

# Comparative Evaluation of Novel Herbal Rice Husk Mouthwash with Kidodent against *Streptococcus mutans*: A Parallel Double-blinded Randomized Control Trial

Raghavendra Havale<sup>1</sup>, Dhanu G Rao<sup>2</sup>, Neha Bemalgi<sup>3</sup>, Omera B Fatima<sup>4</sup>, Sara S Syeda<sup>5</sup>, Namratha Tharay<sup>6</sup>

## ABSTRACT

**Aim:** The purpose of the study is to evaluate and compare the effectiveness of rice husk extract mouthwash (RHM) and Kidodent mouthwash (KM) for reduction in salivary *Streptococcus mutans* count.

**Materials and methods:** After approval from institutional review board and institutional informed consent, 45 children who met the inclusion criteria were divided into three groups. In group A, children received rice husk mouth wash whereas in group B placebo is specified and in group C, intervention being KM. The unstimulated saliva is collected at baseline, 7th, 10th, and 15th days and subjected to microbiological analysis. The data are statistically analyzed using one-way ANOVA and repeated measures analysis of variance (ANOVA).

**Results:** Rice husk mouthwash showed equivalent, significant, and effective reduction in *S. mutans* count similar to KM ( $p < 0.05$ ).

**Conclusion:** The rice husk mouthwash showed potential therapeutic effect in reduction of *S. mutans*.

**Clinical trial registry india (CTRI No) :** CTRI/2020/10/028594.

**Keywords:** Children, Herbal, Kidodent, Mouthwash, Rice husk.

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## INTRODUCTION

Dental caries is the most common, persistent, cumulative, and modern, lifestyle-dependent human disease, and it is a continual process arising from numerous cycles of tooth demineralization and remineralization. The prevalence of dental caries ranges from 60 to 90% of the world's schoolchildren with a profound effect on the quality of life of children including from a decrease in school attendance to as severe as its influence on growth and development of the child.<sup>1</sup>

With the exponential advances in the materials and techniques in dentistry, there is a paradigm shift from the well-known surgical model to the most recent medical model that aims in the prevention of dental caries<sup>2</sup> than its treatment. Chemical plaque control is one of the means of medical management of caries that increases the efficacy of toothbrushing.

Chemical plaque control synergizes the effect of mechanical aids efficacy in the prevention of dental caries. Chlorhexidine gluconate is the most prescribed mouth rinse with antiplaque action. Although it has widespread use, side effects such as discoloration, taste disturbances, paresthesia, and oral mucosal erosions are well documented.<sup>3</sup> So, there is a paradigm shift in testing herbal mouthwashes as an antiplaque agent.

The amount of *S. mutans* in the saliva is related to the number of colonized surfaces in the deciduous, mixed, and permanent dentition. As a result, diminishing the concentration of *S. mutans* in the oral cavity would have a significant impact on the occurrence of dental caries.<sup>2</sup>

Therefore, one such attempt was made by preparing a rice husk extract as an herbal mouthwash which is commonly used as an ingredient in dentifrice powder. As a result, the focus of this research is to see how effective rice husk mouthwash is as an antibacterial agent against *S. mutans* in kids.

<sup>1-6</sup>Department of Pedodontics and Preventive Dentistry, AME's Dental College and Hospital, Raichur, Karnataka, India

**Corresponding Author:** Dhanu G Rao, Department of Pedodontics and Preventive Dentistry, AME's Dental College and Hospital, Raichur, Karnataka, India, Phone: +91 9845031755, e-mail: drdhanu74@yahoo.co.in

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**Conflict of interest:** None

## MATERIALS AND METHODS

### Source of the Data

Children aged 8–12 years were chosen after receiving institutional ethical clearance (AME/DC/216/19-20). Consent was obtained from the residential school principal as well as the study participants. The study period was for 15 days (25th October 2020 to 9th November 2020).

### Study Design

It is a three-arm, parallel design, pragmatic, effectiveness, fixed-size double-blinded placebo-controlled randomized control trial.

### Locations

Aforementioned research was held in the Morarji Desai Residential School of Raichur District, Karnataka in association with the Department of Pharmaceutics and Microbiology, Laxmi Venkatesh Desai College, Raichur District, Karnataka. Subjects were chosen by lottery method.

A total of 55 students were screened for the study of which five did not suffice the inclusion criteria and five could not participate as it was noted that they will not be available during the study period, therefore, 10 students were excluded from the study. After randomization by lottery method 45 students were equally divided.

