

ORIGINAL ARTICLE

Comparison of Antimicrobial Efficacy of Cinnamon Bark Oil Incorporated and Probiotic Blend Incorporated Mucoadhesive Patch against Salivary *Streptococcus mutans* in Caries Active 7–10-year-old Children: An *In Vivo* Study

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ABSTRACT

Introduction: Among the various plants studied, cinnamon has emerged as a potential herbal antimicrobial agent. Besides the medicinal plants, recently probiotics have also been recognized to affect cinnamon bark oil *Streptococcus mutans* (*S. mutans*) and other harmful oral and gut microflora.

Aim and objective: This placebo-controlled study aims to compare the antimicrobial potential of cinnamon bark oil incorporated and probiotic blend [*Lactobacillus plantarum* (TSP-Lp1), and *Lactobacillus rhamnosus* (TSP-Lrh1)] incorporated mucoadhesive patch against salivary *S. mutans* in caries active 7–10-year-old children.

Design: It was a double-blinded placebo-controlled study with $n = 60$. They were randomly allotted into three groups—Group I: Cinnamon patch, group II: probiotic patch, and group III: control patch (placebo) with $n = 20$ in each group.

Materials and methods: The study was carried out in three phases. In the first phase, the minimal inhibitory concentration (MIC) of cinnamon bark oil was determined against *S. mutans* followed by the formulation of cinnamon and probiotic patches. After a washout period of 2 weeks and a collection of baseline saliva samples, these patches were tested on the subjects from respective groups for 14 days with twice a day placement protocol. On the 15th day, saliva samples were collected and cultured, CFU/mL of the saliva of *S. mutans* for each subject was recorded and compared with baseline samples. Feedback in the form of a questionnaire was obtained from the patients.

Statistical analysis: Descriptive statistics, paired *t*-test for intragroup comparison, unpaired *t*-test for intergroup comparison, analysis of variance (ANOVA) for intergroup comparison, and *post hoc* Scheffe's.

Results: The results showed that both cinnamon patch and probiotic patch were comparable to each other in terms of their anti-*S. mutans* activity. The intragroup comparison of the CFU/mL count showed a highly significant reduction from baseline to post-intervention for both the groups ($p = 0.001$).

Conclusion: Both cinnamon and probiotic blend have a strong antimicrobial property owing to their ability to cause significant reduction in salivary *S. mutans* and both the patches showed good patient acceptance.

Keywords: Dental caries, Mucoadhesive patch, Probiotic, *Streptococcus*, *Streptococcus mutans*.

International Journal of Clinical Pediatric Dentistry (2020): 10.5005/jp-journals-10005-1818

INTRODUCTION

Dental caries is one of the most common diseases in the world—second only to the common cold.¹ The notion that dental caries in animals is an infectious transmissible disease was first demonstrated by Keyes.² Dental caries can be prevented by the maintenance of proper oral hygiene with the use of oral care products like toothpaste, toothbrush, and even mouthwashes with antimicrobial and anti-cariogenic properties. However, the incidence of adverse effects from the use of these chemical products has brought about an inclination toward the use of natural remedies for achieving the same. This has led to research and development in the area of functional foods having a potentially positive effect on health beyond basic nutrition. The concept of functional food was introduced long back by Hippocrates with the motto being “let food be your medicine”.³ Moreover, for thousands of years, medicinal plants have been used in many parts of the world as traditional treatments to cure a multitude of ailments including toothache. Among the various plants studied, *Cinnamomum zeylanicum* Blume (cinnamon)^{4,5}

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How to cite this article: Gandhi HA, Srilatha KT, Deshmukh S, et al. Comparison of Antimicrobial Efficacy of Cinnamon Bark Oil Incorporated and Probiotic Blend Incorporated Mucoadhesive Patch against Salivary *Streptococcus mutans* in Caries Active 7–10-year-old Children: An *In Vivo* Study. *Int J Clin Pediatr Dent* 2020;13(5):543–550.

