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A Preliminary Study on Herbal Composite: Comparative Analysis with Commercial Ayurvedic Toothpaste for The Prevention of Dental Pathogens of Dental Caries Cases

KEYWORDS: Dental caries, minimal inhibitory concentration, antibacterial activity, ayurvedic toothpaste, herbal composite

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ABSTRACT *Herbal composite was developed using three different medically significant herbs in the present study. The composite was developed with the aim of inhibiting the growth of dental pathogens that causes dental caries. Different dental pathogens were isolated from outpatients' dental department, Kerala. Dental isolates were subjected to antibacterial activity against different herbs. After screening three herbs, minimal inhibitory concentration of individual herbal extracts were carried out. The MIC of the herbs was compared with the sensitivity pattern of antibiotic disc diffusion assay. Three trials were made using three different concentrations of herbal composites. Finally the prepared herbal composite was compared for its antibacterial activity with commercial ayurvedic toothpaste against the dental isolates. The prepared herbal composite showed good antibacterial activity than the commercial ayurvedic toothpaste against all the dental isolates.*

1.0 INTRODUCTION

Poor oral hygiene results in plaque accumulation which is a major etiological factor in occurrence of dental caries, gingival and periodontal diseases (Omar, 2013). Bacteria form an important group of microorganisms found in both healthy and diseased mouths as more than 300 types of bacteria are found in the oral cavity. Plaque is a complex bio film found on the tooth surface which forms the major reason of the development of dental caries. The accumulation and development of plaque depends upon the outcome of the interactions between the adhesiveness of plaque to the tooth surface and the physical shear forces which serve to dislodge and remove the plaque (Benson et al., 2004). Dental caries is a localized and transmissible pathological infectious process that results in the destruction of hard enamel tissue. Streptococcus mutans, an acidogenic and aciduric microorganism colonizing the oral cavity, is considered to be the main cause of dental caries (Robertson and Smith, 2009).

Chemoprophylactic agents that are used in dental caries prevention include penicillin and vancomycin; cationic agents such as chlorhexidine and cetylpyridinium chloride; and non-ionic agents such as triclosan (Chen and Wang, 2010). Chlorhexidine is one of the most tested compounds and its anti-plaque properties are well-known. In a supragingival biofilm model, chlorhexidine was shown to inhibit bacterial growth and biofilm formation. Because chlorhexidine is positively charged, it binds to various surfaces including enamel pellicle, hydroxyapatite and mucous membranes. However, the retention of chlorhexidine on tooth surface also leads to its an undesirable side-effect which is tooth staining and calculus formation (Anderson, 2003). The strategy for the oral disease is changing to the prevention from the treatment and reconstruction. The population using the preventive remedies such as mouthwash, dental floss and fluoridation have increased (Furgang et al., 2003).

Previous studies have revealed that dental plaque can be controlled by physical removal and use of various pharmaceutical formulations like antimicrobial toothpastes and mouthwashes. Mechanical plaque control methods include tooth brushing and interdental cleansing using oral hygiene aids and professional prophylaxis. Chemical plaque control has been used only as adjunct to mechanical means and not a substitute even though various chemicals are widely used nowadays. Toothpaste is by far the most widespread form of fluoride usage and the decline in the prevalence of dental caries in developed countries is mainly attributed to its increased use. Fluoride therapy has been the cornerstone of caries preventive strategies since the introduction of water fluoridation schemes over five decades ago. Since the 1980s nearly all commercially available toothpaste formulations contain fluoride. The intensive promotion of fluoridated toothpastes by the oral health care industry is a major factor in their increased use (Ramji et al., 2005).

Natural products have been used for several years in folk medicine (Omar, 2013). Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world (Chitme, 2003). Over the last decade herbal medications in both prophylaxis and treatment of various diseases turned to be a popular form of therapy throughout the world. Many side effects associated with traditional medicines have been averted by using herbal medicines and thus they are safer to use. Several natural products are marketed for oral and dental use to satisfy the shift to usage of natural products from pharmaceutical products among the public. These alternative products can be either dental products with natural ingredients or herbal products. Different herbs have been included in dental products as: Bloodroot, Carawy, Chamomile, Echinacea, Peppermint, Rosemary, Thyme, Aloe vera, Green tea, Fennel, Ginger, Salvadora persica (meswak extract), Clove oil, Eucalyptus and Nigella sativa oil. Toothpastes and oral rinses are usually marketed as cosmetic products. Both products are used to maintain good oral hygiene and counteract bad breath (Omar, 2013). According to Omar, (2013) researchers should be encouraged to conduct controlled studies to prove the effectiveness and safety of natural dental products. Those studies will provide

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dentists with sufficient clinical evidence before prescribing promoted natural products for their patients. Considering this conceptual approach, in the present study, herbal composite was prepared using different combinations of medically significant herbs.

