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Original Article

Assessment of *Syzygium cumini* and *Beta vulgaris* L Extract as natural disclosing agent among adults: A randomized crossover design

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Abstract

Background: Plaque disclosing agents are regularly used to identify, quantify, and diagnose dental plaque. Agents such as *Syzygium cumini* (jamun plum) and *Beta vulgaris* L. (red beetroot) have natural staining properties and are rich in purplish-red pigment. This study aimed to assess and compare *S. cumini* extract (jamun plum) and *B. vulgaris* L.(beetroot) as natural disclosing agents among adults.

Methods: A triple-masked randomized crossover study was conducted among 45 participants. The participants were randomly allocated with allocation concealment into three groups, with a two-week washout period. The extracts were freshly prepared using 3% *S. cumini* and 3% *B. vulgaris* extract in 10 mL of ethanol. The disclosing agents were applied using an applicator tip for two minutes. Dental plaque was assessed using the Quigley-Hein index. Statistical analysis involved a one-way analysis of variance (ANOVA) test followed by post hoc analysis.

Results: The results showed a significant difference between the groups, with mean values of 1.6 ± 0.19, 1.49 ± 0.23, and 2.14 ± 0.43 for 3% *S. cumini*, 3% *B. vulgaris* extract, and conventional disclosing agents, respectively. Post hoc analysis showed a significant difference between conventional and natural disclosing agents ($P < 0.05$).

Conclusion: The conventional disclosing agent showed better results than the newer natural agents. However, 3% *S. cumini* and 3% *B. vulgaris* can be used to identify dental plaque.

Keywords: Anthocyanin, Betacyanin, Disclosing agent, Dental plaque, Natural extract

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Introduction

Dental bacterial plaque is the primary etiological agent for periodontal disorders and dental caries.[1,2] This thin layer of dental plaque adheres to the tooth surface and is host to microbial colonies.[3] The dental plaque biofilm contains the bacterial communities embedded in a self-produced matrix of extracellular polymeric substances, and the self-sustainability of dental plaque biofilm is one of its most significant features in initiating oral disease.[4] Dental plaque is clear, resembling translucent white glass; therefore, it is invisible to the naked eye. Dental plaque can be assessed using a disclosing solution, a liquid or gel containing a contrast agent such as a dye.[5]

Various chemical disclosing agents exist, including iodine, Bismarck brown, erythrosine, gentian violet, malachite green, methylene blue, basic fuchsin, food dyes, and two-tone disclosing agents. In 1990, the US Food and Drug Administration limited the use of chemical disclosing agents due to their potential cytotoxicity with excessive use.[6]

Various natural dyes or staining agents are now available. *Syzygium cumini* (jamun plum) native to the Indian subcontinent and a member of the Myrtaceae family, is used as a natural colorant. Some anthocyanin-rich flowers and fruits have been traditionally used as medicine to treat various diseases.[7,8] *S. cumini* has antibacterial and antioxidant properties, and it also strengthens teeth. *S. cumini* leaf extract acts against dental caries-producing organisms.[9] In addition, anthocyanins are water-soluble, active compounds that can attach to bacterial glycoproteins. These anthocyanins are more stable in solutions with lower pH.[7]

Similarly, *Beta vulgaris* L. (red beetroot), rich in purplish-red pigment, contains the betalain dye. Betalain combines the purple pigment betacyanin and the yellow pigment betaxanthin. It is a natural pigment with antioxidant, antibacterial, anticancer, anti-inflammatory, and antiviral properties. Betacyanin is easier to diffuse and disperse in liquid form throughout the oral cavity's restricted areas.[10]

Despite being purely natural and readily available replacements for chemical agents, *S. cumini* and *B. vulgaris* L. have not been employed in any prior research to evaluate tooth plaque as a natural disclosing agent in in-vivo experiments. This study aimed to assess and compare the effect of 3% *S. cumini* extract and 3% *B. vulgaris* L. (beetroot) extracts as natural disclosing agents and 0.8 g of erythrosine in adults to measure plaque score using the Quigley-Hein Index.

