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EVALUATION OF ANTIBACTERIAL POTENTIAL OF HERBAL MOUTH WASH ON LACTOBACILLUS IN COMPARISON WITH CHLORHEXIDINE GLUCONATE - AN IN-VITRO STUDY

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ABSTRACT **BACKGROUND**: Dental caries is one of the most common chronic diseases affecting millions of people globally. It is a major cause of tooth loss, pain and discomfort worldwide. The etiology of dental caries is polymicrobial in nature with majority of the microorganisms being Lactobacillus and Streptococcus. Chlorhexidine mouth wash is one of the most commonly used commercially available antimicrobial agent, but has its own adverse effects. Hence the aim of my study is to evaluate and compare the antimicrobial efficacy of herbal mouth wash with chlorhexidine gluconate. **MATERIALS & METHODS:** An in-vitro study was conducted with isolated colonies of Lactobacillus acidophilus. The tube dilution method was used for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). **RESULT:** The results of our study revealed that herbal mouthwash has got antimicrobial activity equal to the activity of conventional chlorhexidine mouth wash. **CONCLUSION:** It can be concluded that plants extracts have potential to be developed into agents which can be used as a preventive or treatment therapies for oral diseases such as dental caries.

KEYWORDS : Herbal Mouth wash, dental caries, conventional mouth wash, anti-bacterial, Lactobacillus acidophilus

INTRODUCTION:

Dental caries is a pathological process, which cause localized destruction of dental hard tissues. It is recognised as the primary cause for orofacial pain and tooth loss. The etiology of dental caries is more attributed to polymicrobial nature of dental plaque biofilm. (1) The association between lactobacillus and dental caries dates back to a century and it was considered as the leading cause of dental caries prior to 1950's, until streptococcus mutans started to dominate the literature. Lactobacillus are strictly fermentative organism, which sustain on habitats containing carbohydrates. They produce lactic acid as their by-product which lowers the pH of the oral environment. The current paradigm on caries suggests that, dental caries is caused by acidogenic bacteria that produce lactic acid by anaerobic fermentation of carbohydrates thereby reducing the pH (2,3,4) Effective treatments for the prevention of dental caries include, fluoride gels, toothpastes and mouth washes. Studies have shown evidence that commercially available chlorhexidine mouth washes have shown effectiveness against dental caries due to their anti-bacterial activity.(5) Along with antibacterial activity, chlorhexidine also has cytotoxic effects on mucosa, causes tooth discolouration, tartar accumulation, taste alterations and dry mouth. When prescribing chlorhexidine mouth wash its adverse effects also has to be taken into consideration. (6)

Due to adverse effects of most chemical drugs used, there is an increased proclivity of patients towards usage of natural products. Hence this study was conducted with an aim to evaluate and compare the antibacterial potential of an herbal mouthwash with commercially available chlorhexidine gluconate solution. (7)

The following are the components of herbal mouth wash used in the study:

Tulasi (Ocimum sanctum), Lavanga (Syzygium aromaticum), Jatiphala (Myristica fragrans), Misreya (Foeniculum vulgare), Peppermint satva (Mentha spp.), Suryakshara (Potassium nitrate)

- Suryakshara: Naturally derived **Potassium nitrate** (Suryakshara) inhibits pain in hypersensitive teeth through its desensitizing effect on dentinal nerves.
- Clove (Lavanga): It contains eugenol, an anesthetic chemical compound, which anesthetises nerves and stops pain. The essential oil of clove is also an antiseptic, which helps eliminate oral bacteria
- Peppermint oil: Peppermint (Mentha piperita) provides a pleasant, freshening flavor and sensation and antimicrobial properties.
- Myristica Fragrans: Has broad spectrum antibacterial activity. (8)

MATERIALS AND METHODS:

An invitro study was conducted using isolated colonies of *Lactobacillus acidophilus.* The tube dilution method was used for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC).

1 mL of sterile tryptic soy broth (Blood agar, Merck Germany) was added to ten sterile test tubes. Next, 1 mL of various dilutions of herbal mouth wash [1000µl to 1.9µl] was added to all the test tubes. A bacterial suspension of 1.5x108 CFU equal to No. 0.5 McFarland standard was prepared from *Lactobacillus acidophilus*.

