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RESEARCH ARTICLE

EVALUATION OF ANTI-MICROBIAL EFFICACY OF PLANT DERIVED EXTRACT AGAINST PLAQUE ASSOCIATED BACTERIA

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ABSTRACT

Context: Dental plaque are soft deposits that adhere to the tooth surface and acts as a harbor for numerous pathogenic microorganisms. Prevention of plaque accumulation serves to eliminate the microbial colonization. The commercially available anti-plaque agents are associated with certain side effects especially with prolonged use. Hence, the utility of natural plant derived extracts may possess beneficial effects against the pathogenic bacteria.

Aim: The aim of the study was to evaluate and compare the anti-microbial efficacy of herbal extracts and chlorhexidine against plaque associated aerobic micro-organisms.

Materials and Method: Supragingival plaque samples were collected from healthy individuals (n=30) who are free from tobacco/ alcohol habits, any oral microbial diseases and systemic illnesses. Anti-microbial efficacy of curcumin, ginger-garlic paste, clove oil and cinnamon oil were evaluated against plaque associated aerobic bacteria and compared against chlorhexidine by measuring the zone of inhibitionin culture media. Statistical analysis was performed to determine the significance of zone of inhibition amongst the various agents.

Results: Cinnamon oil was found to be more effective than the other herbal extracts as well as chlorhexidine against plaque associated aerobic bacteria.

Conclusion: Cinnamon oil can thus be used as an effective anti-plaque agent either individually or in combination with chlorhexidine. Assessment of the anti-microbial efficacy of naturally available plant extracts against the oral pathogenic microorganisms may enable their clinical utility in preventing microbial colonization thereby preventing common oral diseases.

INTRODUCTION

Oral cavity is a complex ecosystem consisting of oral fluids, immunoglobulins, agglutinins, microflora, carbohydrates, proteins and certain anti-microbial enzymes.[1]The abundance of oral microbes results in the formation of dental biofilm mainly on the tooth surface. The term biofilm describes the relatively indefinable microbial community associated with a tooth surface or any other hard non-shedding material, randomly distributed in a shaped matrix or glycocalyx.[2] Dental biofilms are surface associated colonies of microorganisms embedded in an extracellular polymeric matrix which on contact with the host may result in disturbances in tissue homeostasis and subsequent disease.[3] Dental plaque is described as the most complex dental biofilm which is a primary initiating factor for the commonly occurring oral diseases like dental caries, gingivitis and periodontitis. [4] It is defined as "a specific but highly variable structural entity consisting of micro-organisms and their products embedded in a highly organized intercellular matrix".[5] Streptoccocus mutans is one of the important primary colonizers of the dental plaque which plays a pivotal role in causing dental caries and periodontal diseases. [6] Other organisms commonly found in dental plaque are E. coli, P. gingivalis, A.actinomycetemcomitans, capnocytophaga species and fusospirochetal organisms. [7] Prevention of plaque accumulation and periodic cleansing of oral cavity is a pre-requisite for avoiding accumulation of pathogenic micro-organisms and thereby reducing the incidence of common oral diseases.

Plaque control forms the mainstay of primary and secondary prevention of gingivitis and periodontal diseases. [8]Plaque control is achieved primarily by two methods namely mechanical therapy and the use of anti-microbial agents. [9]Supragingival plaque removal is mainly achieved by mechanical therapy involving home care but is usually insufficient for its complete eradication. [10] Chemical plaque control agents may act as effective adjuvant to the conventional mechanical plaque control thereby interfering

with biofilm composition and its metabolism. These agents may be either bacteriostatic or bactericidal. [11] Several chemical anti-plaque agents such as cetylpyridinium chloride, chlorhexidine, triclosan, Bis-biguanides, pyrimidines, quaternary ammonium compounds, oxygenating agents, halogens, heavy metal salts or antibiotics have been used in the plaque control. [12] Among these chlorhexidine is the most commonly used chemical anti-plaque agent which is a broad-spectrum biocide effective against gram positive, gram negative and mycotic organisms. [13] Despite its popularity, long term and irrational use of chlorhexidine mouthwash was associated with side effects such as bitter taste, light-brown stains on tooth, loss of taste sensitivity thereby limiting acceptability by the patients. [14] The inability to obtain a satisfactory and reliable chemical anti-plaque agent resulted in renewed interest in developing herbal mouthwash from natural plant extracts. The natural phytochemical ingredients of herbal mouthwash possess anti-microbial and anti-inflammatory effect. Theseproducts are advantageous as they do not use alcohol, artificial preservatives, flavor or color; [15] have natural cleansing and healing property to teeth and gums; are gentle for daily use and are less abrasive. [13]Some of the plant derived extracts evaluated for their beneficial role in oral diseases include neem, tulsi, green tea, clove, garlic extract, aloe vera extract and cinnamonamong others. [16, 17]

Thus, the aim of the present study was to evaluate the anti-microbial effect of herbal extract derived from clove oil, ginger garlic paste, cinnamon oil and curcumin oil against plaque associated aerobic microorganisms. Further, the study also compared the effectiveness of these extracts with each other and with that of chlorhexidine mouthwash to assess their clinical usefulness.

