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EFFECT OF TRADITIONAL MEDICINAL PLANTS ON TEETH MICROFLORA

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ABSTRACT The oral cavity provides an ideal environment for the growth of microbiota. Many of the bacterial species in the oral cavity are involved in dental caries. Acidogenic and aciduric Gram positive bacteria, metabolize sucrose to organic acid, which dissolves the calcium phosphate in the teeth resulting in decalcification and finally causing decay. Therapeutic drugs used to treat dental diseases may have many undesirable effects such as diarrhea, vomiting and tooth staining. Therefore, natural phytochemicals isolated from plants used in traditional medicines are considered as a good alternative to synthetic chemicals. Therapeutic and chemical methods are costly but there are many easily available plants which we can use for cleaning our teeth and maintaining our oral hygiene. A study was undertaken to investigate the antimicrobial effect of *Psidium guajava*, *Mangifera indica*, *Anacardium occidentale* and *Azadirachta indica* on oral normal microflora. Ethanolic and aqueous extracts of the leaves were prepared and was tested against the three isolates from the oral cavity using Agar well diffusion method namely Gram positive α-haemolytic cocci in chain, Gram positive non-haemolytic cocci in chain and Gram positive bacilli. *Psidium guajava* and *Anacardium occidentale* was found to be effective against the isolates. From this study *Psidium guajava* and *Anacardium occidentale* have shown great potential to act as natural and cost effective alternative for maintaining oral hygiene.

KEYWORDS : *Psidium guajava, Mangifera indica, Anacardium occidentale, Azadirachta indica*, agar well diffusion method

1.INTRODUCTION

The microorganisms that reside in the oral cavity collectively are referred to as oral microbiome (Deo and Deshmukh., 2019). The oral cavity provides an ideal environment for the growth of microorganisms. The normal temperature of the oral cavity is 37°C which is the optimum temperature for most of the bacterium to grow. The optimum pH for most species of bacteria is provided by the saliva (6.5-7) (Pelczar et al., 2008). *Streptococci, Lactobacilli, Staphylococci* and *Corynebacteria*, with a great number of anaerobic especially bacteriodes constitute the oral microflora (Pelczar et al., 2008).

Periodontitis, dental caries is amongst the most important yet preventable oral diseases included in the list of major health problems that humans face (Rudraiah et al., 2015). *Streptococcus mutans* appears to be major cause of dental caries. *Streptococcus mutans* produces glucan, a highly branched extracellular polymer of glucose, that acts like cement which binds the bacterial cells together and cause them to adhere to the tooth surface. Glucan is formed in the presence of sucrose by means of enzyme glycosyl transferase, located on the surface of the cocci (Pelczar et al., 2008). Sucrose and the other sugars can be fermented by *Steptococci* giving rise to lactic acid, which is responsible for etching of the teeth surface (Park et al., 2003).

Teeth provides opportunities for extensive biofilm formation. The moist and warm environment in mouth favors the growth of many organisms. A biofilm or mass of bacteria that grows on surface within the mouth is called dental plaque. Dental plaque is a sticky substance, which may keep acids in contact with teeth for long time, which may eventually lead to cavitation. In long run it may also lead to gum diseases like gingivitis or periodontitis.

Dental caries is a supragingival condition. In variance, periodontal diseases are subgingival condition that have been linked to anaerobic Gram negative bacteria such as *Porphyromonas gingivalis, Actinobacilli* species, *Prevotella* species and *Fusobacterium* species (Palombo,2011).

Cariogenic bacteria are necessary for the disease process. The acids produced during the metabolism of fermentable carbohydrates by these bacteria readily dissolve the minerals of enamel and dentine. Acidogenic bacteria are the ones which produce acids as a by-product of their metabolism and aciduric bacteria are the ones which are live in the acid environment (Featherstone, 2008). When in the dental plaque the organic acids are produced on the tooth surface, they readily diffuse in all directions and also through the pores of enamel or dentine and into the underlying tissue. This marks the beginning of the dissolution of acid soluble mineral. The end result of this process is a cavity. Cavitation requires many months or years to progress which in turn leads to dental caries (Featherstone, 2008).