1 mL of the dilute suspension was added to each set of 10 tubes that contained TSB medium and mouthwash. The tubes were finally placed in an incubator and were incubated at 37°C for 24 - 48 hours. After a period of 48 hours, the tubes were examined for the presence of turbidity, which indicates microbial growth. The last tube or the last dilution of mouthwash at which turbidity was not observed, was considered as the MIC. The above same procedure was followed for conventional mouth wash and the results were compared in terms of ability to inhibit microbial growth. After 24- 48 hours of incubation, the tubes without turbidity (appeared transparent), which indicated the inhibition of bacterial growth by the respective mouthwash, were transferred to a solid medium (Blood agar) and were evaluated in terms of microbial growth to determine the MBC of both mouthwashes. The last tube, which was negative in terms of culture on solid medium, indicated the minimum bactericidal concentration (MBC) of mouthwashes.

RESULTS:

The MICs of herbal and conventional chlorhexidine mouth wash for *Lactobacillus acidophilus* were 15.62 and 3.9(µg/mL) respectively (Table 1). The MBCs of herbal and conventional chlorhexidine mouth wash for *Lactobacillus acidophilus* were 31.24 and 3.9(µg/mL). The result indicate that, herbal mouthwash has got antimicrobial activity equal to the activity of conventional chlorhexidine mout h wash.

TABLE 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MIB) (µg/mL) of herbal and conventional chlorhexidine mouthwashes against oral pathogenic bacteria determined by the Tube Dilution Method.

	MIC(µg/mL)	MBC(µg/mL)
Herbal mouth wash	15.62	31.24
Conventional mouth wash	3.9	3.9

DISCUSSION:

The results of the present study, showed that herbal mouthwash can cause inhibition of bacterial growth with effects similar to conventional mouth washes. The use of mouthwashes as antimicrobial

agents can help mechanical methods like brushing and tooth flossing, to reduce plaques or biofilms that have an important role in the aetiology of dental caries and periodontal disease. (9) The three main methods in which mouthwashes exhibit antimicrobial effects are apoptosis, inhibition of bacterial growth and/or cell metabolic inhibition; and depending on their concentration bactericidal and/or bacteriostatic properties. (10)

Study conducted by Renuka et al, antibacterial and antiseptic effect was well established for herbs like Tulasi and lavanga. (11)

Natural herbs like, tulsi, neem, clove oil, pudina, and many more plants are used either as whole single herb or in combination and have been scientifically proven to be safe and effective medicine against various oral health problems like bleeding gums, halitosis, mouth ulcers and preventing tooth decay. The major strength of these natural herbs is that their use has not been reported with any side-effects till date. (12)

In the study conducted by Nasreen et al, Lactobacillus showed a zone of inhibition of 11mm, 13mm and 16mm for dilutions of tulsi extracts at 50µl, 100 µl and 150 µl respectively. Zone of inhibition of 9mm, 11mm and 17mm was observed for mint extracts with dilutions of 50µl, 100 µl and 150 µl respectively. This shows the antibacterial efficacy of tulsi and mint against Lactobacillus, which are two of the components used in my study.(13)

Study conducted by Rajalakshmi et al, to evaluate in-vitro antibacterial effect of herbal mouthwash containing Liquorice root, Tulsi leaf, no growth was observed at 100mg/ml concentration aginst Lactobacillus acidophilus.(14)

Thus, many studies have been conducted on the effects of herbal mouthwashes on oral microorganisms and have proven its efficacy on Lactobacillus. Though Chlorhexidine gluconate is proved to be the most superior amongst all mouthwashes, due to its high antimicrobial activity against two major dental caries causing microorganism (lactobacillus and streptococcus). In the present study herbal mouth wash was checked for is antibacterial activity in comparison with chlorhexidine mouth wash and results showed that herbal mouth is as equivalent to conventional mouth wash in use.