Source of support: Nil

Conflict of interest: None

has emerged as a potential herbal antimicrobial agent. The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but

also in traditional and modern medicines. Cinnamon bark oil can inhibit amino acid decarboxylase activity in various oral pathogenic bacteria due to the presence of an active ingredient cinnamaldehyde (highly electronegative), an aromatic aldehyde.⁶ Cinnamon bark oil also contains benzoic acid, benzaldehyde, and cinnamic acid, which too have been recognized to have antimicrobial properties. Also, cinnamon bark oil contains 4.7% eugenol, which depending upon the concentration used is known to be either bactericidal or bacteriostatic agent. An essential oil from cinnamon bark also contains cinnamyl acetate (8.7%), which increases the activity of the parent compound.⁷ Besides the medicinal plants, recently probiotics have also been recognized to affect *Streptococcus mutans* (*S. mutans*) and other harmful oral and gut microflora. The term probiotics was introduced by Lilley and Stillwell in 1965.⁸ Probiotics have been used in combating various diseases, such as, gastrointestinal infections, cancer prevention, constipation, irritable bowel syndrome, periodontal diseases, and dental caries.⁹ The mechanisms of action of probiotics in the human body include normalization of the microbiota, modulation of an immune response, and metabolic effects.⁹ Probiotics in the oral cavity have been used to replace cariogenic organisms like streptococci and *Lactobacillus* species with strains of bacteria that are not cariogenic.¹⁰ Many clinical studies have confirmed that probiotic lactobacilli can reduce the counts of salivary *S. mutans* after the ingestion of *Lactobacillus rhamnosus*-GG, *L. reuteri*, *L. plantarum*, and lactobacilli mix.⁹ Studies have shown that probiotics are as effective as chlorhexidine and safer than chlorhexidine.¹¹ Hence, in the proposed study, probiotics were used as a safer alternative to chlorhexidine in children.

A mucoadhesive patch incorporated with anti-cariogenic and antibacterial agents may help in slowly releasing the drug in the oral cavity where there is a constant circulation of the drug in the oral cavity through saliva¹² and hence it has a potential to be used as an alternative to mouthwash especially in the pediatric age group.

There is no documented literature comparing the efficacy of cinnamon bark oil and probiotic incorporated mucoadhesive patch against *S. mutans*, hence this placebo-controlled study was taken up to test the hypothesis.

MATERIALS AND METHODS

The present study was carried out at the Department of Pedodontics and Preventive Dentistry, JSS Dental College and Hospital, Mysuru. The participants comprised of children aged 7–10 years were selected from St. Matthias Primary School, Bannimantap, Mysuru. The nature of the study, its duration, and objectives were clearly described and explained to the authorities.

Before the start of the study, ethical clearance was obtained from the Institutional Ethical Committee, and written informed consent and assent was obtained from all the parents and the children participating in the study, and the study was carried out under the Helsinki Declaration of 1975, as revised in 2000.

Study Design

Placebo-controlled double-blinded randomized clinical trial.

Sample Size

Using mean and standard deviation from earlier studies with the power of study 80% and a confidence interval of 95%, the sample size estimated was 17. Anticipating the loss of study subjects to

follow-up, the sample size chosen was a total of 60 subjects with 20 subjects in each of the three groups.

$$N = \frac{(Z_{\alpha/2} + Z_{\beta})^2 (\sigma_1^2 + \sigma_2^2)}{L^2}$$

N = sample size

α and β = constants

σ_1 = SD of group I

Z = the power of the study (80%)

σ_2 = SD of group II

L = Difference of means

Sampling Methodology

From a pediatric population from 7 to 10 years of age, 60 children were selected based on the predetermined inclusion criteria and exclusion criteria. The subjects were screened and selected from St. Matthias Primary School, Bannimantap, Mysuru. The subjects were further divided into three groups—cinnamon patch group (group I), probiotic patch group (group II), and control group (group III) by the simple random sampling (lottery method). Healthy children in the age group of 7–10 years who have voluntarily signed the informed assent and consent with deft/DMFT score 3 and <5 and Frankel's behavior rating of "positive" and "definitely positive" were included in the study whereas children with oral or systemic diseases and medically compromising conditions with history of hypersensitivity reactions and under long-term antibiotic therapy were excluded. Children with acute symptoms requiring immediate dental treatment with deep dental caries or grossly decayed teeth were also excluded from the study (Flowchart 1).

Methods

The study was carried out in three phases.