Allocation was implemented by a statistical programmer who was not directly involved in the study, 15 students were allotted in each group.

The students as well as examiner were blinded during the study. The study was completed by all of the participants. A flow diagram of the participants through the study is presented in Figure 1.

### Participants

Inclusion criteria for selection of participants included the following:

- Age group between 8 and 12 years.
- Children with good general health.
- Children devoid of infectious diseases.

Exclusion criteria considered the students who presented the following:

- Children who cannot expectorate completely.
- History of taking antibiotics 1 month before and amid clinical trial.
- Children receiving orthodontic treatment or with any kind of oral prosthesis.
- Presence of any intraoral pathology.
- Children not available for follow-up phase and children who are using any oral hygiene aids.

### Sample Size Estimation

A power analysis was established by G\*power, version 3.0.1 (Franz Fauluniversitat, Kiel, Germany). A total of 45 individuals were used, with 15 in each group yielding 95% power so as to determine the significant difference, with 0.25 of effect size and a 0.05 level of significance.

### Interventions

Having gained institutional ethical clearance and school authority consent, 45 subjects matching the inclusion criteria were divided into three equal groups using block randomization.

- Group A—rice husk extract mouthwash (RHM)
- Group B—distilled water (DWM)
- Group C—Kidodent mouthwash (KM).

A complete oral prophylaxis was done prior to the start of the trial. An hour after breakfast subjects were asked to swish and spit the mouthwash for 30 seconds underclass teacher's guidance. Unstimulated form of saliva was collected in disposable sterile containers after half an hour of mouthwash use, at baseline, 7th day, 10th day, and 15th day and subjected to *S. mutans* microbiological examination.

### Preparation of Mouthwash

After collection of rice husk from rice mills, 400 gm of rice husk is roasted at 100°C and for 15 minutes and allowed to cool down to room temperature. After that, the mixture is grounded into a fine powder. From the entire grounded mixture, each time 40 gm of rice