CONCLUSION:

The medicinal plants find its application in cosmetic, agricultural, pharmaceutical and food industry. The use of herbs with medicinal properties for curing disease has been documented in history of all civilizations. (15) Given the incidence of oral disease, increased resistance by bacteria to antibiotics, adverse affects of some antibacterial agents currently used in dentistry and financial considerations in developing countries, there is a need for alternative options for prevention and treatment that are very safe, effective and economical. While several agents are commercially available, these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhoea and tooth staining. Therefore, the search for alternative products continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives. Plant extracts or phytochemicals inhibit the growth of oral pathogens, reduce the development of biofilms and dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases. From the above study, it can be concluded that plants extracts have potential to be developed into agents which can be used as a preventive or treatment therapies for oral diseases such as dental caries.

REFERENCES:

1. Devi BP, Ramasubramaniaraja R. Journal of Pharmacy Research Vol. 2009 Nov;2(11):1669-75.
2. Caufield PW, Schön CN, Saraithong P, Li Y, Argimón S. Oral lactobacilli and dental caries: a model for niche adaptation in humans. Journal of dental research. 2015 Sep;94(9_suppl):110S-8S.
3. Caufield PW, Schön CN, Saraithong P, Li Y, Argimón S. Oral lactobacilli and dental caries: a model for niche adaptation in humans. Journal of dental research. 2015 Sep;94(9_suppl):110S-8S.
4. Badet C, Thebaud NB. Ecology of lactobacilli in the oral cavity: a review of literature. The open microbiology journal. 2008;2:38.
5. Sicca C, Bobbio E, Quartuccio N, Nicolò G, Cistaro A. Prevention of dental caries: A review of effective treatments. Journal of clinical and experimental dentistry. 2016 Dec;8(5):e604.
6. Müller HD, Eick S, Moritz A, Lussi A, Gruber R. Cytotoxicity and Antimicrobial Activity of Oral Rinses In Vitro. BioMed research international. 2017 Mar 19;2017.
7. Deshmukh MA, Dodamani AS, Karibasappa G, Khairnar MR, Naik RG, Jadhav HC. Comparative Evaluation of the Efficacy of Probiotic, Herbal and Chlorhexidine Mouthwash on Gingival Health: A Randomized Clinical Trial. Journal of clinical and diagnostic research: JCDR. 2017 Mar;11(3):ZC13.
8. L G Vijayaalakshmi, R V Geetha. Comparison of Herbal Mouth Wash with Conventional Mouth Wash in Use in Reducing Streptococcus Mutans -An Invitro Study. J. Pharm. Sci. & Res. Vol. 7(7), 2015, 485-486.
9. Chitsazi M, Shirmohammadi A, Balayi E. Effect of herbal and chemical mouth-rinses on periodontal indices; comparison of matrica, persica and chlorhexidine. Journal of Dentistry, Shiraz University of Medical Sciences. 2008;8(4):54-60.
10. Marin V, Zitelli A, Scandone L, Moretti GF. Bactericidal and bacteriostatic action of mouthwashes used in oral hygiene. Minerva stomatologica. 1982;31(5):583-90.
11. Renuka S, Muralidharan NP. Comparison in benefits of herbal mouthwashes with chlorhexidine mouthwash: A Review. Asian J Pharm Clin Res. 2017;10(2):3-7
12. Nagappan N, John J. Antimicrobial efficacy of herbal and chlorhexidine mouth rinse: a systematic review. J Dent Med Sci. 2012 Nov;2(4):5-10.
13. J Nasreen Banu, V Gayathri. Preparation of Antibacterial Herbal Mouthwash against Oral Pathogens. Int.J.Curr.Microbiol.App.Sci. 2016;5(11): 205-221.
14. T. V. Rajalakshmi Rakshanaa, T. Lakshmi. Antibacterial efficacy of herbal mouthwash against oral microbes - in vitro assay. J Adv Pharm Edu Res 2017;7(1):31-33.
15. Kabra P, Loomba K, Kabra SK, Majumdar DS, Kumar N. Medicinal plants in the treatment of dental caries. Asian Journal of Oral Health & Allied Sciences Volume. 2012 Jan;2(1):13.
16. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evidence Based Complementary and Alternative Medicine. 2009;2011(100):1-15.