Methods

This was a triple-masked randomised crossover study conducted among adults older than 18. This study was reported in compliance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines. Ethical clearance was obtained from the Institutional Ethical Committee (KIDS/IEC/2024/I/002) with the clinical trial registry No. CTRI/2024/03/064012. Informed consent was obtained from the volunteers before the beginning of the study. Participants older than 18 years were included in the study. Participants who had done aesthetic restorations, used mouthwash in the past 30 days, were undergoing antibiotic therapy, had oral lesions, such as oral ulcers, leucoplakia, and candidiasis, or were under orthodontic treatment, were excluded from the study.

The sample size was estimated using G*Power software version 3.1. A fixed effect size of 0.5 was assumed, with a 5% margin of error, a 95% confidence level (CI), and 80 % statistical power. The estimated sample size was 42, which was rounded up to 45. The participants were given concealment numbers 1 to 45 and were randomly allocated to three groups in a 1:1:1 ratio. The study was conducted in three phases, I, II, and III, and each group consisted of 15 participants ($n = 15$ for each group). Group A represented *S. cumini* (jamun fruit), group B represented *B. vulgaris* L. (red beetroot), and group C represented erythrosine.

The present crossover study used a Latin square design to assign the disclosing agents in phase I: Group A ($n = 15$) received *S. cumini* intervention, group B received *B. vulgaris* L. intervention, and group C received erythrosine intervention. A crossover procedure was done in Phase II and Phase III after a two-week washout period, and all three groups received all three interventions (Figure 1).

The disclosing agents *S. cumini* (jamun plum) and *B. vulgaris* L. (beet root) were extracted and prepared by collecting them, washing them thoroughly, and peeling them. The flesh of the red beetroot and jamun fruit was obtained, washed, thinly grated, and air-dried. After obtaining 500 g of *S. cumini* and *B. vulgaris* L., around 3% dried beetroot and 3% jamun fruit were blended and soaked in 100 mL of distilled water with 10 mL of 96% ethanol solvent as a preservative. Then, a maceration process was performed for 72 hours, and the mixture was filtered using Whatman filter paper. To acquire a concentration of red beetroots and jamun plum, the filtrates were stored and then concentrated using a water bath and a rotating evaporator adjusted at ± 50 °C to

