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A Comparative Evaluation of Antimicrobial Activity of *Embelia basal* Extracts, *Morinda pubescens* Extracts, Chlorhexidine 0.2% against Salivary Microflora of Mixed Dentition

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ABSTRACT

Throughout history, from the Bible,Quran,Vedas and other old texts, the medicinal benefits of herbs are quoted.The Western Ghats of India and Srilanka together comprise a biodiversity hotspot and have more than 2000endemic vascular plant species and is a rich source for medicinal plants.In this study a comparative evaluation of antimicrobial activity of embelia basal,Morinda pubescens extracts and chlorhexidine0.2% against salivary microflora of mixed dentition at different concentration is done. The antimicrobial activity was assisted by measuring the inhibition zones by well diffusion method. Saliva was collected from children of age group 6-12 years having DMFT value four or above four. Ten salivary samples were tested for antimicrobial property to determine the Minimum Inhibition Concentration in order to increase the reliability and precision of the study. The results confirmed that both the plants in the study showed the antimicrobial efficacy as compared to the golden standard .Such herbal extracts,with less side effects can be used as a therapeutic remedy for the hard tissue diseases of the oral cavity.

Key words - *Embelia basal, Morinda pubescens*, chlorhexidine0.2%, salivary microflora

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INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China and India but it is doubtless an art as old as mankind [1].

The increasing failure and side effects of popularity used chemotherapeutic agents and appearance of multiple drug resistance phenotypes in pathogenic bacteria led to the search of new compounds with antimicrobial activity. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand on plant based therapeutics is increasing in both developing and developed counties due to growing recognition that they are natural products, non narcotic, easily biodegradable producing minimum environmental hazards, having no adverse side effects and easily available at affordable prices. The problem of microbial resistence is growing and the outlook for use of antimicrobial drug in future is still uncertain. According to WHO medicinal plants would be best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants [2]. Several plants species are known to have helped in cure, treatment of periodontal diseases, particularly in alleviation of tooth aches.

The genus *Embelia* (vidanga) is a shrub from family *'Myrsinaceae'*, an Indian variety, is widely distributed throughout India. It is used as a gargle for sore throats, remedy for toothache and as ointment for treating pleurities. *Embelia* basal has powerful anthelmintic, antioxident and significant anti-microbial properties [3].

Morinda pubescens(bartondi) ,found as weed in the dried region of Maharashtra is used as traditional medicine, commonly known as Indian mulberry and belongs to family *Rubiaceae*(coffee family)[4] .

In the oral cavity, saliva serves as a reservoir for normal commensals as well as pathogenic micro flora causing infectious diseases. 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. Chlorhexidine 0.2% is used as a standard antibacterial agent [5].

This paper reveals the antibacterial efficacy of different concentrations of Embelia basal and *'Morinda pubescens'* and chlorhexidine 0.2% for a therapeutic purpose.

MATERIALS AND METHODS

Plant material

The extract of *Embelia basal and Morinda pubescens are* authenticated by Agharkar Research Institute, Pune, Maharashtra, India. Their authentication no. is AHMA F- 084 and AHMA-21220 respectively and was procured from local market, Pune, Maharashtra, India.

Preparation of acetone Extract

Air shade dried and powdered fruit material (10g) was refluxed with acetone for 18 hrs. The yield of extract was found to be 11.6%. This extract was further used for experiments. Sample of each acetone extract (50 mg) were dissolved in respective solvents (5 ml). The well (8mm) was filled with these extract of different concentrations ranging from 50µg to 800µg per well.

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

Inclusion Criteria

In the present study, patients of 6-12 years of age, in mixed dentition period with DMFT/deft value 4/>4were included.

Exclusion Criteria

Patients with history of antibiotic therapy or use of chemical anti-plaque agents prior to 6 months of study initiation.