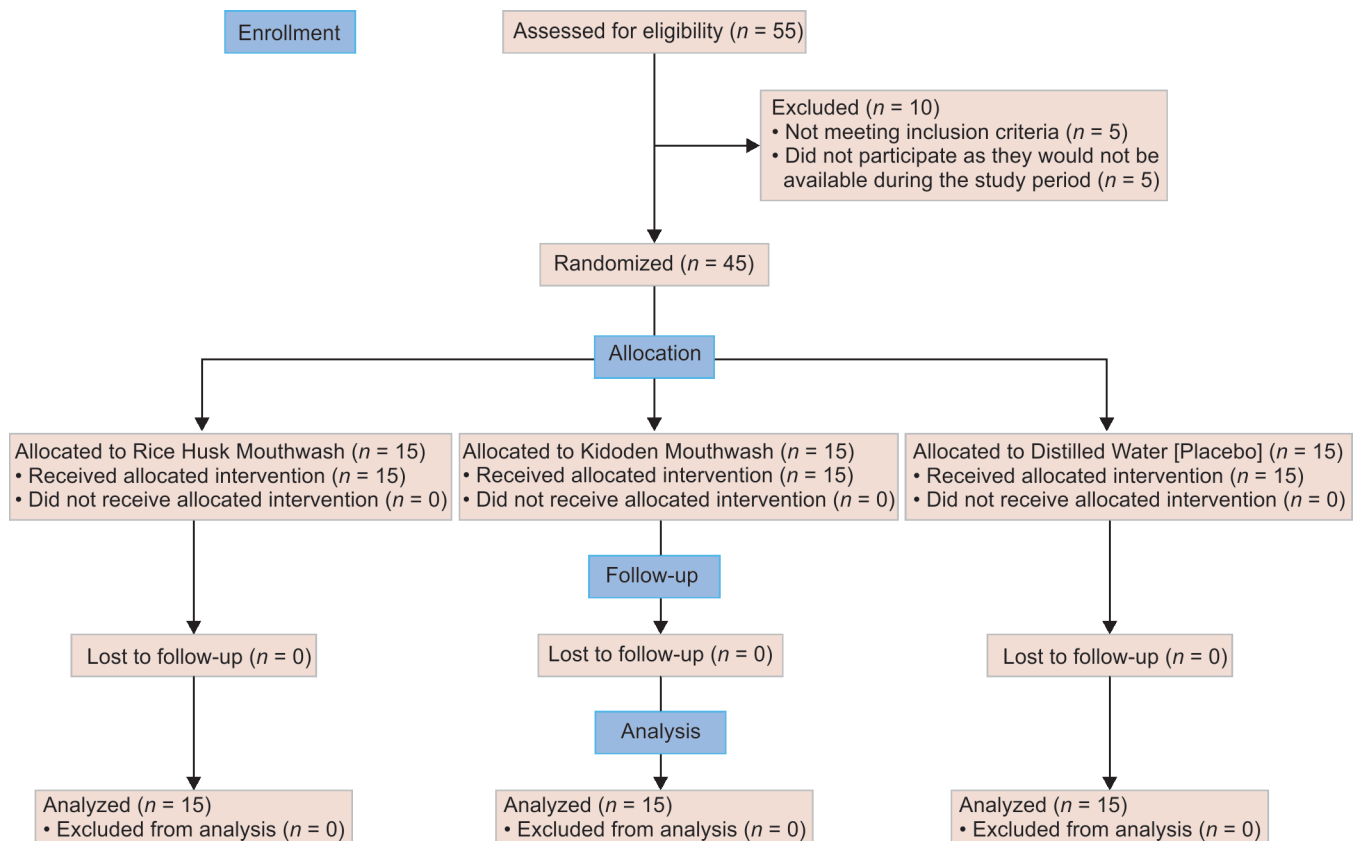


Fig. 1: CONSORT flow chart

husk powder was treated with 30% methanol and boiled for 4 hours by simple reflex method. The resultant extract was filtered using vacuum filtration and later kept on a water bath for the remaining methanol to evaporate. Furthermore, the attained residue is treated with 15 mL of hexane and 15 mL ethyl acetate in a separator.

This is mixed and shook well for an hour and then allowed to settle for an hour. The basal layer with hexane residue is collected in a beaker and placed on a water bath for evaporation. Then, 240 mg of the obtained extract was uniformly mixed with 30 mL of DWM in an ultrasonic vibrator, to this 15 mL of ethyl alcohol was added and was made up to 200 mL with DWM. This solution was filtered using filter paper and 20 mL of it was mixed with 80 mL of DWM, this was dispensed into 100 mL bottles and labeled (Fig. 2).

### Microbiological Procedure

Salivarius mitis agar is a selective media for *S. mutans*. For the media preparation, about 90 gm of salivarius mitis along with 32 gm of agar (type I, for solidification) mixed with 1 L of DWM and autoclaved it at 121°C 15 lbs 15 minutes. Under UV light, the sterile media was put onto 100 × 15 mm Petri dishes in a laminar airflow cabinet. Furthermore, 1 mL of unstimulated saliva samples were taken and serially dilution was done before being inoculated by streak method onto the media using an inoculating loop. After 24 hours incubation period the colony counting was done with a manual colony counter and values were reported as colony forming units per milliliter.

### Statistical Analysis

Graph Pad Prism V.5 was used to tabulate and statistically analyze data. For intergroup comparisons, a one-way ANOVA test is used, whereas for intragroup comparisons, repeated measures ANOVA is used. Finally, the significance of changes for comparisons was

determined using the Bonferroni test. The level of significance is set at 0.05 ( $p < 0.05$ ).

## RESULTS

### Demographic Data

In total of 45 children employed in the research, 21 (60%) were boys and 24 (40%) were girls. The mean age of the participants was  $9.53 \pm 1.31$ .

### Intragroup Analysis

#### Rice Husk Mouthwash

The efficacy of RHM in reducing *S. mutans* count is better on the 15th day then on the 7th day, followed by the 10th day, with mean scores of  $2.46 \pm 0.42$ ,  $1.94 \pm 0.40$ ,  $2.03 \pm 0.46$ , and  $1.82 \pm 0.46$  for baseline, 7th day, 10th day, and 15th day, respectively, which is more significant statistically ( $p = 0.002$ ) (Table 1) (Fig. 3).

#### DWM and KM

When comparing baseline to 15th day, the depletion in a mean score of *S. mutans* is largest on the 15th day ( $1.43 \pm 0.296$  and  $4 \pm 0.236$ ), subsequently the 7th day with mean  $1.66 \pm 0.2$  and  $4.36 \pm 0.52$ , and the 10th day with mean  $1.82 \pm 0.35$  and  $5.48 \pm 0.52$ , respectively. The difference in mean scores was determined to have high significance ( $p = 0.001$ ) (Table 1) (Fig. 3).

