



## Clinical Study

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## Effectiveness of a custom-made natural tooth powder on oral hygiene status of children: A randomized controlled trial

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### ABSTRACT

**Aim:** To evaluate antibacterial, antifungal, antioxidant properties of custom-made natural tooth powder and the effectiveness of custom-made natural tooth powder on the oral hygiene status of children. **Methodology:** Antibacterial and antifungal properties were determined by agar diffusion method against *Streptococcus mutans*, *Candida albicans* and antioxidant properties were determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay. Clinical trial included 60 children aged 8 to 11 years. Oral prophylaxis was done and after a washout period of 10 days, baseline Simplified Oral Hygiene index (OHI-S) scores, plaque scores and *Streptococcus mutans* colony counts were recorded. Children were randomly divided into two groups: Group I children brushed with custom-made natural tooth powder and Group II children used commercially available natural tooth powder twice daily. At the end of 30 days, post intervention data was collected and statistically analyzed. **Results:** In-vitro trial showed that the custom-made natural tooth powder exhibited both antimicrobial and antioxidant properties. Intragroup assessment of clinical trial showed significant reduction in the OHI-S [Group I (p=0.03), Group II (p=0.01)] plaque scores [Group I (p=0.05), Group II (p=0.006)] and *Streptococcus* colony counts [Group I (p=0.000), Group II (p=0.000)] in both the groups. But there was no statistically significant difference between the two groups. **Conclusion:** The newer custom-made natural tooth powder is effective in oral hygiene maintenance and can be a possible alternative to the commercially available natural tooth powder.

**Keywords:** Natural tooth powder, Oral hygiene, Camphor, Clove.

### INTRODUCTION

According to WHO, 80% of the world's population especially those from the developing countries depend on plant derivative medicines for their healthcare and there has been an increased global interest in traditional medicine [1]. Natural products are safe, economical and act as effective alternatives to currently used chemotherapeutics which exhibit increased bacterial resistance and adverse reactions [2]. India, a nation known for its traditional medicinal systems has always been interested in the discovery of alternative treatment options using natural products [3].

One of the oldest human inventions include oral cleansing products. Dental tooth powder was developed around 3000-5000 BC by ancient Egyptians. Variety of products like egg shells, oxen bones are powdered to ashes and has been used as tooth powder [4]. The practice of brushing teeth with charcoal based preparations are regaining popularity mainly because of its detoxifying effect [5]. Bhasma is one of its kind obtained through incineration and calcination of cow dung ash. Bhasma is considered as ancient Indian nano medicine which can neutralize harmful acids, maintain optimum alkalinity and protect from free radical damage [6].

Camphor is a terpenoid organic compound extracted from the bark of *Cinnamomum camphora* and it has antiseptic, anesthetic, antispasmodic, anti-inflammatory and decongestant properties. Camphor was popularly used in ancient Chinese medicine to treat various illness such as sprain, allergies and swellings [7]. Camphor is used in dentistry to relieve pain and tooth sensitivity due to its mild anesthetic action [8]. It was used as an intracanal medicament with 35% parachlorophenol for infected root canals [9, 10]. An herbal tooth powder consisting of *Acacia catechu*, menthol and camphor was used to remove tartar, plaque and stains in tooth surface without producing any abrasion [11].

Natural essential oils have an important role in killing oral pathogens by its antimicrobial action and hence been widely used in dentistry [12]. Essential oils inhibit the bacterial enzymatic activity, prevent

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co-aggregation of colonizing bacteria and thereby reduce dental plaque formation [13]. Essential oil extracted from the buds of clove (*Eugenia caryophyllata*) has been used as an antiseptic and analgesic agent in dental practices. Clove gel could be a potential replacement for benzocaine as a topical agent [14]. A mouthwash containing tea tree oil and clove was found to be effective in reducing oral microbial colony count [15].

Dental caries and periodontal diseases are the two most challenging oral health problems faced worldwide. Epidemiological studies show that 60 to 90% of school children have oral health problems and the periodontal disease has been estimated to be the 11th most prevalent disease globally [16]. Despite the efficacy of many commercially available chemical toothpaste formulations, there is always an increasing societal desire to rely on naturally occurring compounds for oral health care. Hence the present study was planned to prepare a custom-made natural tooth powder and evaluate its antimicrobial and antioxidant properties. This study also aimed to assess the effectiveness of this tooth powder on the oral hygiene status of children.

