

Comparative evaluation of different concentrations of *Mimusops elengi* (L) extract as an antimicrobial agent against salivary micro flora

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Abstract:

Plants used in traditional medicinal systems have been proved to be reliable source of antimicrobial compounds and in the field of dentistry. In this study, the bark extract of *Mimusops elengi* was screened for antimicrobial activity against salivary micro flora collected from children of 6-12 years of age. The study was performed using acetone extracts of the plant by 'paper disc diffusion' method. The results were compared with chlorhexidine, a known chemical antimicrobial agent. The results confirmed the antimicrobial potential of the plant and indicated that the acetone extract can be used in the treatment of infectious diseases caused by salivary micro flora. A concentration of 450µg/disc was found to be inhibitory for the growth of most of the tested salivary micro flora. A dose dependent study on a salivary sample was performed to determine minimum inhibitory concentration and IC₅₀

Keywords : *Mimusops elengi*, chlorhexidine, salivary micro flora, acetone extract

Introduction:

Herbal medicines or herbal drugs are of ancient origin and their use is known in cultures throughout the world [1]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. A movement to identify individual active ingredients in beneficial herbs developed in the 18th century, leading into a transitional period from the use of natural herbs to the use of pharmaceutical drugs. However, during this time of transition, the synthesized, purified or extracted active ingredients of pharmaceutical drugs were observed to exhibit significant adverse side effects. The potential risk of using synthetic form of phytochemicals has been reported[2]. In the oral cavity, saliva serves as a reservoir for normal commensals as well as pathogenic micro flora causing infectious diseases. Dental decay is a chemico-parasitic process in which the oral microorganisms play a very pivotal role. For prophylactic purposes, it seems reasonable to target processes involved in formation of single or mixed bacterial communities that have the potential to cause or favour initiation of dental caries, without perturbing the balance of the normal flora [3].

Mimusops elengi Linn (Family: Sapotaceae) commonly known, as Bakul

or Maulsari in Hindi, is a native of Western Peninsula and also distributed widely throughout India. The plant is used as a gargle in salivation in weak and spongy gums, pyorrhea, stomatitis, ulcerated throat and antibacterial properties [4, 5]. In the study performed by Murudkar A. et. al. the antibacterial activity of bark of *M. elengi* was evaluated against isolated microbial species [6]. But, the properties & antimicrobial resistance of these microbial species vary significantly when they exist in isolated conditions & when in a community [4, 5]. Literature survey revealed that detailed research work has not been carried out to determine antibacterial activity of bark of *M.elengi*, especially in dentistry. The present study was undertaken to investigate the active principle, the potent drug, from active acetone extract against salivary micro flora.

Materials & Methods:

Plant Material

Plant material used in this study was collected from the local market of Pune, Maharashtra, India. It was authenticated at Agharkar Research Institute, Pune, India. Its authentication number is AHMA S/B – 065.

Preparation of extracts

Air shade dried powdered bark material (10g) was extracted using acetone (50ml), by soaking it for 24 hours at room

temperature. The solvent was evaporated under reduced pressure to obtain crude acetone extract (6.6 %).

Criteria for selection of patients

In the present study, patients of 6-12 years of age, in mixed dentition period with three or more decayed teeth were included. These patients had no history of antibiotic therapy or use of chemical anti plaque agents prior to 6 months of study initiation [7].

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 2 minutes and collected using sterile cotton tipped swabs placed in the same area (Swab method) [7]. By following the above mentioned method, 10 samples were collected in the early morning time. These salivary samples were diluted in a sterile vial containing 2 ml of normal saline and were used to inoculate on the agar plates. All samples were refrigerated within 30 minutes, and frozen within 4 hours. (If collection is being carried out in the field, it may not be practical to freeze the samples until the end of the day, but samples should be kept cold until they are returned to the lab)[7].

Anti-microbial Assay

The microbial inhibition assay was done using the paper disc diffusion method [8]. Sterile 5mm diameter filter paper discs were impregnated with the extract of different concentrations ranging from 150µg to 600µg per disc.

The bacterial strains were inoculated on nutrient broth and incubated for 24 hours at 37±0.1°C. Adequate amount of Muller Hinton agar was 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 medium in plates. The agar plates inoculated with these test samples were incubated for one hour before placing the extract impregnated paper discs on the plates. Following this, the sterile discs

impregnated with different extracts were placed on agar plates. The bacterial plates were incubated at 37±0.1°C for 48 hours. After incubation, all the plates were observed for zones 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. Chlorhexidine was used as positive control [9, 10]. The lowest dose required to attain maximum inhibition of a mixed oral micro flora was recorded.

Results and Discussion:

The results of the anti-microbial assay of the acetone extract of *M. elengi* showing average zones of inhibition (mm) are reported in Fig 1. A concentration of 450 µg of acetone extract is found to inhibit most of the salivary samples. The crude extract can be enhanced in activity upon further work.

