

Bacterial analysis on dentistry and the study of antibacterial activity using formulated herbal toothpaste

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ABSTRACT

The current investigation dealt with studies on isolation and identification of oral biofilm forming bacteria and using formulated herbal toothpaste for inhibition. In this study, the bacterial populations were observed, and three were isolated from the used denture and different toothbrushes from various persons. From the collected oral samples, the streptococci, staphylococci, and bacilli were isolated and confirmed by biochemical tests. The increasing awareness of the environment and healthy lifestyles are generating some demand for more natural products such as those containing herbal extracts, so some of the indigenous medicinal plants were identified and formulated as herbal toothpaste; the efficiency of herbal toothpaste in inhibiting the growth of periodontal bacteria were evaluated. The bacterial inhibition rate and culture turbidity were observed in using herbal toothpaste against isolated pathogenic oral bacteria. Results were discussed.

KEY WORDS: Bacteria, dentistry, herbal toothpaste, medicinal plants

INTRODUCTION

The role that the human microbiome plays in health and disease has become a major area of interest and has revealed a number of novel links to disease (Cho and Blaser, 2012). More than 700 bacterial species, of which over 50% have not been cultivated, have been detected in the oral cavity (Aas *et al.*, 2005). Different predominant species typically associated with periodontitis. There is a distinctive predominant bacterial flora of the healthy oral cavity that is highly diverse and site and subject specific. It is important to fully define the human microflora of the healthy oral cavity before we can understand the role of bacteria in oral disease (Aas *et al.*, 2005). The major features that distinguish biofilm forming bacteria from their planktonic counterparts are their surface attachment ability, high population density, extracellular polymeric substances slime, and a wide range of physical, metabolic, and chemical heterogeneities (Beer and Stoodley, 2006).

The oral cavity is comprised many surfaces, each coated with a plethora of bacteria, the proverbial bacterial biofilm.

Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans. In addition, specific oral bacterial species have been implicated in several systemic diseases such as bacterial endocarditis (Berbari *et al.*, 1997). Other investigators have determined the bacterial diversity of saliva (Sakamoto *et al.*, 2000), subgingival plaque of a subject with gingivitis (Kroes *et al.*, 1999), and dent alveolar abscesses (Dymock *et al.*, 1996; Wade *et al.*, 1997). The temporal variability of the human microbiome may be an important factor in determining its relationship with health and disease. It was charted the temporal variability of the salivary microbiome, suggesting that bacterial diversity is stable, but that 16S rRNA gene copy number may be subject to seasonal flux (Cameron *et al.*, 2015). Over half of the species detected have not yet been cultivated, but still only limited information is available on species associated with health. Recently, Mager *et al.* (2003) demonstrated significant differences in the bacterial profiles of 40 oral cultivable species on soft and hard tissues in healthy subjects. They also found that the profiles of the soft tissues were more similar to each other than those of supragingival and subgingival plaques.

Different parts of plants, herbs, and spices have been used for many years for prevention of infections. The herbal preparation symbolizes more beneficial than the synthetics that are regarded as unsafe for human health. A medicinal herb contains a variety of natural chemical constituents, therapeutic agents, and active chemical constituent with different medicinal property (Velmurugan *et al.*, 2011). The increasing demand of plant extracts the use in the cosmetic, food, and pharmaceutical industries suggests that systematic studies of medicinal plants are very important to find active compounds and their use as a medicine for curing various diseases (Nostro *et al.*, 2001). Herbal medicine practice plays an important role in the primary healthcare delivery system in most of the developing countries. Traditional medicines are used by about 60% of the world's population. These are not only used for primary health care just in rural areas but also in developed countries, where modern medicines are predominantly used (Kamboj, 2000). The traditional medicines are derived from medicinal plants. Indian systems of medicine derive many of their curative tools from plants (Kumar *et al.*, 2005) which are used as drugs. Information is often found in the old literature (Atharvaveda, Charak Samhita, Sushruta Samhita, etc.). In spite of the many achievements of allopathic medicines, the Indian Systems of Medicine still continue to provide medical care to the majority of the people on account of their cheaper cost with no side effects (Kokate *et al.*, 2002). Herbal drugs obtained are safer in the treatment of various diseases (Ayyanar and Ignacimuthu, 2005). Tamilnadu has great potential for development of medicinal plants as a commercially viable venture. Its rich biodiversity and varied agro-climate provide a conducive atmosphere for the promotion of medicinal plants as a successful commercial venture. Several workers were reported the utility of plants for the treatment of various diseases by different tribal and rural people inhabiting in various regions of Tamil Nadu (Laila Banu *et al.*, 2007; Shanmugam *et al.*, 2011). Still people are practicing the art of the use of crude herbal products as medicines (Ignacimuthu *et al.*, 2008; Singh and Singh, 2009).

