



COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF VARIOUS NOVEL HERBAL MOUTHWASHES AGAINST STREPTOCOCCUS MUTANS - AN IN VITRO STUDY

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ABSTRACT

Introduction: Major causative agent causing dental caries are the microorganisms such as Streptococci mutans present in the oral cavity. The amount of microorganisms can be decreased on a daily basis through various preventive measures such as dentifrices, mouthwashes at home, fluoride application etc..Mouthwashes play an easy, economic, readily available way of keeping a relatively sterile oral cavity to maintain a good oral hygiene. The demand of herbal mouthwashes has increased in the recent times, due to the widespread awareness of the effect of complementary and alternative medicine. Herbs such as *Calotropis gigantea*, *Acacia catechu* extracts are used in this study. **Aim:** To comparatively evaluate the antimicrobial efficacy of various novel herbal extracts against *Streptococcus mutans* by agar diffusion test. **Material and methods:** Agar diffusion method was used to evaluate the antimicrobial action of mouthwashes. Sixty samples were segregated into four groups with 15 samples in each: Group I: *Acacia catechu* extract, Group II: *Calotropis gigantea* extract, Group III: *Hiora* herbal mouthwash, and Group IV: saline. The inhibition zones against *Streptococcus mutans* were recorded and statistically assessed using one-way analysis of variance (ANOVA) test ($P < 0.05$). **Result:** Significant but comparable antibacterial effect against *Streptococcus mutans* was observed with *Hiora* herbal mouthwash followed by *Calotropis gigantea*, *Acacia catechu* and saline. **Conclusion:** Experimental groups have all inhibited the growth of *Streptococcus mutans* & can be used as effective antimicrobial agents.

KEYWORDS : *Calotropis gigantea*, *Hiora* herbal mouthwash, *Acacia catechu*

Introduction

Dental caries is still one of the major healthcare concern in spite of various preventive measures in the world. Major causative agent causing dental caries are the microorganisms such as *Streptococci mutans* present in the oral cavity. The level of microorganisms play an important role in increasing or decreasing the incidence of caries. The amount of microorganisms can be decreased on a daily basis through various preventive measures such as dentifrices, mouthwashes at home, fluoride application etc..Mouthwashes play an easy, economic, readily available way of keeping a relatively bacteria free oral cavity to maintain a good oral hygiene.

Chlorhexidine gluconate, is one of the widely used mouthwash as of now and is a gold standard in antimicrobial efficacy. It is a very potent chemoprophylactic agent, it has a broad spectrum antibacterial action especially against *Streptococci mutans* group. But it has many side effects such as objectionable taste, tooth discoloration, desquamation and soreness of the oral mucosa.¹

Plant-produced compounds are of more interest as they are a source of safe and more effective substitutes against the synthetically produced antimicrobial agents.² Healing powers of plants have been used since ages in medicine but relatively recently introduced in dentistry. The demand of herbal mouthwashes has increased in the recent times, due to the widespread awareness of the effect of complementary and alternative medicine.³ It is also because herbal products have got less side effects. *Hiora*, commonly used mouthwash across the world is a herbal preparation made up of natural herbs with the benefits of being

anti-cariogenic, antiplaque, anti-inflammatory, anti-bacterial, antimicrobial and antioxidant properties.

Calotropis gigantea commonly known as Madar is a traditional medicinal plant belonging to the family of "Asclepiadaceae," habitat of Asian countries that includes India, Indonesia, Malaysia, Thailand, Sri Lanka, China, and others, and it is bestowed with enormous medicinal property. There are reports of tribal people using this plant parts to cure numerous illnesses such as toothache, ear ache, sprain, anxiety, pain, epilepsy, diarrhea, and mental disorders.⁴

Acacia catechu commonly known as Black cutch, is a medium sized thorny deciduous tree mainly found in India and also found in deciduous forests around the world. The extract of *Acacia catechu* extract have been reported to have various pharmacological effects. It is useful in cold and cough, ulcers, boils and eruptions of the skin, bleeding piles, uterine haemorrhages, chronic bronchitis etc. In stomatitis, halitosis, dental caries and cavities. The leaves, bark, heartwood has many nutritional and medicinal uses.⁵ *Hiora* is a herbal preparation made up of natural herbs with the benefits of being anti-cariogenic and antiplaque, anti-inflammatory, anti-bacterial and antimicrobial, antioxidant properties.

In the light of above knowledge this in-vitro study has been designed to compare and evaluate the antimicrobial efficacy of *calotropis gigantea* and *acacia catechu* against *S. mutans*.

Materials and methods

A total of 60 wells in 15 agar plates of brain heart infusion culture media were prepared for the study, which were divided as follows in four groups having 15 wells in each group.