3.0 MATERIALS AND METHODS

3.1 Isolation of dental pathogens collected from outpatients' dental department (Robertson and Smith, 2009)

A total of 100 clinical samples were collected from out patient's dental department, Unity Hospital, Kunamkulam, Kerala, India for a period of 7 months (August 2008 – Feb 2009). Of the 100 cases, twenty-five cases constituted the control group. The samples were collected from different legions in oral cavity and the carious teeth by excavating the deeper layers. From the collected dental samples, the aerobic and anaerobic bacterial strains were isolated by growing them at 37° C on nutrient broth for 48 hours.

3.2 Extraction and antibacterial assessment of selected herbal plants (Kumar et al., 2007)

3.3.1. Collection of live and healthy herbal plants

Live and healthy herbal plant parts of Banyan leaves (Ficus benghalensis), Neam leaves (Azadirachta indica), Prickly chaff flower (Achyranthes aspera), Babool bark (Acacia nilotica), Clove flower (Syzygium aromaticum), Cardamom seed (Elettaria cardamomum) and Karpuravalli leaves (Coleus amboinicus) were collected from different ayurvedic shops in and around Tamil Nadu and Kerala which were authenticated by the Botanical survey of India, Coimbatore. After harvesting, the selected herbs were extracted using ethanol by the method described by Kumar et al., (2007).

3.3.2. Antibacterial assessment of herbal extracts in comparison with antibiotics (Rojas et al., 2006)

The antibacterial activities of the herbal extracts were compared with the standard antibiotics such as ampicillin, chloramphenicol, tetracycline, roxythromycin, ciproflaxin and co-trimaxazole by well diffusion method against the isolated dental pathogens.

3.3.3. Minimal inhibitory concentration of individual herbal extracts and in combinations (Rojas et al., 2006)

The antibacterial activities of the selected herbal extracts were tested against the isolated dental pathogens by well diffusion method. The herbal extracts with maximum antibacterial activity against the pathogens were selected for the combinatorial studies. Different combinations were prepared with the selected herbs to assess the combinatorial effect on the dental pathogens. The minimal inhibitory concentration of the different herbal combinations was determined by standard well diffusion method.

3.4.2. Antibacterial activity of commercial ayurvedic toothpaste in comparison with developed herbal composite (Okpalugo et al., 2009)

Combination of clove, babool and karpuravalli was selected for the development of herbal composite based on their antibacterial activity and MIC. Three herbs were mixed in the ratio of 1:1:1 for the composite preparation. The composite prepared in three different strengths like 0.5%, 1% and 5% was compared with commercial ayurvedic toothpaste to determine the antibacterial activity. The determination of developed herbal composite and commercial toothpastes against dental isolates was assessed through standard well diffusion method. The isolates were inoculated onto Muller-Hinton agar plates using sterile cotton swab. Wells were punctured (6mm) and loaded separately with developed herbal composite (at a concentration of 1g/ml) and commercial tooth pastes (50 µl). All the inoculated plates were incubated at 37 °C for 24h to observe zone of bacteriostasis (mm) around each well against each of the dental isolates.

4. Results and Discussion

4.1 Isolation of dental pathogens collected from outpatients' dental department

A total of 100 clinical samples were taken from patients at outpatient dental department Unity Hospital, Kerala, India for a period of 7 months. Of the 100 cases, twenty-five cases constituted the control group (based on the non-occurrence of any orodental infection or any oral manifestations). 25 patients each constituted the dental caries, gingivitis and periodontitis. The samples were collected from different legions in oral cavity and from the carious teeth by excavating the deeper layers with the help of a sterile excavator from the patient with dental caries and dental decay.

Table-2: Demographic characteristics of the studied population

S. No.	Variable		No. of patients
1	Sex	Female	69
		Male	31
2	Age group	< 15 years	8
		16 – 20 years	5
		21 – 30 years	6
		31 – 40 years	13
		41 – 50 years	17
		51 – 60 years	24
		61 – 70 years	16
		> 70 years	11
3	Marital status	Married	41
		Single	43
		Free Union	2
		Widowed	8
		Divorced / separated	6

In Table-2, the demographies characteristics of the studied population were presented. Among the 100 selected patients, 69% were female and 31% were male. About 8% of the patients were found belong to the age group of less than 15 years of age. Higher percentage of patients constituted the 51 – 70 years age group. Ages of the patient were ranged from 5 to 92 years (mean ± SD: 30.4 ± 15.2 years).

