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Original Research Article

Efficacy of *Artocarpus heterophyllus* leaf extract mouthwash in gingivitis subjects: a clinico microbiological study

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ABSTRACT

Background: Gingivitis is the inflammatory disease of gingiva that supports the teeth. The prevailing belief regarding the cause of gingivitis is the toxins produced by anaerobic bacteria in oral cavity particularly that cause the formation of plaque. The tropical climacteric fruit *Artocarpus heterophyllus*, commonly known as jackfruit possess anti carcinogenic, antimicrobial, antifungal, anti-inflammatory, wound-healing and hypoglycaemic properties. Hence the objective of this study is to evaluate clinical parameters at baseline and 21 days post non-surgical periodontal therapy after the use of jackfruit leaf extract mouthwash and to evaluate the colony count of *P. gingivalis* in plaque samples at baseline and 21 days after the use of jackfruit leaf extract mouthwash.
Methods: A total of 30 gingivitis subjects were selected and divided randomly into group A and B. Group 1, non-surgical periodontal therapy alone (15). Group 2, non-surgical periodontal therapy followed by jackfruit leaf extract mouthwash (15). The supra gingival plaque samples were collected from both the groups at baseline and 21 days post non-surgical periodontal therapy.
Results: Significant reduction in clinical parameters and colony count of *P. gingivalis* after the use of Artocarpus heterophyllus mouthwash.
Conclusions: Jackfruit leaf extract mouthwash has better promising effect on inflammation in gingivitis subjects when compared to gingivitis subjects with non-surgical periodontal therapy alone.

Keywords: Gingivitis, Non-surgical periodontal therapy, Plaque samples, *Porphyromonas gingivalis, Artocarpus heterophyllus*

INTRODUCTION

Gingivitis is one of the most prevalent clinical conditions in oral cavity, characterized by inflammation of the gingival tissue which is limited to the connective tissue and soft tissues of gingival epithelium. Depending on the cause, intensity, length of infection, and clinical presentation, gingivitis can take various forms. Dental plaque is a thin film that forms by bacteria on the surface of tooth, due to poor oral hygiene and causes inflammation of gingiva and harbours a large number of bacteria.[1,2] *P. gingivalis, A. actinomycetemcomitans, T. forsythia, P. intermedia, T. denticola* are few of the bacteria that cause gingivitis. Plaque and poor oral hygiene are the major causative factors for the progression of the disease. Until early lesions of gingivitis appear, the first signs of the disease i. e., bleeding on probing and gingival redness do not appear.[3]

Usage of mouthwash, toothpaste, irritants, sprays, chewing gum and varnishes are few ways to prevent plaque formation. However, using mouthwash such as

chlorhexidine (CHX) is the most successful among all. Inspite of several uses and advantages, it has certain drawbacks such as tooth discoloration, allergic reactions and burning sensation. The usage of herbal mouthwashes has increased recently as a result of growing knowledge of the benefits of complementary and alternative medicine. Therefore, any herbal mouthwash with more beneficial effects than CHX is required for the patients which show some better results.[4]

Artocarpus heterophyllus commonly known as jackfruit, a member of *Moraceae* family is a tropical climacteric fruit that is native to Western Ghats of India, Africa, and some parts of South America. It is regarded as the world's largest edible fruit which abundantly contains phytochemicals, carbohydrates, proteins, vitamins and minerals. Seeds and flesh of fully ripen jackfruit are edible. Several nations have used pureed jackfruit to create a variety of culinary goods, including ice creams, jams and jellies. The purpose of this study is to encourage the use of jackfruit in commercial food production by spreading awareness about it's nutritional and health benefits. Studies have shown that jackfruit contains potent antibacterial and anti-inflammatory properties that protect against oral microbes such as *P. gingivalis*.[5]

The methanolic extract of stem, root, barks, leaves, fruits and seeds of jackfruit showed a wide range of anti-bacterial and anti-inflammatory activity. Therefore, we aimed to assess and compare the efficacy of jackfruit leaf extract mouthwash on periodontal pathogen, *P. gingivalis* in gingivitis subjects.