The practising dentist fixes caries by drilling and filling instead of intervening therapeutically before cavitation occurs, and while the process of remineralisation is still possible. “White spot lesion” is the earliest clinical sign of the progression of dental caries which human eyes can see. At this stage in the process prior to the cavitation, therapeutic medication can help to arrest or reverse the process by remineralisation. If the carious lesion is in the enamel and non-cavitated, it can be reversed or arrested (Khoroushi and Kachuie.,2017). Remineralisation process is speeded up by fluorides (Featherstone, 2008).

Before or during demineralisation, if fluoride ions are present at the crystal surface in sufficient concentration, they can absorb onto the surface of the crystal and inhibit demineralisation by acids.

There are multiple mechanisms by which fluoride prevents decay. After water fluoridation, there is 30-50% reduction seen in the decay. Fluorides replace hydroxyl group in the tooth crystal, forming fluorapatite. Fluorapatite is genuinely less soluble in low

pH value found in plaque and also remineralizes faster in the intervals between sugar ingestion (Featherstone, 2008). Acid production in most plaque bacteria is inhibited by the presence of 10-100 ppm of fluoride (Khoroushi and Kachuie.,2017).

Many medicines or chemicals or antibiotics can be used to treat dental diseases. But they may have many undesirable side effects such as vomiting, diarrhea and tooth staining (Rudraiah et al.,2015). For e.g., antibiotics used commonly to treat oral infections are penicillin and cephalosporin, erythromycin, tetracycline and its derivatives and metronidazole (Rudraiah et al.,2015). Other antibacterial agents used in the prevention and treatment of oral infections include cetylpyridinium chloride, chlorhexidine, amino fluorides or products containing such agents are reported to exhibit toxicity which result in the staining of the teeth. Ethanol which is commonly found in mouth washes are associated to oral cancer (Rudraiah et al.,2015). Therefore the search for alternative products continues.

Natural phytochemicals isolated from plants, used in traditional medicine are considered as good alternative to synthetic chemicals since therapeutic and chemical methods are costly. There are many easily available plants which we can use for cleaning our teeth and maintaining our oral hygiene. For e.g., Mango, Cashew, Neem, Guava, Miswak sticks, etc. It is used as a primary source of medicine in rural area of developing countries. The natural products derived from medicinal plants are abundant source of biologically active compounds (Rudraiah et al.,2015). Many pathogens are developing resistance to therapeutic agent such as antibiotics and antiviral agents (Rudraiah et al.,2015).

Many studies have shown the ability of Mango, Cashew, Neem, Guava juice or their constituents to prevent the adhesion of oral pathogens to the surfaces and related phenomena, such as production of glucan and fructans, and the formation of biofilms. They have shown to reduce the activity of fucosyl transferase and glucosyl transferase (Varghese etal.,2013).

Several mouth washes with *Psidium guajava* leaves may help to relieve aphthous ulcers, which are extremely painful acute mouth ulcers (Fredinand et al.,2013). Flavonoids extracted from *P. guajava* leaves including Morin-3-O-lyxoside, Morin-3-O-arabinoside, Quercetin and Quercetin-3-O-arabinoside were shown to have strong antibacterial activity. The activity of cell surface hydrophobicity of *Streptococcus sanguinis, Streptococcus mitis and Actinomyces* species was reduced by aqueous extract of *P. guajava* (Fredinand et al.,2013). The plant's leaf extract has numerous chemical compounds like tannins, polyphenolic compounds, flavonoids, ellagic acid, triterpenoids, guaijaverin, quercetin and are known to show anti-inflammatory and analgesic effects (Gayathri and kiruba.,2014), (Fredinand et al.,2013). Quercetin-3-O-aalpha-1-arabinopyranoside (Guajaverin), the active flavonoid compound isolated from *P. guajava* shows high potential antiplaque effect by inhibiting the growth of *Streptococcus mutans* (Fredinand et al.,2013).