## METHODOLOGY

The present study was carried out from the Department of Pedodontics and Preventive Dentistry, KSR Institute of Dental Science and Research (KSRIDSR), Tiruchengode in two phases. First phase of the study included in-vitro trials to assess the antimicrobial and antioxidant properties of the custom-made natural tooth powder. Second phase included a clinical trial to assess its effectiveness on oral hygiene status of children. The study design and protocol was analyzed and approved by the Institutional Review Board and Institutional Ethics Committee of KSRIDSR.

### Tooth powder preparation

The custom-made natural tooth powder was prepared by mixing 2 ml of dabur clove oil, 25gm of thymol crystals, 25gm of raw camphor and 160gm of Himalayan rock salt in a sterile container until it reached a liquid consistency. To this liquid, 1kg of cow dung ash was added, mixed well and placed in an airtight container for a week. After a week the mixture was stirred well again and made ready to use.

### In-vitro trials

The following in-vitro trials were done to assess the antimicrobial and antioxidant activity before the custom-made tooth powder was used for the clinical trial.

#### Antimicrobial activity

The antimicrobial activity of the custom-made tooth powder was determined by agar well diffusion method against *Streptococcus mutans* and *Candida albicans* [17]. In this method, BHI (Brain Heart Infusion) agar plates were seeded with the test organisms for 24 hours. Wells of 5mm diameter was made at equal distance in each plates using a sterile cork borer. Tooth powder dilutions at different concentrations such as 0.25mg/ml, 0.5mg/ml and 0.75mg/ml were introduced into each of the three wells [18]. The plates were incubated at 37°C for 24 hours. Diameter of zone of inhibition was evaluated to determine the antimicrobial activity of each organism.

#### Free radical scavenging activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is a stable free radical assay used to determine antioxidant activity of a material. The tooth powder samples were treated with DPPH radical at different concentrations (75.8mg/ml, 81.2mg/ml, 85.9mg/ml, 95.8mg/ml, 118.4mg/ml). The free radical, stable at room temperature, gets reduced in the presence of an antioxidant molecule, causing a change in the colour of the solution. This change in colour from deep violet to light yellow was noted at 517 nm after 100 minutes of reaction using a UV-VIS spectrophotometer. Ascorbic acid, a known antioxidant was used as the control [19].

### Clinical trial

This double blinded randomized controlled trial was conducted in the panchayat union middle school, Tiruchengode, Tamil Nadu. All children aged 8 to 11 years were screened and those with poor oral hygiene score [Simplified Oral Hygiene Index (OHI-S) score = 3.1 – 6] were selected. The study population included a convenient sample of 60 healthy children (28 boys and 32 girls) in the age group of 8 to 11 years. Informed consent was obtained from the parents/guardians of all the children included in the study. Children with special health care needs, medically compromised children, those under orthodontic therapy and children who had a history of antibiotic therapy in the past 3 months were excluded from the study.

The principal investigator (PI) along with a junior resident performed oral prophylaxis to all the study children prior to the intervention. Modified Bass tooth brushing technique was demonstrated and children were instructed to follow this method of tooth brushing [20]. After a wash out period of ten days, baseline data collection was done by the PI. It included oral hygiene scores, plaque scores and *Streptococcus mutans* colony count. Oral hygiene scores were recorded using OHI-S given by Greene and Vermilion [21] Plaque index demonstrated by Loe H [22] was used to record the dental plaque scores. Commercially available K.P. Namboodiri® (K.P. Namboodiri's Ayurvedics, Thrissur, Kerala, India) natural tooth powder was used as a control [23]. Both the test and the control tooth powders were stored in an identical opaque containers and labelled as A and B respectively by another investigator. The study children were randomly divided into two groups using randomly assigned computer generated numbers with an allocation ratio of 1:1. Group I children (n=30) were advised to brush their teeth twice daily using tooth powder A and Group II children (n=30) were asked to use tooth powder B. All the children were asked to brush only with the prescribed tooth powder twice daily for a period of 2 to 3 minutes. Parents were instructed to monitor their child's tooth brushing daily and ratify the use of the prescribed tooth powder in the tooth brushing chart provided to them. In case of any symptoms such as burning sensation, mouth sores, allergy or swollen gums after the usage of the tooth powders, children were asked to discontinue the use of tooth powder and inform the PI immediately.

Post intervention data was collected by the PI after a trial period of thirty days. The subjects, the PI and the statistician were blinded of the type of tooth powder allocated. A flow diagram summarizing the progress of the subjects through the clinical trial was presented in Figure 1.

