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Comparative evaluation of antimicrobial properties of three different extracts of *Achyranthes aspera* Linn and chlorhexidine against salivary microflora.

Snehal V Shep^{1*}, Rahul R Deshpande^{1,2}, Vishwas Patil¹, Fawaz Siddiqui¹, Anjali Ruikar³, and CD Shendkar³.

¹Dr. D. Y. Patil Dental College and Hospital, Pimpri, Pune-18, Maharashtra India.

²Deenanath Mangeshkar Hospital, Pune-4, Maharashtra, India.

³Dr. T. R. Ingle Research Laboratory, Department Of Chemistry, S. P. College, Pune – 30, Maharashtra, India.

ABSTRACT

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Medicinal plants and their extracts have been analyzed and reported to have significant therapeutic properties. The antimicrobial efficacy of many plants is yet to be verified. In this study, the ethanolic extracts of leaves, stem and root parts of *Achyranthes aspera* Linn are evaluated for antimicrobial activity against salivary microflora. The salivary samples were collected from children of mixed dentition age group with moderate caries activity. Antibacterial assay was carried out using agar diffusion method. The results were comparatively evaluated with 0.2% Chlorhexidine used as a standard chemotherapeutic agent. The results depict that all the extracts have marked activity against the tested samples showing zones of inhibition. Thus, this in vitro study supports its application as a preventive remedy for microbial diseases of hard & soft tissues in the oral cavity.

Keywords- Antimicrobial activity; *Achyranthes aspera* Linn; salivary microflora; Chlorhexidine

**Corresponding author*



INTRODUCTION

The dental community has prided itself on efforts that have reduced dental caries including use of systemic and topical fluorides, toothpastes, sealants, improvements in diet, oral health education and dental care. There are however, recent studies that report alarming increases in caries. The prevalence and pattern of dental caries has changed significantly over centuries. It is a public health problem in India, with a prevalence as high as 60-80% in Indian children. 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].

Caries being an irreversible disease it is needful to focus on the prevention of dental caries. A number of chemotherapeutic agents are used to target the causative factors in oral diseases, among these factors salivary microflora play a pivotal role in dental caries. Chemotherapeutic and antimicrobial agents aiming at these predisposing factors therefore play a significant role in prevention of such oral diseases. The vigorous use of such chemotherapeutic and antimicrobial agents to combat caries led to increased prevalence of side effects and failure of many popular synthetic antimicrobial agents due to development of multidrug resistant strains of micro-organisms [2]. These drawbacks justify the search for new effective anti-cariogenic compounds with minimal or no side that could be employed in caries prevention.

Herbal medicine is in great demand in the developed world for primary health care because of their efficacy, safety and minimal documented side effects. Herbal medicine is both promotive and preventive in its approach. It is a comprehensive system, which uses various remedies derived from plants and their extracts to treat disorders and to maintain good health [3]. There are several studies performed to evaluate medicinal properties of many plants and anti-microbial, anti-fungal, anti-helminthic, anti-inflammatory, wound healing properties are found in these plant extracts [4]. Medicinal plants like *Tulsi*, *neem*, *turmeric*, *Aloe vera* and many others well known and well established plants have been evaluated worldwide by many researchers. There are lot many seasonal plants which are less palpatated.

Achyranthes aspera is one such less palpatated seasonal plant, known as *Aghada* (Marathi), *Latjeera* (Hindi) & Rough Chaff tree (English) languages. There are studies which scientifically validate the traditional use of *A.aspera* as a natural brush for teeth cleaning and state that phytochemicals of this traditionally used dental caries preventive natural chewing stick plant could be harnessed for dental caries and other biofilm mediated disease management [5]. Thus, in this study we evaluated ethanolic extracts of various parts of *Achyranthes aspera* (Aghada) viz., leaves, stem and roots, for its antimicrobial activity against salivary microflora and compared it with the Chlorhexidine mouth wash as a gold standard.

AIM OF THE STUDY

The aim of this study was to evaluate and compare antimicrobial property of extract of *Achyranthes aspera* (Aghada) and 0.2% Chlorhexidine against salivary microflora in mixed dentition age group.

