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Comparative Evaluation of Antibacterial Activity of *E. basal* and *E. laevis* Against Salivary Microflora in Mixed Dentition Age Group.

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ABSTRACT

'Ayurveda', an age old discipline, provides a vast scope for treatment of diseases in a way that is thought to be closer to nature. Our forefathers had known different methods to treat ill-health. A few of those did pass on the generations, but as time passed these started to fade away. Hence to continue the legacy of our ancestors, different experiments have been conducted for the hunt of medicinal properties of plants. Plants are used medicinally in the different countries and are a source of many potent and powerful drugs. Plants having antifungal, antiemetic, antispasmodic and other properties have been discovered, of which, *Embelia basal* and *Ehretia laevis* are thought to have antibacterial property comparable to that of Chlorhexidine. Hence extracts of the above plant species were tested against salivary microflora in mixed dentition age group (6-12 years). The results were obtained based on the measurement of Inhibition zones on various concentrations of the extracts. When compared to the standard (Chlorhexidine) it was found that these extracts can be used as preventive and therapeutic measure in dentistry in different forms with least adverse effects.

Key words: *Embelia basal, Ehretia laevis,* salivary microflora, antibacterial activity, mixed dentition.

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INTRODUCTION

We are blessed to be born in our mother land, India, being the world's third largest bio reserve and the cradle of birth for a wide variety of flora and fauna. The Flora of India is one of the richest in the world showcasing a wide range of climate, topology and environments in the country. There are over 15000 species of flowering plants in India, which account for 6 percent of the total plant species in the world. India covers more than 45,000 species of flora, out of which there are several species that are not found anywhere else. India is divided into main eight floristic regions namely - Western Himalayas, Eastern Himalayas, Assam, Indus plain, Ganga plain, the Deccan, the Malabar and the Andamans [1].

Since the ancient times, use of plants as a source of medicines has been the inherent part of life in India. There are more than 3000 officially documented plants in India that holds great medicinal potential. Ayurveda has proved its wide acceptance and importance in India as well as abroad containing age old facts and mysteries as to what we can procure from the herbs, shrubs and trees we see in our day to day living. Talking of the medicinal values of plants around us, our ancestors had a fine knowledge about the treatment for most of the ill-conditions pertaining to the humans as well as animals, which mostly was collected from the extracts of these plants.

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'. The larger elliptical leaves of the plants are used in combination with ginger, are used as a gargle for sore throats. The dried bark of the root is used as a remedy for toothache and the finely powered berries are formulated as an ointment for treating pleuritis [3]. *E. basal* is highly esteemed in Ayurvedic medicine with powerful antihelmintic and antioxidant properties and also an important constituent of number of formulations [4]. *E. basal* shows significant anti-microbial property [5].

Ehretia laevis is a small tree. It is generally found in Asia and Australian tropics. Literature survey revealed wide biological activity of family Boraginaceae. The inner bark of *E. laevis* is used as food. Leaves are applied to ulcers and in treating headache. Fruit is astringent, antihelmintic, diuretic, demulcent, expectorant and used in infections of urinary passages, diseases of lungs and spleen. Powdered kernel mixed with oil is a remedy in ringworm. Seeds are antihelmintic [6]. The following study aims to showcase the antibacterial efficacy of these two plants in the prevention of dental caries in the mixed dentition age group.

MATERIALS AND METHODS

Plant material

The leaves of *E. laevis* was collected from Pune; Maharashtra, India during the month of July. The taxonomic identification is accomplished with the help of flora of Bombay Presidency and Flora of Maharashtra for identification. It was identified and authenticated at Botanical Survey of India, Pune, Maharashtra, India. Its voucher number is BSI / WC / Tech / 2006 /185.

The fruits of *E. basal* (R & S) A. Dc. Family Myrsinaceae are obtained as a market sample. The fruits are authenticated by Agharkar Research Institute, Pune, Maharashtra, India.