MATERIALS AND METHOD

Study participants

The study was conducted in the department of oral pathology and microbiology at our institution from the regular OPD. The study participantsincluded 30 healthy individuals free from tobacco/ alcohol habits, oral lesions and systemic illness who visited the institution for regular checkup. The participants were informed of the procedure and written informed consent was obtained before collection of the sample. Institutional ethical committee clearance was obtained prior to the commencement of the study.

METHOD

Supragingival plaque was collected from the cervical areas of the teeth using Williams graduated probe. The participants were instructed to avoid food intake at least 30min before sample collection. Prior to obtaining the samples, the participants were asked to rinse their mouth and expectorate to remove any debris accumulated on the tooth surface. The collected samples were then transported in a thioglycolate broth and subjected to microbial analysis for the identification of aerobic bacteria. The sample obtained was then streaked over nutrient agar using an inoculated loop and incubated at 37^0C for 24 to 48 hours to assess the microbial colony growth. Further, the organisms were identified under the microscope using Gram's stain.

Four plant derived extracts namely clove oil (Narendra marketing, India), ginger garlic paste, cinnamon oil (Dr Jain's) and curcumin oil (G2 gold) along with commercially available chlorhexidine (Rexidine mouthwash)were employed in the study. The extracts were dried and ground into fine powder using mortar and pestle. The decoctions were prepared by mixing 10gm powder into 100ml distilled water which was boiled for 15 to 20 minutes, filtered and collected in sterile sample containers and cooled down. All extracts were stored separately in sterile container and labeled.

The next step was the anti-microbial evaluation of the extracts and chlorhexidine performed by disc diffusion method in Mueller-Hinton agar media.Circular discs of 6mm diameter prepared from Whatman filter paper were infused with the extract material and chlorhexidine. The discs were placed on the agar plate after uniformly streaking the plates with sample obtained from the colony growth on the nutrient agar. Ciprofloxacin infused discs were used as positive controls. The culture plates were incubated at $37^0$for 24 hours to assess the sensitivity of the products by measuring the zone of inhibition. The zone of inhibition for each disc was then measured in all the samples and statistically compared. Statistical analysis was done using Microsoft office excel (version 10) using student t-test at confidence interval of 95%.

RESULTS

The participants included in the study were in the age range of 20-35 years.The sex distribution included 20 females and 10 males. The plaque samples obtained from the study participants showed 2-3mm opaque, raised, circular emulsified white to yellowish colored colonies (Fig 1). Gram's stain from the bacterial colonies on nutrient agar predominantly showed gram positive cocci arranged in chains and clusters.The anti-microbial sensitivity of the plant extracts was determined by measuring the zone of inhibition around the respective discs (Fig 2).

Fig 1 Nutrient agar media demonstrating colony growth from the plaque samples

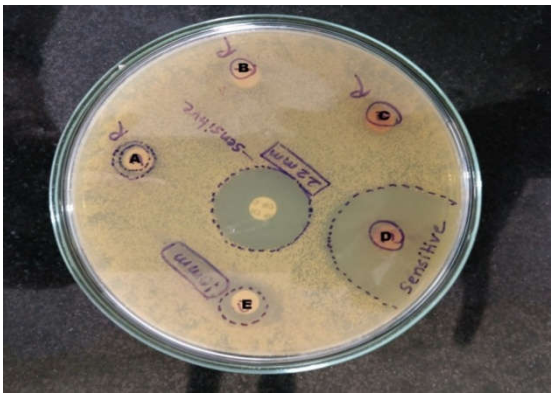

Fig 2 Anti-microbial sensitivity of the herbal extracts and chlorhexidine determined by measuring the zone of inhibition

It was observed that cinnamon oil demonstrated better sensitivity amongst all the extracts(Table 1). On statistical comparison of the mean zone of inhibition cinnamon oil was significantly better than chlorhexidine (Table 2).

