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A Comparative Evaluation of Antimicrobial Activity of Various Extracts of *Embelia basal* Against Salivary Microflora of Mixed Dentition Age Group.

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ABSTRACT

The increasing failure and side effects of popularly used chemotherapeutic and appearance of multiple drug resistant phenotypes in pathogenic bacteria led to the search of new compounds with antimicrobial activity. *Embelia basal* is a medicinal plant used in traditional Indian medicine for the treatment of various ailments. This plant was selected to evaluate its potential antimicrobial activity. The study was performed using acetone, ethanol and methanol extracts of *Embelia basal*at different concentrations by 'well-diffusion' method. The extract of *Embelia basal*was screened for antimicrobial activity against salivary microflora collected from children of 6-12 years of age group having DMFT =4. The results confirmed the antimicrobial potential of *Embelia basal*in acetone extract at all the concentrations were better than ethanol and methanol extracts and can be used as preventive and therapeutic measure in dentistry. This scientific approach has confirmed the antimicrobial potential of *Embelia basal*. Ayurveda can serve as a better alternative to its synthetic counterpart; hence the detailed study of plants like *Embelia* for preventive and therapeutic health care is the need of the hour.

Keywords: *Embelia basal*, acetone extract, methanol extract, ethanol extract.

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INTRODUCTION

Medicinal plants have proved to be significant resources for medicines; documentation of their use in medicine originates from ancient times. Medicinal plant based drugs have the added advantage of being simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action[1]. The genus *Embelia* has been investigated for a variety of purposes in Ayurveda [2]. It is a shrub from family Myrsinaceae, an Indian variety, is widely distributed throughout India and commonly known as '*Vidanga*'[3]. The*Embelia* Species has been tested and proved to possess anti-helmenthic[4], anti-oxidant[5] and antimicrobial [6] properties. Although *Embelia ribes* has been studied for anticariogenic properties[7],no research has been carried out on the *Embelia basal* varieties against salivary micro-flora. The trend of using natural products has increased and the active plant extracts are frequently used for new drug discoveries and for the presence of antimicrobials [8]. Oral diseases are major health problems with dental caries and periodontal diseases, among the most important preventable global infectious diseases. With respect to diseases caused by microorganisms, the increasing resistance in many dental pathogens to currently used antibiotic drugs has led to renewed interest in the discovery of alternative prevention and treatment options that are safe, effective and economical.

Moreover, chemicals like chlorhexidine, amine fluorides or products can and have undesirable side-effects such as teeth and restoration staining, increase in calculus deposition and imbalance of the oral and intestinal flora, thus leading to vomiting and diarrhoea. These drawbacks justify the search for new effective anticariogenic compounds that could be employed in caries prevention. This paper focuses on comparative evaluation of different concentration of *Embelia basal* in acetone, ethanol and methanol extracts against human salivary microflora.

MATERIALS AND METHODS

Plant Material

The fruits of *Embelia basal* (R & S) A. Dc. Family Myrsinaceae are obtained as a market sample. The fruit are authenticated by Agharkar Research Institute, Pune, and Maharashtra, India. Its Authentication No. is AHMA F- 084.

Preparation of acetone Extract.

Air shade dried and powdered fruit material (10 g) was refluxed with acetone for 18 hours. The yield of Acetone extract was found to be 11.6%. This extract was further used for experiments. Sample of acetone extract (50 mg) was dissolved in respective solvents (5 ml). The well (8mm) was filled with these extract of different concentrations ranging from 50µg to 800µg 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. 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. The inhibition zones of acetone, ethanol and methanol extracts were recorded and compared.

RESULTS AND DISCUSSION

The results of the antimicrobial assay of *Embelia basal*t different concentrations (50 µg, 100 µg, 200 µg, 400 µg, 800 µg) compare in acetone, ethanol and methanol extracts are presented in (Table 1, 2 and 3 and Figure 1, 2 ,3). All zones of inhibition are represented excluding the diameter of well. The zones of inhibition significantly increased as the concentrations were increased in the acetone, ethanol and methanol extracts. When the mean zones of inhibition were compared acetone had better values as compared to ethanol and methanol extracts at the different concentrations (Table 4). However, ANOVA- test, clearly indicates that there was no significant difference ($p>0.05$) in the anti-microbial activity of the acetone, ethanol and methanol extracts.

It must be noted that the acetone extract had produced zones of inhibition 20mm, 22mm and 28mm for concentrations 200µg , 400µg and 800µg respectively only in the fifth saliva sample which could be a false positive result.

