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Original Research Article

Antimicrobial efficacy of *Syzygium cumini* leaf mouthwash following non-surgical periodontal therapy in gingivitis subjects: a clinico microbiological study

Bhagyashri N. Vanaki[1], Vijayalaxmi M. Kokatnur[1], Nagaraj B. Kalburgi[1], Shruti Hugar[1]*, Anjaly Roy[1], Jayadev N. Hiremath[2]

[1]Department of Periodontics, PMNM Dental College and Hospital, Navanagar, Bagalkote, Karnataka, India
[2]Hanagal Shri Kumareshwar college of Pharmacy, Bagalkote, Karnataka, India

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***Correspondence:**
Dr. Shruti Hugar,
E-mail: shruti.hugar1994@gmail.com

ABSTRACT

Background: Gingivitis is an inflammatory condition of the gingival tissue caused by bacterial infection and by the microbial plaque deposits located in or close to the gingival sulcus. *Syzygium cumini* commonly known as jamun/jamlang possesses antioxidant, anti-inflammatory, antidiabetic and antibacterial properties and can be used as reasonably safe alternative to synthetic pharmaceuticals. The leaves of jamlang have the ability to inhibit the growth of Gram negative and Gram positive bacteria. Therefore, this study aimed to assess the impact of antimicrobial efficacy of jamlang leaves in gingivitis subjects.
Methods: A total of 40 gingivitis subjects were selected and divided randomly into 2 groups. Group 1- non surgical periodontal therapy alone (20), group 2- non surgical periodontal therapy followed by *Syzygium cumini* mouthwash (20). The supragingival plaque samples of both the groups were collected at baseline and 21 days after scaling. Simplified oral hygiene index, gingival index, plaque index and bleeding on probing were recorded at baseline and 21st day.
Results: Significant reduction in clinical parameters and colony count of *Porphyromonas gingivalis* was seen in both the groups. But comparatively *Syzygium cumini* mouthwash group showed more reduction in BOP and inflammation when compared with group 1.
Conclusions: Leaf extract of *Syzygium cumini* mouthwash showed reduction in various clinical parameters and the colony count of *Porphyromonas gingivalis*. Hence *Syzygium cumini* mouthwash can be used as an adjunct to the mechanical periodontal therapy.

Keywords: Gingivitis, Non-surgical periodontal therapy, *Syzygium cumini* mouthwash

INTRODUCTION

Gingivitis is an inflammatory condition of the gingival tissue, most commonly caused by bacterial infection and by toxins produced by the bacteria in the mouth, particularly the bacteria that causes the formation of plaque. Unlike periodontitis, there is no attachment loss and hence no migration of the junctional epithelium. The condition is limited to the soft-tissue area of the gingival epithelium and connective tissue.[1]

There are various forms of gingivitis based on clinical appearance, duration of infection, severity, and etiology. However, the chronic form of gingivitis that is caused by plaque is considered to be the most frequent variant. Dental plaque plays a major role in the etiology of the

diseases and dental plaque control is important in the prevention of the dental diseases.[1,2]

The initial symptoms of gingivitis are not obvious, and bleeding on probing and redness of the gums do not occur until early lesions of gingivitis are present. It is usually painless, rarely causes spontaneous bleeding, and its clinical symptoms are not obvious enough for most patients to identify the disease. Periodontal tissues become more sensitive to numerous irritants, such as plaque, calculus, and food debris collected in the gingival sulcus.[3]

Some of the bacteria that causes the oral infections includes *Streptococci spp*. (including *S. mutans*), *Porphyromonas spp*., *Bacteroides spp.*, *Staphylococci spp*., *Actinobacillus spp*., *Veillonella parvula*, *Actinomyces naeslundii*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. Formation of dental plaque and lack of maintenance of oral hygiene is the main reasons for the progression of oral disease.[4]