Kumar et al., (2007) demonstrated the demographic characteristics of the age, sex, and ethnic distribution of the 45 subjects whose serum samples were not suitable for analysis for serum markers. The samples were processed to isolate the aerobes and anaerobes from the patients. Meurman et al., (1997) reported in their study that there is increasing evidence that oral infections may also play a role in the pathogenesis of many systemic diseases, and this may occur not only in ill and immunocompromised individuals, but also in those who are healthy. This may be due to biofilm formation of aerobic and anaerobic dental flora. Socransky et al., (2002) provided an evidence to support this statement. The researchers proved that, bacterial populations attached to tooth surfaces consist of biofilm communities' sometimes 50-100 cells in thickness and with a bacterial density of up to 1011 CFU/mg. The biofilms that colonizes tooth surfaces may be among the most complex that exist in nature.

4.2 Isolation of aerobic and anaerobic pathogens using standard culture techniques

Among the 100 selected patients, the study group was divided into four groups each constituting 25 patients. In all the four groups both aerobic and anaerobic organisms were isolated. When compared to control group dental caries and gingivitis patients were found to comprise more number of aerobic isolates. Interestingly more anaerobic organisms were found in case of periodontitis followed by gingivitis. This was very common that both infections were specifically caused by only anaerobic bacterial pathogens. From Table-3, it was observed that totally about 73 isolates were screened from 25 patients with periodontitis, followed by 64 from gingivitis and 62 from dental caries cases. The obtained result thus showed that dental infections were caused by anaerobic organisms than the aerobic organisms.

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S.No	Isolates		Study group (n = 25)			
			Control	Dental caries	Gingivitis	Periodontitis
1	Aerobic isolates		26	29	27	25
2	Anaerobic isolates		21	33	37	48
3	Mono-microbial		3	2	1	0
4	Poly-microbial	2 isolates	14	9	7	8
5		3 isolates	4	13	16	11
6	Total no. of isolates		47	62	64	73

Table-3: Number of aerobic and anaerobic isolates from study groups'

The subject study analysis showed more anaerobic pathogens associated with the dental and oral sites, which could be fall under the genera of different bacteria viz; Prevotella sp, Fusobacterium sp, Streptococcus sp, etc. Robertson and Smith (2009) studied the acute dental abscess caused by similar group of organisms. They reported that the dentoalveolar abscess is polymicrobial comprising various facultative anaerobes, such as the viridans group Streptococci and the Streptococcus anginosus group, and strict anaerobes, especially anaerobic cocci, Prevotella and Fusobacterium species. In support to this, Smith et al., (2001) studied the ecology of Staphylococcus sp in the oral cavity of many patients. They concluded that the most commonly found facultative anaerobes belong to the viridans group Streptococci and the anginosus group Streptococci. Kuriyama et al., (2000) studied the bacteriological features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. They revealed that a complex mix of strict anaerobes and facultative anaerobes were accounted for most infections (59–75%).

4.3 Identification of aerobic and anaerobic pathogens from the study group

In Table-4, different aerobic and anaerobic isolates were identified based on Bergey's Manual. Even though more anaerobic organisms were isolated during the screening process of study groups', interestingly more facultative anaerobic Staphylococcus sp and Streptococcus sp. were identified from control, dental caries, gingivitis and periodontitis groups.

Table-4: Identification of dental isolates from the study group

S.No.	Isolates	Study group			
		Control	Dental caries	Gingivitis	Periodontitis
1	Enterobacter aerogens	0	4	9	10
2	Staphylococcus aureus	10	11	14	15
3	Coagulase negative Staphylococcus	9	10	9	9
4	Streptococcus	16	14	10	19
5	Klebsiella spp.	1	9	1	2
6	Pseudomonas spp.	3	6	0	5
7	Pseudomonas aeroginosa	4	1	2	2
8	Escherichia coli	3	2	4	2
9	Prevotella intermedia	0	3	1	1
10	Porphyromonas gingivalis	0	3	6	7
11	Yeast	1	0	8	1

According to Smith et al., (2001) Staphylococcus species have not been considered to be the members of oral flora or to play a major role in the pathogenesis of oral infections. However, a number of more recent studies have indicated that Staphylococci may indeed be a more frequent colonizer of the oral tissues than previously thought. From the study groups' total of 11 different microorganisms were identified. Among them two significant strict anaerobic organisms were also identified in all three cases of caries, gingivitis and periodontitis. According to Jacinto et al., (2006) the most commonly reported anaerobic Gram-negative bacilli from acute dento-alveolar infections are species from the pigmented Prevotella intermedia group (comprising Prevotella intermedia, Prevotella nigrescens and Prevotella pallens). The researchers also reported that the recovery rates using conventional culture of Staphylococcus aureus from the acute dental abscess range from 0.7 to 15%. Recovery rates of coagulase-negative strains of Staphylococci (usually reported as Staphylococcus epidermidis) are generally higher with figures ranging from 4 to 65%.

