

**COMPARATIVE EVALUATION OF ANTIBACTERIAL PROPERTIES OF DIFFERENT EXTRACTS OF *MIMUSOPS ELENGI* (BAKUL) AND *EHRETIA LAEVIS* (AJAAN) AGAINST SALIVARY MICROFLORA**

MAYURI MUTHA<sup>1\*</sup>, RAHUL R DESHPANDE<sup>1,3</sup>, VISHWAS PATIL<sup>1</sup>, SNEHAL SHEP<sup>1</sup>, NIRMALA R DESHPANDE<sup>2</sup>,  
RASIKA TORNE<sup>2</sup>

<sup>1</sup>Department of Pedodontics and Preventive Dentistry, Dr. D. Y. Patil Dental College and Hospital, Pune - 411 018, Maharashtra, India.

<sup>2</sup>Department of Chemistry, Dr. T. R. Ingle Research Laboratory, Sir Parshurambhau, Pune - 411 030, Maharashtra, India. <sup>3</sup>Department of Dentistry, Deenanath Mangeshkar Hospital, Pune - 411 004, Maharashtra, India. Email: mayurimutha1410@gmail.com

Received: 07 October 2014, Revised and Accepted: 17 November 2014

**ABSTRACT**

**Objectives:** Oral health is an important aspect of the overall health of an individual. The diseases produced by a number of micro-organisms are manifested in the oral cavity. Ayurveda is a branch of medicine, and it is as fresh and useful to humans today as it was in the ancient times yet more relevant and applicable in these modern times. Its use provides a holistic approach to our daily lives. The potential of higher plants as a source for new drugs is still largely unexplored. Evaluation of antimicrobial properties of different concentration of extracts of *Mimusops elengi* and *Erethia laevis* extracts against the human salivary microflora.

**Method:** Microbial inhibition assay was prepared using the agar "well-diffusion" method. Sterile 8.0 mm diameter of the well were impregnated with the extract of different concentrations.

**Results:** This study compares antimicrobial activities of "*M. elengi* and *E. laevis*." The zone of inhibition are measured by excluding the diameter of well. These zones of inhibition are directly proportional to the concentration.

**Conclusion:** The results confirmed the antimicrobial potential of the plants at different concentrations and were comparable with chlorhexidine and can be used as preventive and therapeutic measure in dentistry.

**Keywords:** *Mimusops Elengi*, *Ehretia Laevis*, Antimicrobial.

**INTRODUCTION**

Ayurveda is a branch of medicine that originated and is practiced in India for more than 5000 years. It is as fresh and useful to humans today as it was in the ancient times yet more relevant and applicable in these modern times. Its use provides a holistic approach to our daily lives. The potential of higher plants as a source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents [1].

Oral health is an important aspect of the overall health of an individual. The diseases produced by a number of micro-organisms are manifested in the oral cavity. In recent years, prevalence of dental caries in most western countries is steadily declined. By contrast, studies carried out in some developing countries such as Zambia, Indonesia, Sudan, Nigeria, Thailand have indicated a marked increase in dental caries [2]. According to the National Oral Health Survey in 2004 caries prevalence in India was 51.9%, 53.8%, 63.1% at ages 5, 12, 15 years respectively [3].

Several antibiotics such as ampicillin, chlorhexidine, sanguinarine, entridazole, phenolicantiseptics and quaternary ammonium-antiseptics, among others, have been very effective in preventing dental caries [4,5]. However, various adverse effects such as tooth and restoration staining, increasing of calculus formation, diarrhea, and disarrangements of the oral and intestinal flora have been associated with the use of these chemicals [5,6]. Furthermore, there is an increase in prevalence of multidrug-resistant strains of bacteria. 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.

This paper focuses on comparative evaluation antimicrobial properties of different concentration of extracts of *Mimusops elengi* and *Erethia laevis* extracts with 2% chlorhexidine gluconate against the human salivary microflora.

**METHODS****Inclusion criteria**

Patients of 6-12 year-old in mixed dentition age group with moderate caries (decayed, missing, filled teeth=3-4) (modified WHO criteria 2003) having good general health [7].

**Exclusion criteria**

Patients with a history of antibiotic and oral drug therapy, chemical anti-plaque agents prior to 6 months of study initiation, physically and mentally handicapped patients were excluded from the study.

**Plant extracts**

Plant materials used in this study were procured from the local market of Pune, Maharashtra, India. The plant materials of *M. elengi* was authenticated at Agharkar Research Institute, Pune, India with authentication number AHMA S/B - 065. The plant material of *E. laevis* was authenticated at Botanical Survey of India, Pune, Maharashtra, India. Its voucher number is BSI/WC/Tech/2006/185.

**Preparation of extracts**

Air shade-dried powdered bark material (10 g) of *M. elengi* was extracted using acetone (50 ml), by soaking it for 24 hrs at room temperature. The solvent was evaporated under reduced pressure to obtain crude acetone extract (6.6%).

Air shade-dried and pulverized material (60.0 g) of *E. laevis* was charged with methanol (360 ml) at room temperature for 18 hrs. The

solvent was recovered in vacuum under reduced pressure to yield crude methanol extract (5.83%).

#### 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 (3 ml) 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 1 ml of normal saline and were used to inoculate on the agar plates.

#### Antimicrobial assay

The microbial inhibition assay was prepared using the agar "well-diffusion" method. Sterile 8.0 mm diameter of well were impregnated with the extract of different concentrations.