The human oral cavity consists of a number of well-defined areas (tongue, tooth, buccal epithelium etc), which have been shown to have distinct microbiomes (Aas *et al.* 2005). Surprisingly, diminutive is known about the microflora of the healthy oral cavity. Our purposes were as follows: (i) Isolation and identification of bacteria from different oral samples (ii) Formulation of antibacterial

toothpaste using different medicinal plants and (iii) To evaluate the efficacy of herbal toothpaste of bacterial deterring activity.

MATERIALS AND METHODS

Collection of Samples

For the present investigation, used toothbrushes and used denture were collected from different people and randomly selected three toothbrushes and one denture for an isolation of oral bacteria. The experiments were conducted in the Department of Botany and Microbiology Laboratory of A.V.C. College, Mannampandal, Tamil Nadu, India.

Preparation of Nutrient Broth

Beef extract 0.3 g, Peptone 0.5 g, and sodium chloride 0.5 g mixed with 100 ml double distilled water and pH was maintained in 6.4. The samples which were collected for bacterial isolation were inoculated in the nutrient broth and incubated 37°C for 24 h for isolation.

Turbidity Test

To test the turbidity and find the quantity of the bacteria present in the different used brushes and used denture, the fresh broth was taken and kept all the samples individually in the broth for 24 h. After 24 h samples inoculated broth were taken, and bacterial growth was observed by colorimeter at 590 nm against fresh nutrient broth as blank.

Biochemical Test for Identify the Bacteria

For identification and confirmation, different biochemical tests such as gram staining technique, methyl red test, Voges-Proskauer test, indole test, litmus test, and carbohydrate test were used for this analysis.

Preparation of Nutrient Agar Medium

Nutrient agar medium was used for the culture of the organism, beef extract 0.6 g, peptone 1 g, sodium chloride 1 g, agar 3 g, DW 200 ml, and pH 6.4. All the ingredients mixed thoroughly after sterilization the medium was poured into the sterile petri plates and were allowed to solidify. 1 ml culture broth was inoculated in petri plate containing solidified medium by spread plate technique. 5 mm diameter Whatman No. 1 filter paper disc moisture with different dilution of herbal paste to analysis inhibition rate.

Formulation of Herbal Toothpaste

Serial number	Species	Family	Useful parts	Quantity (g)
1	<i>Azadirachta indica</i> L.	Meliaceae	Leaves	20
2	<i>Curcuma longa</i> L.	Ziniziberaceae	Rhizome	15
3	<i>Phyllanthus niruri</i> L.	Euphorbiaceae	Root	10
4	<i>Ocimum sanctum</i> L.	Lamiaceae	Leaves	5
5	<i>Mentha piperita</i> L.	Lamiaceae	Leaves	10
6	<i>Ficus benghalensis</i> L.	Urticaceae	Bark	10
7	<i>Crocus sativus</i> L.	Irideae	Flower	2
8	<i>Myrtus caryophyllus</i> L.	Myrtaceae	Fruit, Flower	2+3
9	<i>Acacia nilotica</i> L.	Meliaceae	Bark	8
10	Chalk powder			15
	Total			100

The plant materials were taken and washed thoroughly with running water thrice. All the medicinal plant materials shade dried and cut into small pieces. These plant materials were powdered and sieved through a 40-mesh screen. The fine powder was stored in air tight containers and left in the refrigerator. Chalk powder and plant powder mixed thoroughly and double distilled water was added to the substance to get into paste form.