4.4 Extraction and antibacterial assessment of herbal plants collected from different ayurvedic shops in and around Tamil Nadu and Kerala

4.4.1 Antibacterial assessment of herbal extracts in comparison with antibiotics

The selected herbal ethanol extracts when subjected to antibacterial activity against tooth decaying isolates such as S. aureus, E. coli, Proteus sp., Pseudomonas sp. and Klebsiella sp., showed varied inhibitory zones ranging from 10mm to 18mm. The variation was due to antibacterial susceptibility and resistant patterns of the isolates for the selected herbs. Interstingly no inhibitory zones were detected for Achyranthes aspera (Prickly chaff) when tested against all five dental isolates. Among the seven herbs, Babool bark (Acacia nilotica), Clove flower (Syzygium aromaticum) and Karpuravalli leaves (Coleus amboinicus) showed maximum inhibitory zones against the four isolates except Proteus sp. In Table-5, the zone of bacteriostasis of the selected herbs against the isolates was recorded.

Table-5: Antibacterial activity of selected herbs against the dental isolates

S.No	Name of the Herb	Zone of Bacteriostasis (mm) against tooth decaying isolates				
		S. aureus	E. coli	Proteus Sp.,	Pseudomonas Sp.,	Klebsiella Sp.,
1.	Banyan leaves	16	10	0	0	0
2.	Neem leaves	15	10	0	0	10
3.	Prickly chaff	0	0	0	0	0
4.	Babul	18	17	0	15	12
5.	Clove	10	13	0	10	10
6.	Cardamom	0	10	0	0	0
7.	Karpuravalli	12	13	0	10	11

The antibacterial activity of selected herbs against the dental isolates was compared with standard antibiotics. In Table-6, the bacteriostatic zone was recorded for all the antibiotics against dental isolates. Ciprofloxacin showed maximum inhibitory zones against all the pathogens when compared to other antibiotics. The isolates also showed sensitivity to tetracycline and roxythromycin next to ciprofloxacin. The sensitivity pattern of ciprofloxacin, tetracycline and roxythromycin emphasized that the dental isolates were

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sensitive to babool, clove and karpuravalli extracts. The antibacterial activity of the herbs thus provided sufficient justification for the formulation and development of herbal composite.

Table-6: Antibacterial activity of commercial antibiotics against dental pathogens

S. No.	Antibiotics	Test Isolates			
		S. aureus	E. coli	Klebsiella sp	Pseudomonas sp
1	Ampicillin	10	15	24	02
2	Chloram phenicol	9	24	29	05
3	Tetracycline	15	26	32	09
4	Roxythromycin	20	18	16	12
5	Ciprofloxacin	18	20	32	18
6	Co-trimoxazole	9	25	32	02

4.4.2 Minimal inhibitory concentration of individual herbal extracts

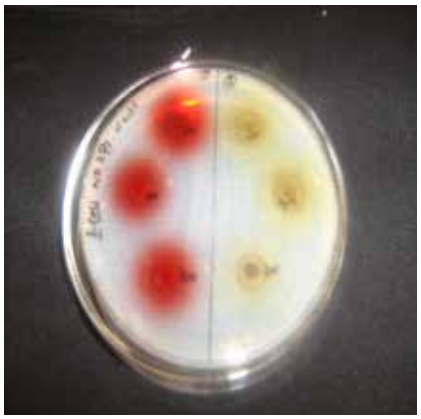
In Table-7, zone of bacteriostasis against dental isolates were presented for three different known concentrations of herbal extracts (20, 40, 60µg/ml). From these three concentrations, MIC of selected herbs was screened based on the inhibitory zones. Eventhough certain inhibitory zones were measured, based on the significant level of inhibitory zones, MIC values were recorded for all the herbs. Significant inhibitory zones of 12mm, 22mm, 10mm and 10mm against S. aureus, E. coli, Pseudomonas sp and Klebsiella sp. respectively were obtained for 60µg/ml of Babool extracts. Similarly, minimal inhibitory concentration of 60µg/ml was obtained for karpuravalli extract against all the dental isolates. Whereas, 40µg/ml was considered significant MIC for clove extract with maximum inhibitory zones of 13mm, 11mm, 12mm and 10mm against S. aureus, E. coli, Pseudomonas sp and Klebsiella sp. respectively.

