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IN VITRO STUDY ON THE EFFICACY OF HERBAL MOUTHWASH/MOUTHRINSE AGAINST SELECTED ORAL PATHOGENS

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ABSTRACT

Oral hygiene is the practice of keeping the Fauces clean to prevent oral problems, most commonly, dental cavities, gingivitis, and halitosis. All cavities occur from acid demineralisation of teeth, where chewing, food trapped on teeth. The dead and dying bacteria release a sulphur compound which has an unpleasant odour giving out bad breath. To evaluate in vitro, efficacy of a herbal mouthwash formulation against Streptococcus mutans and Candida albicans, the normal microflora which turns to opportunistic pathogens, causing dental problems. Twelve herbs viz., Azadirachta indica (leaf), Cinnamomum verum (bark), Citrus lemon (fruit rind), Emblica officinalis (fruit), Ocimum sanctum (leaf), Piper longum (fruit), Piper nigrum (fruit), Punica granatum (fruit rind), Syzyium aromaticum (Bud), Terminalia chebula (fruit), Terminalia bellirica (fruit), Zingiber officinale (Rhizome) were extracted in ethanol by soxhlet extraction. The individual herbal extract, polyherbal extract and the same incorporated in Mouthwash formulation were tested for its antimicrobial efficacy against S. mutans MTCC 890 and C. albicans MTCC 3017 culture by Zone of inhibition test. Mean and standard deviation were used for statistical analysis. The herbal extract (100 microlitre (µl)) was showing 20 ± 0.5 milli meter (mm) diameter against S. mutans. The Mouthwash formulation showed Zone of inhibition of 10±2 mm against S. mutans. Base formulation without herbal extract was also not showing zone of inhibition. There is no antifungal activity against C. albicans. Study reveals that the extract in the formulation was effective against the S. mutans, a causative agent for dental caries.

KEYWORDS: Dental caries, Plaque, C. albicans, S. mutans, Antimicrobial, Mouthwash, Mouth rinse, Phytotherapy.

INTRODUCTION

Maintenance of good oral hygiene is the key to the prevention of dental diseases. The primary etiological factor for dental diseases is dental plaque. The formation of plaque on the tooth surface is characterized by the progression from a limited number of pioneer microbial species to the complex flora of mature dental plaque. This progression involves initial adherence of bacteria to the salivary pellicle and subsequent accumulation by growth and inter-bacterial adherence. Ultimately, the tooth surface gets coated with a dense, complex micro-community that ends up in the destruction of hard enamel tissue.[1] In India as in other developing countries, a very significant proportion of dental problems are due to microbial infections. Dental problems are of three types, formation of dental plaques, dental caries and periodontal diseases.[2]

Dental caries is a localised, transmissible infectious process that ends up in the destruction of hard dental tissue. It results from accumulation of plaque on the surface of the teeth and the biochemical activites of complex micro-communities. S. mutans is one of the main opportunistic pathogens of dental caries, which plays a central role in fermenting carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel.[1]

In addition, other microflora like Escherichia coli and Candida are also associated with active caries lesions. C. albicans is the most common yeast isolated from the oral cavity. It is by far the fungal species most commonly isolated from infected root canals, showing resistance to intercanal medication. Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities.[3]

Periodontal diseases are bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar bone). The endotoxins, hydrolytic enzymes and toxic bacterial metabolites are involved in this disease. Gingivitis, an inflammatory condition of gum, is the most common form of periodontal disease. Serious forms of periodontal disease that affect the periodontal membrane and alveolar bone may results in tooth loss. Streptococci, spirochetes and bacteroides are found to be the possible pathogens responsible for the disease.[4]

Since some chemical materials including chlorhexidine can cause brown staining of the teeth.[5,6] tongue and silicate and resin restorations transient impairment of taste perception,

toxic effects on connective tissues, dryness and soreness of oral cavity,[5] allergic reactions in patients.[7] and oral desquamation in children, use of herbal agents can be a useful alteration.[8]

The use of plants for treating diseases is as old as the human civilization. There are many plants which have been in use as traditional medicine, so they are called as medicinal plants. The use of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics.[9] Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids found to have antimicrobial properties. Many of the spices and herbs used today have been valued for their antimicrobial activity in addition to their flavor and fragrance properties.[10]

There is a continuous urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases contrary to the synthetic drugs, antimicrobial of origin are not associated with many side effects and have an enormous therapeutic potential to treat many infectious diseases.[11] The use of natural antimicrobials may contribute to control the disordered growth of oral microbiota, thus overcoming problems caused by species resistant to conventional antimicrobials.[12,13]

Hence an approach was made to formulate a mouthwash/mouthrinse formulation incorporated with polyherbal extract.