Phase 1: Antimicrobial testing and minimal inhibitory concentration (MIC) determination—The antibacterial effect of various concentrations (2.5, 5, 10, and 20 μ L/mL) of the cinnamon bark oil against *S. mutans* was determined using the broth microdilution method. The inoculums of test strains were adjusted to an optical density of 0.1 (0.5 McFarland standard) which corresponds to 1.5×10^8 CFU/mL by diluting it with sterile brain heart infusion (BHI) broth using a spectrophotometer. One hundred microliters of each test concentration and bacterial inoculum were added into each of the wells of the 96-well tissue culture plate in an orderly predecided manner. After 24 hours of incubation under anaerobic conditions, the antibacterial activity was recorded using visual confirmation and reading of absorbance using a multimode plate reader (Tables 1 and 2). Minimal inhibitory concentration of cinnamon bark oil (76.8% cinnamaldehyde-active ingredient, Cinnamon Vogue, Las Vegas, Nevada) was estimated to be 20 μ L/mL (Fig. 1). The strength of the probiotic blend of *L. plantarum* (TSP-Lp1) and *L. rhamnosus* (TSP-Lrh1) (Triphase Pharmaceuticals Pvt. Ltd., Mysuru) was determined taking into consideration the literature available on the use of safe and effective strength of probiotic strains in children from a dental point of view which ranges from 10^5 to 10^9 with 10^9 being most effective.^{3,9,11,13–15}

Phase 2: Formulation of cinnamon bark oil incorporated, probiotic blend [*L. plantarum* (TSP-Lp1) and *L. rhamnosus* (TSP-Lrh1)] incorporated and placebo mucoadhesive patch—The mucoadhesive patches were formulated at the Department of Pharmaceutics at JSS College of Pharmacy, Mysuru and were

Flowchart 1: Flow diagram showing methodology of the study

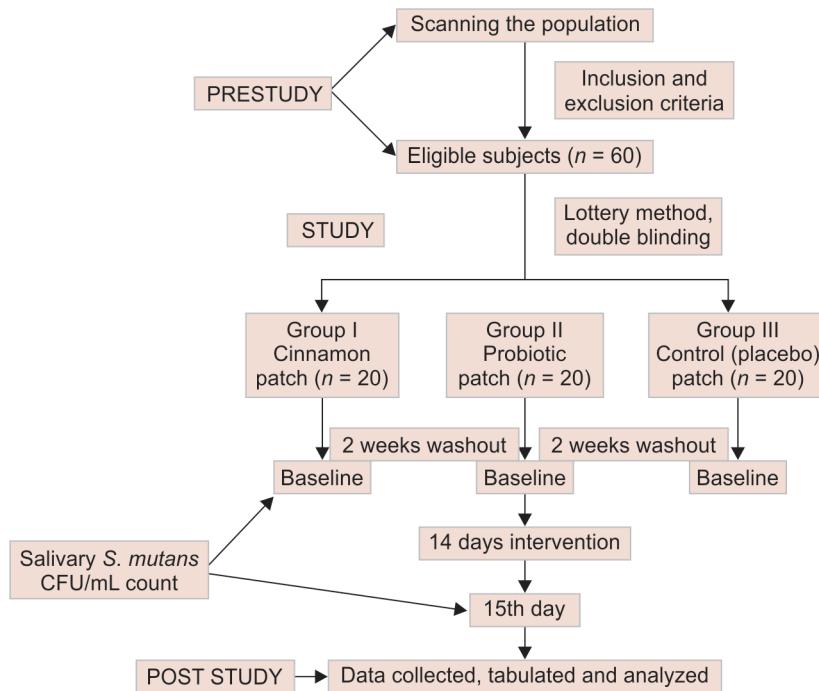


Table 1: Mean optical density (OD) values of the different concentrations of cinnamon bark oil against *Streptococcus mutans*

		Blank broth (negative control)	0.2% CHX with bacterial broth (positive control)	Test concentrations of cinnamon oil			
Bacterial broth	Blank DMSO			2.5 µL/mL	5 µL/mL	10 µL/mL	20 µL/mL
0.34	0.30	0.08	0.16	0.23	0.23	0.20	0.16

Table 2: Percentage reduction in the optical density (OD) values of the different concentrations of cinnamon bark oil against *Streptococcus mutans*

Concentration of cinnamon bark oil	2.5 $\mu\text{L}/\text{mL}$	5 $\mu\text{L}/\text{mL}$	10 $\mu\text{L}/\text{mL}$	20 $\mu\text{L}/\text{mL}$
Percentage reduction in OD values	30.47	31.96	39.78	51.20

optimized for various formulation ingredients before making the final batches of mucoadhesive patches. Mucoadhesive patches were prepared using the solvent casting technique.¹⁶ All the materials used for the patch preparation are approved by the FDA and come under the category of GRAS (Generally regarded as safe).¹⁷ Carbopol 934P and Hydroxypropyl methylcellulose (HPMC) K15LV were used as the mucoadhesive polymers and polyethylene glycol (PEG) 4000 was used as the plasticizer. Stevia was used as a sweetening agent. 1.5 L of the cinnamon bark oil (20 µL per patch) and 10 mg of the probiotic blend [5 billion of *L. plantarum* (TSP-Lp1) and 5 billion of *L. rhamnosus* (TSP-Lrh1)] was required for a batch of 75 patches. Mucoadhesive patches were cut in 1 × 1 cm size and evaluated for thickness uniformity, weight uniformity, and Ph.

