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Screening of Antimicrobial Activity of Active Compound of *Embelia basal*, Chlorhexidine and Amoxicillin against Salivary Microflora of Mixed Dentition Age Group

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ABSTRACT

Dental caries (decay) is ubiquitous and is one of the most prevalent infectious diseases of man. It is a localized, progressive demineralization of the hard tissues of the crown (coronal enamel, dentine) and root (cementum, dentine) surfaces of teeth. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in the different countries and are a source of many potent and powerful drugs. In this study the Antimicrobial activity of active compound of '*Embelia basal*' were compared with Chlorhexidine, Amoxicillin 125mg & Amoxicillin 250mg, against human salivary microflora at different concentrations. The antimicrobial activity was assisted by measuring the inhibition zones by well diffusion method. Saliva was collected from children of age group 6-12 years having DMFT value four or above four. Ten salivary samples were tested for antimicrobial property to determine the Minimum Inhibition Concentration in order to increase the reliability and precision of the study. The results confirmed the antimicrobial potential of active compound of 'Embeiia Basal' plant at different concentrations are comparable with chlorhexidine and can be used as preventive and therapeutic measure in dentistry in the form of different mouthwashes, irrigating solutions, gum paints etc.

Keywords: *Embelia basal*, chlorhexidine, amoxicillin, salivary microflora

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INTRODUCTION

Ayurveda is a branch of medicine which originated and is practiced in India for more than 5000 years. It is as fresh and useful to humans today as it was in the ancient times yet more relevant and applicable in these modern times. Its use provides a holistic approach to our daily lives. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000 - 500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents.

In recent years, prevalence of dental caries in most western countries is steadily declined. By contrast, studies done in some developing countries such as Zombia, Indonesia, Sudan, Nigeria, Thiland have indicated a marked increase in dental caries. [2] At the individual level, caries is a preventable disease. Given its dynamic nature the disease, once established, can be arrested or reversed prior to significant cavitation taking place.[3] As prevention is always better than cure one should be more interested in curing the disease before it occurrence. Oral cavity is mirror of general health so it important to take good care of oral cavity and preventing sever dental diseases like dental caries, periodontitis etc. As said earlier now a day focus is changing to herbal medicine from synthetic drugs because of many common reasons like naturally availability & unlimited source, minimum side effects of herbal products. So one should think of using herbal products to take care of oral cavity and to produce herbal dental medicine one should know medicinal value of some plants.

In this study we are investigating the antimicrobial properties of active compound of '*Embelia basal*' with chlorhexidine at increasing concentration against saliva in mixed dentition age group.

Materials and methods

Plant material

The fruits of *Embelia basal* (R & S) A. Dc. family Myrsinaceae were obtained as a market sample. The taxonomic identification was accomplished with the help of flora of Bombay Presidency and Flora of Maharashtra for identification. The fruits were authenticated by Agharkar Research Institute, Pune, Maharashtra, India. Its voucher specimen No. is AHMA F-084.

Experimental

The dried pulverized material (100g) was steam distilled. The distillate was extracted by using diethyl ether. During the removal of diethyl ether under reduced pressure, a crude mass was obtained. It was found to be 0.036% by the weight of dried material. Purification of the crude mass was executed using mixed solvent system of chloroform and hexane. Repeated

crystallization yielded white crystalline needle shape solid of 2-(2',4',5'-trihydroxy-3'-oxocyclohexa-1',5'-dienyloxy)-3,5,6-trihydroxycyclohexa-2,5- diene-1,4-dione.

The same active principle was isolated from acetone extract of the same plant material by employing different chromatographic technique followed by repeated recrystallization. The compound (1 mg) was dissolved in respective solvents (5 ml). The well (8mm) was filled with these extracts of different concentrations i.e. 5µg, 10µg, 20µg, 40µg, 80µg per well.

Criteria for selection of patients

In the present study, patients of 6-12 years of age, in mixed dentition period with DMFT value four or more were included. These patients had no history of antibiotic therapy or use of chemical anti-plaque agents prior to six months of study initiation.

Method of saliva collection and storage

The subjects were told to rinse with water; saliva was allowed to accumulate in the floor of the mouth for approximately two minutes and by asking the subject to spit in the funnel, saliva (3ml) was collected in a vial . By following the above mentioned method, 10 samples were collected in the early morning time. These salivary samples were diluted (3:1 ratio) in the sterile vials containing 1ml of normal saline and were used to inoculate on the agar plates. All samples were refrigerated within 30 minutes and frozen within 4 hours.

Antimicrobial Assay

The microbial inhibition assay was prepared using the agar well diffusion method. Sterile 8.0mm diameter of well were impregnated with the extract of different concentrations ranging from 50µg to 800µg per well. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and allow solidifying under aseptic conditions. The test samples of saliva (0.1ml) were inoculated with a sterile spreader on the surface of solid Muller Hinton Agar medium in plates. After the media was solidified; a well was made in the plates with the help of a cup-borer (8.0mm). The well was filled with different concentrations of the extract (50µg to 800µg/ well) and plates were incubated at 37 ± 0.1^{o}C for 24 hours. After incubation, the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimetres by using bacterial inhibition zone reading scale. All the tests were performed under sterile conditions. Chlorhexidine was used as positive control. The lowest dose required to attain maximum inhibition of a mixed oral micro flora was recorded. The dose dependent maximum inhibition zones of a mixed oral micro flora was recorded.

