Comparative Evaluation of Antimicrobial Properties of Active Extracts of *Morinda pubescens* and *Ehretia laevis* against Salivary Microflora

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**Abstract**

There has been an increase in the demand for newer antimicrobial agents to combat prevalent microbial diseases of oral tissues due to rapid emergence of multidrug-resistant pathogens and many drawbacks of synthetic chemotherapeutic drugs. This study aims to comparatively evaluate the antimicrobial efficacies of herbal extracts of *Morinda pubescens* and *Ehretia laevis* against salivary microflora and with a standard synthetic chemotherapeutic agent, to support use of natural herbs’ formulations as preventive and therapeutic agents. Their efficacy was evaluated by performing antimicrobial assay on patient saliva samples. It was observed that all the extracts have marked activity against the tested microorganisms with the acetone extract of *M. pubescens* and derived Compound 5 of ethanol extract of *E. laevis* showing maximum antimicrobial activity with larger zones of inhibition at used concentrations. This paper primarily focuses on a comparison between these two extracts, and it shows that extracts of *E. laevis* have higher antimicrobial activity than *M. pubescens* extracts at lower concentrations, but at higher concentrations *M. pubescens* shows larger zones of inhibitions. The effect of these plants on more pathogenic organisms, evaluation of further higher concentrations for toxicological investigations and further purification however needs to be carried out.

**Citation:**


1. Introduction

In the view of dentistry caries, an irreversible and multi-factorial disease of the calcified tissues of 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 (Bali et al., 2004). Several antibiotics such as ampicillin, chlorhexidine, sanguinarine, entmizadole, phenolicantiseptics and quaternary ammonium-antiseptics, among others, have been very effective in preventing dental caries (Chugh et al., 2006; Tsui et al., 2008). However, various adverse effects such as tooth and restoration staining, increasing of calculus formation, diarrhea and disarrangements of the oral and intestinal flora has been associated with the use of these chemicals (Vickers et al., 1999). These drawbacks justify the search for newer effective herbal antimicrobial compounds that could be employed in oral preventive measures with minimal or no adverse effects.

Mother Nature is an inexhaustible storehouse for the plants which have proved to be a significant resource for medicinal purposes since times immemorial. These plants are floripotent in nature and hence offer a great therapeutic umbrella for the health of the mankind. The use of plants for healing purposes predates human history and forms the origin of much modern medicine. 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 (More et al., 2008; Wee et al., 1992).

*Morinda pubescens* is one of popular medicinal plant which has been used in traditional medicine. *Morinda pubescens* commonly known as Indian mulberry belongs to family Rubiaceae. The plants are found as weed in the dried region of Maharashtra. Another species Morinda citrifolia commonly called as ‘Noni’ has been used for several years for its therapeutic and nutritional value (Joshi et al., 2000).

*Ehretia laevis* is a small tree. It is generally found in Asian 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 astrigent, 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 antihelmintic (Deshpande et al., 2013).

In this sense, efforts have been made to evaluate & compare the antimicrobial properties of active extracts of *Morinda pubescens* and *Ehretia laevis* with 2% chlorhexidine gluconate as a gold standard against human salivary microflora. Furthermore, the effectiveness of acetone extract of *M. pubescens* and derived Compound 5 of ethanol extract of *E. leavis* at various concentrations is also comparatively evaluated.

2. Objective of Research

The increase in prevalence of side effects of many synthetic antimicrobial agents and the recent appearance of microbial strains with reduced susceptibility to presently being used antibiotics raises the spectrum of untreatable bacterial infections and adds urgency to the search for newer chemotherapeutic agents. Herbal medicines are in great demand in the developed world for primary health care because of their efficacy, safety and minimal documented side effects. This research aims at finding a natural antimicrobial agent which would prove to be an effective alternative and eventually substitute the available synthetic medications.

3. Materials and Methods

This research would provide a scientific basis for the use of antimicrobial potentials of the herbal extracts of *Morinda Pubescence* and *Ehretia leavis* as effective antimicrobial formulations in combating oral diseases.

The antimicrobial potentials herbal extracts of the above said plants will be tested against salivary microflora obtained from human saliva samples by an antimicrobial assay.

2.1 Plant Material

The plant material *Morinda Pubescence* was collected from Pune, Maharashtra; India. It was authenticated at Agharkar Research Institute, Pune Maharashtra, India. Its authentication no. Is AHMA-21220 (Cook T., 1958).