Table-7: MIC of selected herbs against dental isolates

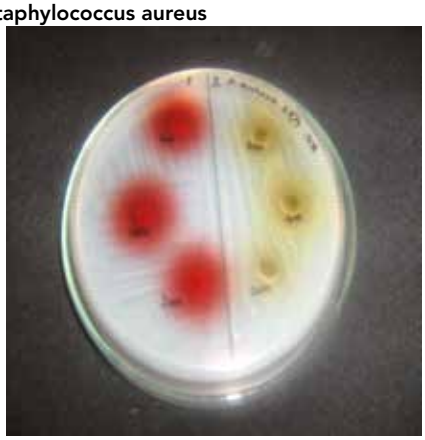
S. No.	Name of the Herb	Concentration (µg/ml)	Zone of Bacteriostasis (mm) against tooth decaying isolates			
			S. aureus	E. coli	Pseudomonas Sp.,	Klebsiella Sp.,
1	Babool	20	7	0	0	0
		40	8	0	0	0
		60	12	22	10	10
2	Clove	20	10	9	0	0
		40	13	11	12	10
		60	13	17	15	17
3	Karpuravalli	20	0	0	0	0
		40	0	0	0	0
		60	13	14	12	13

Figure-1: MIC of selected herbs against dental isolates

Escherichia coli



Staphylococcus aureus



Pseudomonas aeruginosa

Klebsiella sp

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4.5 Antibacterial activity of commercial toothpaste in comparison with developed herbal composite

In Table-9 and Figure-2 the antibacterial activity of prepared herbal composite was analyzed against the significant dental isolates. Among the three trials developed 5% herbal composite relatively more antibacterial activity than the first and second trial. Third trial showed good antibacterial activity with inhibitory zones, 16mm, 16mm, 21mm and 19mm against Staphylococcus sp., E. coli, Klebsiella sp. and Pseudomonas sp. respectively. The obtained inhibitory zone in mm was compared to sensitivity pattern of standard antibiotic disc assay. When compared it was observed that the zone of inhibition was considered significant to retard growth or biofilm formation of dental pathogens.

Table-9: Assesment of antibacterial activity of formulated herbal tooth paste

S. No	Selected pathogen	Zone of inhibition (mm)			Commercial toothpaste			
		Trail I	Trail II	Trail III	1	2	3	4
1	Staphylococcus sp.	15	14	16	15	12	14	14
2	E. coli	13	16	16	09	10	08	09
3	Klebsiella sp.	12	18	20	06	09	11	10
4	Pseudomonas sp,	14	16	18	09	08	08	07

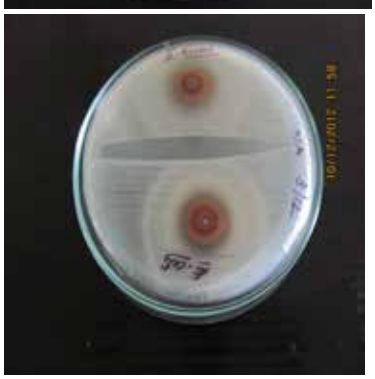
1: Dabur Red, 2: Meswak, 3: Dabur babool, 4: Himalaya herb

Figure-2: Assesment of antibacterial activity of herbal composite

(Trail-1)

(Trail-2)

(Trail-3)



Conclusion

Dental infections were found common in all age groups of people due to several routine activities. Flouride based toothpaste eventhough used widely with chemical base and mouthwash, still it may not found satisfactory in many people as one of the best to inhibit dental pathogens or biofilm formation. This was mainly due to increase in microbial resistance to the chemical based toothpaste. Hence in this study, herbal composite was developed using three different medically significant herbs, babool, clove and karpuravalli. All the selected herbs were already proved to possess good activity against dental organisms. So combination of the three herbs was done at a combinatorial ratio to exhibit the properties of individual herbs which was found satisfactory for the formulation and development of herbal toothpaste in future. The developed toothpaste was also found good in inhibiting the growth of dental isolates in the present study. Optimization of several other ingredients with different set of herbal extracts could be good commercial toothpaste which can be studied in near future.

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