

COMPARATIVE STUDIES ON ANTIBACTERIAL ACTIVITY OF HERBAL DENTIFRICES AGAINST *STREPTOCOCCUS MUTANTS****Rachana Pachori, Dipali Gore, Nikhilesh Kulkarni and Prithviraj Sadar**

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College, Washim, (M.S),
India.**ABSTRACT**

Tooth decay and periodontal diseases are one of the most common bacterial infections infecting the world population. In India, a very significant proportion of dental problems are due to microbial infections. A wide range of chemicals mainly antimicrobial agents have been added to toothpaste in order to produce a direct inhibitory effect on plaque formation. Now- a- days, herbal tooth paste and tooth powder has gained more attention due to the side effects caused by chemicals generally used in the preparation of dentifrices. However, there is no alteration in the rate of periodontal diseases as well as tooth decay among the population also many herbal dentifrices claim to have

antimicrobial properties and give assurance for dental protection. Hence, the present study was carried out to investigate the antibacterial efficacy of different locally available herbal toothpaste and toothpowders against *Streptococcus mutans* which is reported to be the most predominant oral pathogen associated with dental diseases. The findings suggest that the dentifrice containing Akarkara was found to possess good antibacterial activity against the test pathogen. It is also suggested that brushing the teeth for 15 minutes will allow enough contact time for toothpaste/powder to act on oral microbes and importantly pathogens for maximum result of good oral hygiene. Hence, herbal dentifrices could be exploited for teeth protection.

KEYWORDS:- Periodontal diseases, antibacterial activity, *Streptococcus mutans*, Akarkara, herbal dentifrices.

INTRODUCTION

Dental caries and periodontitis is the second most prevalent infection affecting all age groups globally. It ranked the second most prevalent disease in humans as well as the common

chronic childhood disease of children aged 5 to 17.^[1-2] Dental problems are of three types, formation of dental plaques, dental caries and periodontal diseases.^[3] In India, a very significant proportion of dental problems are due to microbial infections. Among the wide variety of oral microflora, *Streptococcus mutans* is frequently reported to be the prime organisms associated with plaque formation. The pathogenicity of *Streptococcus mutans* is explained by its strong affinity for the tooth surface. It adheres to salivary acquired enamel pellicles and contributes to dental biofilm maturation.^[4]

The American Dental Association (ADA) describes toothpaste as a paste or dentifrice used with the aid of toothpaste to cleanse and maintain the aesthetic and well being of the buccal cavity (tooth). Toothpaste and brushes are among the physical forces that remove plaque. Brushing of teeth using toothpaste is the most commonly practiced form of oral hygiene in most countries.^[5] It serves as an abrasive which helps in removing dental plaque and food particles from the teeth, assist in suppressing halitosis and release active ingredients mainly fluoride.^[6] The success of any toothpaste, in part, lies on its ability to eliminate pathogenic oral microflora.^[7] Dentifrices need to contain various antimicrobial agents in order to reduce, control and prevent different kinds of dental diseases.

Many different herbal as well as synthetic products in the form of paste, powder or gel are currently marketed that promise to provide consumers with fresh breath. In the last decade many consumers have switched over to herbal dentifrice in order to avoid synthetic and artificial flavors commonly found in regular toothpastes. Many herbal dentifrices claim to have antimicrobial properties and give assurance for dental protection. However, very little research has been traced to investigate the antimicrobial activity of those claims. Hence, the present study was initiated to determine the antimicrobial efficacy of herbal toothpaste/toothpowder against *Streptococcus mutans*.

MATERIALS AND METHODS

Collection of clinical sample

The pus samples (n=10) were collected in sterilized containers from patients infected with dental abscess and tooth decay using sterile cotton swab from local dental clinic located in Washim city area and transported immediately to Microbiology Research Laboratory, R. A. College, Washim.

Enrichment and isolation of *Streptococcus mutans*

The collected pus samples were enriched in nutrient blood broth fortified with 10 % serum v/v. and incubated anaerobically at 37°C for 48 hours. After incubation period, the enriched cultures were further inoculated on blood agar medium for isolation of *Streptococcus mutans*.

