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Research Article

# SCREENING OF ANTIMICROBIAL ACTIVITY OF ACTIVE COMPOUND OF EMBELIABASAL, CHLORHEXIDINE AND S-FLOAGAINST SALIVARY MICROFLORA OF MIXED DENTITION AGE GROUP

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**ABSTRACT**

Dental caries, also known as tooth decay or a cavity, is an infection, usually bacterial in origin, that causes demineralization of the hard tissues (enamel, dentin and cementum) and destruction of the organic matter of the tooth, usually by production of acid by hydrolysis of the food debris accumulated on the tooth surface. Approaches to the prevention of dental caries involve attempts to reduce the microbiological burden, reduce the availability of refined sugars, increase the resistance of teeth, or some combination of these approaches. Reducing the microbiological burden is the focus of interventions using antimicrobial rinses and dentifrices and behavioural interventions to improve oral hygiene and thus remove the bacterial plaque coating tooth surfaces. Behavioural interventions are also used to reduce the availability of fermentable carbohydrates through changes in the composition of the diet and frequency of ingestion of refined sugar. Increasing the resistance of teeth is typically achieved through the use of sealants and fluorides.

The word, Ayurveda in Sanskrit roughly means the "science or knowledge of life." Other ayurvedic experts however, contend that Ayurveda is even more accurately translated as the "science or knowledge of longevity." As such, it focuses on comprehensively addressing the body and preventing disease by reestablishing equilibrium. When balance is achieved, longevity – living a long, healthy, active and productive life – can be obtained[1].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[2].

In this study the Antimicrobial activity of active compound of 'Embelia Basal' were compared with Chlorhexidine, 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:** Embeliabasal, Chlorhexidine, S-Floagainst Salivary Microflora

## INTRODUCTION

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated250,000-500,000 plant species, only a small percentage hasbeen investigated phytochemically and the fractionsubmitted to biological or pharmacological screening iseven smaller. Thus, any phytochemical investigation of agiven plant will reveal only a very narrow spectrum of itsconstituents. Historically pharmacological screening ofcompounds of natural or synthetic origin has been thesource of innumerable therapeutic agents. Random screening as tool in discovering new biologically activemolecules has been most productive in the area of antibiotics[3,4].

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[5].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[6].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 dayfocus 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.

Considering the potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen theantibacterial activity from Embelia Basal plant.

Embelia Basal is a shrub from family 'Myrsinaceae', an Indian variety, is widely distributed throughout India and commonly known as 'Vidanga'. E.basal shows significant antimicrobial property[7]. 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°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.

**RESULTS AND DISCUSSION**

This stud compares antimicrobial activity of active compound of Embelia Basal with 0.2% chlorhexidine and S-flo. 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 and S-flo 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 80µgand 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 and S-float five different concentrations. Results were obtained after 24 hours of incubation. Number 1 and 2 in Fig 2 represents zone of inhibition of chlorhexidine and S-florespectively. 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 |

S1 to S10 are codes given to ten samples.

**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 |
| S-flo | 16 |


**Fig. 1: Here '1' and '2' represents zone of inhibition of standard antimicrobial agent 0.2% Chlorhexidine, S- florespectively.**

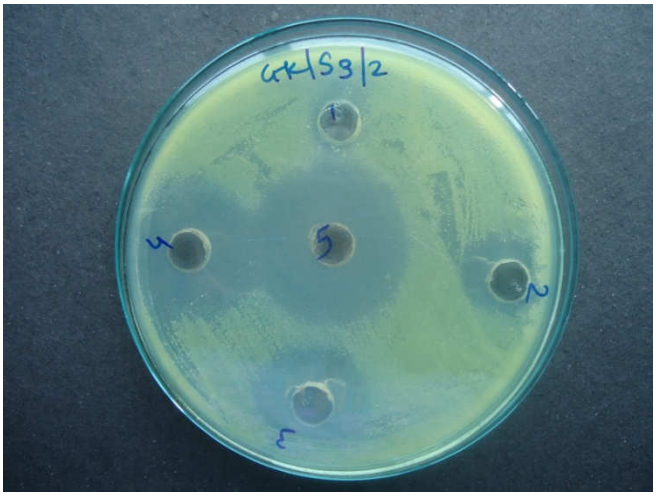


**Fig. 2: Zone of inhibition of active compound of embelia basal at five different concentrations.**

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 proveantimicrobial activity of active compound of Embelia Basal with chlorhexidine and S-flo we need to take further higher concentration, because mean of zone of inhibition of chlorhexidine & S-flo are 20mm & 16mm respectively and mean of zone of inhibition at 80µg is 15mm.

## CONCLUSION

The antimicrobial activity of embelia basal at higher concentration is comparable with 0.2% chlorhexidine. 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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