ORIGINAL ARTICLE

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A Comparative Evaluation of Antimicrobial activity of Herbal Extracts and Chemically Synthesized Chlorhexidine Mouthwash against Salivary Microflora of Children in Mixed Dentition age Group- An Overview of Eight Different Medicinal Plants

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ABSTRACT:

Background- Plants are the oldest source of pharmacologically active compounds and have provided man with many medically useful substances for centuries and their extracts have been previously analyzed and were found to have significant therapeutic properties. The role of many such medicinal plants in the field of dentistry has been confirmed by many researchers. This paper overviews a long term research work performed to analyze the antimicrobial potential of plants namely *Mimusops elengi, Juglans regia, Morinda pubescens, Embelia basal, Ehretia leavis, Cassia auriculata, Achyranthes aspera* and *Artemisia pallens*. These studies have been performed to evaluate antimicrobial potential of phytochemicals of above mentioned plants extracts against salivary microflora.

Methods- Saliva samples are collected from children of age group 6-12yrs who were moderately prone to dental caries. The derived extract of each of the plants was screened for its growth inhibition potential against microflora in salivary samples. The microbial assay was performed using agar diffusion method in the laboratory. The zones of microbial growth inhibition produced by each plant extract were measured. The obtained results were compared with chlorhexidine, the gold standard antimicrobial agent used in dentistry.

Results- The antimicrobial analysis observed exhibition of significant zone of microbial growth inhibitions by the analyzed plants ranging from zero to 16 mm for the different extracts. This was comparable to that of 0.2% Chlorhexidine mouthwash which exhibited a zone of inhibition of 20 mm against the microbial growth in the test samples of human saliva.

Conclusion- Results obtained exhibited presence of significant antimicrobial potential in the studied extracts of the above mentioned plants. This indicates that an active bio-molecule is present in each of the extracts and the antimicrobial potential of these plant extracts can be used in the prevention and treatment of microbial diseases of the oral cavity.

Keywords: Antimicrobial Activity, Dental Caries, Medicinal Plants, Salivary Microflora.

INTRODUCTION

Dental caries is one of the most prevalent diseases affecting children as well as adult population. The National Health Survey 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.[1] Dental caries is a chemo-parasitic process in which the oral microorganisms play a very vital role. The human saliva serves as a reservoir for useful as well as pathologic microflora causing various microbial diseases of the oral cavity such as dental caries. For prophylactic processes, it

seems reasonable to target the microorganisms involved in the process of caries initiation and progress of dental caries without perturbing the balance of the normal oral microflora.

Several antibiotics such as Ampicillin, Chlorhexidine, Sanguinarine, Entrnidazole, Phenolic antiseptics and Quaternary ammonium-antiseptics, among others, have long been used in preventing dental caries.[2] 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] Hence there is a need to search for an alternative to currently being used synthetic drugs against oral microbial diseases.

Over the past 100 years, the development and mass production of chemically synthesized drugs have revolutionized health care in most parts of the world. However, large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care.[4] The World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care which exposes them to lesser known side effects and risks caused by the chemically synthesized pharmacological drugs.[5] Hence bioactive extracts of medicinal plants and their herbal drug formulations form a feasible alternative to the commonly used chemically synthesized drugs.

Thus considering the increased prevalence of dental caries, the role of salivary microflora in caries process and increased side effects of the chemically synthesized therapeutic agents these studies were designed. The objective of each of the studies was to search for natural preventive and therapeutic antimicrobial agents against salivary microflora which can be used as an alternative to the currently being used synthetic antimicrobials such as 0.2% chlorohexidine mouthwash. For this conventionally derived herbal extracts of eight different plants namely; *Mimusops elengi*, *Juglans regia, Morinda pubescens, Embelia basal, Ehretia leavis, Cassia auriculata, Achyranthes aspera* and *Artemisia pallens* have been evaluated for the presence of antimicrobial activity against salivary microflora.