The herbal paste was weighed 5, 10, 15, and 20 g, respectively, in the aseptic condition in a UV chamber. Thus, 5%, 10%, 15%, and 20% dilution of herbal paste were prepared separately. The disc was placed in the petri plates containing medium, each petri plates had one disc of each dilution. Plates were incubated 37°C for 24 h. The filter paper disc moisture in sterile distilled water served as control. The extent of inhibition zones was formed; all the zones were measured, and the results were presented as an area of inhibition zone in centimeter. Different concentrations of formulated herbal toothpaste solution were prepared, and the used denture was immersed in the toothpaste solution. After the inoculation of used denture, the broth containing bacterial colony was evaluated through spectrophotometer in 590 nm.

RESULTS AND DISCUSSION

The microbes are present in the human oral cavity. The mouth is the entrance to the digestive system; it provides an environment that supports a large and varied microbial populations. The present study reveals that certain streptococci, staphylococci, and bacilli species are found in the oral cavity and the formulated herbal toothpaste significantly reduce the microbial population which causes periodontal disease. From the present investigation, among the sources of samples more bacterial population were observed and recorded from the used denture (Figure 1) followed by different used toothbrushes (Figure 2) which were collected from various persons. Three different bacterial species were identified; they were streptococci,

staphylococci, and bacilli (Figures 3-5) and confirmed by Gram's staining method and biochemical tests. The efficiency of formulated herbal toothpaste was evaluated; the maximum inhibition zone was observed in 20% dilution of formulated herbal toothpaste (Figures 6 and 7).

Bacterial Growth Analysis

After identification, the samples were cultured in the fresh nutrient broth among the two oral samples more number of bacterial populations were observed and recorded in used denture and used a brush (Figure 8). Effect of herbal toothpaste on the microbial population of used denture was reduced when compared to control. It was 0.850 in treated with herbal toothpaste, and untreated



Figure 1: Oral sample denture



Figure 2: Oral sample used toothbrushes



Figure 3: Bacterial culture

(control) was 0.978 (OD value at 590 nm), in 24 h of culture reduced bacterial growth, it was recorded in herbal toothpaste treated denture. The results indicated that the reduced bacterial activity in the sample due to the antimicrobial activity of formulated herbal toothpaste.

The similar results of antibacterial activity observed in Neem stick extract, and the gallotannin-enriched extract from *Melaphis chinensis* inhibited insoluble glucan synthesis. Incubation of oral streptococci with the Neem stick extract resulted in a microscopically observable bacterial aggregation. These data suggest that Neem stick extract can reduce the ability of some streptococci to colonize tooth surfaces (Wolinsky *et al.*, 1996). However, little is known about the antimicrobial effects of *C. longa* on methicillin-resistant *Staphylococcus aureus*. *C. longa* used in oriental folk medicines to treat infectious diseases (e.g. sinusitis and cough), cholecystitis, and cholangitis and used as a therapy for hepatic disorders, rheumatism, and anorexia (Kim *et al.*, 2005).

The similar results of antibacterial activity were observed in the various scientific analysis, Ekwenye and Njoku, 2006 had described the antibacterial activity of *Phyllanthus niruri* L. Tulsi (*Ocimum sanctum* L.) fixed oil showed good antibacterial activity against *S. aureus*, *Bacillus pumilus* (Singh *et al.*, 2005). The dimethyl sulfoxide extracts of *P. niruri* L. showed a