Preparation of Methanol Extract

Air shade dried and pulverized material (60.0 g) was charged with methanol (360 ml) at room temperature for eighteen hours. The solvent was recovered in vacuum under reduced pressure to yield a greenish black thick viscous mass (K, 5.83 %). The details are shown in table1. For the evaluation of the active principle(s), various fractions of K were screened by performing broad fractionation using gradient polarity of solvents (Hexane to methanol). Total three fractions were collected. Fraction I (i. e. fraction L) was a mixture of Compound 1 along with unidentified compounds, tested for the activity. Rechromatography of fraction L was achieved using gradient polarity of solvents (Hexane to ethanol) and collected Fraction II (i.e. sub-fraction M) was studied for its activity.

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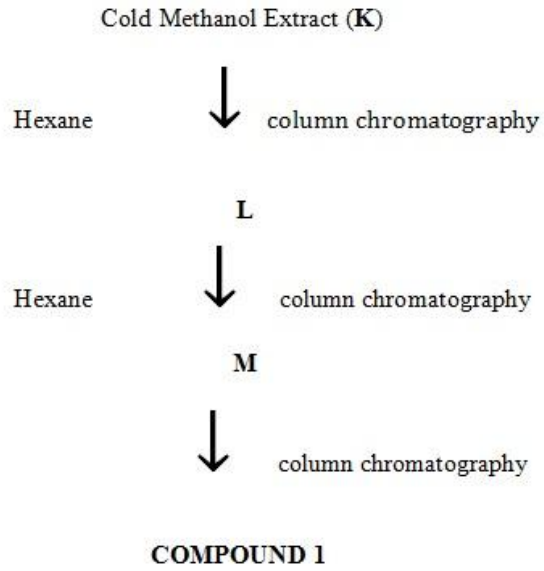
Table 1



Preparation of acetone extract

Air shade dried and powdered fruit material (10g) was refluxed with acetone for 18 hrs. The yield of extract was found to be 11.6%. This extract was further used for experiments. Sample of each acetone extract (50 mg) were 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 Hilton 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 Hilton 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 millimeters 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 were recorded.

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RESULT AND DISCUSSION

Chlorhexidine, a synthetically derived compound, is used widely in dentistry as a mouthrinse, irrigating solution, gum paints etc. This study is a comparison of synthetically derived compound with naturally derived extracts of *E. basal* and *E. laevis.*

The experiments are performed with various concentrations of the *E. laevis and E. basal*. The results depict that all the extracts have marked activity against the tested microorganisms. Results of test samples are reported after twenty four hours and indicate its dose dependent activity. The study of crude methanol extract (K) along with its fraction (L), sub-fraction (M). Zone of inhibition starts from concentration of 200µg/ ml and increases from K to M. It is observed that the zones of inhibition are directly proportional to the increase in concentrations of extracts. Extract M reveals maximum zone of inhibition at 400µg/ ml, as compared to K and L extract with very slight increase in activity at 800µg/ ml.

On comparison, it was observed that *E. basal* gave better results than the extracts of *E. laevis* under same concentrations as the zones of inhibition of *E. basal* measured greater than *E. laevis.*

Chlorhexidine being synthetically derived compound has several side effects some of them being dysguesia, discoloration, ulceration, staining, and tissue necrosis unlike the naturally derived extracts.

Figure 1: Zones of inhibition at 5 concentrations of acetone extract where numbers 1 to 5 indicates 50µg, 100µg, 200µg, 300µg, 400µg, 800µg concentrations respectively

Figure 2: Number 1 to 5 represents different concentrations of methanol extract from 50µg, 100µg, 200µg, 400µg, 800µg and different zones of inhibition respectively