Method of saliva collection and storage

The subjects were told to sit upright and rinse with water; saliva was allowed to accumulate in the floor of the mouth for approximately 2 minutes and by asking the subject to spit in a sterile funnel, saliva (3ml) was collected in a vial. By following the above mentioned method, 10 samples were collected in the early morning time. These salivary samples were diluted (3:1 ratio) in the sterile vials containing 1ml of normal saline and were used to inoculate on the Muller Hinton agar plates. All samples were refrigerated within 30 minutes and frozen within 4 hours.

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 well for *Embelia Basal* and from 62.5µg to 4000µg per well for *Morinda pubescens*. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and allow solidifying 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/ 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 millimetres 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. The dose dependent maximum inhibition zones of a mixed oral micro flora were recorded.

RESULTS AND DISCUSSION

Ten samples were evaluated for the their zone of inhibitionat different concentration for both the plants and their mean values were tabulated. (Table 1).

A dose dependant evaluation of extracts on salivary microflora was analysed and was reported in concentration of 800 µg of acetone extracts is found to inhibit most the salivary samples showing maximum zone of inhibition i.e.14mm(Morinda pubescens)and 13.2mm(Embelia basal)Chlorhexidine 0.2% shows the maximum zone of inhibition(20mm)(fig.4).

The graphical representation of the result obtained of acetone extracts of both the plants.It is noted that the average zone of inhibition is increased from 50 µg to 800 µg.(fig.1)

Comparative evaluation of the results of the two plants shows that there is a linear increase in antimicrobial activity of both at increasing concentrations. Also it is seen that the antimicrobial activity of *Embelia basal* is more than that of *Morinda pubescens* at all concentrations except at 800 (µg).This exception may be due to a threshold level reached by the antimicrobial activity at concentration of 800(µg).But for statistical significance further research has to be conducted with a larger sample size. The zones of inhibition significantly increased as the concentrations were increased in the acetone extract (Figure 2,3), but comparison in between the groups it is seen that Embelia basal extract has high antimicrobial activity as

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compared to *Morinda pubescens* extract with an exception in concentration of 800µg where the zone of inhibition of Morinda pubescens is more than that of *Embelia basal*(Table1)

Table1

Extracts Concentration (µg)	Zone of Inhibition(mm)				
	50	100	200	400	800
Morinda pubescens	4	5.2	7.2	9.2	14
Embelia basal	4.1	6.4	9.5	11	13.2
Chlorhexidine 0.2%	20				

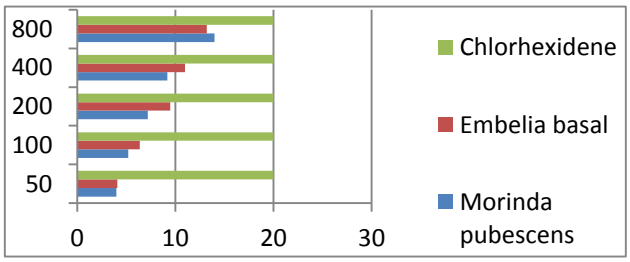

Figure 1: where the numbers 50-800 represent the extract concentration in µg and 0-30 represent zone of inhibition in mm.

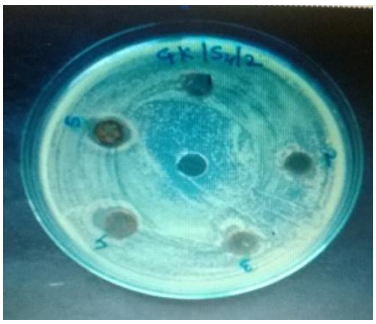

Figure 2: 1 to5 as different concentrations of acetone extract from 50 µg, 100µg, 200µg, 400µg, 800µg and different zones of inhibition respectively.

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

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Figure 4: Here '1' represents zone of inhibition of standard antimicrobial agent Chlorhexidine0.2%

CONCLUSION

Both the plants in the study showed the antimicrobial efficacy as compared to the golden standard. Such herbal extracts, with less side effects can be used as a therapeutic remedy for the hard tissue diseases of the oral cavity. Further research with higher sample size is required for the authentication.

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