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Anti-microbial Efficacy of Commercially Available Herbal Dentifrices on *Streptococcus mutans* and *Candida albicans*

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Authors' contributions

This work was carried out in collaboration among all authors. Author NKB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IMA and RPK managed the analyses of the study. Author DSS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Dental caries is a multi-factorial disease .Various chemically derived antimicrobial agents are incorporated to dentifrices to curb cariogenic microorganisms. There has been renewed interest in naturally occurring products in recent years, the side effects encountered with the use of synthetic formulations led to the surge for novel alternatives. To compare the efficacy of herbal dentifrices on oral microflora using antibiotic susceptibility tests. Two strains of microorganisms- *Streptococcus mutans*, and *Candida albicans* were taken and incubated in Mutans media, Sabouraud Dextrose agar respectively. Different dilutions (1:5, 1:10, 1:15) of several brands of toothpastes with different compositions were made. Sterile discs were incorporated with equal amounts of prepared

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toothpaste formulations. Then these discs were placed equidistant to each other followed by plates were incubated for 24 hours. Zone of inhibition is found to be higher in Ayush (30 mm), followed by Dant kanti, Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 26 mm, 24 mm, 17 mm and 14 mm respectively. Results show that herbal toothpaste formulations were effective in controlling the oral microflora. The antimicrobial properties of the six dentifrices concluded that almost all the commercially available herbal dentifrices had antimicrobial activity to some extent.

Keywords: Antibacterial activity; fluoride; herbal; micro-organisms; toothpaste.

1. INTRODUCTION

Dental health has been defined by the WHO, as a state of complete normality and functional efficiency of the teeth and supporting structures, and that of the surrounding part of the oral cavity, and the various structures related to mastication and the maxillofacial complex. Dental caries is a multifactorial disease and is the outcome of a multiple complex process involving factors like diet, microorganisms, trace elements, saliva, genetic predisposition and tooth morphology.

Oral cavities harbour a wide variety of microorganisms, these are considered crucial for the initiation and progression of dental diseases [1]. Over 400 species of microbes inhabit as commensals in the oral cavity of a healthy adult. An aberration to this ecology due to dietary habits, improper oral hygiene or systemic factors lead to increased cariogenic microorganisms [1-3].

Cariogenic microorganisms like *Streptococcus mutans* and lactobacillus acidophilus are the primary causative microorganisms for the development of dental caries. These cariogenic microorganisms encourage the accumulation and adherence of plaque biofilm by metabolizing sucrose into sticky glucan. The microorganisms in dental plaque degrade the dietary carbohydrates producing lactic acid leading to localized demineralization and the eventual formation of dental caries [3–5]. Plaque – induced caries is a local disease, therefore, the local use of antimicrobial agents is more efficient than their systemic use [6].

Poor oral hygiene is one of the reasons for accumulation of microbes and their harmful activities [6–8]. Dental diseases are effectively avoidable by simple inexpensive and easy to practice personal hygiene habits [9,10]. Tooth paste is used to promote oral hygiene. Triclosan is a common antibacterial agent in the tooth paste.

Besides, the use of chemical agents has been proposed as a potential prophylactic method of reducing plaque-mediated disease [11].Most effective of them is the tooth brushing habit. Of various factors of this practice, antibacterial efficacy of the tooth paste has a major role to play in the outcome [12,13].

Recently, there has been increased interest in the various medicinal plants which have demonstrated anti-cariogenic activity in various studies. Herbal products contain antimicrobial agents and these formulations may be more appealing as they do not require alcohol, artificial preservatives, flavors or colours for their activity.

Considering the overflowing number of brands of numerous toothpastes in the market, the efficacy of these toothpastes in controlling the bacterial count has to be scientifically analyzed. Rising popularity of herbal and natural products has mandated dental professionals to evaluate the effectiveness of these products and provide evidence-based suggestions to their patients to make a better choice [14]. Also there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world over the last decade.

Antibiotic susceptibility testing is the method employed for testing the effectiveness of agents against specific microorganisms [15]. A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. Disk-diffusion assay offers many advantages over other methods such as simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided. The minimum inhibitory concentration is the minimum (lowest) concentration of an antibiotic that will inhibit the growth of a bacterial strain. According to the

epidemiological triad for the dental caries causes, there is a requirement to evaluate the host factors such as oral health knowledge, oral hygiene practices, dental visits, and eating habits among the school going children. We have successfully completed numerous epidemiological studies [16–23] and in vitro studies [24-27] for the betterment of our community [28-33]. Hence, the objective of the study was to compare the antimicrobial efficacy of herbal dentifrices on *Streptococcus mutans* and *Candida albicans*.