- GROUP 1 Acacia Catechu Extract (n= 15)
- GROUP 2: Calotropis Gigantea Extract (n= 15)
- GROUP 3: Hiora herbal Mouthwash (n= 15)
- GROUP 4 :Saline (n=15)

Preparation Of Calotropis Gigantea Extracts

Calotropis gigantea powder weighing 20gms was soaked in 200 ml of ethanol for about 24 h. The supernatant that formed was filtered through muslin cloth & then evaporated in sunlight and dissolved in distilled water to get the required concentration of 2.5%.

Preparation Of Acacia Catechu Extracts

Acacia catechu powder weighing 10gms was soaked in 100ml of ethanol for about 24 h. The supernatant that formed was filtered through muslin cloth. The filtrate was then evaporated in sunlight and dissolved in dimethyl sulphoxide to get the required concentration of 100mg/ml.

Commercial preparation of Hiora herbal mouthwash & saline were used.

Agar Diffusion Test

The bacterial stock culture streptococcus mutans was obtained and culture was grown in BHI broth and inoculated in BHI agar plates. Inoculation was performed by using sterile swab brushed across the media. 4 wells of 4mm depth was punched in each agar plate and mouthwashes were added to the wells. Agar Plates were incubated at 37 degree celsius for 24 hours in a incubator. The diameter of bacterial inhibition zones around each well was recorded using vernier caliper (fig:1). The results were tabulated & statistically analyzed.

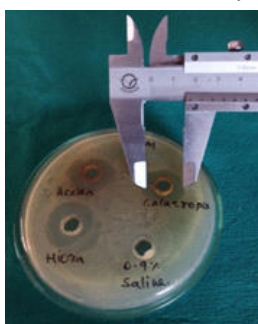


Figure 1: Measurement of inhibition zone using vernier caliper

Results

On statistical analysis group 3 Hiora herbal mouthwash showed the best result with the mean value 18.66 followed by group 2 Calotropis gigantea with a mean value of 18.26 followed by group 1 Acacia catechu with a mean value of 18.133 & group 4 with a mean value of 0.00.(Table :1)

Table 1: Mean values of inhibition zone in various groups

Groups	N (Sample Size)	Mean	Standard deviation	Minimum	Maximum
Group I	15	18.133	0.833	16.00	19.00
Group II	15	18.266	0.961	17.00	21.00
Group III	15	18.666	0.723	18.00	20.00
Group IV	15	0.000	0.000	.00	.00

Group I-Acacia, Group II-Calotropis, Group III-HiOra, Group IV -Saline

On applying one way anova we found that inhibition zone in all the groups was statistically significant(p=0.001).

The pair wise intergroup comparison was done using the independent T test. The intergroup comparison was non- significant between all the groups except when group IV was compared with group I, group II and group III, group III and group IV, (Table:3).

Table 2 : Pair wise Intergroup comparison

Groups		Mean Difference (I-J)	Std. Error	Sig.
Group I	Group II	-.13333	.26726	0.620 (Non-Sig)
	Group III	-.53333	.26726	0.061 (Non-Sig)
	Group IV	18.13333*	.26726	0.001 (Sig)
Group II	Group III	-.40000	.26726	0.140 (Non-Sig)
	Group IV	18.26667*	.26726	0.001 (Sig)
Group III	Group IV	18.66667*	.26726	0.001 (Sig)

Discussion

It has been well known that mouthwashes are effective means of reducing the amount of microorganisms in the oral cavity which are responsible for causing dent caries. Mouthwashes are thus recommended for small kids as they are easy to use, do not require manual dexterity and are proven to show antimicrobial activity, due to which they are beneficial for children.

Use of mouthwashes for the prevention of dental caries in children and adolescents was established as a mass prophylactic method in the 1960s and has shown average efficacy of caries reduction between 20-50%.6 Nowadays, many different types of mouthwashes are available in the market & various new formulas of mouthwashes with different antiplaque agents are being tried and tested continuously, so that we can make use of the new and safe mouthwashes with not only high efficacy, but also high effectivity and efficiency.

Herbal mouthwashes had been used in India and South Asia to clean and fight bacterial and fungal infections. Since ages complementary alternative medicine³, natural, unconventional, integrative and holistic are the terms used to describe medical and dental treatments that do not conform to the western or conventional allopathic regimes. The public's interest in alternative health care, including use of natural or herbal health care products has grown dramatically in the past few years. Common herbal ingredients in oral health care products range from cariostatic agents to analgesics to microbials to bleaching agents. According to Dalirsani et al (2011)⁷ and Anupama et al (2010)⁸, herbal mouthwashes have advantages such as decreased side-effects and are more economical when compared to allopathic commercial mouthwashes like Chlorhexidine.