Aim of the study was to investigate the antimicrobial and anti-inflammatory activity of jackfruit leaf extract mouthwash on gingivitis subjects.

METHODS

Type of study

It is an original study which was performed in July 2024 to September 2024.

Study design

A total of 30 subjects diagnosed with gingivitis aged 18-40 years, were randomly selected from the outpatient department of periodontics, P. M. N. M dental college and hospital, Bagalkot. Keeping alpha error at 5%, β error 15%, power of the study at 85%, the sample size estimated was approximately 10 in each group.

For follow-up study, to avoid loss due to attrition we have taken 15 subjects in each group.

Subjects were randomly divided into 2 groups: Group 1: 15 subjects who undergone non-surgical periodontal therapy alone. Group 2: 15 subjects who undergone non-surgical periodontal therapy followed by jackfruit leaf extract mouthwash.

Ethical approval was taken from the institutional ethical committee for the study.

Inclusion criteria

It includes male and female healthy subjects from 18 to 40 years of age, who are untreated with gingivitis and were not under any medication within last 6 months which might alter the periodontal status.

Exclusion criteria

It includes subjects with systemic diseases that could influence periodontal conditions and who have undergone periodontal therapy in the past 6 months. Subjects on any systemic antibiotics, anti-inflammatory, hormonal therapy or corticosteroid therapy for any other reasons which affect the periodontal status and who had known history of allergy to jackfruit were also excluded.

Method of preparation of jackfruit leaf extract mouth wash

Jackfruit leaves were washed thoroughly, cut into pieces and shade dried. The dried leaves were crushed finely and sieved to obtain fine powder. The 630 grams of leaf powder was weighed and maceration was carried out with 2.5 L of 70% ethanol followed by filtration using a Buchner funnel and filter paper. The filtrate was collected and stored at room temperature. To this filtrate, another 2.5 L of 70% ethanol was added until the residue was buried in the jar and left undisturbed for 24 hours. The extract was filtered for second time using a filter paper. Then the first and second filtrate were mixed and evaporated using a rotary evaporator for 2 hours. To the final filtrate, 5% glycerine as base, 4.2% Stevia as sweetening agent, 1.6% sodium benzoate as preservative were added to obtain *Artocarpus heterophyllus* mouthwash (Figure 1).[6,7]

Clinical examination to assess the periodontal condition

Clinical examination was performed on all the subjects using gingival index, plaque index and bleeding on probing at baseline and 21 days post nonsurgical periodontal therapy. Using a sterile curette, supragingival plaque samples were collected from the buccal surface of first molar of every quadrant on day 0 and day 21. It was combined with 500 µl of reduced transport fluid (RTF) in a sterile Eppendorf tube and later the samples were sent to microbiological analysis for bacterial culture to check the colony count of *P. gigivalis*.[8] Following the collection of baseline data, oral prophylaxis was conducted, and participants were advised to utilize the provided mouthwash and refrain from using any other oral hygiene products other than toothpaste and toothbrush.

The collected data was analysed using Chi-square test, independent t test or Mann-Whitney U test for comparison of two groups and descriptive statistics with frequency, percentage, mean SD etc. All participants were explained clearly regarding the need and design of study and consent was obtained.

