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Comparative Evaluation of Antimicrobial Properties of Two Different Extracts and One Derived Compound of Ehretia Laevis and Chlorhexidine against Salivary Microflora.

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ABSTRACT

Dental caries is the most prevalent disease of hard tissues in oral cavity in humans. Salivary microflora plays a major role in the caries process. Vigorous use of various preventive and therapeutic measures to control caries led to development of multidrug resistant strains to the synthetic antimicrobial agents and increased demand for new effective and efficient alternatives with minimal side effects. Since centuries plants and their extracts have been analyzed and reported to have significant therapeutic properties. The antimicrobial efficacy of many plants is yet to be verified. In this study, the methanol and ethanol extract of *Ehretia laevis* and derived Compound 5 of crude ethanol extract are evaluated and compared for antimicrobial 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 in lab. The results are compared with Chlorhexidine as standard. The results depict that all the extracts have marked activity against the tested microorganisms with the Compound 5 showing larger zones of inhibition. Thus, this in vitro study supports its application as a preventive remedy for microbial diseases of hard & soft tissues in the oral cavity.

Keywords- Antimicrobial activity; *E. laevis*; salivary microflora; Chlorhexidine

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INTRODUCTION

In the view of dentistry caries, an irreversible and multi-factorial disease of the calcified tissues of the teeth is the most prevalent disease in children & among several chronic dental problems in all age groups. National Health Survey conducted in 2004 throughout India has shown dental caries in 51.9% in 5 year-old children, 53.8% in 12 year-old children and 63.1% in 15 year-old teenagers [1]. Caries being an irreversible disease it is needful to focus on the prevention of dental caries. A number of chemotherapeutic agents are used to target the causative factors in oral diseases, among these factors salivary microflora play a pivotal role in dental caries and periodontal disease. Chemotherapeutic and antimicrobial agents aiming at these predisposing factors therefore play a significant role in prevention of such oral diseases. The vigorous use of such chemotherapeutic and antimicrobial agents to combat caries led to increased prevalence of side effects and failure of many popular synthetic antimicrobial agents due to development of multidrug resistant strains of micro- organisms. This justifies the search for new effective and efficient alternatives to synthetic antimicrobial agents with minimal or no side effects that could be employed as preventive measures in oral diseases.

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originated from plant sources, examples include aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) [2]. There are several studies performed to evaluate medicinal properties of many plants and anti-microbial, anti-fungal, anti-helminthic, anti-inflammatory, wound healing properties are found in these plant extracts [3, 4]. *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 infections of urinary passages, diseases of lungs and spleen. Powdered kernel mixed with oil is a remedy in ringworm. Seeds are anthelmintic [5]. This paper focuses on comparative evaluation of different concentrations of *E. laevis* in crude methanol, ethanol extracts and derived Compound 5 of crude ethanol extract and 0.2% chlorhexidine against human salivary microflora.

MATERIALS AND METHODS

Plant Material

The leaves of *E. laevis* were 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 [6]. 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 (60.0g) 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 %). This methanol extract K was used for the assessment of antimicrobial activity.

Preparation of ethanol extract

Air shade dried and pulverized material (150.0 g) was refluxed with ethanol (360 ml) at room temperature for eighteen hours. The solvent was recovered in vacuum under reduced pressure to yield a thick viscous mass O (7.33 %). This ethanol extract O was used for the assessment of antimicrobial activity. Purification of this viscous mass was accomplished with column chromatography to acquire the Compound 5. This Compound 5 was used for the estimation of antimicrobial activity. The details are reported in Table.

Standard antimicrobial agent

The zones of the inhibition of each plant extract are compared with 0.2% Chlorhexidine using it as a gold standard [7].

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Patient Selection Criteria

In the present study, patients of 6-12 years of age, in mixed dentition period with DMFT four or above four 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 for Saliva Collection

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 funnel, saliva (3ml) was collected in vial. 10 samples were collected in the early morning time. These salivary samples were diluted (3:1) in a sterile vial containing 1ml of normal saline and were used to inoculate on the agar plates [8].

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 allowed to solidify 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° 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 millimeters by using bacterial inhibition zone reading scale. All the tests were performed under sterile conditions. 0.2% Chlorhexidine was used as positive control.

RESULTS

The evaluation is performed with various concentrations *viz*. 50, 100, 200, 400 and 800 µg/ml for each methanol extract (K), ethanol extract (O) and Compound 5 of the *E. laevis* and it is compared with 0.2% chlorhexidine. The results depict that all the three plant extracts show significant antimicrobial activity against salivary microflora at higher concentrations and mean values of zones of inhibition significantly increase as the concentrations were increased (Table 1). In the study of these extracts and Compound 5 it appears that crude ethanol extract O is less active than crude methanol extract K. The ethanol extract O is almost inactive in all the studied samples up to 400 µg/ ml. It shows feeble activity at 400 and 800 µg/ml concentrations in most samples. Whereas, Compound 5, isolated from crude ethanol extract O exhibits significantly higher zones of inhibition as compared with ethanol extract O and methanol extract K. The antimicrobial activity of all the three extracts is lesser as compared with standard agent chlorhexidine (Table 2). Figure.1 below shows graphic bars representation of the values of zones of inhibition which significantly increase as the concentrations were increased.

