

COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACTS OF 'MORINDA PUBESCENS' IN DIFFERENT CONCENTRATION ON HUMAN SALIVARY MICROFLORA

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Received: 16 May 2013, Revised and Accepted: 18 Jun 2013

ABSTRACT

Objective: *Morinda Pubescens*' has been used in this study with different extracts and concentrations to test the antimicrobial properties against human salivary microflora.

Method: Human salivary microflora collected from children of age group 6-12 years having DMFT four or above four. The acetone extracts of *Morinda Pubescens*' have been compared by 'well diffusion' method. Ten salivary samples were tested for antimicrobial property to determine the MIC in order to increase the reliability and precision of the study.

Result: The result proved the antimicrobial properties of *Morinda Pubescens*' with maximum activity were found to be in acetone extracts.

Conclusion: Acetone extract was found to be more effective than ethanol and acetate extract as antimicrobial against the oral microflora.

Keywords: 'Morinda Pubescens', Extracts, Antimicrobial, DMFT.

INTRODUCTION

Dental caries is the most prevalent oral disease. It's very high morbidity potential has brought this disease into the main focus of the dental health profession. There is practically no geographic area in the world whose inhabitant does not exhibit some evidence of dental caries. It affects both the sexes, all races, all socioeconomic status and all age groups[1]. A very extensive and comprehensive National Health Survey[2], 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. Dental caries is microbial disease and multifactorial in origin. There are several other oral diseases like periodontitis, dental caries etc needs antimicrobial medicines.

Several antibiotics such as ampicillin, chlorhexidine, sanguinarine, enridazole, phenolic antiseptics and quaternary ammonium-antiseptics, among others, have been very effective in preventing dental caries[3,4]. 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[3,5]. 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.

In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet healthcare needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. *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[6].

Morinda Pubescens' has been used in this study with different extracts and concentrations to test the antimicrobial properties against human salivary microflora collected from children of age group 6-12 years having DMFT four or above four.

MATERIALS AND METHOD

Plant material

The plant material *Morinda Pubescens* was collected from Pune, Maharashtra; India. It was authenticated at Agharkar Research Institute, Pune Maharashtra, India. Its authentication No. is AHMA-21220

Preparation of extracts

Air shade dried and pulverized leaves material (25g) for each solvent was refluxed with chloroform, ethyl acetate, acetone and ethanol for 18 hours. Solvents were recollected under reduced pressure to obtain crude extracts. Exactly weighed amounts of dried extracts (50 mg) were dissolved in respective solvents (5ml). Thus hot solvent extracts were analyzed for their antibacterial capacity against six bacterial strains and a yeast strain.

Criteria for selection of patients

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 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 two minutes and by asking the subject to spit in funnel, saliva (3ml) was collected in vial. By following the above mentioned method, 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. 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).

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 ranging from 62.5µg to 4000µg per well. 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 (62.5µg to 4000µg/well) and plates were incubated at 37 ± 0.1°C for 24 hours. After incubation, the plates were observed for zones of growth of inhibition 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. Chlorhexidine was used as positive control. The lowest dose required to attain maximum inhibition of a mixed oral micro flora was recorded.

This is an procedure for the extract as discussed with Hon. Deshpande Madam.

Plant materials and preparation of extracts

Morinda Pubescence was collected from Western Pune, Maharashtra, India. The taxonomic identification was carried out with the help of Flora of Botany Presidency and Flora of Maharashtra and herbaria were prepared by following standard methods. The specimens were also compared with the authentic herbaria of BSI, Western circle Pune, Maharashtra, India for confirming the identification, its

number is BSI/WC/Tech /2009/95. Air shade dried and pulverized plant material was used. Extracts were prepared using exact weighed sample powder in the measured volume of solvents like, acetone, ethanol. Vacuum dried extracts are used for the experiment. Solvents used after distillation

RESULTS

Table.1 represents average zone of inhibition of the ethanol, acetone, acetate extracts at five different concentrations. Results were obtained after 24 hours of incubation. Figure 1, 2, 3 shows zones of inhibition at 5 different concentrations of Acetone, Ethenol, Acetate extracts. Amongst which acetone extract has shown maximum zone of inhibition in comparism to other two extracts i.e. ethanol and acetate extracts. The zone of inhibition were measured by excluding the diameter of well. In all the extracts the zone of inhibition increased with increasing concentration. In acetone extract maximum zone of inhibition was of 14mm diameter at 800µg, acetate extract showed the least zone of inhibition with compared to other two extracts.