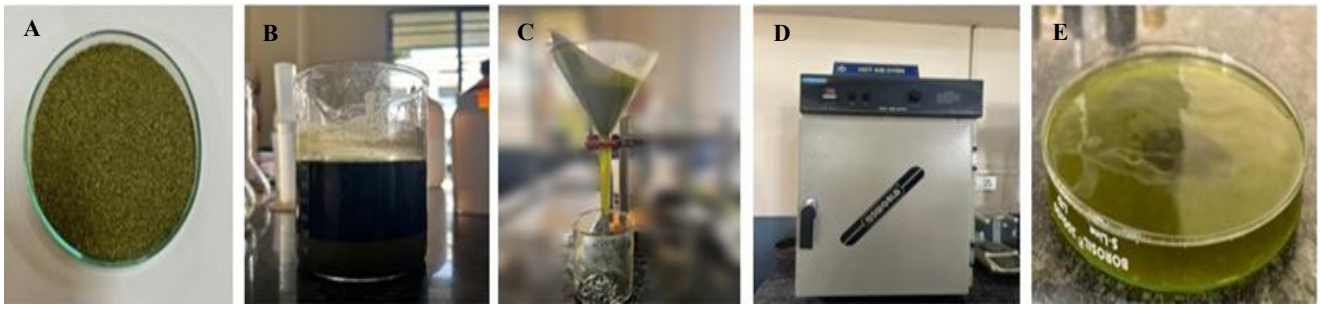

Figure 1 (A-E): Method of preparation of jackfruit leaf extract mouthwash.

RESULTS

The mean percentage of gender is about 65.00 in group 1 and 35.00 in group 2. The mean value of age group is 25.65 in group 1 and 25.75 in group 2 and the $p<0.05$. The results of the study showed that there was a statistical difference in colony forming unit of *P. gingivalis* and clinical parameters in group B compared to group A after 21 days.

The *P. gingivalis* count was significantly lower in the group who received scaling and jackfruit mouthwash than in the group that only received scaling with mean difference of 18.90 in group A and 18.55 in group B by independent t test (Figure 2).

Gingival index score showed a mean difference of 0.49 in group 1 from base line to day 21 and group 2 showed a mean difference of 0.64 from base line to day 21. Plaque index score showed a mean difference of 0.35 in group 1 from base line to day 21 and group 2 showed a mean difference of 0.70 from base line to day 21. Bleeding on probing score showed a mean difference of 0.38 in group 1 from base line to day 21 and group 2 showed a mean difference of 0.57 from base line to day 21 (Table 1).

The scores of changes in all parameters except CFU from baseline to day 21 in group 1 and 2 did not follow normal distribution. Therefore, non-parametric tests were applied. Otherwise, parametric tests would have been applied (Table 2).

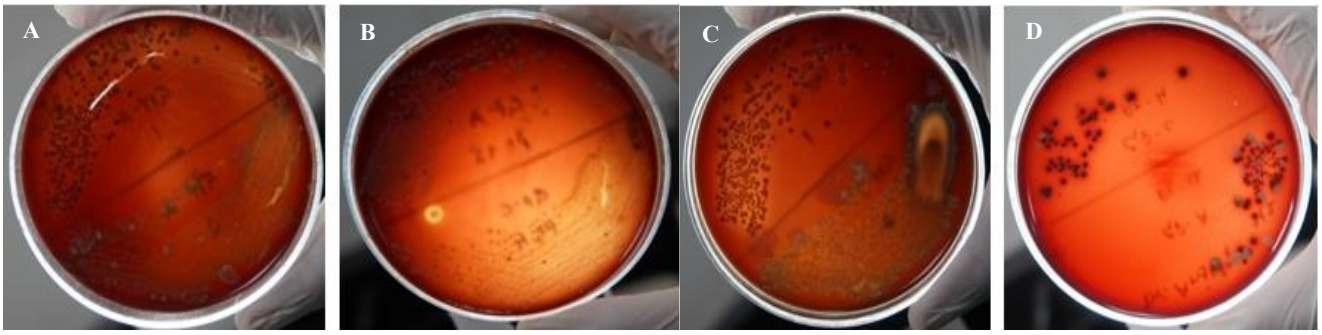

Figure 2 (A-D): Count of *P. gingivalis* in group 1 and 2 at baseline and after 21 days.

Table 1: Comparison of group 1 and 2 with GI, PI and BOP scores at baseline and day 21 treatment time points by Mann-Whitney U test.

Time points	Group 1			Group 2			U value	Z value	P value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Gingival index									
Baseline	1.16	0.34	23.33	1.08	0.27	17.68	143.5	1.5148	0.1298
Day 21	0.67	0.19	27.50	0.28	0.20	13.50	60.0	3.7735	0.0002*
Difference	0.49	0.41	18.18	0.64	0.28	22.83	153.5	-1.2443	0.2134
Plaque index									
Baseline	1.06	0.20	21.03	0.93	0.32	19.98	189.5	0.2705	0.7868
Day 21	0.71	0.11	30.10	0.36	0.17	10.90	8.0	5.1801	0.001
Difference	0.35	0.16	13.53	0.70	0.32	27.48	60.5	-3.7600	0.0002

Continued.