MATERIAL AND METHODS

Test organism: Streptococcus mutans MTCC 890 and MTCC 3017 were procured from IMTECH, Chandigarh. Cultures were then subcultured and maintained in Nutrient broth (Streptococcus mutans) and Sabouraud's dextrose broth (Candida albicans) (Himedia).

Inoculum preparation: Inoculum of Streptococcus mutans MTCC 890 and Candida albicans MTCC 3017 were prepared by inoculating in five ml of Nutrient broth and Sabouraud dextrose broth (Himedia, Mumbai, India). The inoculum size was adjusted to 0.5 MacFarland standard measuring 10^8 cfu/ml.

Preparation of the extracts: Twelve herbs viz., Azadirachta indica (leaf), Cinnamomum verum (bark), Citrus lemon (fruit peel), Emblica officinalis (fruit), Ocimum sanctum (leaf), Piper longum (fruit), Piper nigrum (fruit), Punica granatum (fruit peel), Syzyium aromaticum

(Bud), Terminalia chebula (fruit), Terminalia bellirica (fruit), Zingiber officinale (Rhizome) were collected from local market and authenticated. The samples were washed, dried in shade and were powdered. These samples were separately suspended in 300 ml of Ethanol (Et OH) in a soxhlet apparatus for 6 hours. Then the extract was filtered and evaporated on water bath at 60-80°C. The above extracts were tested individually for antimicrobial activity by zone of inhibition (ZOI) and minimum inhibitory concentration (MIC). Similarly all the twelve herbs were taken together and suspended in 300 milli litre of Ethanol (Et OH) in a soxhlet apparatus for 6 hours. Then the extract was filtered and evaporated on water bath at 60-80°C which is used as polyherbal extract.[14]

And the same was incorporated in Mouthwash formulation as given in table 1. Then the formulations were tested for its antimicrobial efficacy against Streptococcus mutans MTCC 890 and Candida albicans MTCC 3017 cultures by Zone of inhibition test.

Minimum Inhibitory Concentration (MIC) Test: MIC was determined by incorporating various concentrations of the extract (0.5-10 mg/ml) in ten ml of Mueller Hinton agar (Himedia) and SDA (Himedia). The medium with extract was mixed thoroughly and was allowed to solidify at room temperature. 100 µl of the inoculum was inoculated on each plate. The plates were incubated for 24-48 hrs at 35 - 37ºC for S. mutans and for 5 days at 28°C for C. albicans. Negative control with solvents (ethanol) was maintained. The platings were done in triplicates and the mean values were taken.[15]

Agar well diffusion method: Mueller Hinton agar (Himedia) and SDA plates inoculated with respective cultures by spreading on the surface of the media. A well was made in the center of the medium and from the formulation stock (100 milli gram dissolved in one ml of sterile distilled water) 50 and 100 µl was loaded in the well. Comparison of formulation was made with market available sample, Listerine cool mint. Tetracycline (10 µg) for S. mutans and Ketoconazole (10 µg) (Himedia) for C. albicans were maintained as positive control. The plates were incubated at their respective growth conditions as given in MIC. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm), and the platings were done in triplicates. Their mean values with standard deviation was taken.[15]

Table 1: Mouthwash Formulation

S.No	Item Name	Base	Formulation
1	Propylene glycol	5 g	5 g
2	Ethanolic extract	-	5 g
3	Ethanol	10 g	-
4	Polysorbate 80	1.8 g	1.8 g
5	Sodium benzoate	0.2 g	0.2 g
6	Honey	1 g	1 g
7	Water	82 g	87 g
pH		6.5	6.5

RESULTS

Ethanolic extract of C. verum and S. aromaticum extracts were showing better MIC value of 1 mg/ml than other extracts against S. mutans. Similarly C. verum and S. aromaticum extracts were showing better MIC value of 0.5 mg/ml, against C. albicans. The compiled results were tabulated in Table 2.

The polyherbal extract (100 µl) was showing 20 ± 0.5 mm diameter against S. mutans and 10±2 mm against C. albicans. The Mouthwash formulation showed zone of inhibition of 10±2 mm against S. mutans and no zone against C. albicans. Listerine was not showing zone against S. mutans and C. albicans. Base formulation without herbal extract was also not showing any zone of inhibition. The results were tabulated in Table 3 and Figures from 1-3. Positive control Tetracycline showed 26±2 mm diameter against S. mutans and Ketoconazole was showing 35±4 mm diameter against C. albicans.