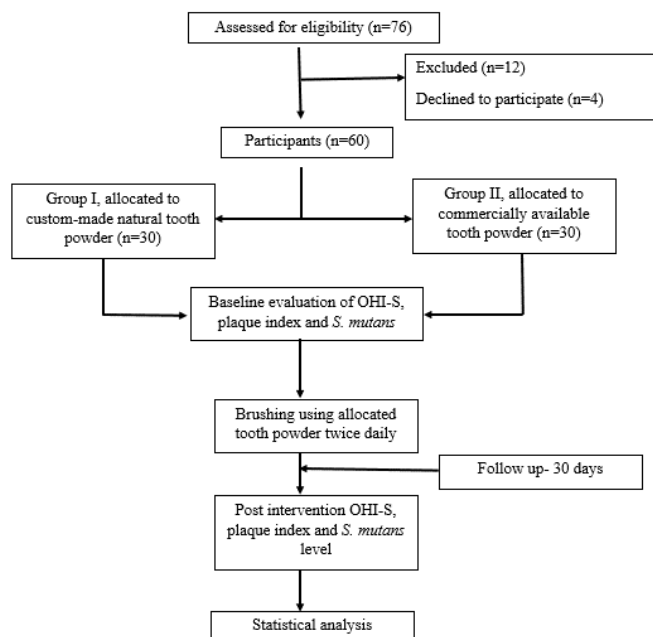


Figure 1: Clinical trial flow diagram

### Statistical analysis

Data were tabulated and analysed using IBM SPSS Statistics for Windows (Version 22.0, IBM Corp., Armonk, New York, USA). The statistical significance was considered to be at  $p \leq 0.05$ . Normality distribution of the mean values were analyzed using Kolmogorov-Smirnov test. Student t test and Mann Whitney test were used appropriately.

## RESULTS

### In-vitro trial

Custom-made natural tooth powder exhibited both antibacterial and antifungal activity. Highest zone of inhibition of 2.5mm was seen against *Streptococcus mutans* at 0.75mg/ml and 3mm against *Candida albicans* at 0.75mg/ml. (Table 1).

Table 1: Antimicrobial activity of custom-made tooth powder at different concentrations against test organisms

S.No	Test organisms	Zone of inhibition (mm)		
		0.25mg/ml	0.50mg/ml	0.75mg/ml
1	<i>Streptococcus mutans</i>	1.5mm	2.1mm	2.5 mm
2	<i>Candida albicans</i>	1.8mm	2.4mm	3.0 mm

Table 2: Radical scavenging activity of custom-made natural tooth powder at different concentrations

S. No.	Concentration of tooth powder (mg/ml)	Radical scavenging activity (%)
1	75.8	17.74
2	81.2	20.35
3	85.9	27
4	91.2	31.24
5	118.4	35.27

Table 2 illustrates the radical scavenging activity of custom-made natural tooth powder which exhibited mean inhibition of 26.32% at an average concentration of 90.5mg/ml.

### Clinical trial

In this study, a total of 60 children with 30 children in each group (14 boys and 16 girls) participated. The mean age of the children was  $9.5 \pm 1.5$  years. Table.3 demonstrates the OHI-S and plaque scores of two groups at baseline and post intervention. It reveals that there was no statistically significant difference in intergroup comparison at baseline and post intervention. Table.4. shows the intragroup comparison of OHI-S, plaque score and *Streptococcus mutans* colony count. There was a significant reduction in OHI-S scores in both group I ( $p=0.03$ ) and group II ( $p=0.01$ ). There was also a significant reduction in plaque scores in both group I ( $p=0.05$ ) and group II ( $p=0.006$ ). *Streptococcus mutans* colony count was reduced as well in both group I ( $p=0.000$ ) and group II ( $p=0.000$ ).

Table 3: Baseline and post intervention comparison of OHI-S and plaque index between Group I and II

Indices	Subjects (n=60)	Baseline (Mean $\pm$ SD <sup>†</sup> )	P-value	Post intervention (Mean $\pm$ SD <sup>†</sup> )	P-value
OHI-S	Group I (n=30)	0.93 $\pm$ 0.44	0.34*	0.59 $\pm$ 0.27	0.33*
	Group II (n=30)	0.97 $\pm$ 0.38		0.59 $\pm$ 0.39	
Plaque index	Group I (n=30)	1.16 $\pm$ 0.52	0.45**	0.45 $\pm$ 0.26	0.45 <sup>†</sup>
	Group II (n=30)	1.30 $\pm$ 0.55		0.42 $\pm$ 0.24	

\*Mann-Whitney test, <sup>†</sup>Student t test, <sup>‡</sup>Standard deviation

Table 4: Intragroup comparison of OHI-S, Plaque score and *Streptococcus mutans* colony count