Time points	Group 1			Group 2			U value	Z value	P value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Bleeding on probing									
Baseline	0.96	0.35	21.83	0.87	0.28	19.18	173.5	0.7033	0.4819
Day 21	0.58	0.24	26.50	0.42	0.12	14.50	80.0	3.2325	0.0012
Difference	0.38	0.39	17.53	0.57	0.26	23.48	140.5	-1.5960	0.1105

*P value significant.

Table 2: Normality of changes in all parameters from baseline to day 21 in group 1 and 2.

Parameters	Groups	Kolmogorov-Smirnov	Df	Sig.	Shapiro-Wilk	Df	Sig.
GI	Group 1	0.1370	20	0.2000	0.9500	20	0.3630
	Group 2	0.1800	20	0.0890	0.9040	20	0.0500*
PI	Group 1	0.1780	20	0.0980	0.9390	20	0.2280
	Group 2	0.2000	20	0.0350*	0.9500	20	0.3600
BOP	Group 1	0.1730	20	0.1190	0.9110	20	0.0670
	Group 2	0.2900	20	0.0001*	0.7830	20	0.0001*
CFU	Group 1	0.1570	20	0.2000	0.9670	20	0.7010
	Group 2	0.1420	20	0.2000	0.9480	20	0.3340

*P value significant.

DISCUSSION

The most prevalent cause of gingivitis, an inflammatory disease of the gingival tissue, is bacterial infection. In contrast to periodontitis, there is no loss of attachment, which means that the junctional epithelium does not migrate. Dental plaque is recognized as the primary cause of caries, gingivitis and periodontal disease. Effective plaque control is essential in preventing the occurrence of the disease. An antibacterial effect can be produced throughout the oral cavity by combining mechanical plaque removal with a chemical plaque control treatment, such as mouthwash. In dentistry, CHX is one of the most often prescribed chemical plaque management agents. Its mainly utilized due to its antiseptic and antibacterial properties. Oral rinse containing CHX gluconate can dramatically lower plaque bacteria and stop from developing gingivitis.[9] CHX's ability to manage gingivitis is linked to its capacity to manage both the overall amount of plaque and the periodontopathic germs that are present in it.[10]

Ahmad et al in their studies verified how the long-term usage of CHX mouthwash causes tooth stains, allergies, and mouth burns.[11] Nagesh Bhat et al in their studies explained clearly about the potential side effects of CHX

mouthwash like slight discoloration, dry mouth and burning sensation. As natural herbs contain natural phytochemicals, they can be used to create alternative medications in place of synthetic medications. Hence people are aware of the benefits of complementary and alternative medicines, the use of herbal mouthwash has increased recently.[12]

Due to some disadvantages of CHX mouthwash, now a days people are preferring herbal mouthwashes which are widely accepted and have very less side effects, among which jackfruit is one of them. Several studies are available which explains the antibacterial property of Jackfruit leaves in various forms in gingivitis and periodontitis.

The jackfruit is commonly referred to as 'poor man's food.' It has many properties like antibacterial, anti-diabetic, antioxidant and anti-inflammatory effects. Jackfruit leaves are rich in vitamins and minerals.[13]

Prakash et al investigated the anti-inflammatory effect of jackfruit leaf extract-based gel in periodontitis subjects and the results have showed that the gel has high potential in anti-inflammatory activity and shows less cytotoxic activity. At baseline, three months, and six months, clinical measures such as gingival index, plaque index, probing pocket depth, and clinical attachment level were measured. All clinical parameters significantly improved in both groups, according to statistical analysis, with the group that received jackfruit leaf extract hydrogel demonstrating further advantages. Because of its antibacterial and anti-inflammatory qualities, the hydrogel has showed great promise in reducing the symptoms of chronic periodontitis. Positive patient feedback and few side effects point to its viability and adoption as a therapeutic option. According to these results, jackfruit leaf extract hydrogel shows promise as a localized drug delivery method for periodontal therapy.[14]