Phase 3: Clinical trial—From caries active pediatric population of 7–10 years of age, 60 children were selected based on the predetermined inclusion criteria and exclusion criteria. They were

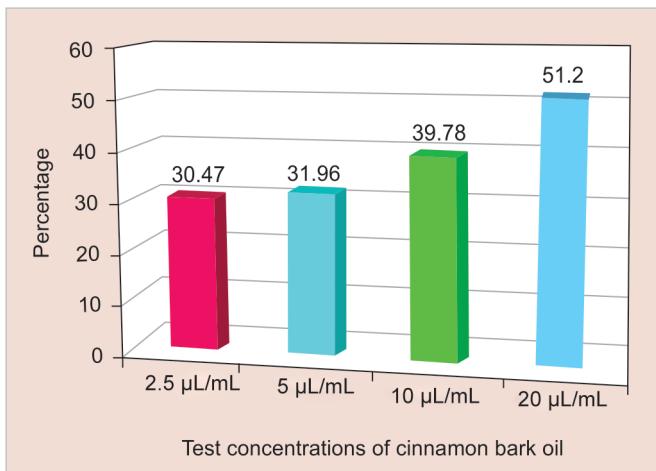


Fig. 1: Percentage reduction in the optical density values of the different concentrations of cinnamon bark oil against *Streptococcus mutans*

randomly allotted into three groups—Group I: cinnamon patch, group II: probiotic patch, and group III: control patch (placebo) (Fig. 2). A washout period of 2 weeks was observed. The clinical study commenced with the recording of case history followed by the collection of baseline saliva samples. Patches were placed on the palatal slope (Fig. 3) every day 1 hour post breakfast and they were also given patches in a sterile sealed pouch to be placed 1 hour post-dinner under parent's supervision for 14 days⁹ followed



Fig. 2: (A) Cinnamon bark oil incorporated; (B) Probiotic blend incorporated; (C) Control/placebo patch

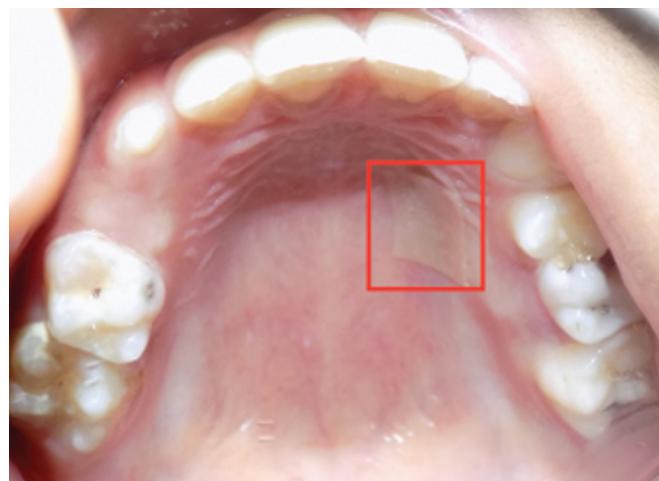


Fig. 3: Position of patch placement

Table 3: Descriptive statistics for colony-forming units per mL (CFU/mL)

Group	N	Mean of baseline ± SD (CFU/mL)	Minimum	Maximum	Mean of post-intervention ± SD (CFU/mL)	Minimum	Maximum
Cinnamon patch group	0	39,650 ± 20,606.99	7,800	74,400	416.5 ± 241.30	30	800
Probiotic group	0	37,113 ± 21,151.80	8,400	78,400	602 ± 387.16	100	1,600
Control group	0	33,971 ± 21,328.34	7,800	76,000	19465 ± 11,603.01	3,200	44,000

by saliva sample collection on the 15th day. A record was maintained for all subjects to ensure the daily placement of mucoadhesive patches. Any adverse effects experienced by subjects demanded immediate notification from the parents and was recorded. Salivary *S. mutans* count was checked at baseline and post-intervention and compared for all three groups.