### Intergroup Analysis

At baseline, RHM colonies had a count of  $2.46 \pm 0.424$ , whereas DWM and KM have colony counts of  $5.99 \pm 0.480$  and  $2.12 \pm 0.439$ , respectively. On the 7th day, the RHM, DWM, and KM mean scores drop to  $1.94 \pm 0.407$ ,  $4.36 \pm 0.521$ , and  $1.66 \pm 0.201$ , respectively.



Fig. 2: Preparation of rice husk extract mouthwash

On the 10th and 15th days, the mean scores of RHM, DWM, and KM are  $2.03 \pm 0.466$ ,  $5.48 \pm 0.528$ ,  $1.82 \pm 0.353$ , and  $1.82 \pm 0.404$ ,  $4 \pm 0.236$ ,  $1.43 \pm 0.296$ , respectively. When analyzed statistically by ANOVA, at baseline, 7th day, 10th day, and 15th day, the difference between the three groups is highly significant ( $p < 0.001$ ) (Table 2) (Fig. 4).

Moreover, when RHM and KM were compared to DW using the *post hoc* Bonferroni test, RHM and KM were shown to be more effective than DW at all time intervals, which is highly significant ( $p = 0.001$ ). However, when RHM and KM were set side by side, the effectiveness of both mouthwashes was nearly

**Table 1:** Comparison within the group at different time intervals using repeated measures ANOVA

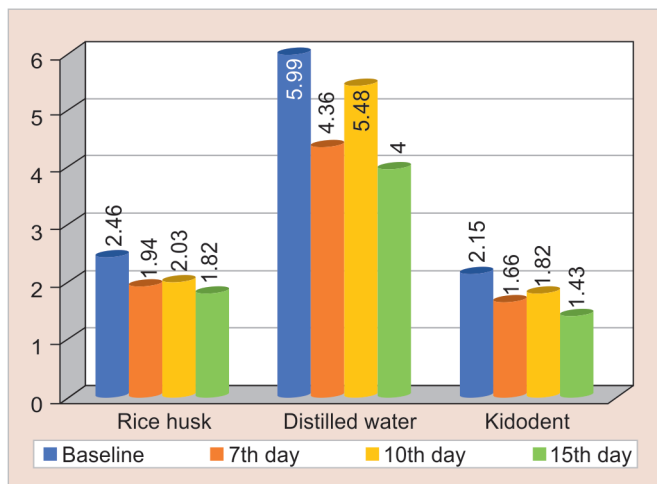
		N	Mean	Std. deviation	p-value
Rice husk	Baseline	15	2.46	0.424	0.002*
	7th day	15	1.94	0.407	
	10th day	15	2.03	0.466	
	15th day	15	1.82	0.404	
Distilled water	Baseline	15	5.99	0.480	0.00*
	7th day	15	4.36	0.521	
	10th day	15	5.48	0.528	
	15th day	15	4.00	0.236	
Kidodent	Baseline	15	2.15	0.439	0.00*
	7th day	15	1.66	0.201	
	10th day	15	1.82	0.353	
	15th day	15	1.43	0.296	

\*Significant

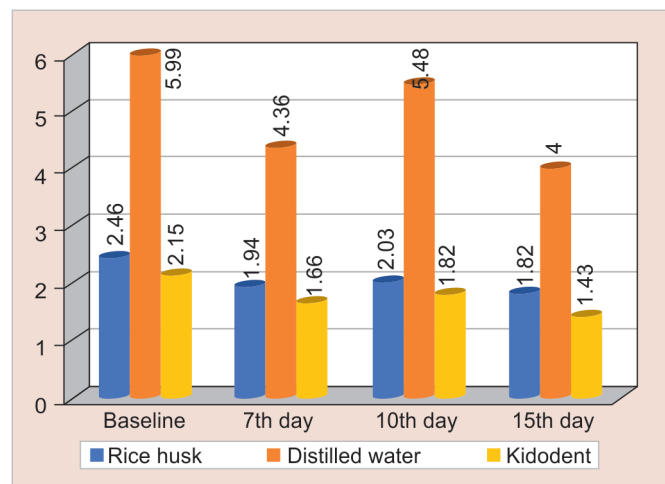
**Table 2:** Comparison among the groups at different time intervals using ANOVA

		N	Mean	Std. deviation	p-value
Baseline	Rice husk	15	2.46	0.424	0.00*
	Distilled water	15	5.99	0.480	
	Kidodent	15	2.15	0.439	
7th day	Rice husk	15	1.94	0.407	0.00*
	Distilled water	15	4.36	0.521	
	Kidodent	15	1.66	0.201	
10th day	Rice husk	15	2.03	0.466	0.00*
	Distilled water	15	5.48	0.528	
	Kidodent	15	1.82	0.353	
15th day	Rice husk	15	1.82	0.404	0.00*
	Distilled water	15	4.00	0.236	
	Kidodent	15	1.43	0.296	