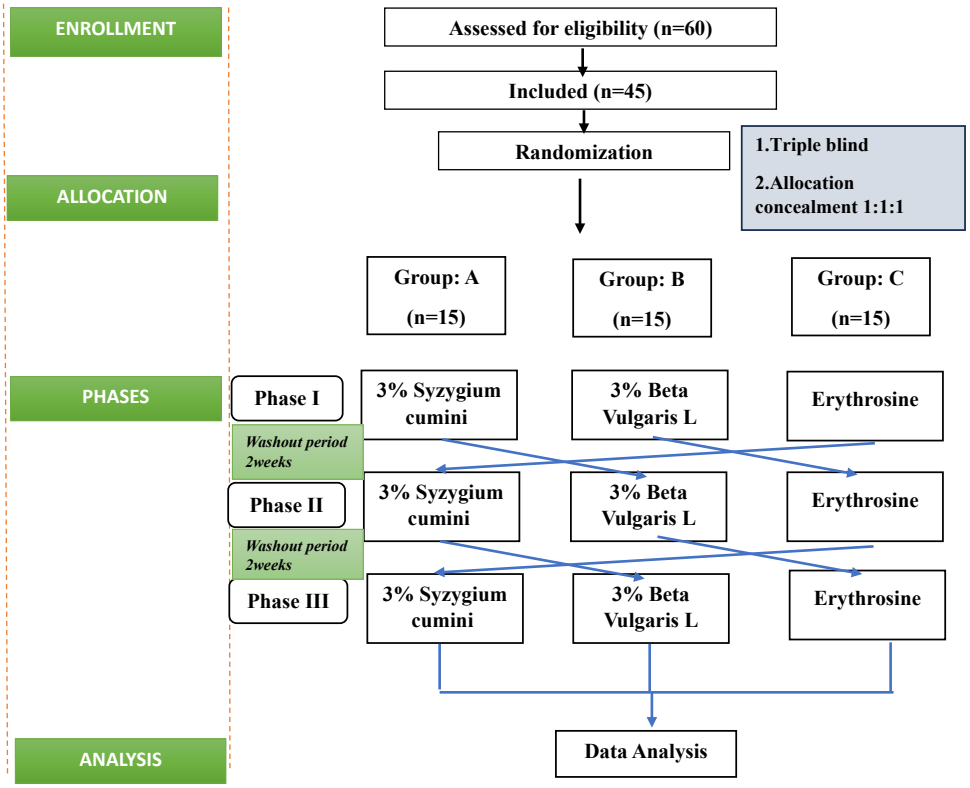

Figure 1. CONSORT flow diagram of crossover design

evaporate the solvent. Subsequently, 30 mL of glycerine was added to thicken the agent and serve as a sweetener.[10]

The disclosing agent was applied to all tooth surfaces using a microbrush and left undisturbed for two minutes. The patients were asked to rinse their mouths for approximately 30 to 60 seconds. Data were collected using the Quigley-Hein Index (G. A. Quigley and J. W. Hein in 1962)[11] to obtain the plaque score.

Statistical analysis

Data were compiled in a Microsoft Excel sheet and analysed using SPSS version 22 software. The data were checked for normality using the Kolmogorov-Smirnov test, and normal distribution was confirmed; therefore, parametric analysis was performed. Intergroup comparisons among the three disclosing agents were done using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A *P* value of < 0.05 was considered statistically significant.

Results

In a crossover trial, analysis of the data can be done using the following two methods; in our study, both methods were used:

1. Compiling, summarizing, and comparing the observations on the total sample concerning each intervention (each disclosing agent).
2. Comparing the observations done on each group individually at different trial phases.

Overall, the results among the total sample of each group ($n = 45$) showed a statistically significant difference between the three groups. Erythrosine showed a higher stability to dental plaque and a higher mean value (2.06 ± 0.38). The post hoc test for individual analysis among the three disclosing agents showed that erythrosine had a higher mean value and statistical significance ($P < 0.001$) (Table 1, Table 2, and Figure 2).

Table 1. Overall comparison between groups (phases I + II + III) by comparing the observations done on the total sample with each intervention.

Groups	Mean ± SD	Standard error	*f*-value	*P* value
Group A ($n = 45$) 3% *Syzygium cumini*	1.47 ± 0.26	0.03	56.85	**0.001***
Group B ($n = 45$) 3% *Beta vulgaris* L.	1.46 ± 0.24	0.03		
Group C ($n = 45$) Erythrosine	2.06 ± 0.38	0.05		

ANOVA *P* value < 0.05, statistically significant.

Table 2. Post hoc comparisons using Tukey's test comparing the observations done on the total sample with each intervention

Groups	Mean difference	Standard error	*P* value
Group A vs group B	0.01	0.06	0.97
Group A vs group C	-0.59	0.06	0.001*
Group B vs group C	-0.59	0.06	0.001*

Post hoc test *P* value < 0.05, statistically significant.

In each phase, groups A, B, and C (n = 15 per group) received all three interventions in a crossover pattern across different trial phases and demonstrated statistically significant differences. The erythrosine disclosing agent showed a higher mean value than the *S. cumini* and *B. vulgaris* L. No significant difference was observed between *S. cumini* and *B. vulgaris* L. in the post hoc test at different phases (Table 3, Table 4).