Adequate amount of Muller-Hinton Agar was dispensed into sterile plates and allow solidifying under aseptic conditions. The test samples of saliva (0.1 ml) 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 (8.0 mm). The well was filled with different concentrations of the extract and plates were incubated at 37±0°C for 24 hrs. After incubation, the plates were observed for zones of inhibition of growth and the diameters of these zones were measured in millimeters using bacterial inhibition zone reading scale.

#### RESULTS

The results of the anti-microbial assay of the acetone extract of *M. elengi* showed average zones of inhibition (mm) as in reported in Table 1. A dose-dependant evaluation of the extract on a salivary microflora was analyzed and recorded. It was noted that zone diameter is increased from 100 µg to 250 µg and remain steady. The crude extract can be enhanced in activity upon further work.

The experiment was performed with various concentrations of the *E. laevis*. The results depicted in Table 2 shows that all the concentrations have marked activity against the tested microorganisms. Results of test samples are reported after 24 hrs and indicate its dose-dependent activity. It appears that zone of inhibition increases at 400 µg/ml from K to M. Extract M reveals maximum zone of inhibition at 400 µg/ml, when compared to K and L extract. The results watched carefully materialized that there is very slight increase in activity at 800 µg/ml.

#### DISCUSSION

Alternatives to available antibiotics for disease management are increasingly felt due to an increase in the resistance of bacterial

**Table 1: Mean value of zones of inhibition of *M. elengi* extract at different concentrations**

Concentration of the <i>M. elengi</i> extract (µg/ml)	Mean
100	6.25
150	9.50
200	8.25
250	10.00
Control	18.50

*M. elengi*: *Mimusops elengi*

**Table 2: Mean value of zones of inhibition of *M. laevis* extract at different concentrations**

Concentration of <i>E. laevis</i> (µg/ml)	Mean
100	00
200	0.4
400	4.8
800	6.7
Control	18.50

*E. laevis*: *Ehretia laevis*

isolates. Awareness for misuse of antibiotics and also the potential risk of using a synthetic form of phytochemicals have been reported. This has necessitated the requirement of second and third-line drugs.

In Germany and France, many herbs and herbal extracts are used as prescription drugs and their sales in the countries of European Union were around \$ 6 billion in 1991 and may be over \$ 20 billion now. In USA, herbal drugs are currently sold in health food stores with a turnover of about \$ 4 billion in 1996 which is anticipated to double by the turn of the century. In India, the herbal drug market is about \$ 1 billion and the export of plant-based crude drugs is around \$ 80 million. 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. 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.

From this study, it was evident that the methanol extract of *E. laevis* and the acetone extracts of *M. elengi* has antimicrobial activity. This antimicrobial activity may be compared with other "synthetic" antimicrobial agents. It may have fewer side effects as it falls in the category of natural medicine. These plant extract can be formulated in the form of a dentifrices, mouthwashes, gum paints or as an intracanal medicament where an antimicrobial agent is required. The leaves of *E. laevis* and bark of *M. elengi* have been conclusive in demonstrating antimicrobial action. It may be interesting to obtain other active ingredients from the same plant or from different parts such as stem, fruits etc. to assay its active ingredient and other properties and compared against each other. Natural product of higher plants may provide a new source of antimicrobial agents with possibly primordial prevention type of mechanism of action [8,9]. 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 [8,10]. Thus, efforts need to be made for the primary prevention of dental caries initiation, rather than its treatment, throughout the life.

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.

#### CONCLUSIONS

The resistance in many dental pathogens to currently used antibiotic drugs is ever increasing. The ingredients derived from *E. laevis* and *M. elengi* plants used in this study are herbal, they are eco-friendly and do not produce any side-effects as well as are effective and economical, when compared to the synthetic drugs. 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.

#### REFERENCES

- Jadhav MV, Deshpande RR, Dadpe M, Mahajan P, Kakade P, Kamble G, et al. Screening of antimicrobial activity of active compound of *Embelia basal*, chlorhexidine and amoxicillin against salivary microflora of mixed dentition age group. Res J Pharm Biol Chem Sci 2012;3(4):1334.
- Kidd AM, Joyston-Bechal S. Essentials of Dental Caries: The Disease and its Management. 3<sup>rd</sup> ed. Oxford: Oxford University Press; 2005.
- National Oral Health Programme Implementation Strategies. DGHS, MOH & FW. Govt. Of India; 2004.
- Chung JY, Choo JH, Lee MH, Hwang JK. Anticariogenic activity of macelignan isolated from *Myristica fragrans* (nutmeg) against *Streptococcus mutans*. Phytomedicine 2006;13(4):261-6.

5. Tsui VW, Wong RW, Rabie AB. The inhibitory effects of naringin on the growth of periodontal pathogens *in vitro*. *Phytother Res* 2008;22(3):401-6.
6. More G, Tshikalange TE, Lall N, Botha F, Meyer JJ. Antimicrobial activity of medicinal plants against oral microorganisms. *J Ethnopharmacol* 2008;119(3):473-7.
7. Vieira AR, Marazita ML, Goldstein-McHenry T. Genome-wide scan finds suggestive caries loci. *J Dent Res* 2008;87(5):435-9.
8. Hamill FA, Apio S, Mubiru NK, Mosango M, Bukenya-Ziraba R, Maganyi OW, et al. Traditional herbal drugs of southern Uganda. Part III: Isolation and methods for physical characterization of bioactive alkanols from *Rubus apetalus*. *J Ethnopharmacol* 2003;87(1):15-9.
9. Motsei ML, Lindsey KL, van Staden J, Jäger AK. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *J Ethnopharmacol* 2003;86(2-3):235-41.
10. Katz BP, Huntington E. Statistical issues for combining multiple caries diagnostics for demonstrating caries efficacy. *J Dent Res* 2004;83:C109-12.