Syzygium cumini commonly known as jamun belongs to the kingdom: Plantae as Angiosperms, order: Myrtales, family: Myrtaceae and genus: Syzygium. It is most commonly grown in India. It is found in Thailand, Philippines, Madagaskar and some other countries. The plant has been successfully introduced into many other tropical countries such as West Indies, East and West Africa and some subtropical regions including Florida, California, Algaria and Israel. It consists of four parts-seeds, leaves, fruits, bark. Research in India shows that the stems, leaves and fruit of *Syzygium cumini* possesses antiallergic, gastroprotective, anti-diabetic, anti-inflammatory, anti-diarrhoeal, antiplaque, antipyretic, antibacterial and antioxidant properties.[5] Excessive free radicals can trigger a variety of degenerative diseases, such as cancer and heart disease (cardiovascular). Jamblang leaf contains flavonoid, glycoside, quercetin, myricetin 3-O-4 acetyl-L-rhamnopyranoside, triterpenoids and tannins that has antibacterial activity. The emergence of degenerative diseases by free radicals can be inhibited or prevented by antioxidant compounds.[6] Therefore, the body needs an important substance that is antioxidants to capture free radicals so that it cannot induce other diseases. Food rich in antioxidant plays essential role in the prevention of diseases. Hence the leaf of *Syzygium cumini* have the ability to inhibit the growth of Gram positive and Gram negative bacteria.[4]

Several studies have highlighted the promising potential of *Syzygium cumini* leaf against few Gram positive and Gram negative bacteria. Further clinical studies are needed to particularly show the efficacy of leaf extract of *Syzygium cumini* as mouthwash. Therefore, we aimed to assess and compare the effect of *Syzygium cumini* leaf extract mouthwash on various clinical parameters and also its antimicrobial effect on potent periodontal pathogen, *Porphyromonas gingivalis* in gingivitis subjects.

METHODS

Type of study

It was an original study which was performed in August 2024 to October 2024.

Study design

A total of 40 subjects diagnosed with gingivitis aged 18-45 years, were randomly selected from the outpatient department of periodontics, PMNM Dental College and Hospital, Navanagar, Bagalkote. Keeping alpha error at 5%, β error 20% and power of the study at 80%, the sample size estimated was approximately 16 in each group. For follow-up study, to avoid loss due to attrition 20 subjects were taken in each group.

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

Subjects were randomly divided into 2 groups: group A: non surgical periodontal therapy alone. Group B: non surgical periodontal therapy followed by *Syzygium cumini* mouthwash.

Inclusion criteria

Systemically healthy both male and female subjects were included from 18 to 45 years of age, who were untreated with gingivitis and subjects who were willing to participate in the study and given written consent for the same.

Exclusion criteria

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 jamlang were also excluded.

Method of preparation of the 5% Syzygium cumini mouth wash[5,7]

Jamlang leaves collected, rinsed with water and shade dried for 15 days. The dried leaves were crushed to obtain a fine powder. Later powdered leaves were extracted and maceration was carried out by soaking in distilled water with 1:10 ratio (powder:water) and mixture was prepared. To avoid bacterial growth 10 ml of chloroform was added for 1 liter of mixture. The mixture was allowed to stand for 48 hours without disturbing. After 48 hours, the mixture was filtered using Whatman No.1. The collected filtrate solution was dried in hot air oven at 40°C. The dried extract was then triturated in a uniform direction using mortar and pestle to form a uniform mixture. Water was added to obtain the desired quantity of mouthwash.

To this stevia was added as sweetening agent, sodium benzoate as preservative and menthol as coolant were added to prepare it to a final 5% *Syzygium cumini* mouthwash (Figure 1).

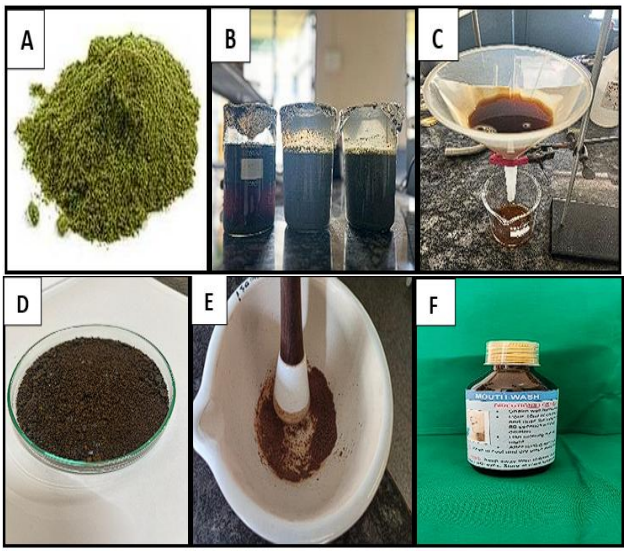

Figure 1: Method of preparation of Jamlang leaf extract mouthwash: (A) jamlang leaf powder (B) maceration, (C) filtration, (D) extract (E) trituration (F) mouthwash.