Identification of *Streptococcus mutans*

The developed colonies on blood agar medium showing γ -hemolysis were putatively considered as *Streptococcus mutans* and further confirmed on the basis of conventional methods viz. morphological, cultural and biochemical characteristics adopting Bergey's Manual of determinative bacteriology.

Selection of dentifrices

Three locally formulated herbal dentifrices viz. one containing cow dung ash (CDA), second with Vajradanti (VJD) and third containing Akarkara (AKK) as a principal ingredient were selected for observing the antibacterial activity against *S. mutans*. The dentifrices were used in the form of paste as well as powder for the present research work.

Preparation of toothpaste /toothpowder Samples

The toothpastes/toothpowder slurry was prepared by diluting them at 1:10 w/v concentrations in distilled water.

Antibacterial activity Testing

The antibacterial activity of the selected toothpaste/ toothpowder was determined by both disk diffusion method^[8] as well as well diffusion method.^[9]

Determination of Rideal walker coefficient

The effective antibacterial toothpaste/toothpowder was selected for determining the Rideal walker coefficient test with slight modification.^[10]

Time kill test

The time killing test with slight modification was performed using the effective concentration of toothpaste with reference to 10% phenol.^[11]

RESULTS

Table 1 represents the comparative findings on antibacterial activity of different dentifrices against *Streptococcus mutans* by disc and well diffusion method. It was found that the

antibacterial testing technique affects antibacterial activity of the dentifrices. In case of AKK paste (18 mm), VJD paste (12mm) and AKK paste/ powder (21 mm) VJD paste/powder (10mm) decreased zone of inhibition was observed in Disc diffusion method as compared to Well diffusion technique while in case of CDA paste(14mm) CDA powder(10mm) AKK paste + powder (13mm) same zone of inhibition was observed by both the techniques. However, AKK paste (19 mm) as well as VJD paste (16 mm) and AKK mix(28mm) and VJD mix (15mm) showed increased zone of inhibition and AKK powder showed (12mm) and VJD Powder (10mm) showed decreased Zone of inhibition in well diffusion method as compared to disc diffusion technique. Hence, the mean of inhibition zone obtained by both the techniques was considered for selecting the effective Toothpaste against *Streptococcus mutans*.

The findings on comparative Studies on antibacterial activity of different dentifrices showed that AKK paste/powder mix showed highest zone of inhibition in disc as well as well diffusion method. The mean zone of inhibition was found to be (24mm) followed by AKK paste (18mm), AKK powder (15mm). The zone of inhibition (14mm.) exhibited by VJD and CDA paste was found to be at par against *Streptococcus mutans*. The CDA paste/ powder mix showed zone of inhibition 13mm. followed by VJD paste/ powder mix(12mm, VJD powder(11mm.) and least antibacterial activity against *Streptococcus mutans* was found in CDA powder (10mm). Hence, both AKK paste as well as powder was considered to be the most effective dentifies against *Streptococcus mutans*.

Table 1: Comparative Studies on antibacterial activity of different denitrifies against *Streptococcus mutans* by disc and well diffusion technique.

Sr. No.	Toothpaste name	Zone of inhibition by Disk Agar Diffusion Method	Zone of inhibition by Well agar diffusion method	Mean
Paste	VJD paste	12 mm	16 mm	14mm
	AKK paste	18 mm	19mm	18mm
	CDA paste	14 mm	No zone	14mm
Powder	VJD powder	12 mm	10 mm	11mm
	CDA powder	10 mm	10mm	10mm
	AKK Powder	19 mm	12 mm	15mm
Powder+ Paste	AKK paste + Powder	21 mm	28mm	24mm
	CDA paste + Powder	13 mm	13mm	13mm
	VJD Paste + powder	10 mm	15 mm	12mm

Table 2 represents the findings on determination of Rideal walker coefficient of AKK paste/powder mix. From the table it was observed that in 6% AKK paste/powder mix solution *Streptococcus mutans* showed growth in 5 min. However, growth was inhibited in 10 min. which is comparable with 5% phenol concentration. Hence, 6% AKK paste/powder mix concentration was considered for determining the Rideal walker coefficient using the formula:

DC= Concentration of toothpaste killing bacteria in 10 min but not in 5 min

Concentration of phenol killing bacteria in 10 min but not in 5 min

$$DC = \frac{6}{5} = 1.2$$

The Rideal walker coefficient of AKK paste/powder mix was found to be 1.2 which represents that it possess effective disinfecting property and can be used against dental problem especially caused due to *Streptococcus mutans*.

