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## Comparative Evaluation of Antimicrobial Properties of Different Extracts of '*Ehretia laevis*' Against Salivary Microflora.

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### ABSTRACT

Nature is an inexhaustible storehouse of riches. One of these is the therapeutic effect of various medicinal plants that have been described in traditional medicinal practice. 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. 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. In this study, the leaves extract of '*Ehretia laevis*' in methanol and its two fractions were evaluated and compared for antibacterial activity against salivary microflora. The salivary samples were collected from children of 6-12 years of age with moderate caries. Antibacterial assay was carried out using paper disc diffusion method. The results are compared with 0.2% Chlorhexidine as standard. The results depict that the extract have marked activity against the tested microorganisms with the sub-fraction of methanol extract showing highest zones of inhibition.

**Keywords:** antimicrobial activity; *E.laevis*; salivary microflora.

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## INTRODUCTION

Dental decay, also known as dental caries, is defined as a disease of the hard tissues of the teeth caused by the action of microorganisms, found in plaque, on fermentable carbohydrates (principally sugars) [1]. It is a chemico-parasitic process in which the oral microorganisms play a very pivotal role [2].

The prevalence of dental caries in industrialized countries like India is on the rise. As the treatment is very costly and requires a lot of manpower, the prevention at the primary level is the solution of choice [3]. It is reported that about 80% of the world population is dependent (wholly or partially) on plant-based drugs .

Conventional preventive methods such as the use of alcohol or antibiotics, e.g. chlorhexidine, erythromycin, ampicillin and penicillin, have proven effective in preventing dental caries [4].

However, various adverse effects such as tooth and restoration staining, increasing of calculus formation, diarrhoea, and disarrangements of the oral and intestinal flora has been associated with the use of these chemicals [5,6].

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies [7]. These drawbacks justify the search for new effective and herbal antimicrobial compounds that could be employed in oral preventive measures with minimal or no adverse effects. 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].

*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 headache. Fruit is astringent, anthelmintic, diuretic, demulcent, expectorant and used in affections of urinary passages, diseases of lungs and spleen. Powdered kernel mixed with oil is a remedy in ringworm. Seeds are anthelmintic [9].

This paper focuses on comparative evaluation of *E.laevis* in methanol sub- fraction extract against human salivary microflora

### Objective of Research

This research aims at finding a natural antimicrobial agent which could eventually substitute the available synthetic medications. 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.

## MATERIALS AND METHODS.

### Plant Material

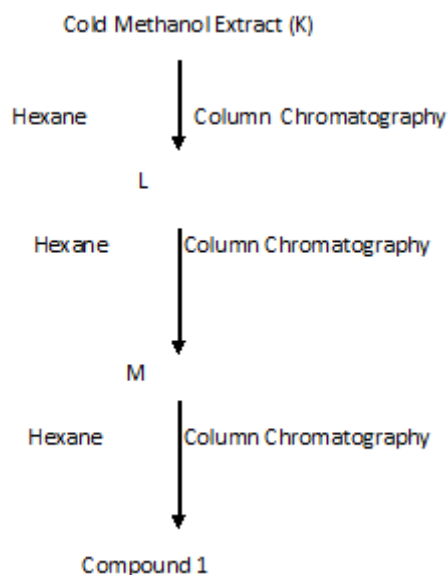
The leaves of *E. laevis* was collected from Pune; Maharashtra, India during the month of July. The taxonomic identification is accomplished with the help of flora of Bombay Presidency and Flora of Maharashtra [Cook T. 1958] for identification. It was identified and authenticated at Botanical Survey of India, Pune, Maharashtra, India. Its voucher number is BSI / WC / Tech / 2006 /185.