RESULTS

The results showed that both cinnamon patch and probiotic patch were comparable to each other in terms of their anti-*S. mutans* activity (Table 3 and Fig. 4). The intragroup comparison of CFU/mL count showed a highly significant reduction from baseline to post-intervention for all three groups (Table 4). One-way analysis of variance (ANOVA) revealed a significant difference in mean CFU counts of groups, i.e., cinnamon patch group (group I), probiotic patch group (group II), and control patch group (group III). F value of 53.30 was found to be significant at $p = 0.001$. Mean CFU count at post-intervention was 416.5, 602, and 19,465 CFU/mL for group I, group II, and group III, respectively (Table 5). Furthermore, Scheffe's *post hoc* test revealed that there was no significant mean difference between the cinnamon patch group (group I) and the probiotic patch group (group II). However, there was a significant mean difference between the cinnamon patch group (group I) and control patch group (group III) and probiotic patch group (group II) and control patch group (group III) with less CFU/mL reduction seen in control patch group ($p = 0.05$) (Fig. 5). A questionnaire was used to assess patient compliance in terms of the presence of adverse effects if any like displeasure in taste, alterations in the breath, teeth staining, nausea, and any other symptoms. Ten percent of the subjects in the probiotic patch group experienced

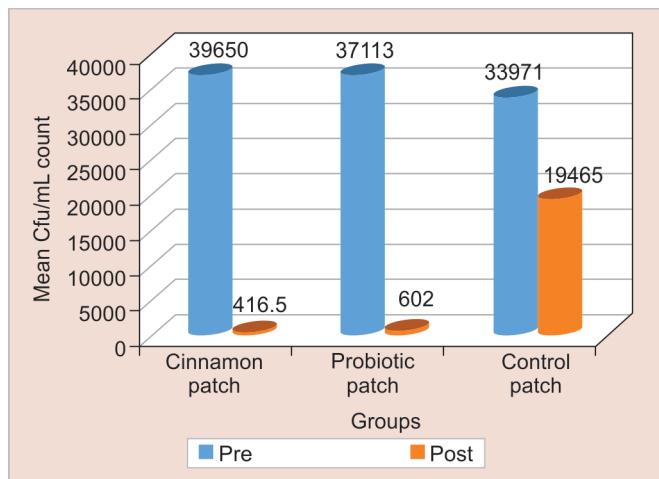


Fig. 4: Intergroup comparison of colony-forming units in the cinnamon patch group, probiotic patch group, and control patch group

displeasure in terms of taste. Five percent of the subjects in all the three groups complained of nausea on the placement of the patch. However, none of the subjects reported any difficulties with regards to alteration of breath, teeth staining, irritation, burning, or any other symptoms (Table 6).

DISCUSSION

Streptococcus mutans has been implicated as the major causative organism in the etiology of caries.^{18,19} Salivary *S. mutans* levels are a reflection of the number of tooth sites that have been colonized as

Table 4: One-way ANOVA

		Sum of squares	df	Mean square	F	Sig.
Baseline	Between groups	323730493.33	2	161865246.67	0.37	0.7
	Within groups	25211951400.00	57	442314936.84		
	Total	25535681893.33	59			
Post-intervention	Between groups	4791283543.33	2	2395641771.67	53.3	0.05*
	Within groups	2561919675.00	57	44945959.211		
	Total	753203218.33	59			

*p value—significant; p > 0.05—not significant

Table 5: Post hoc Scheffe's test

Post-intervention (a)			
Group	N	Subset for $\alpha = 0.05$	
		1	2
Cinnamon patch	20	416.5000	
Probiotic patch	20	602.0000	
Control patch	20		19,465.0000

Change (b)			
Group	N	Subset for $\alpha = 0.05$	
		1	2
Cinnamon patch	20	14,506.0000	
Probiotic patch	20		36,511.0000
Control patch	20		39,233.5000