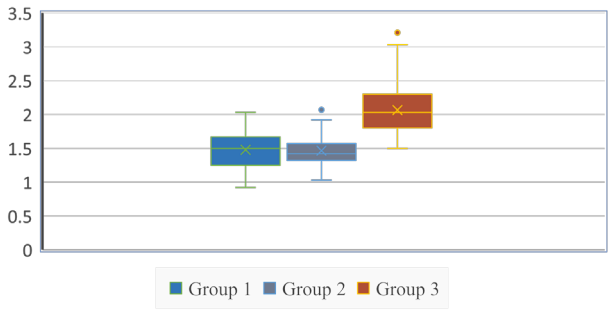

Figure 2. Box and whisker plot comparing the mean value between *Syzygium cumini, Beta vulgaris* L., and erythrosine

Table 3. Comparison of observations done on each group individually at different phases

Group	*n*	Mean ± SD	SE	*f*-value	*P* value
Phase I – 3% *Syzygium cumini*	15	1.60 ± 0.19	0.50		
Phase II – 3% *Beta vulgaris* L.	15	1.49 ± 0.23	0.09	19.95	0.001*
Phase III - Erythrosine	15	2.14 ± 0.43	0.04		
Phase I – 3% *Beta vulgaris* L.	15	1.42 ± 0.35	0.05		
Phase II –Erythrosine	15	2.04 ± 0.30	0.02	15.52	0.001*
Phase III - 3% *Syzygium cumini*	15	1.52 ± 0.32	0.08		
Phase I –Erythrosine	15	2.00 ± 0.40	0.62		
Phase II - 3% *Syzygium cumini*	15	1.40 ± 0.17	0.07	25.15	0.001*
Phase I – 3% *Beta vulgaris* L.	15	1.38 ± 0.12	0.10		

ANOVA *P* value < 0.05, statistically significant.

Table 4. Post hoc comparisons using Tukey's test by comparing the observations done on each group individually at different phases

Group	Mean difference	SE	*P* value
Phase I		0.11	
Group A vs group B	0.11		0.58
Group A vs group C	-0.54		0.001*
Group B vs group C	-0.65		0.001*
Phase II		0.11	
Group A vs group B	0.10		0.672
Group A vs group C	-0.51		0.001*
Group B vs group C	-0.62		0.001*
Phase III		0.09	
Group A vs group B	0.59		0.959
Group A vs group C	0.62		0.001*
Group B vs group C	0.02		0.001*

Post hoc test *P* value < 0.05, statistically significant.

Discussion

The study's primary goal was to assess and compare the effect of 3% *S. cumini* extract and 3% *B. vulgaris* L. (beetroot) extract as natural disclosing agents and 0.8 g of erythrosine in adults to measure dental plaque scores.

Plaque disclosing agents are used to assess the quality and quantity of dental plaque. As dental plaque is difficult to visualize with the naked eye, plaque disclosing agents are used in dental plaque indices to measure the amount of plaque accumulated on tooth surfaces.[12] One of the simplest and fastest methods for diagnosing dental plaque is using dyes, which facilitates the plaque's subsequent removal under permanent management.

In the present study, among the three different disclosing agents, the conventional disclosing agent showed better results compared to the natural disclosing agents. However, the *S. cumini* and *B. vulgaris* L. extracts were also able to stain and differentiate the plaque. Both natural disclosing agents adhere to dental plaque due to the polarity imbalance between the dyes and the components of dental plaque, allowing dental plaque to retain the dye compounds. Hydrogen bonds (polysaccharides) and electrostatic interactions (proteins) bind the particles to the plaque surface.[13]

The betacyanin content in beetroot has been used as a natural disclosing agent in vitro studies. The betacyanin content in red beetroot is 2.4535 mg/100 g.[14] *B. vulgaris* betalains have two distinct types of compounds: betaxanthins (mostly vulgaxanthin-I), which are yellow, and betacyanins, which are reddish-violet. Betanin, or betanidin-5-O-β-glucoside, is the main betalain found in red beets. It has phenolic and cyclic amine groups, which function as antioxidants.