Table 1: Zones of Inhibition of Methanol extract (K), Ethanol extract (O) and Compound (5) (in millimeter)

Mean values of zones of inhibition of Methanol extract (K), Ethanol extract (O) in mm and Compound (5) in mm			
Concentration of extracts (µg/ml)	Methanol extract (K)	Ethanol extract (O)	Compound (5)
50	-	-	**>10**
100	-	**0.2**	**>10**
200	**1.6**	**0.2**	**>10**
400	**4.2**	**1.2**	**>10**
800	**5.2**	**2.6**	**>10**

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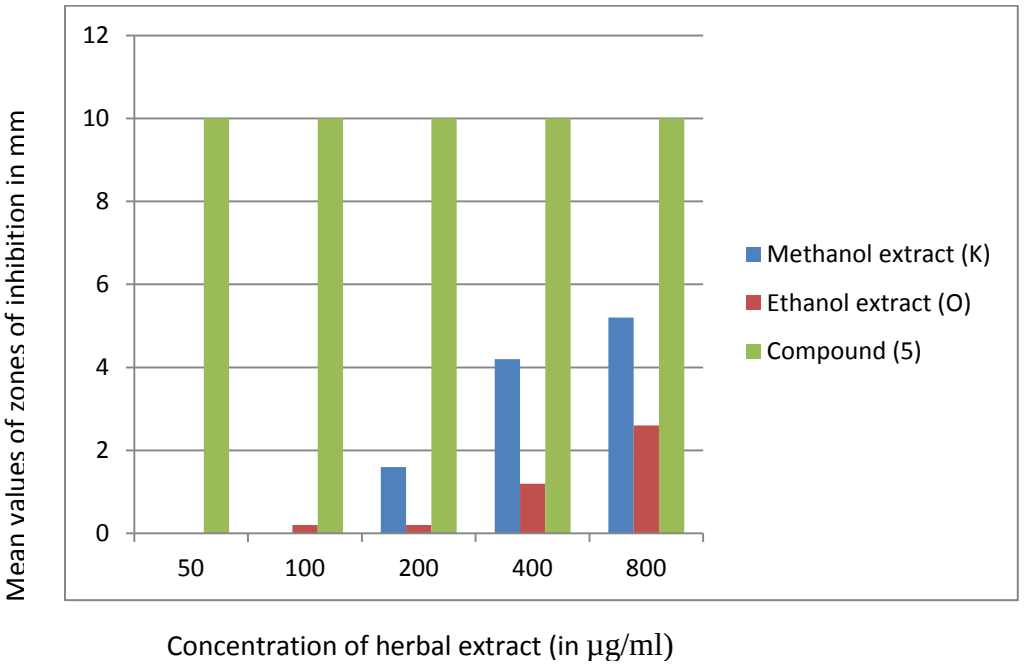
Table 2: Comparison of Zones of Inhibition of Methanol extract (K), Ethanol extract (O) and Compound (5) with 0.2% Chlorhexidine (in millimeter)

Mean values of zones of inhibition of Methanol extract (K), Ethanol extract (O) and Compound (5) in mmwith 0.2% Chlorhexidine				
Concentration of extracts (µg/ml)	Methanol extract (K)	Ethanol extract (O)	Compound (5)	0.2% Chlorhexidine
50	-	-	**>10**	**20 mm**
100	-	**0.2**	**>10**	
200	**1.6**	**0.2**	**>10**	
400	**4.2**	**1.2**	**>10**	
800	**5.2**	**2.6**	**>10**	

Figure 1: Diagrammatic representation of the values of zones of inhibition which significantly increase as the concentrations were increased.



DISCUSSION

The WHO has indicated that as many as 80% of all people living in the world make use of herbal medicine as their main source of healthcare [9]. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects [4]. From this study, it was evident that the plant extracts and especially the derived compound 5 of crude ethanol extract show significant antimicrobial activity in the tested samples. 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 *E. laevis*. The demonstration of antimicrobial activity by various extracts provides the scientific basis for the use of this plant as preventive and therapeutic measure in traditional treatment of oral diseases. It may have fewer side effects as it falls in the category of natural medicine. The effective plant extracts can be formulated in the form of a dentifrices, mouth washes, gum paints or as an intracanal medicament where an antimicrobial agent is required. The leaves of *E. Laevis* have been conclusive in demonstrating antimicrobial action. It may be interesting to obtain other active ingredients from the same plant or from different parts like stem, fruits etc. to assay its active ingredient and other properties and compared against each other.

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, evaluation of further higher concentrations for toxicological investigations and further purification however needs to be carried out.

CONCLUSION

This study indicates that the derivative compound 5 of crude ethanol extract obtained from leaves extract of *Ehrita laevis* 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 and soft 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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