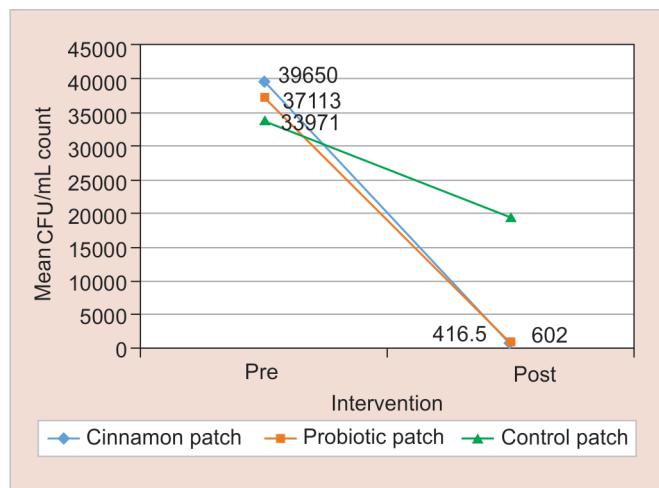


Fig. 5: Intergroup comparison of reduction in colony-forming units pre- and post-intervention in the cinnamon patch group, probiotic patch group, and control patch group

well as their proportion in dental plaque.^{20,21} A positive correlation has been quoted about the concentration of *S. mutans* in saliva and dental caries by Lenander-Lumikari and Loimaranta,²¹ Emilson,²² and Salonen et al.²³ Saliva samples are most often preferred for quantification of *S. mutans* according to Köhler et al.²⁴ The number of mutans streptococci in saliva can be used for the evaluation of caries risk and is also useful for monitoring the level of colonization of the individual (Krasse, 1984).²⁵ Hence, in the present study, the salivary *S. mutans* levels were assessed to evaluate the anti-caries effect exhibited by three different mucoadhesive patches.

Keeping in mind the evidence of an alarming increase in the global spread of resistant clinical isolates, the need to find alternate,

Table 6: Feedback with respect to patient compliance and adverse effects

Adverse effects	Cinnamon patch	Probiotic patch	Control patch (placebo)
Displeasure in taste, teeth staining, nausea, burning, other	10%		

newer antimicrobial agents has become of paramount importance. Also, the past record of rapid and widespread emergence of resistance to newly introduced synthetic antimicrobial agents indicates that even newer drugs might suffer the same fate.²⁶ This has prompted researchers to study herbal products and functional foods in the hope of combating this rising tide of resistant bacteria safely and efficiently.

Among the various plants studied, *Cinnamomum zeylanicum* Blume (cinnamon)^{4,5} has emerged as a potential herbal antimicrobial agent. The bark of cinnamon is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicine. According to the US Department of Health, the recommended safe dose is 6 g daily for 6 weeks or less, at this level cinnamon is considered safe.²⁷ Although antimicrobial properties of cinnamon bark oil have been reported recently, scientific evidence on the action of cinnamon bark oil on specific pathogenic oral bacteria, particularly pathogens related to periodontal disease and dental caries, are still scarce.

Besides the medicinal plants, recently probiotics have also been recognized to affect *S. mutans* and other harmful oral and gut microflora. Grover and Luthra reported that the hypothetical mechanism of probiotic action in the oral cavity is by various direct and indirect means.²⁸ Various indirect actions include modulation of systemic immune function, enhancement of local immunity, regulation of mucosal permeability, and its effect on the non-immunological defense mechanism. It also functions as an antioxidant and neutralizes free electrons, thus preventing plaque formation. The possible direct interactions include binding of oral microorganisms to protein, production of chemicals that inhibit oral bacteria, action on plaque formation, and its complex ecosystem by competing and intervening with bacterial attachments.²⁸ Probiotics in the oral cavity have been used to replace cariogenic organisms like streptococci and *Lactobacillus* species with strains of bacteria that are not cariogenic.¹⁰ Bacterial strains that have been tested for probiotic action in the oral cavity and *in vitro* against oral pathogens include lactobacilli species (*Lactobacillus acidophilus*, *L. rhamnosus* GG, *Lactobacillus johnsonii*, *Lactobacillus casei*, *L. rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus reuteri*, *Lactobacillus paracasei*, *Lactobacillus plantarum*), *Bifidobacterium* species (*Bifidobacterium bifidum*, *Bifidobacterium*

longum, *Bifidobacterium infantis*, *Bifidobacterium animalis* strain DN-173 010), and others (*Streptococcus salivarius*, *Weissella cibaria*).¹⁵ Clinical studies by Näse et al.,¹³ Caglar et al.,²⁹ Haukioja et al.,³⁰ Cildir et al.,³¹ Jindal et al.,⁹ Harini and Anegundi,¹¹ Khanafari et al.,¹⁴ Saha et al.,³² and Chinnappa et al. (2013),³³ have confirmed that probiotic lactobacilli can reduce the counts of *S. mutans* after ingestion of *L. rhamnosus*-GG, *L. reuteri*, *L. plantarum*, and lactobacilli mix of which *L. rhamnosus* and *L. plantarum* were found to have better antimicrobial efficacy against *S. mutans*^{9,13,14,30,32} and hence it was decided to use the blend of pure strains of the probiotic bacteria *L. rhamnosus* (TSP-Lrh1) and *L. plantarum* (TSP-Lp1) for the formulation of probiotic mucoadhesive patch.