In the present study, the highest zone of inhibition was seen with Hiora herbal mouthwash against s. mutans which was higher but comparable to the other two experimental mouthwashes used in this study. Hiora mouthwash contains herbal products like Pilu (Salvadorapersica), Bibhitaka (Terminalia bellerica), Nagavalli (Piper betel), Gandhapurataila, Ela, Peppermint satva and Yavanisatva. So, the synergistic effect of all herbal products might be responsible for its better antimicrobial efficacy. Evidence suggests that S. persica contains a number of antimicrobials including fluorides, alkaloids, sulfur compounds and volatile oils such as benzyl isothiocyanate, which can alter the characteristics of the early plaque settlers and make them less adherent. Whereas the anionic components of S. persica has an antimicrobial activity against Streptococcus mutans and several other cariogenic bacteria (Kaur R K 2014, Kaur S 2014)⁹.

Hydrochavicol in Piper betel inhibits expression of pro-inflammatory cytokine, tumor necrosis factor- α , disrupts the permeability barrier of the microbial membrane of S. mutans to an extent and also exhibits an astringent action.¹⁰ Rest of the contents like Terminalia bellerica, Gandhapurataila, Ela, Peppermint satva and Yavanisatva does not have any antibacterial property but they impart astringent action and flavour to the mouthwash.

Pathan M M, Bhat K G and Joshi V M compared the efficacy of Hiora herbal mouthwash and chlorhexidine mouthwash on periodontal pathogens by determining the minimum inhibitory concentration (MIC) using broth dilution method. Results showed that both the mouthwashes demonstrated effectiveness without any significant difference. This study had similar results as Bhat et al who compared CHX and the Hiora herbal mouthwash against supragingival dental plaque. The hiora herbal mouthwash showed comparable antibacterial effectiveness to CHX.¹¹

Ethanollic extract of Calotropis gigantea commonly known as madar

showed antibacterial activity lower than that of Hiora herbal mouthwash & higher but comparable to that of Acacia catechu. The antibacterial efficacy of *Calotropis gigantea* might be due to its presence of alkaloids, flavonoids, and tannins that are also found in Hiora. These phytochemical constituents are secondary metabolites of plants that provide the defence mechanism against herbivorous, insects & microorganisms. (Sharma et al 2015).¹²

Tannins form irreversible complexes with proline-rich protein 33 resulting in the inhibition of cell protein synthesis. Alkaloids are one of the largest groups of phytochemicals in plants; they have remarkable effects on decreasing pain perception in humans; this has led to the development of powerful painkiller medications.² Flavonoids, another constituent, exhibits a wide range of biological activities such as antimicrobial, anti-inflammatory, analgesic, antiallergic, cytostatic, and antioxidant properties. Hiora's antibacterial activity is synergistic effect of a multiple components which is absent in *Calotropis gigantea* mouthwash, this might be the reason for its lesser antibacterial activity. Sharma et al , 2015 evaluated the antibacterial potential of 2.5 %*Calotropis gigantea* extract against *Streptococcus mutans*. Study was designed to evaluate the anticariogenic efficacy of natural product of plant *C. gigantea* extract in mouth rinse form against salivary *S. mutans* and compare with two other commonly used artificial mouth rinses 0.2% chlorhexidine and Listerine. *C. gigantea* extract showed a significant reduction in salivary *S. mutans* .

In another invitro study conducted by Sharma et al (2017)*C. gigantea* has shown antimicrobial activity against *S. mutans* and *Lactobacilli*. It was found to be effective at as low as 1.25% concentration also.²

Acacia catechu commonly known as khair or black cutch showed antibacterial efficacy which was lower but comparable to that of *Calotropis gigantea* and Hiora herbal mouthwash. Antibacterial activity of Acacia catechu is primarily due to presence of components like Taxifolin & other flavinols. Hiora's and *Calotropis gigantea*'s antibacterial activity is due to multiple components present in them. This might be the reason for lesser but comparable antimicrobial activity of Acacia catechu.

Ahamed T S, Lakshmi T evaluated the anti-microbial activity of taxifolin extracted from Acacia catechu against *Streptococcus mutans* and *Lactobacillus acidophilus*. Results showed that Taxifolin inhibits *Streptococcus mutans* significantly when compared to *Lactobacillus acidophilus*.

Another study was done by Dinahar S to evaluate the antibacterial activity of heartwood extract of Acacia catechu willd. The results showed that the ethanolic extract had antibacterial activity against *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Bacillus subtilis* & was concluded that the Acacia catechu willd heart wood extracts contain antibacterial activity.

Group four saline showed no antimicrobial efficacy against *S. mutans*, thereby authenticating the validity of the present study.

Therefore, it can be concluded that *Calotropis gigantea* & Acacia catechu mouthwashes are comparable to Hiora herbal mouthwash in terms of antibacterial efficacy. Since these mouthwashes contain single ingredients, they might require less armamentarium for preparation thus cost effective than Hiora. Clinical trials should be made so as to check other properties in the intraoral environment. We recommend further studies to authenticate these results.

CONCLUSION

All the experimental groups showed inhibition of growth of *Streptococcus mutans* thus it can be concluded that herbal mouthwashes can be safely used as antibacterial agents for prevention of dental caries.

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