Clinical examination to assess the periodontal condition

Clinical examination was performed on all the subjects using simplified oral hygiene index (Greene and Vermillion, 1964), gingival index (Loe and Silness, 1963), plaque index (Silness and Loe, 1964), bleeding on probing (Muhlemann and Son, 1971).

All groups were instructed to follow same oral hygiene instructions except allocated mouthwashes. Clinical parameters were recorded along with plaque samples collection on the day of examination and 21 days after *Syzygium cumini* mouthwash use. Oral prophylaxis was be carried out after baseline data collection and participants were instructed to use the given mouthwash and not to use any other oral hygiene aids except toothbrush and toothpaste. Subjects were instructed to rinse 10 ml of assigned mouthwash twice a day. On day 0 and 21st day supragingival plaque was collected using sterile curette from the buccal surface of first molar in each quadrant and then it was mixed in sterile Eppendorf tube containing 500 µl of reduced transport fluid (RTF).[8] The plaque samples were subjected to bacterial culture to check the counts of *Porphyromonas gingivalis* bacteria pre and post use of *Syzygium cumini* mouthwash. Sample collection was done by single investigator and analysis of plaque sample was done by a microbiologist.

The data collected were analysed using chi-square test, independent t-test or Mann-Whitney U test for comparison of two groups, Wilcoxon matched pairs test and descriptive statistics with frequency, percentage, mean SD etc. All the participants were clearly explained regarding the need and design of the study. A duly signed written informed consent was obtained from all the subjects willing to participate in the study.

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 our study showed that there was a statistical difference in colony forming unit of Porphyromonas gingivalis scores between both groups at baseline and day 21.

The group who received scaling along with *Syzygium cumini* mouthwash has got a significant reduction in *P. gingivalis* count compared to the other group who received on scaling (Table 1, Figure 2). The comparison of OHI-S score showed a mean difference of 1.27 in group 1 from base line to day 21 and group 2 showed a mean difference of 1.86 from base line to day 21. 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 2).

Table 1: Comparison of group 1 and group 2 with CFU counts at baseline and day 21 treatment time points by independent t-test.

Time points	Group 1		Group 2		t-value	P value
	Mean	SD	Mean	SD		
Baseline	33.75	7.93	28.95	9.95	1.6869	0.0998
Day 21	14.85	4.36	10.40	4.32	3.2433	0.0025*
Difference	18.90	4.70	18.55	7.07	0.1843	0.8548

*p<0.05

Table 2: Comparison of group 1 and group 2 with OHI-S, 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			
OHI-S									
Baseline	2.44	0.46	20.23	2.55	0.94	20.78	194.5	-0.1353	0.8924
Day 21	1.17	0.37	27.38	0.70	0.28	13.63	62.5	3.7059	0.0002*
Difference	1.27	0.62	17.33	1.86	0.88	23.68	136.5	-1.7042	0.0884
GI									
Baseline	1.16	0.34	23.33	1.01	0.27	17.68	143.5	1.5148	0.1298
Day 21	0.67	0.19	27.50	0.37	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
PI									
Baseline	1.06	0.20	21.03	1.00	0.32	19.98	189.5	0.2705	0.7868
Day 21	0.71	0.11	30.10	0.30	0.17	10.90	8.0	5.1801	0.0001*
Difference	0.35	0.16	13.53	0.70	0.32	27.48	60.5	-3.7600	0.0002*
BOP									
Baseline	0.96	0.35	21.83	0.92	0.28	19.18	173.5	0.7033	0.4819
Day 21	0.58	0.24	26.50	0.35	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<0.05

Table 3: Normality of changes in all parameters from baseline to day 21 in group 1 and group 2.

