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Research Article

SCREENING OF ANTIMICROBIAL ACTIVITY OF HERABAL EXTRACT OF "*MORINDA PUBESCENCE*", CHLORHEXIDINE & AMOXICILLIN AGAINST SALIVARY MICROFLORA OF MIXED DENTITION AGE GROUP.

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ABSTRACT

Objectives : In this study the Antimicrobial activity of active "*Morinda Pubescence*"in acetone extracts were compared with Chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg against human salivary microflora at different concentrations. Method : 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. Result: This study compares antimicrobial activity of "*Morinda Pubescence*"with 0.2% chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg. 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 "*Morinda Pubescence*"plant at different concentrations in acetone extracts are comparable with chlorhexidine and Amoxicillin and can be used as preventive and therapeutic measure in dentistry.

Keywords:

INTRODUCTION

By definition, 'traditional' use of herbal medicines implies substantial historical use,and this is certainly true for many products that are available as 'traditional herbal medicines'. 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 traditionalpractice, herbal medicines have often maintained their popularity for historical andcultural reasons. Such products have become more widely available commercially, especially in developed countries.

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds.[1]The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies.[2] Plants and herbs have attained a significant role not only as therapeutic agent but also as health maintaining agent.

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]. Primary prevention among adolescents is aparticularly important issue in India, due to high population numbers and wide economic, social and health disparities among its population.

So the aim of our study was to evaluate the antimicrobial activity of one of the important medicinal plant named "*Morinda Pubescence*" and commonly used antimicrobial agents like chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg against human salivary microflora. Chlorhexidine is commonly used in dentistry for different purposes like root canal irrigants, in case of stomatitis, in the form of mouthwash etc. Amoxicillin is a moderate-spectrum, bacteriolytic, β-lactam antibiotic used to treatbacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Amoxicillin is one of the most common antibiotics prescribed for children.

MATERIALS AND METHOD

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

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 mention method, 10 samples were collected in the early morning time. These salivary samples were diluted (3:1) in a sterile vile 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 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 (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 extracs are used for the experiment.Solvents used after distillation

RESULTS AND DISCUSSION

This study compares antimicrobial activity of "*Morinda Pubescence*"with 0.2% chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg. The zone of inhibition are measured by excluding the diameter of well. The mean value of average zone of inhibition of "*Morinda Pubescence*" with 0.2% chlorhexidine and S-flo in ten salivary samples has taken for comparison. These zones of inhibition are directly proportional to the concentration.

Table 1. represents the mean value of average zone of inhibition of the "*Morinda Pubescence*" at five different concentrations. Table 2.represents the mean value of average zone of inhibition of chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg at five different concentrations. Results were obtained after 24 hours of incubation. Number 1,3 and 4 in Fig 2 represents zone of inhibition of chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg respectively. Fig 1 represents average zones of inhibition (mm) of "*Morinda Pubescence*".

The demand for plant based medicines, healthproducts, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that thenatural products are non-toxic, have less side effects and easily available at affordable prices.[4]The global market for herbal medicines currently stands at over $60 billion annually. The sale of herbal medicines is expected to get higher at 6.4% an average annual growth rate.[5]

Tooth decay disease is caused by specific types of bacteria that produce acid in the presence of fermentable carbohydrates such as sucrose,fructose, and glucose.[6,7]Dental caries (tooth decay) is the single most common chronic disease of childhood.[8]

Dental caries is an infectious disease of bacterial origin. Therefore, it seems rational to use an antimicrobial approach to prevent and control dental caries. Chlorhexidine is an antimicrobial agent that has been studied extensively over the last 30 years for its ability to suppress the levels of mutans streptococci in the mouth and for its potential to prevent and control dental caries.

This study proves that the antimicrobial activity of "*Morinda Pubescence*" at higher concentration is comparable with 0.2% chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg.Statistically, Kruskal-Wallis test followed by post-hoc test proved that all results are comparable as the p value is 0.0001 which is significant (p< 0.5).

TABLES

Table 1: Mean value of Zones of inhibition of "Morinda Pubescence"

Concentration (µg)	Mean value of average zone of inhibition (mm)
50	4
100	5.2
200	7.2
400	9.2
800	14

Table 2: Mean value of Zones of inhibition of standard antimicrobial agent

Antimicrobial agent	Mean value of average zone of inhibition
0.2% chlorhexidine	20,0000
Amoxicillin 125mg	404.000
Amoxicillin 250mg	48.4000

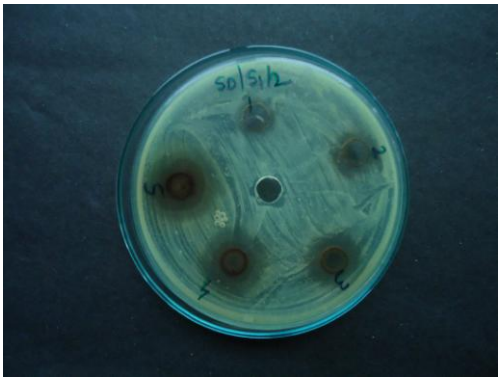

Fig 1: average zones of inhibition (mm) of "*Morinda Pubescence*"

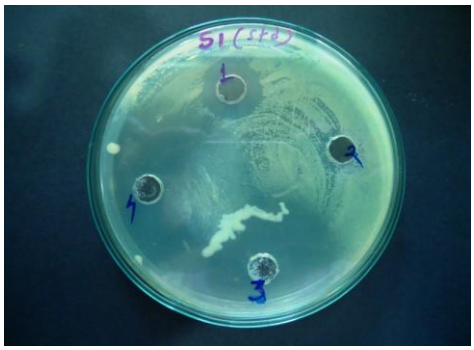

Fig 2: Here '1', '2' and '3' represents zone of inhibition of standard antimicrobial agent 0.2% Chlorhexidine, amoxicillin 125mg and amoxicillin 250mg respectively.

CONCLUSION

The antimicrobial activity of "*Morinda Pubescence*" at higher concentration is comparable with 0.2% chlorhexidine. This study need to increase concentration to confirm the antimicrobial activity of active compound of"*Morinda Pubescence*" againsed Amoxicillin. This study has confirmed the antimicrobial potentials of the plant, thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

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