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 (Deshpande et al., 2014). It was identified and authenticated at Botanical Survey of India, Pune, Maharashtra, India. Its voucher number is BSI / WC / Tech / 2006 /185.

2.2 Preparation of Acetone Extract of *Morinda Pubescence*

Air shade dried and pulverized leaves material (25g) for was refluxed with acetone for 18 hours. Solvent was recollected under reduced pressure to obtain crude extracts. Exactly weighed amounts of dried extracts (50 mg) were dissolved in respective solvent (5ml). Thus acetone solvent extracts were
analyzed for their antibacterial capacity against salivary microflora (Cook, 1958).

2.3 Preparation of ethanol extract of *Ehretia laevis*
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 (7.33 %). 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 (Deshpande et al., 2014).

2.4 Standard antimicrobial agent
The zones of the inhibition of each plant extract are compared with 0.2% Chlorhexidine using it as a gold standard (Jones et al., 1997).

2.5 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.

2.6 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 (Deshpande et al., 2013).

2.7 Antimicrobial assay
The microbial inhibition assay was prepared using the agar ‘well-diffusion’ method. Sterile 8.0mm diameter of well were impregnated with the extracts of different concentrations ranging from 50µg , 100µg, 200µg, 400µg and 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 [Deshpande et al., 2013].

4. Results and Discussion
The evaluation is performed with various concentrations viz. 50, 100, 200, 400 and 800 µg/ml for each acetone extract of *Morinda pubescence* and Compound 5 of the *E. laevis* and it is compared with 0.2% chlorhexidine. The results depict that these extracts show significant antimicrobial activity against salivary microflora as compared with 0.2% Chlorhexidine (Table 1).

The antimicrobial activity of all the extracts is lesser as compared with standard agent 0.2% chlorhexidine (Table 1). It appears that Compound 5 of crude ethanol extract of *E. laevis* is

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<th>Concentration of extracts (µg/ml)</th>
<th>Mean values of Zones of Inhibition (in millimeter)</th>
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<td>Acetone extract of <em>M. Pubescence</em></td>
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<tr>
<td>50</td>
<td>4</td>
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highly active than acetone extract of *M. pubescence* even at lower concentrations of 50, 100, 200 µg/ml showing values of zones of inhibition greater than 10mm whereas the acetone extract of *M. pubescence* shows feeble activity at 50, 100, 200 µg/ml concentrations in studied samples. However, acetone extract of *M. pubescence* show a significant increase in mean values of zones of inhibition ranging from 9.2 mm to 14 mm as the concentrations were increased to 400 and 800µg/ml (Table 2).

Since times immemorial Mother Nature is an inexhaustible storehouse for the plants which have proved to be a significant resource for medicinal purposes. 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. 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 [WHO, 2008]. From this study, it was evident that each acetone extract of *Morinda pubescence* and Compound 5 of the *Ehretia levis* 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 these plants. 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.

This investigation has opened up the possibility of the use of these plants in drug development for human consumption possibly for the preventive and therapeutic needs in dentistry. The effect of this plant on more pathogenic organisms is yet to be evaluated. In this study the herbal extracts of said medicinal plants are tested at lower concentrations, evaluation of further higher concentrations for toxico logical investigations and further purification however needs to be carried out.

### Conclusion

This comparative study indicates that the derivative compound 5 of crude ethanol extract obtained from leaves extract of *Ehretia laevis* was found to be more effective anti-microbial agent at lower concentrations than highly active acetone extract of *Morinda pubescens*, against the salivary microflora. At higher concentrations though, both plant extracts might show similar activity, which can be evaluated with further assay of these extracts. The study also confirmed the antimicrobial potentials of both the plants, 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 these plants by the traditional healers.

### Research Highlights

This research aims to develop newer effective and efficient antimicrobial agents with minimal side effects which would prove to be an alternative to currently being used synthetic antimicrobials. In the dentistry, these effective plant extracts can be formulated into preparations such as dentifrices, mouth washes, gum paints or as an intra-canal medicament and may be used where an antimicrobial agent is required. This research would help support the use of natural herbs, as medicine, which is already in a great demand in developing world.

### Limitations

This investigation opens up the possibility of the use of these plants in drug development for human consumption possibly for the preventive and therapeutic needs in dentistry. The effect of these plants on more pathogenic organisms is yet to be evaluated. In this study the herbal extracts of said medicinal plants are tested at lower concentrations, evaluation of further higher concentrations for toxico logical investigations and further purification however needs to be carried out.

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### References