Table 1 Evaluation of the sensitivity of the herbal extracts in MH media

Herbal extract	Zone of inhibition in Mueller Hinton agar media
Clove oil	Inconsistent
Ginger garlic paste	Inconsistent
Curcumin oil	Inconsistent
Cinnamon oil	Sensitive
Chlorhexidine	Sensitive

Table 2 Student t test to evaluate the significance of the zone of inhibition between cinnamon oil and chlorhexidine

Group	N	Mean ± SD	p-value
Cinnamon oil	30	36.00±12.96	0.02*
Chlorhexidine	30	22.56±11.96	
*- Significant			

DISCUSSION

Periodontal disease is one of the important oral disease affecting mankind since early civilization with dental plaque being the primary etiological factor. Prevention of plaque accumulation is a primary requisite for prevention of periodontal diseases. While mechanical plaque control is the mainstay in management of periodontal diseases; the adjuvant utility of chemical plaque control agents holds significance. Prolonged use of chemical plaque control agents was associated with anti-microbial resistance, staining of teeth and altered taste. The use of natural herbal extracts to supplement the beneficial effects of the routinely used anti-plaque agents may offer clinical benefits.

Herbal medicines have garnered increased attention recently to overcome the deleterious side effects of commercial preparations. Herbal extracts have been tried as anti-plaque agents as they contain natural phytochemicals that possess anti-microbial property.[18] In addition, these products also have anti-inflammatory and analgesic property.[19]Some of the plant derived extracts studied to evaluate their anti-microbial role against pathogenic oral microflora includes clove oil, curcumin, neem, ginger, garlic, cinnamon oil, eucalyptus oil among others. Based on the available literature, this study was performed with the objective of identifying the potential anti-microbial role of curcumin, ginger-garlic paste, cinnamon oil and clove oil against aerobic microorganisms associated with dental plaque.

The study results showed maximum anti-microbial efficiency by cinnamon oil, the results of which were statistically significant when compared to other plant extracts. The efficiency of other plant extracts as determined by the zone of inhibition was not consistent. Also, the effectiveness of cinnamon oil was significant when compared to chlorhexidine. While our study did not demonstrate effectiveness of other extracts, several studies have found good results. A study by RajiniKanth *et al* (2016) showed maximum antimicrobial efficacy of clove oil and ginger garlic paste against microorganisms isolated from dental caries. [20] Minimal literature data exist regarding the role of ginger-garlic paste against plaque associated micro-organisms. Curcumin is reported to possessanti-oxidant property, but the anti-microbial role is not well elicited. Cinnamon oil has demonstrated promising anti-microbial effect as found in this study.

Cinnamon is a member of the Lauraceae family. The bark of the cinnamon tree contains an essential oil called cinnamonaldehyde, which is responsible for the characteristic flavor and aroma of cinnamon. [21] Cinnamon oil is composed of eugenol, eugenol acetate, cinnamaldehyde and benzyl benzoate.[22, 23]It is used as potent antibacterial, antifungal, anti-inflammatory and antioxidant agent. [24]The anti-microbial effect of cinnamon oil is due to its high electronegative charge which interferes with the biological processes involving electron transfer and its reaction with nitrogen containing components.[25]Cinnamon is known to be active on gram positive as well as gram negative bacteria.[26]A study to evaluate the effects of cinnamon and chlorhexidine on dental plaque reported an equal effectiveness of both cinnamon and chlorhexidine as an anti-plaque agent. [21]In a study by Gupta *et al* (2015) using a combination of cinnamon and *T. chebula*, a significant reduction in plaque was observed thereby indicating its utility as an effective anti-plaque agent[27]. Similar results were also obtained in several other studies. [20, 28] Evaluation of antimicrobial effects of essential oils in combination with chlorhexidine gluconate by Filoche *et al* (2005) found that cinnamon oil exhibited the greatest anti-microbial potency. Also, the combination effect of cinnamon with chlorhexidine showed greater activity against plaque biofilm while reducing the quantity of chlorhexidine required to inhibit oral pathogens. [29]

CONCLUSION

To conclude, the findings of this study have shown that cinnamon oil is an efficient anti-microbial agent and the efficacy of cinnamon oil was better than that of chlorhexidine. Thus, cinnamon oil may be used either as an adjuvant or a substitute to chlorhexidine against dental plaque. Considering the harmful effects associated with chlorhexidine or other chemical plaque control agents, it would be prudent to establish the efficacy of various natural plant derived extracts for their clinical use either alone or in combination with the routinely used chemical plaque control agents.

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