Parameters	Groups	Kolmogorov-Smirnov	df	Sig.	Shapiro-Wilk	df	Sig.
OHI-S	Group 1	0.1860	20	0.0500*	0.9430	20	0.2780
	Group 2	0.1460	20	0.2000	0.9510	20	0.3770
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<0.05

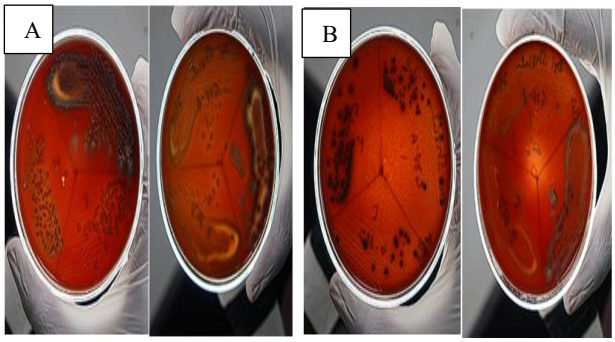

Figure 2: Culturing for *Porphyromonas gingivalis* on kanamycin blood agar: A) group A baseline and 21 days; B) group B baseline and 21 days.

In the present study none of the participants complained of stained teeth or altered taste sensations throughout the study period.

DISCUSSION

The antibacterial activities of leaf extract of 5% *Syzygium cumini* mouthwash on gingivitis was investigated in this study.

Using a chemical plaque control agent (like a mouthwash) to supplement mechanical plaque removal can produce an antimicrobial effect throughout the oral cavity. CHX is one of the most commonly prescribed chemical plaque control agents in dentistry.[9] Ahmad et al, in their study, confirmed the effect of long-term use of CHX mouthrinse on increasing the dental stains, allergy, and burning mouth. In recent times, the use of herbal mouthwash is on the rise due to the spread in the awareness of the effect of complementary and alternative medicine.[10]

In another study Tahir et al demonstrated the antibacterial activity of leaves extract of indigenous species of *Syzygium cumini* on dental caries causing pathogens. The results showed methanol, hexane, aqueous and ethyl acetate extract proved to be active against all the bacterial strains. The bactericidal activity of the *S. cumini* leaves is may be due to their flavonoid and tannin content.[11]

Ruan et al investigated the antioxidant activity of *Syzygium cumini* leaves. The results showed total phenolic and flavonoid content of the methanolic extract of *S. cumini* leaves have potent antioxidant activity according to the DPPH and FRAP assay.[12]

In our study all the plaque samples collected in RTF immediately brought to laboratory and centrifuged and diluted as 1:10. Culturing for *P. gingivalis* was done on kanamycin blood agar and incubated at 37°C for 3-4 days in anaerobic jar these are strictly anaerobe. Then after completion of incubation the plates are removed and noted the colony characters of the required organism and also the colony forming units (CFU/ml) was done for quantification. These organisms were confirmed by grams staining and key biochemicals. In group 1, the mean difference observed from baseline to day 21 was 18.90 with p value of 0.0001 and in group 2, the mean difference observed from baseline to day 21 was 18.55 with p value of 0.0001. Similarly, for OHI-S score, group 1 showed the mean difference 1.27 from baseline to day 21 with p value of 0.0001 and group 2 showed the mean difference of 1.86 with p value of 0.0001.

GI index score showed a mean difference of 0.49 in group 1 from base line to day 21 with p value of 0.0001 and group 2 showed a mean difference of 0.64 from base line to day 21 with p-value of 0.0001. Similarly plaque index score showed a mean difference of 0.35 in group 1 from base line to day 21 with p value of 0.0001 and group 2 showed a mean difference of 0.70 from base line to day 21 with p value of 0.0001. Bleeding on probing score showed a mean difference of 0.38 in group 1 from base line to day 21 with p value of 0.0022 and group 2 showed a mean difference of 0.57 from base line to day 21 with p value of 0.0002.

All the results indicate the statistical difference in the various clinical parameters among both the groups.

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

CONCLUSION

Herbal mouthwashes have a promising role in dentistry; having proper knowledge and their effects on teeth would prove them as a successful dental therapeutic agent. This study has explained the role of leaf extract of 5% *Syzygium cumini* mouthwash in reducing various clinical parameters and the colony count of *Porphyromonas gingivalis*. Hence, we can conclude that as *Syzygium cumini* mouthwash can be easily prepared and economical, it can be used as an adjunct to the mechanical periodontal therapy.

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

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