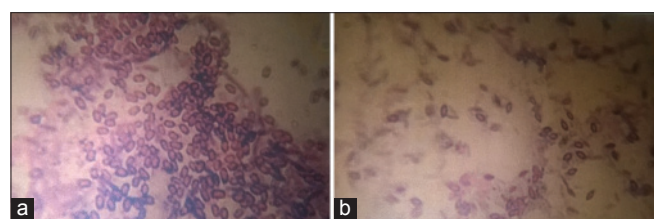


Figure 4: (a and b) *Coccus* bacteria isolated from different oral samples

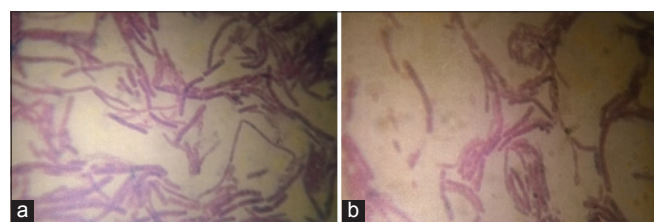


Figure 5: (a and b) *Bacillus* bacteria isolated from different oral samples

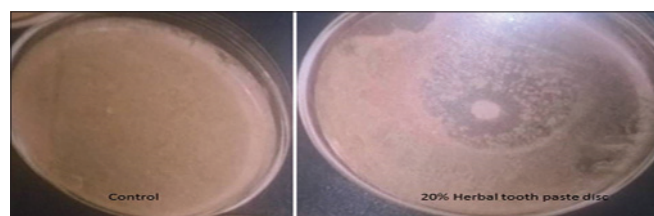


Figure 6: Inhibition zone using herbal toothpaste

remarkable antibacterial effect against *Salmonella typhi*, *Escherichia coli*, and *S. aureus* (Sumathi and Parvathi, 2010). At the 4% concentration of Tulsi extract, a zone of inhibition of 22 mm was obtained. Tulsi extract demonstrated an antimicrobial property against *Streptococcus mutans* (Agarwal and Nagesh, 2010). The antimicrobial activity of the extracts was assayed against *Streptococcus viridans*, *S. aureus*, *E. coli*, *Bacillus subtilis*, and *Shigella sonnei*. The plant extract exhibited antimicrobial activity against all the test microorganisms. *Acacia nilotica* L. was performed as a potential source of antimicrobial agents (Banso, 2009). *Azadirachta indica* L. was used as traditional medicine for house hold remedy against various human ailments, from antiquity (Aslam *et al.*, 2009). Neem has been extensively used in Ayurveda, Unani, and Homeopathic medicine (Kausik *et al.*, 2002). *C. longa*, commonly known as “turmeric,” is widely used as a spice and coloring agent and is well known for its medicinal properties (Luthra *et al.*, 2001). The juice of leaves of *Mentha piperita* exhibited highest antibacterial activity, juice of stem exhibited least antibacterial activity (Sabahat and Perween, 2005). In *Ficus benghalensis*, it was found to be reported to have antimicrobial activity (Aswar *et al.*, 2008). *Crocus sativus* L. Saffron is greatly appreciated for its abilities of coloring and flavoring, aromatic and for bacterial strength (Pintado *et al.*, 2011).

The present study reveals the uses of formulated herbal toothpaste (prepared from different herbal extracts) maximally reduce the microbial population which causes periodontal disease. Although a number of antimicrobial agents have been shown to help control plaque growth chemically and reduce gingival inflammation, only a few

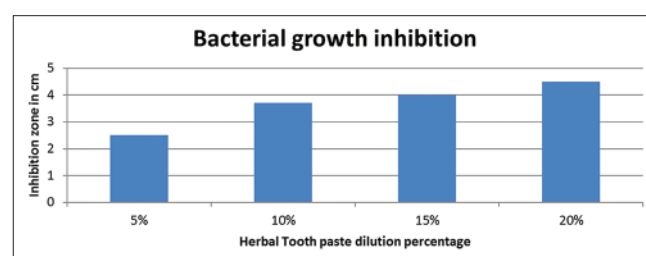


Figure 7: The efficiency of formulated herbal toothpaste inhibiting the growth of oral bacterial culture

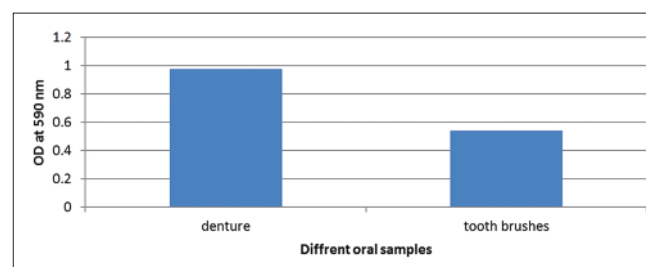


Figure 8: Turbidity analysis using different oral samples

of this are incorporated into marketed herbal oral products the increasing awareness of the environment and health lifestyles are generating some demand for more natural products such as those containing herbal extracts.

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