Betalains are also used as food colorants.[15] An *in vitro* study by Nurul et al reported that beetroot extract (*B. vulgaris* L.) with 100% red color concentration showed more effectiveness than the erythrosine dye for staining plaque.[16] The effectiveness of *B. vulgaris* L. as a disclosing solution (plaque identification material) has been assessed by Fatmasari et al, who reported that beet root extract was an adequate replacement for other chemical-based disclosing solutions for assessing plaque.[17] In comparison to the other formulae, 3% red beetroot extract was the most effective at staining the plaque because of its superior stability and physical characteristics.[10]

This study was the first to use *S. cumini* as a naturally occurring disclosing agent. In other *in vitro* research, the anthocyanin pigments found in purple potatoes, rambutan leaves, and mulberry fruit have been employed as natural disclosing agents. Anthocyanin pigments are also present in the jamun plum. Anthocyanin is an important natural dye. It is an active substance that readily binds to the glycoprotein of bacteria accumulated in the plaque.[18] Ghosh et al reported that 100 g of jamun pulp contained 195.58 ± 6.15 mg anthocyanins. These naturally occurring colors are highly desirable for the food industry as they are water-soluble.[19] The fruit of *S. cumini* (jamun plum) contains anthocyanin, which has an antimicrobial effect on *Streptococcus mutans*.[20] Hamid et al reported that the extract of the *S. cumini* could suppress dental plaque formation.[21] The benefit of employing a disclosing agent is that it allows patients to evaluate their dental plaque independently, become aware of the need to clean their teeth, and locate areas of plaque to optimize cleaning.[22]

Under certain conditions, the stability of anthocyanin pigment on dental plaque is poor. Several factors affect anthocyanin stability, including pH, temperature, light, and oxygen. The stability of anthocyanins can be affected by temperature, making them colorless; pH levels also impact anthocyanin color absorption levels. The absorption value increases with a decrease in pH. However, a pH of 5 or higher may damage the anthocyanin pigment, causing it to become colorless. The color of the extract partially adheres to the plaque due to the color instability of anthocyanins.[23,24]

Strengths and Limitations

This crossover design, also known as the Latin square design, constitutes the study's strength because all three disclosing agents were given to all participants ($n = 45$). The primary objective of the natural disclosing agents is to help individuals self-assess the condition of their dental plaque. Using natural disclosing agents is risk-free, and the Quigley-Hein index is a sensitive test for accurate detection, leading to better results. A limitation of this study was the absence of a baseline value for the plaque score; however, this has been mitigated by the crossover study design. Conducting further research among all population age groups is recommended.

Conclusion

The conventional disclosing agent, erythrosine, showed a higher effect on dental plaque than the newer natural disclosing agents. The disclosing agents 3% *S. cumini* and 3% *B. vulgaris* L. can be used as natural disclosing agents as they showed promising effects on plaque identification.

Authors' Contribution

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Data curation: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson.
Formal analysis: Nandhini Ramesh, Mahesh Jagadeson.
Investigation: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson.
Methodology: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson, Indra Priyadharshini Ganesan, Karthikayan Ravi, Revanth Meenatchi Prabhakaraa.
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Resource: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson.

Software: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson.
Supervision: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson, Indra Priyadharshini Ganesan.
Validation: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson.
Visualization: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson.
Writing-original draft: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson, Indra Priyadharshini Ganesan, Karthikayan Ravi, Revanth Meenatchi Prabhakaraa.
Writing-review & editing: Nandhini Ramesh, Vishnu Prasad Subramanian, Mahesh Jagadeson, Indra Priyadharshini Ganesan, Karthikayan Ravi, Revanth Meenatchi Prabhakaraa.

Competing Interests
The authors declare no competing interests.

Ethical Approval
Institutional Ethical Committee – Karpaga Vinayaga Institute of Dental Sciences (IEC – KIDS) KIDS/IEC/2024/I/002.

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