### 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 table 1. 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

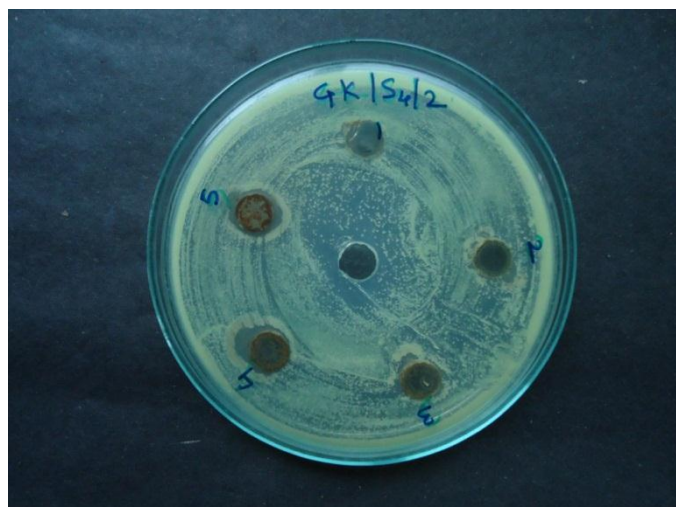
achieved using gradient polarity of solvents (Hexane to ethanol) and collected FractionII (i. e. sub-fraction M) was studied for its activity.

**Table 1**



**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 ml.
- 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 per/ml) and plates were incubated at 37 ± 0.1°C for 24 hours.
- After incubation, the plates were observed for zones of inhibition of growth and the diameters of these zones were measured in millimetres by using bacterial inhibition zone reading scale.



**Figure 1:** number 1 to 5 represents Different concentrations of methanol extract from 50µg,100µg,200µg,400µg,800µg and different zones of inhibition respectively.

**RESULTS AND DISCUSSION**

The experiments are performed with various concentrations of the *E. laevis* for each extract. 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 zone of inhibition are measured by excluding the diameter of well.. Compound 1 obtained from methanol extract of *E.Laevis* is an aromatic ester which comes out to be very potent moiety.

The mean value of average zone of inhibition of active compound of *Ethretia Laevis* is >10 mm at five different concentrations as compared to 0.2% chlorhexidine in which the zone of inhibition is 20 mm in ten salivary samples.

The antimicrobial activity of sub-fraction of *ethretia laevis* has proved to be lower at five different concentrations but remains constant at all the concentrations as compared to 0.2% chlorhexidine in the ten salivary samples.

Primary prevention among children and adolescents is of particular importance in India, due to high population numbers and wide economic, social, and health disparities amongst its population [10]. Oral and dental health awareness has improved tremendously over the last century but the prevalence of dental caries in children remains a significant clinical hurdle [11].

The leaves of *ethretia laevis* have proven to be an optimum source of antimicrobial activity. Dental caries and root canal infections being polymicrobial in nature [12], can be delayed in their process via using a susceptible antimicrobial agent which acts against specific bacteria respectively. Though, Chlorhexidine is used as standard antimicrobial drugs for such use and hurdling the carious process [13], medicinal plants like *ethretia laevis* due to their herbal properties have the dual action of having fewer side effects actively pronounced by the chemical agents which are the prime ingredients of chlorhexidine as well as being an effective antimicrobial product [14].

No	Saliva sample	Concentration	Zone of inhibition (mm)
1	Sample 1	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
2	Sample 2	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
3	Sample 3	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
4	Sample 4	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
5	Sample 5	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
6	Sample 6	Compound 1	

		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
7	Sample 7	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
8	Sample 8	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
9	Sample 9	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10
10	Sample 10	Compound 1	
		5	>10
		10	>10
		20	>10
		40	>10
		80	>10

**Table 2: Concentration of different saliva samples and their respective inhibition zones**

ANTIMICROBIAL AGENT	MEAN VALUE OF AVERAGE ZONE OF INHIBITION
0.2 % Chlorhexidine	20.00 mm

**Table 3: Concentration of chlorhexidine and its inhibition zone.**

### CONCLUSION

The antimicrobial activity of the active compound of *ethretia laevis* has proved to be lower at five different concentrations but remains constant at all the concentrations as compared to 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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