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RESULTS AND DISCUSSION

The demand on plant based therapeutics is increasing in both developing and developed counties due to growing recognition that they are natural products, non narcotic, easily biodegradable producing minimum environmental hazards, having no adverse side effects and easily available at affordable prices. Therefore researchers are progressively more turning their attention to natural products, looking for new leads to develop better drugs against microbial infections and screening of several medicinal plants for their potential antimicrobial activities [4].

This stud compares antimicrobial activity of active compound of *Embelia basal* with 0.2% chlorhexidine, amoxicillin 125mg and amoxicillin 250mg. The zone of inhibition are measured by excluding the diameter of well. The mean value of average zone of inhibition of active compound of *Embelia basal* with 0.2% chlorhexidine, amoxicillin 125mg and amoxicillin 250mg in ten salivary samples has taken for comparison. These zones of inhibition are directly proportional to the concentration means as the concentration increases the diameter of zone of inhibition also increases i.e. antimicrobial activity is more for greater concentrations (Table1). represents the diameter of zones of inhibition of ten salivary samples (S1 to S10) at increasing concentration. As we can see diameter of zone of inhibition is increasing as the concentration is increasing in each patient. So it indicates that active compound of *Embelia basal* has higher antimicrobial activity at 80 µg and to establish its exact greater maximum activity we need to increase more concentration and evaluate it (Table 2). represents the mean value of average zone of inhibition of chlorhexidine, amoxicillin 125mg and amoxicillin 250mg at five different concentrations. Results were obtained after 24 hours of incubation. Number 1, 2 and 3 in Fig 2 represents zone of inhibition of chlorhexidine, amoxicillin 125mg and amoxicillin 250mg respectively. Fig 1 represents average zones of inhibition (mm) of active compound of *Embelia basal*.

Table 1: Diameter of zone of inhibition.

Conc. (µg)	Diameter of zone of inhibition(mm)									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
5	2	10	7	10	10	6	6	5	5	6
10	5	12	17	12	12	10	10	7	7	7
20	6	13	20	15	15	11	11	11	10	8
40	8	15	18	17	17	12	12	12	12	9
80	10	18	18	19	19	12	15	15	14	10

Table 2: Mean value of Zones of inhibition of standard antimicrobial agent in salivary samples.

Antimicrobial agent	Mean value of average zone of inhibition
0.2% chlorhexidine	20.00
Amoxicillin 125mg	40.4000
Amoxicillin 250mg	48.4000

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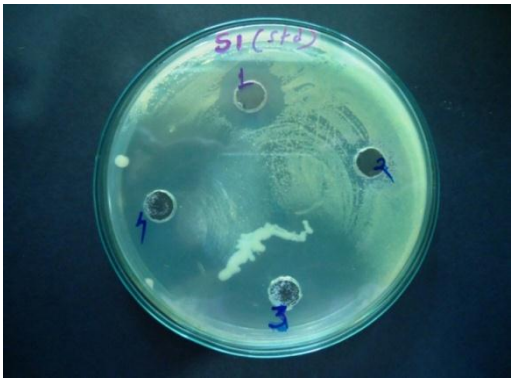

Figure 1: Here '1', '2' and '3' represents zone of inhibition of standard antimicrobial agent 0.2% Chlorhexidine, amoxicillin 125mg and amoxicillin 250mg respectively.

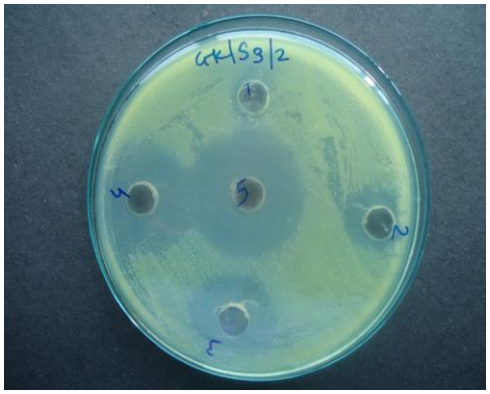

Figure 2: Zone of inhibition of active compound of *Embelia basal* at five different concentrations.

E. basal is highly esteemed in Ayurvedic medicine as a powerful anthelmintic [5] and also an important constituent of number of formulations [6,7]. In addition decoction is widely used in the treatment of insanity and heart diseases [7]. *Embelia basal* also shows antimicrobial activity & thus supporting its promising possibility of finding new clinically effective natural source of bioactive compounds [8].

This study proves that the antimicrobial activity of *Embelia basal* at higher concentration is comparable with 0.2% chlorhexidine and S-flo. Statistically, Kruskal-Wallis test followed by post-hoc test proved that all results are comparable as the p value is 0.0001 which is significant ($p < 0.5$). But to prove antimicrobial activity of active compound of *Embelia basal* with chlorhexidine, amoxicillin 125mg and amoxicillin 250mg we need to take further higher concentration, because mean of zone of inhibition of chlorhexidine, amoxicillin 125mg and amoxicillin 250mg are 20mm, 40.40mm and 48.40 respectively and mean of zone of inhibition at 80 µg is 15mm.

CONCLUSION

The antimicrobial activity of *Embelia basal* at higher concentration is comparable with 0.2% chlorhexidine. But to prove antimicrobial activity of active compound of *Embelia basal* with amoxicillin 125mg and amoxicillin 250mg we need to take further higher concentration. This study has confirmed the antimicrobial potentials of the plant, thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

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