2. MATERIALS AND METHODS

2.1 Study Type

In-vitro study.

2.2 Study Organisms

Streptococcus mutans and *Candida albicans.*

Streptococcus mutans is a facultative anaerobic, gram-positive coccus (round bacterium) commonly found in the human oral cavity and is a significant contributor to tooth decay. This bacterium, along with the closely related species Streptococcus sobrinus, can co- habit the mouth. Both contribute to oral disease, and the expense of differentiating them in laboratory testing is often not clinically necessary. Therefore, for clinical purposes they are often considered together as a group, called the mutans streptococci.

Candida albicans causes oral thrush in which curd-like white patches form inside the mouth, on the tongue and palate and around the lips [15,34]. It may also cause cracked, red, moist areas of skin at the corner of the mouth. Candida species causes infections in immuno-compromised patients, individuals on drug therapy, and the chronically ill [35].

The study was scheduled in the month of October 2017.

2.3 Preparation of Dentifrices

Solutions of selected dentifrices were made by mixing 1 gram of dentifrices in 4 ml of distilled water to give 1:5 dilutions, in a sterile container. Further dilutions were made by mixing 1 gram of tooth paste with 9 ml and 14 ml to give 1:10 and 1:15 dilutions respectively.

2.4 Agar well Diffusion for Anti-microbial Assay

Two strains of microorganisms –*Streptococcus mutans* and *Candida albicans* were taken. The media used were Mutans media and Sabouraud dextrose agar media respectively for each microorganism respectively. These bacteria were inoculated in their respective medium by swab method.

2.5 Agar Disc Diffusion Method

Sterile discs were incorporated with equal amounts of toothpaste formulations using micropipette. Then the discs were placed equidistant to each other following which these plates were incubated for 24 hours.

2.6 Measurement of Zone of Inhibition

The test plates were held in front of a desk lamp, and the zones were measured with a ruler held against the back of the Petri plate. The diameters of the zones of inhibited growth were measured to the nearest whole millimeter.

3. RESULTS AND DISCUSSION

Results show that the zone of inhibition of various types of herbal dentifrices against *Streptococcus mutans*. Zone of inhibition is found to be higher in Ayush (30 mm), followed by Dant kanti, Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 26 mm, 24 mm, 17 mm and 14 mm respectively. Only Dantajeevan shows inhibition at 1:10 and 1:15 dilutions respectively. It also shows the zone of inhibition of various types of herbal dentifrices against *Candida albicans* shows that zone of inhibition is found to be higher in Ayush and Dant kanti with 18 mm zone of inhibition followed by Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 12mm and 10 mm respectively. Only Dant kanti shows inhibition at 1:10 dilution respectively.

Microbial biofilms are the cause for the dental caries and periodontal disease; therefore it is very important to control these biofilms by mechanical debridement which can be achieved with the use of adjunctive antimicrobials in the tooth pastes. Poor oral hygiene is solely responsible for prevalence of dental caries in our community [36]. Key to the prevention of dental

diseases is maintenance of good oral hygiene. Herbal extracts have received special attention because of being non-chemical and non-synthetic in nature, and have been used in traditional medicine [36-39]. Interest in natural-based toothpastes has increased since 2014 [40].

As there is a paucity of literature regarding the efficacy of different types oral microbial flora herbal dentifrices against oral microflora, this in-vitro study was conducted to test the antimicrobial efficacy of different types of herbal dentifrices against *Streptococcus mutans* and *Candida albicans*.

Antimicrobial efficacy of six different herbal dentifrices against *Streptococcus mutans* and *Candida albicans* were tested. Three different dentifrice formulations 1:5,1:10 and 1:15 were made by mixing distilled water with the dentifrice. The media used were Mutans media and Sabouraud dextrose agar media respectively for each microorganism respectively by swab method. The antimicrobial efficacy is then tested using a disc diffusion method.

In our study, antimicrobial efficacy formulations of dentifrice formulations against *Streptococcus mutans* were measured as zones of inhibition. The zone of inhibition is found to be higher in Ayush (30 mm), followed by Dant kanti, Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 26 mm, 24 mm, 17 mm and 14 mm respectively. Only Dantajeevan shows inhibition at 1:10 and 1:15 dilutions respectively. Similar results were obtained in the study done by Sabiha Shaheen et al. [41] in which herbal toothpastes such as Dant kanti show maximum antimicrobial efficacy. Also, few studies show that herbal toothpastes have similar and better antimicrobial activity. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world [42].