Mangifera indica leaves have various biomedical applications including anti-inflammatory, anti-oxidation or free radical scavenging, anti-allergic, cardio protective, anticancer or analgesic hepatoprotective and immune modulator activity (Shah et al.,2010). The presence of phenol constituents flavonoids, triterpenes, phytosterols and polyphenols in the leaves of *Mangifera indica* has been demonstrated by phytochemical studies. A decoction of the leaves can be used to gargle for the prevention of halitosis. A persistent, unpleasant odour in exhaled breath, usually not serious, commonly called bad breath is known as halitosis. Herbal mouthwashes are prepared from *Mangifera indica* leaves (Shah et al.,2010). The twigs and leaves of *Mangifera indica* used to clean teeth are said to be beneficial against gums and the bark is useful for toothache. Various parts of the plants are used as dentifrice in toothache. *Mangifera indica* has Mangiferin, a xanthone glycoside as major bioactive constituent, Isomangiferin, tannins and gallic acid derivatives. The plant also contains chemicals like polyphenols, flavonoids and triterpenoids. The bark contains protocatechuic acid, mangiferin, catechin, glycine, alanine, kinic acid, etc (Shah et al.,2010).

Anacardium occidentale possess anti-inflammatory, analgesic, anti-mutagenic, antimicrobial, hypoglycemic, antioxidant activities (Chaithra et al.,2013). Studies have shown that the cashew leaves' extract has potential to inhibit cariogenic flora. The bark and leaf extract of *Anacardium occidentale* is effective in inhibiting *P. gingivalis* and leaves exerts antibacterial effect against *P. gingivalis* and *P. intermedia* (De Souza Leite et al.,2016). The phytochemicals present in the cashew leaves such as anacardic acid, tannins and flavonoids are also responsible for antimicrobial activities and inactivation of microbial adhesion enzymes (Aderiye et at.,2015). Flavonoids are responsible for the inhibition of DNA gyrase thereby inhibiting DNA synthesis. Phenolic compounds like cardols, anacardic acid, triterpenoids, xanthoprotein, methyl cardol and cardanols inhibits the bacterial cell wall synthesis and block the DNA, RNA and protein pathways (Aderiye et at.,2015).

Another plant used in the present study is *Azadirachta indica.* It also has antibacterial properties which are useful in dentistry for curing gingival problems and maintaining oral health in a natural way. Neem twigs are used as oral toothache relievers and for cleaning of teeth (Yerima et al.,2012).

2. MATERIALS AND METHODS

2.1. Isolation

Using sterile toothpicks, teeth scraping was taken, suspended into 5 mL of saline and then vortexed.

Under aseptic conditions, the above suspension was streaked onto nutrient agar plates.

The plates were incubated at 37°C for 48 hours.

Three isolates were obtained from the nutrient agar plates and their colony characteristics (Table 1), Gram character and type of haemolysis on blood agar were studied. The three isolates were then preserved on nutrient agar slants for antibacterial activity studies.

2.2. Preparation of extract

10g fresh leaves each of *Psidium guajava, Mangifera indica, Anacardium occidentale* and *Azadirachta indica* were weighed individually and washed thoroughly using sterile distilled water.

Ethanolic and aqueous extracts of the leaves were prepared separately using ethanol and water as a solvent respectively. The extracts were filtered using sterile filter paper.

Two concentrations i.e. 100% and 50% of the leaves extract were used for the study.

Likewise, 50% and 100% of aqueous concentration of dried tobacco leaves extract and commercially available herbal toothpaste were also prepared and used for the study. The entire procedure is carried out under aseptic condition.

2.3. Antimicrobial activity

The antimicrobial activity of all the leaves extracts and herbal toothpaste (50% & 100%) was tested against the isolates using Agar well diffusion method.

3. RESULTS AND DISCUSSION

The three isolates were as follows:

Isolate '**a**': Gram positive cocci in chains - non haemolytic

Isolate '**b**': Gram positive cocci in chains - α-haemolytic

Isolate '**c**': Gram positive bacilli - non haemolytic

The results are compared statistically.