MATERIALS AND METHODS

Study design-

This is a long term study in which we have evaluated phytochemicals of 8 different plants overtime in their derived extracts. These extracts of each of the plants have been screened for antimicrobial activity against salivary micro flora in whole saliva samples using agar diffusion method. Saliva samples are collected from children of age group 6-12 yrs with moderate caries activity. The obtained results were compared with 0.2% chlorohexidine mouthwash, commonly used synthetic antimicrobial agent in dentistry.

As mentioned earlier all the plants have been studied individually for a period of over 7 years now. The study design (Table No. 1) for each plant varied in the aspects of the dilution media used in preparation of the plant extracts, concentrations of the plant extracts used in antimicrobial assay and the sampling of the tested groups.

Material

1) Plant material- The authenticated extracts of all the studied plants were prepared conventionally using various dilution media viz., acetone, ethanol, acetate and methanol. These derived extracts were procured from Dr. T. R. Ingle Laboratory, Department of Chemistry, S.P. College, Pune, and Department of Rasashastra and Bhaishajya Kalpana, Dr. D. Y. Patil Ayurvedic College and Hospital, Pimpri, Pune, Maharashtra, India.

2) Standard antimicrobial agent- Chlorhexidine mouthrinse- 0.2% Chlorhexidine gluconate

3) Laboratory materials-

1. Diagnostic instruments- Mouth Mirror, Probe, Explorer, Tweezer
2. Sterilized Glass Vial of 5ml and funnel for collection of saliva
3. Insulated container with ice packs
4. Instruments as per the lab specifications

Table 1: The study design for each plant depending upon the dilution media and concentrations used in the selected plant extracts

SR NO.	Plant's name	Study Design
Study - 1	*Mimusops elengi*	Evaluation of average zone of microbial growth inhibition depicted by 150 µg, 200 µg, 250 µg, 300 µg and 450 µg concentrations of acetone extract of *Mimusops elengi* against microflora in saliva samples.
Study – 2	*Juglans regia*	Evaluation of average zone of microbial growth inhibition depicted by 150 µg, 200 µg, 250 µg and 300 µg of acetone extract of *Juglans regia* against microflora in saliva samples.
Study – 3	*Morinda pubescens*	Evaluation of average zone of microbial growth inhibition depicted by 50 µg,100 µg, 200 µg, 400 µg and 800 µg concentrations of acetone, ethanol and methanol extracts of *Morinda pubescens* against microflora in saliva samples.
Study - 4	*Embelia basal*	Evaluation of average zone of microbial growth inhibition depicted by 50 µg,100 µg, 200 µg, 400 µg and 800 µg concentrations of acetone, ethanol and methanol extracts of *Embelia basal* against microflora in saliva samples.
Study – 5	*Ehretia laevis*	Evaluation of average zone of microbial growth inhibition depicted by 50 µg,100 µg, 200 µg, 400 µg and 800 µg concentrations of ethanol and methanol extracts of *Ehretia laevis* against microflora in saliva samples.
Study – 6	*Cassia auriculata*	Evaluation of average zone of microbial growth inhibition depicted by 125 µg, 250 µg, 500 µg, 1000 µg, 2000 µg and 4000 µg concentrations of acetone and methanol extracts of *Cassia auriculata* against microflora in saliva samples
Study – 7	*Artemisia pallens*	Evaluation of average zone of microbial growth inhibition depicted by 10 µl, 20 µl, 40 µl, 60 µl and 80 µl concentrations ethanol extracts of *Artemisia pallens* against microflora in saliva samples.
Study – 8	*Achyranthes aspera*	Evaluation of average zone of microbial growth inhibition depicted by 80 µl concentrations ethanol extracts of leaf stem and root parts of *Achyranthes aspera* against microflora in saliva samples.

Selection of participant

1) Inclusion Criteria- In the study, patients of 6-12 years of age, in mixed dentition period with DMFT 4 or above were included for collection of saliva samples to be tested.

2) Exclusion Criteria- These patients had no history of antibiotic therapy or use of chemical anti-plaque agents prior to six months of study initiation and no presence or history of systemic illness.