	Groups	Baseline (Mean value $\pm$ SD <sup>†</sup> )	Post intervention (Mean value $\pm$ SD <sup>†</sup> )	P-value
OHI - S	Group I	1.16 $\pm$ 0.52	0.59 $\pm$ 0.27	0.03*
	Group II	1.30 $\pm$ 0.55	0.59 $\pm$ 0.39	0.01*
Plaque Score	Group I	0.93 $\pm$ 0.44	0.45 $\pm$ 0.26	0.05*
	Group II	0.97 $\pm$ 0.38	0.42 $\pm$ 0.24	0.006*
S.mutans colony count	Group I	52.28 $\pm$ 63.66	2.28 $\pm$ 5.21	0.000*
	Group II	50.14 $\pm$ 52.75	2.14 $\pm$ 4.64	0.000*

\*Student t test, <sup>†</sup>Standard deviation.

## DISCUSSION

Oral cavity is the mirror of our body. Proper oral hygiene maintenance is mandatory for our systemic well-being. Controlling the quantity and quality of the oral biofilm is the foremost in preventing gingivitis and caries. The present study came up with the unique composition to render a solution to the oral health problems.

### In-vitro trial

In the present study custom-made natural tooth powder exhibited antioxidant property and antimicrobial action against *Streptococcus mutans* and *Candida albicans*. This may be due to the actions of camphor, ash and clove oil present in the tooth powder. Camphor shows

growth inhibition of gram positive and gram negative organisms (*Escherichia coli*, *Staphylococcus aureus*) [24]. Production of superoxide dismutase and lipid peroxidase by camphor attribute to its antioxidant and antimicrobial action [25]. Camphor oil has a significant antifungal activity and the effectiveness of camphor increased when combined with other essential oils [26]. High carbon to nitrogen ratio in ash has the ability to absorb toxins and hence could be used as a target base drug delivery system with less or no side effects along with immunomodulatory benefit. Highly alkaline pH (11.7) of ash prevents the bacterial growth and possess potential antimicrobial activity against *Cyanobacteria*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* [27].

### Clinical trial

The study included children aged 8 to 11 years belonging to concrete operational stage according to Jean Piaget's theory. This age group of children can apply logical ideas to carry out their work. In the daily practice of oral hygiene maintenance across the globe, toothpaste is the most commonly used agent. A study by Khan MK [28] revealed that tooth powder was superior in controlling plaque and gingivitis compared to tooth paste.

The present study used a unique composition of ingredients which has not been used in any other studies. However, ingredients like bhasma, camphor, clove of this natural custom-made tooth powder has been separately used in other tooth powders like K.P. Namboodiri®, Payorin manjan® (Kamdhenu Laboratories, Jaipur, Rajasthan, India), UDM® (KLE Ayurveda pharmacy, Belgaum, Karnataka, India). Commercially available control tooth powder K.P. Namboodiri® used in this study contains bhasma, pepper, clove and amla. None of the children in this study reported any allergic reactions and there were no drop outs in the 30-days study period.

In this study there was a significant reduction in the OHI-S and plaque scores in both the groups. Agrawal *et al.* [29] in their study using Payorin manjan® unani tooth powder concluded that both tooth powder and tooth paste were efficient in reducing the gingival and plaque index. Both the study tooth powder and unani tooth powder had camphor as a significant composition and they both showed significant reduction in plaque scores. Similarly, the study by Kadam *et al.* [30] using UDM® tooth powder containing camphor showed reduction in plaque scores. Dashana Samskara choorna (K.P. Namboodiri®) which contained camphor and clove oil was efficient in the reduction of gingivitis but it was not significant compared to K.P. Namboodiri® tooth paste [23].

Literature search showed no studies on the effectiveness of tooth powder on the *Streptococcus mutans* colony count. However, in a study by Patil S *et al.* [31] *Streptococcus mutans* colony count was evaluated after the usage of herbal tooth paste which showed the significant reduction in the total colony count. The current study showed a significant reduction in the *Streptococcus mutans* colony count post intervention which can be attributed to the antimicrobial activity of the tooth powder.

Limitations of the study includes the use of a convenient sample which may not represent the general population. Larger sample size of children with different caries status should be included to get a precise evidence on the effectiveness of the custom-made tooth powder. Long term effects of the tooth powder on oral health status was not assessed.

### CONCLUSION

Within the limitations of the study, the newer custom-made natural tooth powder was found to have antimicrobial and antioxidant properties and found to be effective in oral hygiene maintenance. It could be an alternative to the commercially available natural tooth powder. In the changing era of 'back to the basics' this natural tooth powder could be a novel approach in the field of dentistry.

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