TABLE 1: COLONY CHARACTERISTICS OF ISOLATES

	Isolate '**a**'	Isolate '**b**'	Isolate '**c**'
Media	NA	NA	NA
Time	48hr	48hr	48hr
Temp	37°C	37°C	37°C
Size	2mm	3mm	1mm
Colour	Yellow	White	White
Shape	Round	Round	Round
Margin	Entire	Entire	Entire

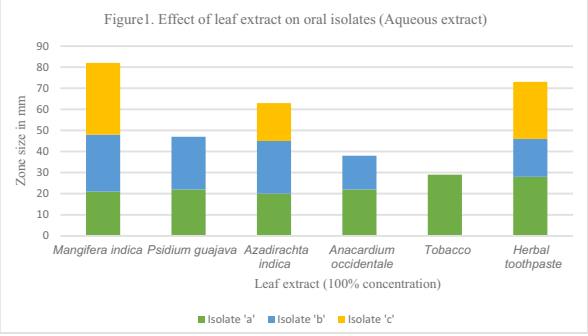
Elevation	Raised	Raised	Raised
Consistency	Butyrous	Butyrous	Butyrous
Opacity	Opaque	Opaque	Opaque
Surface texture	Rough	Smooth	Smooth
Gram character	Gram positive	Gram positive	Gram positive

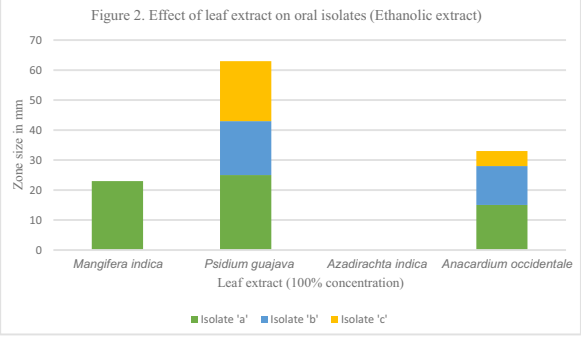
TABLE 2: ZONE OF INHIBITION (AQUEOUS EXTRACT)

Leaf extract	Isolate **'a'**		Isolate **'b'**		Isolate **'c'**		Control
	50%	100%	50%	100%	50%	100%	
Mangifera indica	17mm	21mm	16mm	27mm	18mm	34mm	-
Psidium guajava	17mm	22mm	22mm	25mm	-	-	-
Azadirachta indica	-	20mm	-	25mm	-	18mm	-
Anacardium occidentale	-	22mm	-	16mm	-	-	-
Tobacco	23mm	29mm	-	-	-	-	-
Herbal toothpaste	28mm	28mm	17mm	18mm	27mm	27mm	-

TABLE 3: ZONE OF INHIBITION (ETHANOLIC EXTRACT)

Leaf extract	Isolate **'a'**		Isolate **'b'**		Isolate **'c'**		Control
	50%	100%	50%	100%	50%	100%	
Mangifera indica	20mm	23mm	-	-	-	-	-
Anacardium occidentale	12mm	15mm	12mm	13mm	11mm	13mm	-
Psidium guajava	23mm	25mm	18mm	18mm	20mm	20mm	-
Azadirachta indica	-	-	-	-	-	-	-





Since ancient times, natural products and herbal medicines have been reported to be effective in management of many infections. Some of these like *Psidium guajava, Mangifera indica, Anacardium occidentale* and *Azadirachta indica* have been assessed for their antimicrobial potential against oral isolates. These plants were selected in view of their easy availability and being indigenous plants of Goa.

During the isolation phase, there were 5 different colonies observed, from which 3 colonies were selected and used for further studies which are as follows: Gram positive cocci in chains - non haemolytic (isolate **'a'**), Gram positive cocci in chain - α haemolytic (isolate **'b'**), and Gram positive bacilli - non haemolytic (isolate **'c'**). The present study showed that aqueous extract was more effective against the oral isolates (Figure 1 and 2).

The ethanolic extract of *Psidium guajava* and *Anacardium occidentale* showed antimicrobial activity against all three oral isolates (Figure 2). Aqueous extract of *Psidium guajava* was found to be effective against isolate **'a'** and **'b'** and that of *Anacardium occidentale* was effective against isolate **'a'** and **'c'** (figure 1).

The ethanolic extract of *Anacardium occidentale* showed to have greater antimicrobial potential against oral isolates than the aqueous extract.

P. guajava contains guajaverin, Psidiolic acid and other essential oil constituents such as 1, 8-cineol, monoterpenes ρ-cimen and acetate of alpha- terpenil, which contributes to antimicrobial effects (Ferdinand et al., 2013).