Table 2: Determination of Rideal walker coefficient of Toothpaste:

Toothpaste	concentrations	Growth of test organisms in subculture after exposure for		
		5min	10 min	15 min
AKK paste	1%	G	G	G
	2%	G	G	G
	3%	G	G	G
	4%	G	G	G
	5%	G	G	G
	6%	G	NG	NG
	7%	NG	NG	NG
	8%	NG	NG	NG
	9%	NG	NG	NG
	10%	NG	NG	NG
Phenol	5%	G	NG	NG

NG= No Growth G= Growth.

Table 3 represents the findings on surface time kill test. It was found that in control, the growth inhibition was not observed upto 15 min. However, when the test organism was treated for 15 min. with AKK paste/powder mix, no growth was observed which indicates that the contact time for AKK paste/powder mix is 15 min. Hence, the Mouth should be treated with AKK paste/powder mix for the period of 15 min for decontamination so as to prevent from dental problems.

Table 3: Determination of Time kill test

Treatment	Growth of test organisms in subculture after exposure for		
	5min	10 min	15 min
Control (without Toothpaste)	G	G	G
Test (AKK paste)	G	G	NG

NG= No Growth G= Growth

DISCUSSION

Dentifrices are generally used for the removal of germs and for cleaning purpose. Dentifrices usage is very common and now a day's especially antibacterial Toothpaste are very popular. According to company's claim their antibacterial Dentifrices are bacteria killers. So with the use of antibacterial dentifrices we can get dual functions, removal as well as killing of bacteria. The purpose of the study was to determine the bactericidal activity of herbal toothpaste being used in our daily life. Among the tested herbal toothpaste, Akarkara containing toothpaste as well as powder was considered to be more effective in inhibiting *Streptococcus Mutans* isolated from oral specimens of patient infected from dental abscess. This study also suggests that the AKK paste/powder possess effective disinfecting property as compared to Phenol against *Streptococcus Mutans* bacteria. The contact time is also one of the important parameter in considering the antibacterial activity of dentifrices.

The herbal toothpaste/ powder usually contains combination of different herbs and some ayurvedic ingredients viz. Akarkara, Neem, Babool, Tomar, Pudina, Laung, Pippli, Vajradanti, Bakul, Vidang, Haldi, Pilu, Tulsi Majuphal etc.^[12] All the herbs are reported to be effective in maintaining the strength of teeth and gums. They also help to stop the bleeding and inflammation of the gums. The dentifrices provide a refreshing smell and can help people get rid of bad breath. It helps to fight against dental problems like pyorrhea, dental carries, bleeding & swollen gums, sensitivity and yellowing of teeth, Gingivitis, Toothache, Bad Breath, Spongy & Bleeding Gums. Secondary metabolites such as alkaloid, fluorides and polyphenols present in the herbs used for the production of herbal dentifrices are responsible for their antibacterial activity.^[13] The synergistic interaction between the herbs and some ayurvedic ingredients may help to increase the effectiveness of herbal dentifrices. However, this synergistic activity needs to be established. The findings of present study are in accordance with the researchers working on same line of research.^[3,14-16] Hence, the findings of present research investigation suggest that herbal dentifrices especially Akarkara

containing toothpaste/ powder are effective against dental carries and can be used without side effects. The brushing should be done for at least 15 min. with both toothpaste/ powder for achieving good antibacterial activity.

CONCLUSIONS

The problem of dental caries is increasing at faster rate and is encountered frequently. The bacteria *Streptococcus mutans* is recognized as the chief etiological agent in human dental caries.

Dentifrices are one of the most important agents in preventing the dental caries. It has been prepared in several forms such as powders, pastes and gels. The present research analysis recommends potential use of herbal dentifrices in defending the tooth deterioration and other dental complications as well for sustaining healthy oral cavity.

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