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Table 1: *Embelia basal* acetone extract

sr no	concentration	S1(mm)	S2	S3	S4	S5	S6	S7	S8	S9	S10
1	50µg	2	0	0	2	4	7	7	8	7	4
2	100µg	4	4	0	4	6	9	11	10	9	7
3	200µg	8	4	4	6	20	10	12	12	11	8
4	400µg	4	10	5	8	22	12	14	13	12	10
5	800µg	4	6	8	10	28	15	15	15	16	15

Table 2: *Embelia basal* ethanol extract

sr no	concentration	S1(mm)	S2	S3	S4	S5	S6	S7	S8	S9	S10
1	50µg	0	0	0	0	2	4	10	5	7	7
2	100µg	2	2	2	0	2	7	11	10	11	9
3	200µg	4	4	4	2	2	10	11	11	10	11
4	400µg	6	6	6	10	12	12	14	15	12	13
5	800µg	8	6	8	8	4	15	16	15	13	15

Table 3: *Embelia basal* methanol extract

sr no	concentration	S1(mm)	S2	S3	S4	S5	S6	S7	S8	S9	S10
1	50µg	0	0	0	0	0	4	7	8	6	6
2	100µg	0	0	0	0	2	6	12	12	10	9
3	200µg	2	2	4	2	2	10	12	13	12	13
4	400µg	4	4	6	4	4	12	14	14	13	14
5	800µg	4	4	6	4	8	15	16	16	15	15

Table 4: Mean zones of inhibition

Concentrations (µg)	acetone (mm)	Ethanol (mm)	Methanol (mm)
50	4.1	4.2	3.2
100	6.4	6.3	4.3
200	9.5	6.2	6.3
400	11	8.3	7.6
800	13.2	11.4	10.4

Figure 1: Acetone extract

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Figure 2: Ethanol extract

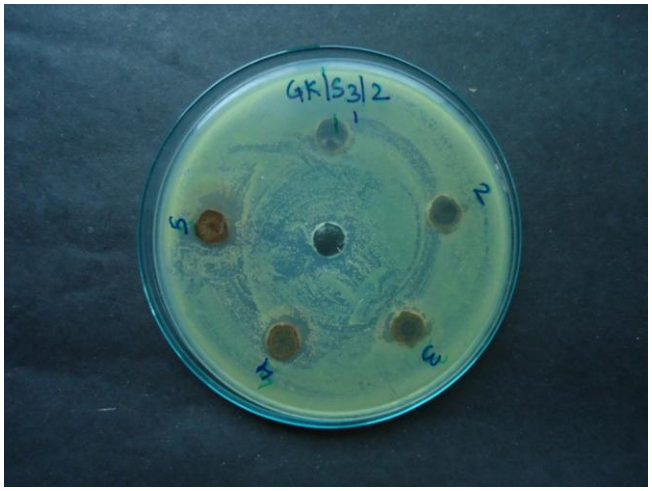
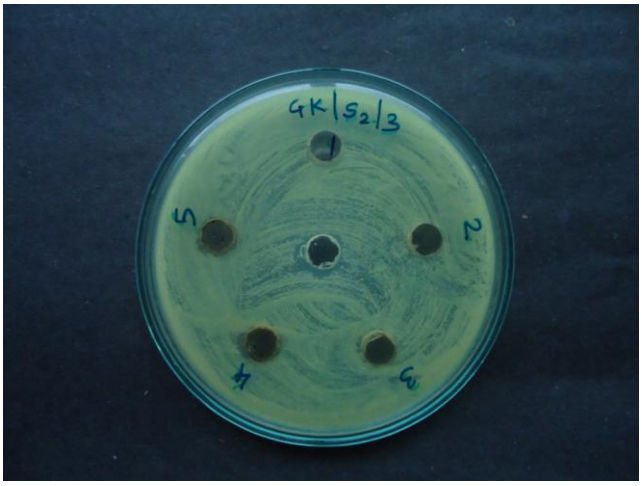


Figure 3: Methanol extract



The significant zones of inhibition indicates that an active molecule must be present in the plant and further studies need to be carried out in order to confirm and isolate the active ingredients of *Embelia basal* . The demonstration of antimicrobial activity by acetone extract provides the scientific basis for the use of this plant as preventive and therapeutic measure in traditional treatment of oral diseases. Natural product of higher plants may provide a new source of antimicrobial agents with possibly primordial prevention type of mechanism of action [9, 10]. In this study highest concentration of acetone extract of *Embelia basal* used was 800 µg. For toxicological investigations there is a need to study concentrations of *E.basal* above 800 µg. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the preventive and therapeutic needs in dentistry. The effect of this plant on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out. This study paves the way to research, wherein these herbal agents could be compared to the available gold standards like chlorhexidine or fluoride mouth washes available commonly in the market.

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CONCLUSION

This study indicates that the acetone extract followed by ethanol extract and methanol extract obtained from fruits of the medicinally important extract of '*Embelia basal*' was found to be an effective anti-microbial agent against the salivary micro flora. The study also confirmed the antimicrobial potentials of the plant, thus supporting its folklore application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity. The findings of the present investigation offer a scientific support to the ethnomedicinal use of the plant by the traditional healers.

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