The ethanolic extract of *Azadirachta indica* did not show inhibition zone against the oral isolates at 50% and 100% concentration but its aqueous extract was effective against oral isolates at 100% concentration.

Similar study was carried out by Adyanthaya et al. at Yenepoya University, Mangalore, to check antimicrobial potential of the extract of twigs of *Azadirachta indica* using different types of solvents such as petroleum ether, Dichloromethane, Ethyl acetate, Methanol and water at 500 mg/mL concentration. They used standard test organisms for studies, i.e. *S. mutans, S. mitis, S. salivarius, Lactobacillus species, P. Intermedia* and *C. albicans.* In their study, *Lactobacillus* species showed highest zone of inhibition against the methanolic extract of *Azadirachta indica.* Similar findings have been observed in other studies, where methanol extract of *A. indica* leaves was reported to have highest antibacterial activity compared to chloroform extract which exhibited moderate to good antibacterial activity (Koona and Budida., 2011).

Although published literature demonstrated the antimicrobial efficacy of *Azadirachta indica*, our studies did not support the above fact for its ethanolic extract. The extract of *Azadirachta indica* has been claimed to contain active ingredients like alkaloids, saponins, flavonoids, sterols, resins, tannins, oil, gum, chloride, fluoride, silica, sulphur and calcium (Adyanthaya et al., 2014). It has been recorded that the presence of fluoride offers anticariogenic benefits; silica acts as an abrasive agent to prevent accumulation of plaque; oil exerts analgesic, antiseptic, and carminative effects; and tannins acts as astringent and form protective coating on the tooth enamel that aids in dental caries prevention (Adyanthaya et al., 2014).

The aqueous extract of *Mangifera indica* showed greater potential and efficiency against the isolates under study than their ethanolic extract. The antimicrobial efficacy of *Mangifera indica* was assigned to the presence of bitter gums, resins, and tannins (Shah et al., 2010), (Nikhal and Mahajan., 2010). The tannin and resins have an astringent effect on the mucous membrane and are claimed to form a protective layer on the enamel (Shah et al., 2010), (Nikhal and Mahajan., 2010).

Dried tobacco leaves are roasted and used for cleaning teeth by some people, hence tobacco was considered to check its effectivity against oral isolates. Tobacco extract was effective against isolate **'a'** only (Figure 1). Though it is a known fact that constant chewing of tobacco can cause oral cancer, roasting of the tobacco may help to inactivate its carcinogens while retaining its antibacterial components thus making it effective for oral utility.

Many commercially available toothpastes claim to be effective in cleaning teeth and maintaining oral hygiene. Antimicrobial activity of herbal toothpaste was tested against the oral isolates. Herbal toothpaste showed zone of inhibition for all three isolates (Figure 1). Nonetheless, herbal toothpastes do contain chemicals and preservatives to maintain their shelf life which in long run may have an undesirable effect on the teeth.

Comparing the results obtained for leaves extract and herbal toothpaste, we found that the mean diameter of zone of inhibition for herbal toothpaste is similar to that of ethanolic extract of *P. guajava* and aqueous extract of *M. indica*. Herbal toothpaste contains ingredients such Babhul, Jambhul, lavang, Manjishtha, Dalchini, Vajradanti, Trifala which were anciently used by our ancestors for cleaning their teeth and as a remedy for toothache.

Overall, it was observed that aqueous extract of *M. indica* has shown greatest antimicrobial potential against all three oral isolates.

4. CONCLUSION

The use of herbal extracts for cleaning teeth and maintaining oral hygiene is considered an interesting alternative to synthetic antimicrobial due to their lower negative impact. The studies have shown that leaves extract of *Psidium guajava, Mangifera indica, Anacardium occidentale* and *Azadirachta indica* have antimicrobial efficacy against the isolates from the oral flora. The aqueous extract was found to be more effective as compared to ethanolic extract. These traditional medicinal plants are easily available, natural and cost effective thus making them ideal agents for oral hygiene.

The effect of commercially available herbal toothpaste and tobacco was also tested against oral isolates. Herbal toothpaste showed favourable results due to its natural ingredients. Tobacco showed antimicrobial effectivity only against isolate '**a**'.

The overall results were quite satisfactory. Plants used for the present studies are indigenous plants of Goa and since they are natural, they have less side effects.

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