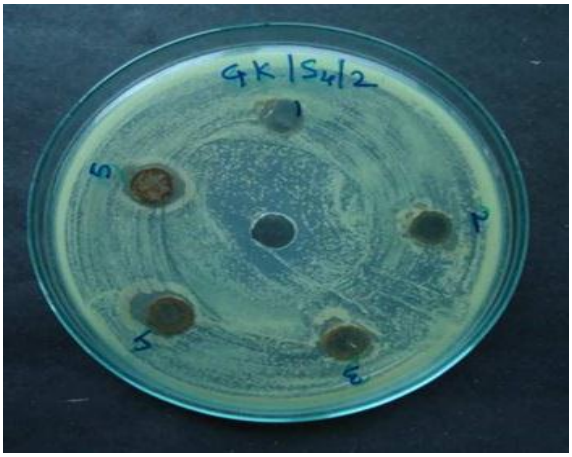

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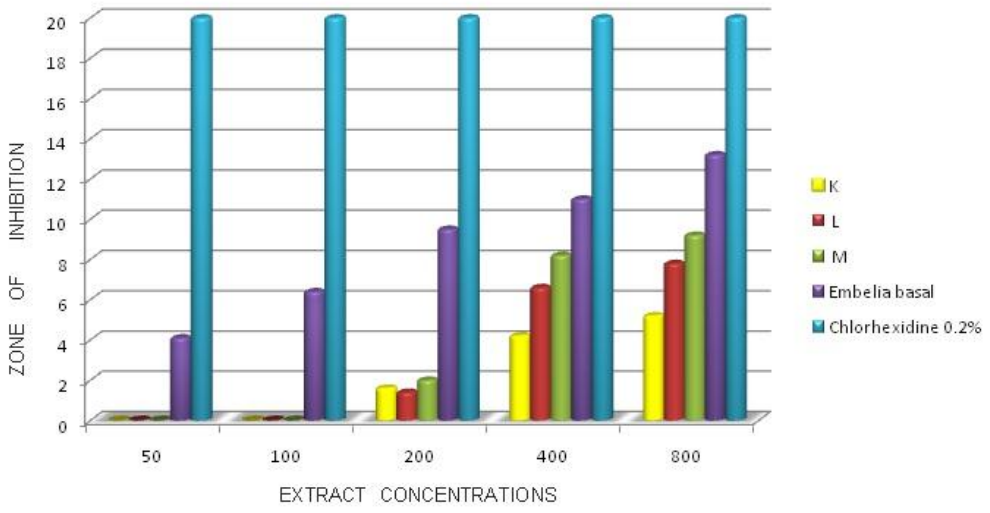
Figure 3: Here '1' represents zone of inhibition of standard antimicrobial agent Chlorhexidine 0.2%

Table 2: Comparison between concentrations of *E. basal* and *E. laevis* extracts with zone of inhibition

Extracts concentration (µg)		Zone of inhibition (mm)				
		50	100	200	400	800
Ehretia laevis	K	-	-	1.6	4.2	5.2
	L	-	-	1.4	6.6	7.8
	M	-	-	2.0	8.2	9.2
Embelia basal		4.1	6.4	9.5	11.0	13.2
Chlorhexidine 0.2%		20	20	20	20	20

Graph 1: where the numbers 50-800 represent the extract concentration in µg and 0-30 represent zone of inhibition in mm.



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CONCLUSION

Hence with comparable antibacterial properties *E. laevis* and *E. basal* can potentially be used as preventive and therapeutic measure in dentistry.

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REFERENCES

[1] http://en.wikipedia.org/wiki/Flora_of_India
[2] Agarwal V S., Drug Plant of India, Kalyani publishers, Ludhiyana 1997; 1:353.
[3] http;//www.botanical.com/botanical/mgmh/e/embili10.html.
[4] Gayatri S Kamble, Rasika C Torane, Kavita S Mundhe, Nirmala R Deshpande and Jyoti P Salvekar. *J Chem Pharm Res* 2011; 3(2):465-471.
[5] Gayatri S Kamble, Rasika C Torane, Asha A Kale, Nirmala R Deshpande and Jyoti P Salvekar. J Pharm Res 2011; 4(6):1640-1641.
[6] Joshi SG. 2000. Medicinal Plants 102.