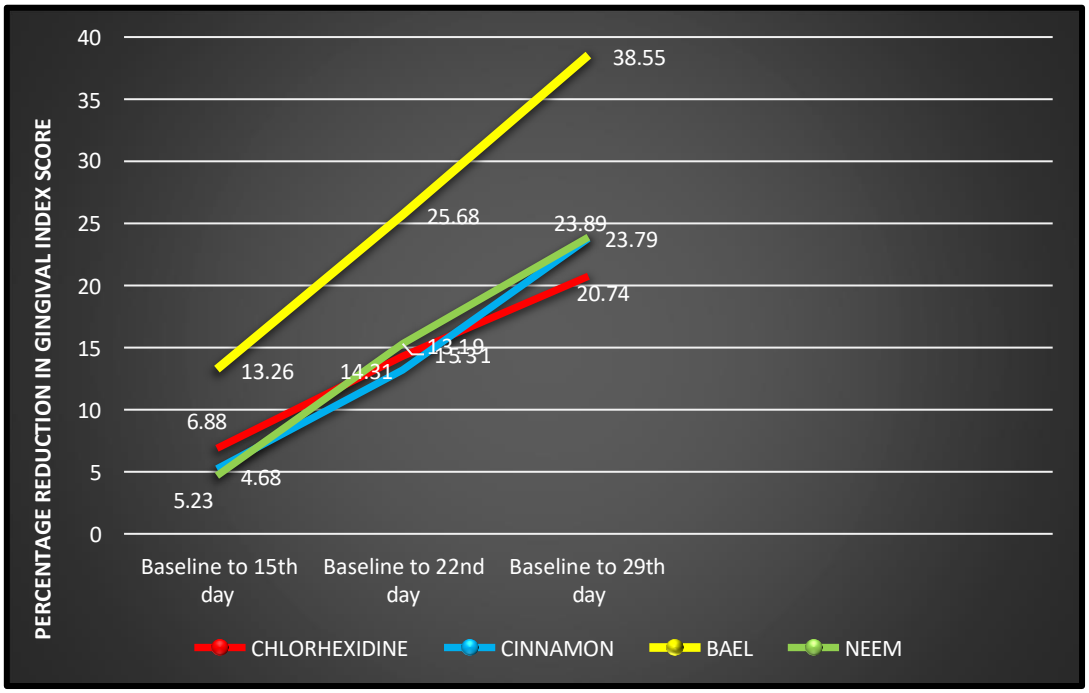
Graph 2 - Mean gingival index scores from baseline to 4 th week in test groups and control group



Graph 3 - Percentage reduction of mean dental plaque scores fr om baseline to 4 th week in test groups and control group



Graph 4 - Percentage reduction of mean gingival index scores from baseline to 4 th week in test groups and control group



CLINICAL RELEVANCE

Scientific rationale for study:
- Paucity of literature on the antimicrobial activity of Bael ag ainst oral microorganisms.

Principal findings:
- Bael proved to be effective than Cinnamon, Neem and Chlorhexidine. in reducing the gingivitis.

Practical implications:
According to the principles of primary health care, development of appropriate technology for maintenance of health should be encouraged.
Common risk/ health factor approach can be used where both general and oral health is promoted by using cost effective and world- wide accepted indigenous products

FIGURES AND GRAPHS:

Figure 1a, 1b: Palm leaf manuscript revealing the medicinal uses of Bael (*Aegle marmelos*)
Front View of the manuscript

Back View of the manuscript

Figure 2: Palm leaf manuscript revealing medicinal uses of Cinnamon (*Cinnamomum zeylanicum)*

Figure 3: Palm leaf manuscript revealing medicinal uses of Neem (*Azadirachta indica)*

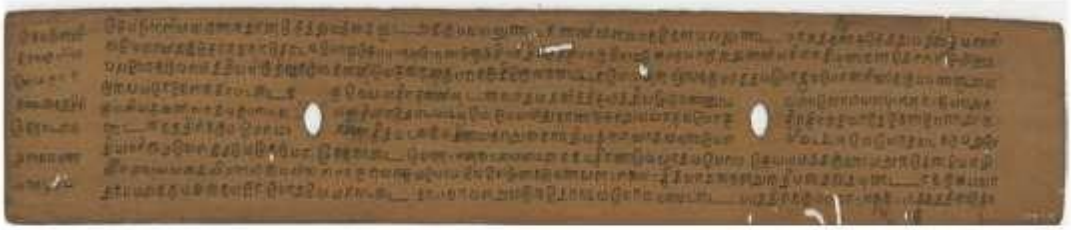


Figure 4: PLANTS and BARK PROFILE

4.a. Bael (*Aegle marmelos)*

4.b Neem (*Azadirachta indica)*

4.c Cinnamon (*Cinnamomum zeylanicum)*

Figure 5: PREPARATION OF THE EXTRACTS

5.1 Drying of the Leaves and Bark

(Bael) **(Neem)** **(Cinnamon)**

5.2 Coarsely Powdered Leaves and Bark

5.3Addition of Hydro alcohol solution to each of the Powdered products (L – R : Cinnamon , Neem, Bael)

5.4Subjecting the solutions to

5.5 Filtration of the solutions Orbital shaking incubator

5.6 Distillation

5.7 Extracts of all the three products (L-R : Bael 22.5 g, Cinnamon 25 g, Neem 22.6 g)

Figure 6: PREPARATION OF MOUTHWASH

6.1 Armamentarium used

6.2Weighing the ingredients to distilled water

6. 3 Adding the ingredients

6.4Stirring the mixtures

6.5 Final Mouthwashes

Figure 7: MOUTHWASHES AFTER ALLOCATION CONCEALMENT

Figure 8: ARAMAMENTARIUM FOR ORAL EXAMINATION

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