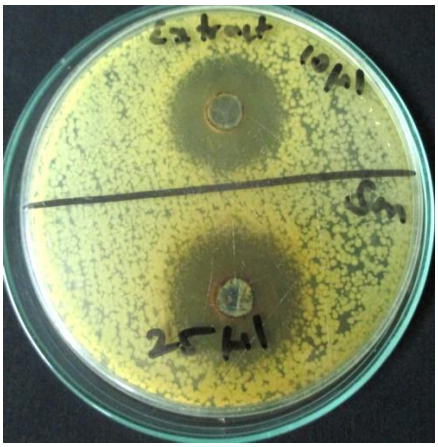

Figure 1: ZOI for polyherbal extract against S.mutans

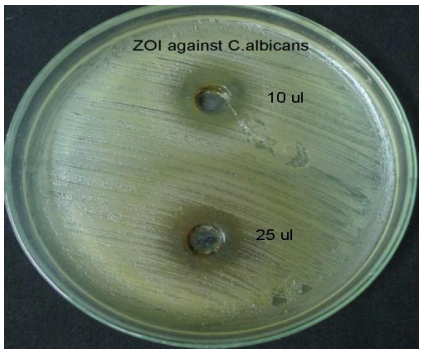

Figure 2: ZOI of polyherbal extract against C. albicans

Figure 3: ZOI for Listerine & Mouthwash Formulation 1

S.No	**Plant name**	**Part used**	MIC against *S. mutans* (mg/ml)	MIC against *C. albicans* (mg/ml)
1	*Azadirachta indica*	Leaf	5	5
2	*Cinnamomum zeylanicum*	Bark	1	0.5
3	*Citrus lemon*	Fruit peel	5	5
4	*Emblica officinalis*	fruit	4	5
5	*Ocimum sanctum*	Leaf	5	5
6	*Piper longum*	seed	2	4
7	*Piper nigrum*	seed	5	4
8	*Punica granatum*	Fruit peel	5	4
9	*Syzygium aromaticum*	bud	1	0.5
10	*Terminalia bellerica*	fruit	5	5
11	*Terminalia chebula*	fruit	2	5
12	*Zingiber officinale*	Rhizome	4	5
13	Poly herbal extract	_	5	Not active upto 10 mg/ml

Table 2: MIC of ethanolic extracts of plants against S. mutans and C. albicans

Table 3: Antimicrobial activity of Mouthwash formulation

Sample	**ZOI against S. mutans**	**ZOI against C. albicans**
Polyherbal extract	20 ± 0.5	10±2
Base Formulation	-	-
Formulation 1	10±2	-

Listerine (cool mint)	-	-
Ketoconazole (10 µg)	-	35±4
Tetracycline(10 µg)	26±2	-

DISCUSSION

The mouthwash/rinse formulation shows good antimicrobial activity, as it has Neem leaves containing tetracyclic triterpenoids which is proven for its efficacy in plaque management, anticaries and antioxidant effects.[16,17,18] Cinnamon bark found to be rich in highly electronegative compounds interfering in biological processes, thus reacting with nitrogen containing components, like proteins and nucleic acids, and inhibiting microbial growth.[19] Lemon peel is rich in alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz ., leaves, stem, root and flower) of Lemon against clinically significant bacterial strains has been reported.[20] Triphala have nitric oxide scavenging effects.[21,22] The therapeutic potential of Tulsi has been found to be largely due to eugenol, a major constituent of the essential oil, which is a phenolic compound (l–hydroxy–2–methoxy–4- allylbenzene). The other important constituents include ursolic acid and carvacrol which also has antimicrobial activity.[23]

The phytochemical analysis of pepper showed the presence of alkaloids, volatile oil, mono- and polysaccharides and resins. The alkaloids like piperine, piperidine, volatile oil and resins which could be responsible for the antibacterial activity[24] P. longum is reported to possess antiasthmatic, hepatoprotective, hypocholesteromic, antiamoebic, antiinflammatory and antibacterial.[25] P. granatum peel consists of cyanidin and pelargonidin that have antimicrobial effects and have been studied for their use in periodontitis and halitosis.[26] S. aromaticum buds contains eugenol used extensively in dental care for relieving toothache, sore gums and oral ulcers. Gargling with clove oil can also aid in sore throat conditions and bad breathe.[27] The main active phytochemicals present in ginger are gingerols, shogaols and paradols, and they have strong antioxidant and chemopreventive properties.[28] Ginger extracts have been extensively studied for a broad range of biological activities including antibacterial, anticonvulsant, analgesic, antiulcer, gastric antisecretory, antitumor, antifungal, antispasmodic, antithrombotic, hypocholesterolemic, antiallergic, antiserotonergic, anticholinergic and other beneficial activities.[29]

The antimicrobial activity of the herbs is due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, and lectins.[30] Since the extracts we have used are rich

in alkaloids, flavonoids, terpinoids, tannins etc., our formulation was found to be effective against S. mutans.

CONCLUSION

The extract and mouthwash/rinse formulation was found to be active against the oral pathogen S. mutans. Hence the formulation could be a boon for oral problems like dental plaque and caries.

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