The suppression of caries causing organisms like *S. mutans* by local administration of chlorhexidine has been well established. The most commonly used and recommended oral health care product, chlorhexidine, also considered as a "gold standard" for oral antimicrobial agents, was among the best range of bisbiguanides synthesized *ad hoc* as bactericides by Davies et al. in 1954.^{34,35} However, the use of chlorhexidine has depleted in recent years which can be attributed to its various side effects, like brown staining of teeth, altered taste sensation, soreness of the oral mucosa after use, and most importantly, the fear of the development of resistant bacterial strains which might eventually render the product less effective.³⁶ A study conducted by Harini and Anegundi concluded that probiotics are as effective as chlorhexidine and safer than chlorhexidine.¹¹ Hence, in the proposed placebo-controlled study, probiotics were used as a safer alternative to chlorhexidine in children.

A mouth rinse can be an effective chemotherapeutic agent used as adjuvant to routine home care remedy to maintain oral hygiene as well as prevent dental caries by targeting the cariogenic microflora but when it comes to its use in children below 10 years, compliance is less. A mucoadhesive patch incorporated with anti-cariogenic and antibacterial agents may help in slowly releasing the drug in the oral cavity resulting in a constant circulation of the drug in the oral cavity through saliva; moreover, some amount of it also gets absorbed in the systemic circulation¹² and eventually facilitates its permanent installation as an inherent component of the saliva enhancing its antimicrobial potential and thus can be used as an effective adjuvant to a daily oral hygiene maintenance routine. Mucoadhesive patches are commonly used in the field of dentistry as a local drug delivery vehicle for treating oral mucosal lesions, periodontal problems, and reducing dental pain.³⁷⁻⁴⁰ To the best of our knowledge, its potential as an alternative to mouthwash especially in pediatric patients has not been explored.

There is no documented literature on the efficacy of cinnamon bark oil incorporated and probiotic incorporated mucoadhesive patch against salivary *S. mutans*; hence, this placebo-controlled study with newly formulated cinnamon bark oil incorporated and probiotic incorporated mucoadhesive patch was taken up to test the hypothesis.

In the first phase, the antimicrobial efficacy and MIC of cinnamon bark oil were evaluated against a pure strain of *S. mutans* (MTCC 890). Although antimicrobial properties of cinnamon bark oil have been reported recently, scientific evidence on the action of cinnamon bark oil on specific pathogenic oral bacteria, particularly pathogens related to periodontal disease and dental caries, are still scarce. Keeping this in mind, it was decided to assess the antimicrobial efficacy and MIC of cinnamon bark oil against *S. mutans*. The antibacterial effect of cinnamon bark oil against

S. mutans was determined using the broth microdilution method. Minimal inhibitory concentration of the cinnamon oil was estimated to be 20 µL/mL and it was under the results obtained in the study conducted by Chaudhari et al.⁵ and Zainal-Abidin et al.⁴¹

The strength of probiotic was determined taking into consideration the literature available on the safe and effective strength of probiotic strains in children from a dental point of view which ranges from 10^5 to 10^9 CFU/g with 10^9 CFU/g being most effective.^{13,41-45} As per the safety guidelines for probiotics use in children, a healthy child can safely take up to 10 billion per day.^{46,47} In the current study, a probiotic blend of 10 billion was used composed of 5 billion CFU/gm *L. rhamnosus* (TSP-Lrh1) and 5 billion CFU/gm of *L. plantarum* (TSP-Lp1).