Zone of inhibition of various types of herbal dentifrices against *Candida albicans* shows that Zone of inhibition is found to higher in Ayush and Dant kanti with 18 mm zone of inhibition followed by Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 12mm and 10 mm respectively. Only Dant kanti shows inhibition at 1:10 dilution respectively. Similar results were obtained in the study conducted by Farogh Gabraiel et al. [7]; in which herbal toothpastes gave maximum zones of inhibition against Candida albicans. Okpalugo et al. [43] reported that the toothpaste brands considered for their study were not effective in reducing the oral microorganisms whereas the results of our study showed inhibition by all brands.

Herbal dentifrices show antimicrobial activity against the oral microflora hence it becomes necessary to preserve this traditional system of medicine by proper documentation and identification of species [44].

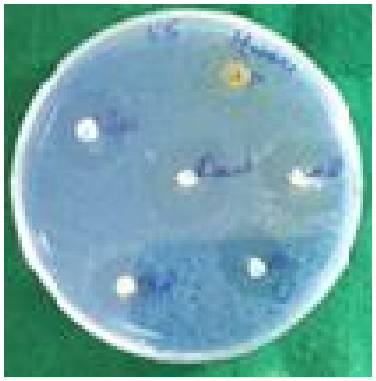

Fig. 1. Zone of inhibition of various types of herbal dentifrices against Streptococcus mutans. Zone of inhibition is found to be higher in Ayush (30 mm), followed by Dant kanti, Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 26 mm, 24 mm, 17 mm and 14 mm respectively. Only Dantajeevan shows inhibition at 1:10 and 1:15 dilutions respectively

Fig. 2. Zone of inhibition of various types of herbal dentifrices against Candida albicans. Zone of inhibition is found to be higher in Ayush and Dant kanti with 18 mm zone of inhibition followed by Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 12mm and 10 mm respectively. Only Dant kanti shows inhibition at 1:10 dilution respectively

Table 1. The inhibition zone of various types of herbal dentifrices against *Streptococcus mutans*. Zone of inhibition is found to be higher in Ayush (30 mm), followed by Dant kanti, Colgate vedshakti, Meswak and Dabur red showing inhibition zones of 26 mm, 24 mm, 17 mm and 14 mm respectively. Only Dantajeevan shows inhibition at 1:10 and 1:15 dilutions respectively

Serial Number	Dentifrices	Zones of Inhibition (mm)		
		1:5 Dilution	1:10 Dilution	1:15 Dilution
1.	Dantajeevan	14	12	10
2.	Dant kanti	26	0.00	0.00
3.	Colgate vedshakti	24	0.00	0.00
4.	Meswak	17	0.00	0.00
5.	Ayush	30	0.00	0.00
6.	Dabur red	14	0.00	0.00

Table 2. The inhibition zone of various types of herbal dentifrices against *Streptococcus mutans*. Zone of inhibition is found to be higher in Ayush and Dant kanti with 18 mm zone of inhibition followed by Colgate vedshakti, Meswak, Dantajeevan and Dabur red showing inhibition zones of 12mm and 10 mm respectively. Only Dant kanti shows inhibition at 1:10 dilution respectively

Serial number	Dentifrices	Zones of Inhibition (mm)		
		1:5 Dilution	1:10 Dilution	1:15 Dilution
1.	Dantajeevan	10	0.00	0.00
2.	Dant kanti	18	8	0.00
3.	Colgate vedshakti	12	0.00	0.00
4.	Meswak	12	0.00	0.00
5.	Ayush	18	0.00	0.00
6.	Dabur red	10	0.00	0.00

4. CONCLUSION

The present study was based on in-vitro experiments. Results of this study have shown that herbal toothpaste formulations were effective in controlling the oral microflora. The antimicrobial properties of the six dentifrices concluded that almost all the commercially available herbal dentifrices had antimicrobial activity to some extent to benefit dental health or antiplaque action.

Based on the results of the present study, it can be concluded that all herbal dentifrices exhibited antimicrobial activity against the selected oral microorganisms, with Ayush and Danth Kanthi being the most effective. Hence, it can be inferred that herbal dentifrices can also be recommended like the conventional formulations. However, future research efforts are needed for the evaluation of quality and efficacy of the herbal dentifrices for its regular use in the oral hygiene products. Further studies on the safety and efficacy of such products need to be established. Thus turning to nature can be as good as the latest advances.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Prior to the start of the study ethical clearance was obtained from the institutional ethics committee, Saveetha University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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