\*Significant



**Fig. 3:** Comparison within the groups at different time intervals



**Fig. 4:** Comparison among the groups at different time intervals

**Table 3:** Post hoc Bonferroni analysis among the groups

		Rice husk		Distilled water		Kidodent	
		Mean diff	p-value	Mean diff	p-value	Mean diff	p-value
Baseline	7th day	0.51	0.004*	1.63	0.00*	0.48	0.007*
	10th day	-0.42	0.010*	-0.51	0.09 <sup>NS</sup>	-0.33	0.15 <sup>NS</sup>
	15th day	0.63	0.014*	1.99	0.00*	0.72	0.00*
7th day	10th day	-0.09	1.00 <sup>NS</sup>	-1.11	0.00*	-0.15	0.82 <sup>NS</sup>
	15th day	-0.12	1.00 <sup>NS</sup>	-0.36	0.19 <sup>NS</sup>	-0.23	0.014*
10th day	15th day	0.21	0.86 <sup>NS</sup>	1.47	0.00*	0.38	0.063 <sup>NS</sup>

\*Significant; <sup>NS</sup>nonsignificant

equal at three intervals, but KM is superior to RHM ( $p = 0.006$ ) on the 15th day (Table 3).

## DISCUSSION

Oral health is an important part of general health of an individual which in turn influences the quality of life. Dental plaque is the predominant causative factor for oral diseases. Plaque formation on the tooth is caused by the production of glucan binding proteins and glucan polymers catalyzed by glucosyltransferases.<sup>4</sup> The increase in initial bacterial adhesion, along with subsequent growth and interbacterial adherence, promotes the formation of a complex micro-community on the tooth surface, eventually leads to the breakdown of hard enamel tissue.<sup>5</sup>

The residential school was chosen for this study to avoid bias from dietary variances because all the subjects are on similar dietary regime, and *S. mutans* count will be modified with the diet. And the other advantage is that the follow-up of the subjects will not be lost.

Rice husk is the by-product of rice milling and, henceforth, turning rice husk into useful goods effectively is an aid to the economy and the ecosystem. Several studies have shown that phenolic compounds may be the preponderant factor in antioxidant ability.<sup>6</sup>

Well documented literature has described the *in vitro* antioxidant activity and phenolic compounds of rice husk<sup>7</sup> and also about its flavonoid content. Bioactive constituents, including phenolic acids, gamma-oryzanol, and tocopherols, were present in higher proportions in the external layers; higher antioxidant activity was also seen in the bran and husk fractions.<sup>8</sup>

A systematic review by James et al., concluded that irrespective of the concentration of chlorhexidine, high-quality evidence indicates the reduction of dental plaque when chlorhexidine is used as an adjuvant along with mechanical oral hygiene aids for 4–6 weeks.<sup>9</sup> And also, the use of chlorhexidine beyond 4 weeks can cause tooth discoloration. To overcome the disadvantages, there is a search for alternative mouth rinses and several clinical trials have been conducted assuming the herbal extracts would be beneficial than the traditional mouth rinse.

*In vitro* and *in vivo*, several plant extracts show promising antimicrobial properties.<sup>10</sup> Hence, many herbal extracts as high molecular weight components of cranberry,<sup>11</sup> pomegranate, guava and grape seed extracts,<sup>12</sup> Tulsi and Black myrobalans fruit extracts,<sup>13</sup> aloe Vera and tea tree oil<sup>14</sup> which showed a promising result in decreasing the counts of *S. mutans*.

Hedge and Kamath compared chlorhexidine mouthwash with 0.5% green tea extract laterally with a combination of chlorhexidine and sodium fluoride. The reduction in the counts of green tea extract is less than the combination and chlorhexidine

mouthwash while the reduction of the *S. mutans* and *lactobacillus* counts were comparable to the combination mouthwash. In the same way, in the current research when compared on the 7th and 10th days, RHM is superior to KM, but on the 15th day, KM mouthwash is superior to RHM.<sup>15</sup>

Despite the results of the current research where rice husk's antimicrobial activity is equivalent to chlorhexidine, longer span researches with a cross-over study design and bigger sized population are required to fully assess rice husk's efficacy.