A pediatric population of caries active children were chosen based on a systematic review done by Leal et al. in 2010^{48,49} with deft/DMFT score between 3 and 5. A study conducted by Sudha et al.⁵⁰ on 5–13 years age group showed a higher prevalence of dental caries in 5–7 years age group compared with that of 8–9 and 11–13 years age group. In the present study, we have taken up children of 7–10 years as at this age the permanent teeth are erupting, so there are chances that the new surfaces would be colonized by pathogenic bacteria. Hence, preventive measures taken at this time might be helpful in the long run. Also, positive compliance could be expected from a child of this age group. According to Jean Piaget, 7 years of age largely correspond to an increase in cognitive development. Therefore, an opportunity existed and prompted us to take up the present study with an age group of 7–10 years.

The collection of saliva was done as mentioned in the study done by Westergren and Krasse.⁵¹ The microbial growth was recorded in terms of colony-forming units/mL. The range of colony-forming units/mL of salivary *S. mutans* is under the findings of Jindal et al.⁹ The pre-intervention (baseline) CFU/mL was in the range of 10^4 CFU/mL. The intragroup comparison of CFU/mL from baseline to post-intervention showed a highly significant reduction ($p = 0.001$) in salivary *S. mutans* counts for all three groups with CFU count reduction being less in the control group compared with the cinnamon patch group and probiotic group (Figs 4 and 5). Reduction in salivary *S. mutans* count in the cinnamon patch group was under a previous study done by Al-Joubori et al.⁵² wherein cinnamon extract mouth rinse was used. Clinical trials conducted using cinnamon against *S. mutans* are very scarce. The CFU count reduction seen in the probiotic patch group was under previous studies by Näse et al.,¹³ Ahola et al.,⁵³ Jindal et al.,⁹ and Siddiqui et al.³ wherein significant reduction was seen *S. mutans* count compared with control group ($p < 0.05$), whereas our results were in contrast to the study done by Montaldo et al.⁵⁴ that found an increase in lactobacilli count but *S. mutans* count remain unchanged. In all these studies, either probiotic curd or milk or cheese was used.

To the best of our knowledge, there is no documented literature on cinnamon bark oil incorporated mucoadhesive patch or probiotic incorporated mucoadhesive patch. The intergroup comparison between all three groups showed a non-significant difference at baseline indicating that baseline was well standardized (Table 4, and Figs 4 and 5). Post-intervention there was a significant mean difference in the CFU count between group I and group III and between group II and group III, whereas there was no significant mean difference in the post-intervention CFU count between group I and group II. This shows that cinnamon bark oil incorporated mucoadhesive patch and probiotic incorporated mucoadhesive patch have a comparable effect against salivary *S. mutans*. The

overall percentage reduction of salivary *S. mutans* was more in the cinnamon patch group and probiotic patch group compared with the control patch group (Tables 3 to 5, and Figs 4 and 5). In our study, the control patch which is a placebo patch has also shown a fairly significant reduction in salivary *S. mutans* count post-intervention indicating some amount of antimicrobial effect of the mucoadhesive polymers used for the formulation and at the same time throughout the course of the study children followed the instructed oral hygiene maintenance protocols under the strict supervision of the examiner and the parent.

We can thus infer that both cinnamon bark oil incorporated mucoadhesive patch and probiotic incorporated mucoadhesive patch have proven to be very effective in reducing salivary *S. mutans*.

CONCLUSION

We can conclude that:

- Both cinnamon bark oil extract incorporated mucoadhesive patch and probiotic incorporated patch have rather strong antimicrobial property owing to its ability to cause a highly significant reduction in salivary *S. mutans*.
- Cinnamon bark oil incorporated mucoadhesive patch is comparable to the probiotic incorporated patch due to its similarity in the reduction of salivary *S. mutans* counts.
- Both the patches had good patient acceptance.

LIMITATIONS OF THE STUDY

In this placebo-controlled study, we have studied the effect of newly formulated cinnamon bark oil incorporated mucoadhesive patch and probiotic incorporated mucoadhesive patch against salivary *S. mutans*. We have not compared it with any commercially available product or gold standard as there are no mucoadhesive patches available commercially that affect caries causing bacteria to be used in the pediatric population.

FUTURE DIRECTIONS OF THE STUDY

- Further research can be done to assess the effect of mucoadhesive patches used as an alternative to mouthwash.
- Bacteriocin released by probiotics can be utilized in the future for newer formulations.
- Further studies can be done to check its efficacy to keep the oral bacterial load in check and also acceptance in special children with poorly developed motor skills and cognition and children who are chronically hospitalized or bedridden where oral hygiene maintenance becomes difficult.

ACKNOWLEDGMENTS

Triphase Pharmaceuticals Pvt. Ltd., Mysuru, India.

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