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. Samples were collected in the early morning time and transported to the laboratory within 2 hours. In laboratory 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 for the further procedure of antimicrobial analysis.

Antimicrobial assay

The microbial inhibition assay was prepared using the agar well-diffusion method. 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 (5.0mm). Sterile 5.0mm diameter of well was impregnated using a certain concentration of a plant extract and plates were incubated at 37 ± 0° C for 24 hours. After incubation, the plates were observed for zones of microbial growth 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. 0.2% Chlorhexidine mouthwash was used as a standard to compare with all the obtained results.

RESULTS

The antimicrobial analysis observed exhibition of significant zone of microbial growth inhibitions by the analyzed plants ranging from zero to 16 mm for the different extracts. The concentrations used varied for each study as per the study design for each plant. In all the extracts the zone of inhibition increased with increasing concentration (Table 2).

The results of the anti-microbial assay of the acetone extract of *Mimusops elengi* showing average zones of inhibition (mm) are reported in Study No. 1. An average zone of inhibition of 2 mm is depicted by a concentration of 450 µg of acetone extract and it is found to inhibit most of the salivary samples.[6] The results of the Study No. 2 which is an antimicrobial assay of the acetone extracts of *Juglans regia* show that a concentration of 250 µg /disc is found to inhibit the growth of most of the test samples of saliva with an average zone of inhibition of 5.3 mm.[7] In another study (Study no. 3) five different concentrations of acetone, ethenol, acetate extracts of *Morinda pubescens* were evaluated, amongst which acetone extract has shown maximum zone of inhibition of 14mm in diameter at 800µg.[8] The results of the antimicrobial assay of *Embelia bas*al (Study No. 4) showed that the acetone extracts had higher growth inhibition potential (13.2 mm) as compared to ethanol (11.4mm) and methanol (10.4 mm) extracts at the 800 µg concentration.[9] The evaluation performed with methanol extract of *Ehretia laevis* (Study No. 5) shows feeble activity in most saliva samples at 800 µg concentrations which is upto 5.2mm.[10] Among the studied plants, acetone and methanol extracts of *Cassia auriculata* (Study No. 6) showed maximum zone of inhibition of 16.2mm and 13.6mm respectively at 4000 µg concentration.[11] It was comparable to that of 0.2% Chlorhexidine mouthwash which exhibited a zone of inhibition of 20 mm against the microbial growth in the test samples of human saliva. Crude ethanolic extracts of *Artemisia pallens* failed to exhibit any significant zone of microbial growth inhibition at the tested concentrations ranging from 10 µl to 80 µl (Study No. 7) and higher concentration with other solvents are under evaluation process for the plant. The evaluation of ethanolic extracts of leaf, stem and roots of *Achyranthes aspera* (Study No. 8) is performed using 80µl concentration. The results depict that all the three plant extracts show significant antimicrobial activity against salivary microflora at the tested concentrations and mean values of zones of inhibition for leaf, stem and root extracts were 8.4 mm, 6.2 mm and 4.2 mm respectively.[12]

DISCUSSION

Herbal medicine provides a source of phytochemicals and active biomolecules having antimicrobial properties which can conventionally be extracted using various dilution media. The current state of knowledge concerning the potential biological effects of herbal medicinal products basically is relevant on their antimicrobial activity, inhibition of exopolysaccharide synthesis and inhibition of bacterial adherence.[13] In these above mentioned studies we have successfully evaluated the antimicrobial activity of eight different plants and their extracts specifically by analyzing the inhibition of growth and metabolism of acidogenic and aciduric organisms in the tested saliva samples using agar diffusion method. The exact mechanism by which this occurs remains to be evaluated. The demonstration of antimicrobial activity by various extracts of the studied plants provides scientific basis for the presence of medicinally active compounds in these plants which can be implemented in drug formulations. These can further be used as preventive and therapeutic

measure in the hard and soft tissue diseases of the oral cavity.