## CONCLUSION

The RHM is effective in reducing colony forming unit of *S. mutans* as it is similar to the standard KM. The following conclusions can be made of the current study, taking into account the limitations:

- Rice husk extract mouthwash has antimicrobial activity equivalent to KM. As a result, RHM can be used instead of commercially available KM.
- As RHM is prepared from the end product, it can be an economical method to prevent dental caries, particularly among people from weak socioeconomic backgrounds.

## REFERENCES

1. Jürgensen N, Petersen PE. Promoting oral health of children through schools—results from a WHO global survey 2012. *Community Dent Health* 2013;30(4):204–218. DOI: 10.1922/CDH\_3283Petersen15
2. Rupesh S, Winnier JJ, Nayak UA, et al. Comparative evaluation of the effects of an alum-containing mouthrinse and a saturated saline rinse on the salivary levels of *Streptococcus mutans*. *J Indian Soc Pedod Prev Dent* 2010;28(3):138–144. DOI: 10.4103/0970-4388.73780
3. Kandaswamy SK, Sharath A, Priya G. Comparison of the effectiveness of probiotic, chlorhexidine-based mouthwashes, and oil pulling therapy on plaque accumulation and gingival inflammation in 10- to 12-year-old schoolchildren: a randomized controlled trial. *Int J Clin Pediatr Dent* 2018;11(2):66–70. DOI: 10.5005/jp-journals-10005-1487
4. Patel RV, Thaker VT, Patel VK. Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. *Asian Pac J Trop Biomed* 2011;1(1):S58–S61. DOI: 10.1016/S2221-691(11)60124-X
5. Prashant GM, Chandu GN, Murulikrishna KS, et al. The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: an *in vitro* study. *Indian J Dent Res* 2007;18(4):148–151. DOI: 10.4103/0970-9290.35822
6. Liu RH. Whole grain phytochemicals and health. *J Cereal Sci* 2007;46(3):207–219. DOI: 10.1016/j.jcs.2007.06.010
7. Lourith N, Kanlayavattanukul M. Appraisal of Thai glutinous rice husk for health promotion products. *J Cereal Sci* 2013;57(3):343–347. DOI: 10.1016/j.jcs.2012.12.005

8. Butsat S, Siriamornpun S. Antioxidant capacities and phenolic compounds of husk, bran and endosperm of Thai rice. *Food Chem* 2010;119(2):606–613. DOI: 10.1016/j.foodchem.2009.07.001
9. James P, Helen V, Worthington HV, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. *Cochrane Database Syst Rev* 2017;3(3):CD008676. DOI: 10.1002/14651858.CD008676.pub2
10. Banas JA, Vickerman MM. Glucan-binding proteins of the oral streptococci. *Crit Rev Oral Biol Med* 2003;14(2):89–99. DOI: 10.1177/154411130301400203
11. Abu-Obaid E, Salama F, Abu-Obaid A, et al. Comparative evaluation of the antimicrobial effects of different mouthrinses against *Streptococcus mutans*: an *in vitro* study. *J Clin Pediatr Dent* 2019;43(6):398–407. DOI: 10.17796/1053-4625-43.6.7
12. Singla A, Malhotra R, Nd S, et al. Antibacterial efficacy of mouthwash prepared from pomegranate, grape seed and guava extracts against oral streptococci: an *in vivo* study. *J Clin Pediatr Dent* 2018;42(2):109–113. DOI: 10.17796/1053-4628-42.2.5
13. Megalaa N, Thirumurugan K, Kayalvizhi G, et al. A comparative evaluation of the anticaries efficacy of herbal extracts (Tulsi and Black myrobalans) and sodium fluoride as mouthrinses in children: a randomized controlled trial. *Indian J Dent Res* 2018;29(6):760–767. DOI: 10.4103/ijdr.IJDR\_790\_16
14. Kamath NP, Tandon S, Nayak R, et al. The effect of aloe vera and tea tree oil mouthwashes on the oral health of school children. *Eur Arch Paediatr Dent* 2020;21(1):61–66. DOI: 10.1007/s40368-019-00445-5
15. Hegde RJ, Kamath S. Comparison of the *Streptococcus mutans* and *Lactobacillus* colony count changes in saliva following chlorhexidine (0.12%) mouth rinse, combination mouth rinse, and green tea extract (0.5%) mouth rinse in children. *J Indian Soc Pedod Prev Dent* 2017;35(2):150–155. DOI: 10.4103/JISPPD.JISPPD\_13\_17