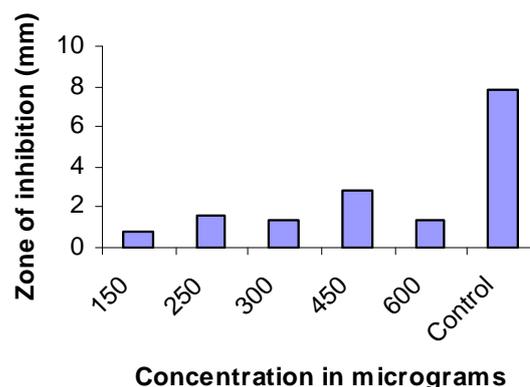


Figure 1: A concentration of 450 µg of acetone extract is found to inhibit most of the salivary samples.

A dose dependant evaluation of extract on a salivary micro flora is analyzed and is recorded in Fig 2. From the graph (Fig 2) it is noted that zone diameter is increased from 150 µg to 250 µg and remain steady. It indicates that 250 µg is the lowest dose required to attain maximum inhibition of a mixed oral micro flora of fungi and bacteria of the patients saliva. The graph indicates that 130 µg is the dose required to attain 50 % inhibition of mixed micro flora. During the GC-MS study of acetone extract one of the compounds is detected

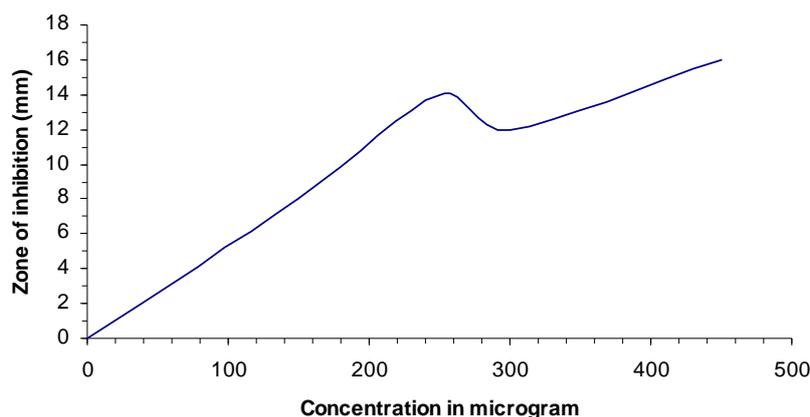


Figure 2: A dose dependant evaluation of extract on a salivary micro flora is analyzed and is recorded..

as cubebin (Fig. 3). The same is isolated from the extract. The structure of the molecule is confirmed by modern spectral

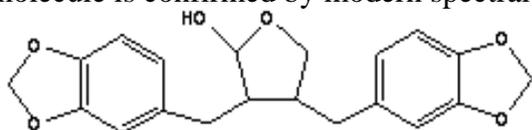


Figure 3: Molecular structure analysis (Mass, IR, NMR etc.). It was tested against the salivary micro flora under the same set of conditions and is found to be comparable as chlorhexidine, which is used as a control.

Caries is a more gradual disease process, with demineralization and remineralization occurring over time. Thus, the process is not a step function where surfaces or teeth show transition, instantly, from sound to cavitations. Neither visual examination nor various radiographic methods can adequately describe the process. During the timeframe from 1968 till today, significant advances have been made with respect to our understanding of the molecular basis of caries and our ability to measure earliest enamel demineralization changes and thus, caries progression [3,11] Thus, efforts need to be made for the primary prevention of dental caries initiation, rather than its treatment, throughout the life. Natural products of higher plants may provide a new source of antimicrobial agents with possibly primordial prevention type of mechanism of action. [11-13]

Also alternatives to available antibiotics for disease management are increasingly felt due to the increase in the resistance of bacterial isolates. Awareness for misuse of antibiotics and also the potential risk of using synthetic form of phytochemicals have been reported. This has necessitated the requirement of second and third line drugs. In the literature, *M. elengi* have been referred to as 'dantarogahara' in Ayurveda [14]. As described earlier, the plant finds an important place in the indigenous system of medicine [15]. The active principle is thought to be cubebin which needs to be further confirmed. However its similarity to reports from other groups [cubebin isolated from *Piper cubeba*] [16] point out that this could be taken as a lead molecule for further development against dental caries.

Conclusion:

Acetone extract of *Mimusops elengi* was found to be effective as anti-microbial against the oral micro flora. Possible target molecule cubebin is implicated in this preliminary study and need to be confirmed. The scientific approach has confirmed the antimicrobial potential of the plant extract thus adding weight to its use as a preventive remedy for various microbial diseases of hard tissues in the oral cavity in traditional medicine. The study provides a lead molecule which can be further developed against dental caries.

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