The objective behind the variation in study design for each of the eight plants was to analyze the effect of dilution media viz., acetone, acetate, ethanol, methanol on the activity of the extracts. Further the concentration of the extracts was useful in several studies to determine the dose dependency, the ranges at which the plant extracts exhibited increased antimicrobial activity and minimal inhibitory concentration. The effect of these plants and their extracts on more pathogenic organisms, evaluation of higher concentrations for toxicological investigations and further purification however needs to be carried out. Evaluation of higher concentrations of the plant extracts and different dilution media can enhance the medicinal activity of this extract. These properties can be used in prevention and treatment of diseases of the oral tissues of microbial origin such as dental caries.

CONCLUSION

Many advances in the treatment and prevention of dental caries have been introduced over the past century. Medicinal plants are pluoripotent in nature and hence offer a great therapeutic aid for the health sciences. Combining the strengths of the knowledge based traditional herbal medicine with the scientific methods of drug preparation can provide new functional leads to reduce time, money and toxicity - the three main hurdles in drug development.

Table 2: Average zone of inhibition (mm) at different concentrations depicted by various plant extracts against whole salivary samples

Study – 1		***Mimusops elengi***					
Concentration (µg)		150	200	250	300	450	-
Average zone of inhibition (mm)	Acetone extract	0	0.5	0.5	0	2	-
Study – 2		***Juglans regia***					
Concentration (µg)		150	200	250	300	-	-
Average zone of inhibition (mm)	Acetone extract	1.3	3.3	4	5.3	-	-
Study – 3		***Morinda pubescens***					
Concentration (µg)		50	100	200	400	800	-
Average zone of inhibition (mm)	Acetone extract	4	5.2	7.2	9.2	14	-
	Ethanol extract	0	0.2	2	1.6	2	-
	Acetate extract	0	0	2	1.6	2	-
Study – 4		***Embelia basal***					
Concentration (µg)		50	100	200	400	800	-
Average zone of inhibition (mm)	Acetone extract	4.1	6.4	9.5	11	13.2	-
	Ethanol extract	4.2	6.3	6.2	8.3	11.4	-
	Methanol extract	3.2	4.3	6.3	7.6	10.4	-
Study – 5		***Ehretia laevis***					
Concentration (µg)		50	100	200	400	800	-
Average zone of inhibition (mm)	Methanol extract	0	0	1.6	4.2	5.2	-
	Ethanol extract	0	0.2	0.2	1.2	2.6	-
Study – 6		***Cassia auriculata***					
Concentration (µg)		125	250	500	1000	2000	4000
Average zone of inhibition (mm)	Acetone extract	0.2	7.4	10.4	12.4	13.1	16.2
	Methanol extract	0	2	4	4.8	8.7	13.6
Study – 7		***Artemisia pallens***					
Concentration (µl)		10	20	40	60	80	-
Average zone of inhibition (mm)		0	0	0	0	0	
Study – 8		***Achyranthes aspera***					
Various Extracts in ethanol		Leaf Extract		Stem Extract		Root Extract	
Average zone of inhibition (mm) at 80 µl concentration		8.4		6.2		4.2	
Standard		**Chlorhexidine mouthwash**					
0.2% Chlorhexidine digluconate		20					

ACKNOWLEDGEMENTS

Department of Rasashastra and Bhaishjya Kalpana, Dr. D. Y. Patil Ayurvedic College and Hospital, Pimpri, Pune. Dr. Anjali Ruikar, Dr. C D Shendkar, Dr. Rasika Torane, Dr. T. R. Ingle Research Laboratory, Department Of Chemistry, S. P. College, Pune – 30, Maharashtra, India. TECHNEXT Lab, Pune. NAFARI Lab, Pune

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How to cite this article: Deshpande RR, Shep SV, Deshpande NR, Rao P, Sabale Y, Umarkar P. A Comparative Evaluation of Antimicrobial activity of Herbal Extracts and Chemically Synthesized Chlorhexidine Mouthwash against Salivary Microflora of Children in Mixed Dentition age Group- An Overview of Eight Different Medicinal Plants. Arch of Dent and Med Res 2017;3(2):1-6.