Statistically, ANOVA test proved that acetone extract has more antimicrobial activity than ethanol extract as the p-value was found to be 0.00 (< 0.05) as represented.

Table 1: Diameter of zone of inhibition.

| Conc. (µg) | Average Zone of Inhibition of 3 Extracts (mm) | | |
|------------|---|-----------------|-----------------|
| | Ethanol Extract | Acetone Extract | Acetate Extract |
| 50 | 0 | 4 | 0 |
| 100 | 0.4 | 5.2 | 0 |
| 200 | 2 | 7.2 | 2 |
| 400 | 1.6 | 9.2 | 1.6 |
| 800 | 2 | 14 | 2 |



Fig. 1: Zones of inhibition at 5 concentrations of acetone extract. Where numbers 1 to 5 indicates 50 µg, 100µg, 200µg, 300µg, 400µg, 800µg concentrations respectively.

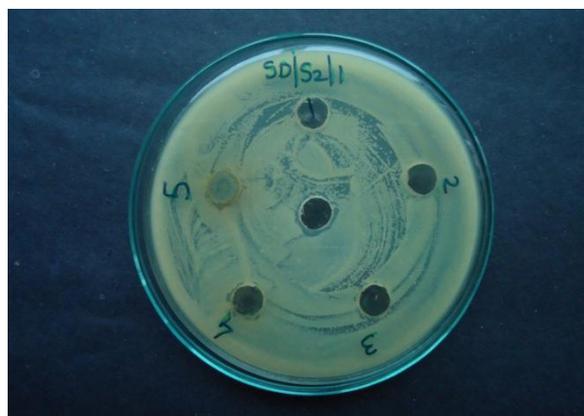


Fig. 2: Zones of inhibition at 5 concentrations of ethanol extract. Where numbers 1 to 5 indicates 50 µg, 100µg, 200µg, 300µg, 400µg, 800µg concentrations respectively.

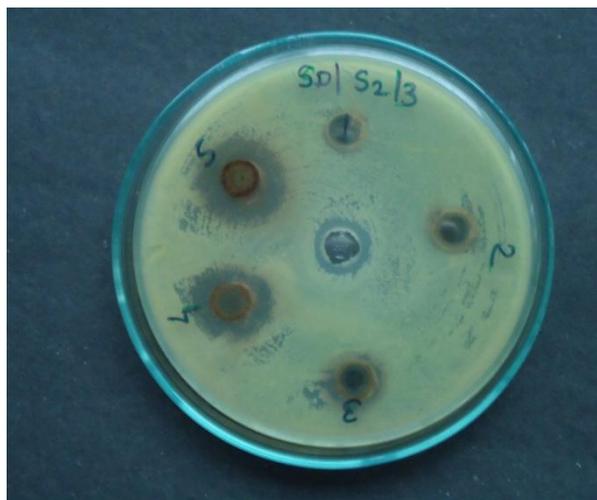


Fig. 3: Zones of inhibition at 5 concentrations of acetate extract. Where numbers 1 to 5 indicates 50µg, 100µg, 200µg, 300µg, 400µg, 800µg concentrations respectively

DISCUSSION

From this study, it was evident that the acetone extract has a better antimicrobial activity than the ethanol and acetate extract. This antimicrobial activity of *Morinda Pubescens* may be compared with other 'synthetic' antimicrobial agents. A clinician can exploit this antimicrobial agent as a prophylactic measure in Dental Caries, Intracanal Medicaments and in prevention of Periodontal Disease.

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[7]. 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.[8] There is a need to focus on the prevention of Dental Caries as the proverb goes 'Prevention is always better than cure'.

Antimicrobial activity of *Morinda Pubescens* was higher in acetone extract at 800µg and lower at 50µg which indicates that antimicrobial activity of this plant increases with increasing concentration.

This study compares the antimicrobial properties obtained by a plant and which is easily available to the common man. It may have fewer side effects as it falls in the category of natural medicine. The Acetone extract can be formulated in the form of a mouth wash, or as an intracanal medicament where an antimicrobial agent is required. We may use the ethanol and acetate extract as well, but this study indicates that the acetone extract has a superior antimicrobial activity.

CONCLUSION

Acetone extract was found to be more effective than ethanol and acetate extract as antimicrobial against the oral microflora. This study has confirmed the antimicrobial potentials of the *Morinda Pubescens*, thus supporting its application as a preventive remedy for various microbial diseases of hard and soft tissues in the oral cavity.

ACKNOWLEDGEMENT

Deshpande's Oral Health Clinic.

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