In this study, the supragingival plaque samples were collected in RTF were promptly taken to the lab, centrifuged, and diluted in the ratio of 1:10. *P. gingivalis* were cultured on Kanamycin blood agar and incubated for 48-72 hours in a CO_2 jar. Bacterial growth and colony count were determined after the incubation period. CFU count has shown a mean difference of 18.90 in group 1 from base line to day 21 and 18.55 in group 2 from base line to day 21 with a p=0.0001.

In this study, GI index score showed a mean difference of 0.49 in group 1 from base line to day 21 and group 2 showed a mean difference of 0.64 from base line to day 21 with a p=0.0001. Similarly, Nagaraj et al conducted a study on anti-microbial efficacy of Myristica fragrans mouthwash in gingivitis subjects and concluded that gingival index score showed a mean difference of 0.71 in group 1 from base line to day 21 and group 2 showed a mean difference of 0.63 from base line to day 21 with a p=0.001.[15]

Similarly, Bhat et al conducted a study on the antiplaque effect of herbal mouthwash in comparison to CHX in human gingival disease: a randomized placebo controlled clinical trial and concluded that the mean gingival index at baseline was statistically nonsignificant between the three mouthwashes (p=0.92). However, the mean gingival index after 1 month was highly significant between CHX group and saline group (p=0.001), statistically significant between herbal group and saline group (p=0.003), whereas the herbal and CHX group were potentially equivalent in reducing gingivitis (p=0.229).[13]

Similarly, PI index score showed a mean difference of 0.35 in group 1 from base line to day 21 and group 2 showed a mean difference of 0.70 from base line to day 21 with a p=0.0001. Gupta et al conducted a study on antiplaque efficacy of aloe vera mouthwash and concluded that there was a significant reduction on plaque in patients with aloe vera and CHX groups and no statistically significant difference was observed among them (p>0.05).[16]

Similarly, Prasad et al conducted a study on anti-plaque efficacy of herbal and 0.2% CHX gluconate mouthwash: a comparative study in which parameters gingival index and plaque index were taken and analysed between three groups i.e., saline, CHX group and Hiora among the patients and concluded that there was significant difference in the mean GI and PI scores (p=0.002) respectively and post-hoc test showed that the mean PI was significantly higher for saline followed by Hiora with least being CHX group.[18]

Bleeding on probing score showed a mean difference of 0.38 in group 1 from base line to day 21 and group 2 showed a mean difference of 0.57 from base line to day 21 with a p=0.0002. All the results indicate the statistical difference in the various clinical parameters and CFU among both the groups at baseline and after 21 days. Vaish et al conducted a study on comparative evaluation of 0.2% CHX versus herbal oral rinse on plaque induced gingivitis and concluded that both CHX and herbal mouthwash were found to be helpful in reducing bleeding score but results were not significant statistically. This substantial reduction in bleeding score in herbal group might be because of its ingredients *Mimusops elengi*, *Acacia catechu* and *Mentha spicata* which reduce bleeding because of their astringent action.[18]

Limitations

The consistency and reproducibility of the mouthwash may be differed by manufacturers in extract process (drying, maceration, ethanol extraction). Clinical parameters like gingival index, plaque index and bleeding on probing are dependent on examiner interpretation and if not calibrated, may create observer bias.

CONCLUSION

Recently in dentistry, the use of herbal mouthwashes has proved their effectiveness by improving the clinical outcome. This study has explained the anti-inflammatory role of jackfruit leaf extract mouthwash in reducing various clinical parameters and the colony count of *P. gingivalis* in gingivitis subjects. Due to the beneficial properties of jackfruit leaves mentioned above, it has shown improvement in various clinical outcomes. But still, further clinical studies are required to prove the anti-inflammatory property of jackfruit leaves in gingivitis and periodontitis subjects.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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