MATERIALS AND METHODS

Plant Material

The ethanolic extracts of leaf, stem and roots of *A. aspera* were procured from Dr. D. Y.Patil Ayurvedic College and Hospital, Pimpri, Pune, Maharashtra, India.

Standard antimicrobial agent

The zones of the inhibition of each plant extract are compared with 0.2% Chlorhexidine gluconate using it as a gold standard [6].

Patient Selection Criteria

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 for 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 (3ml) 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 1ml of normal saline and were used to inoculate on the agar plates [7].

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 were impregnated with the extract of 80 µl concentration and plates were incubated at 37 ± 0° C for 24 hours. After incubation, the plates were observed for zones of inhibition of growth 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 was used as positive control.

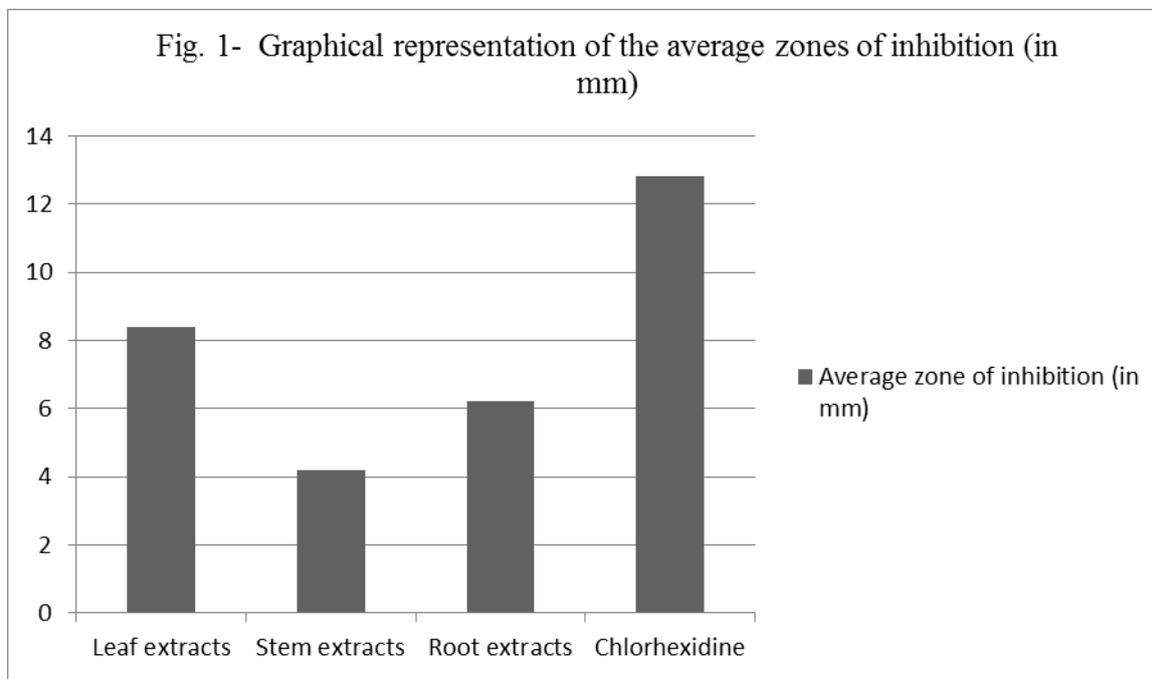
RESULTS

The evaluation is performed using 80µl concentration of the ethanolic extracts of leaf, stem and roots of *A. aspera* and it is compared with 0.2% chlorhexidine. 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. In this study ethanolic leaf extracts of *A.aspera* showed maximum zone of inhibition compared to the other parts but was less than that of 0.2% Chlorhexidine. Mean zone of inhibition of 0.2% Chlorhexidine was 12.8 mm.the results of the individual samples are depicted in the Table no. 1 and Fig.1 below shows graphical representation of the values of zones of inhibition of the tested plant extracts and the standard. A dose dependent evaluation of extracts on salivary microflora was analysed and was reported in concentration of 80µl of all the plant extracts leaf extract is found to inhibit most of the salivary samples showing maximum zone of inhibition 8.4 mm (*Achyranthes aspera*). This results reveals that this plant show anti microbial activity and show a linear equation.

Table no. 1- Zone of inhibition (mm) at 80µl concentration of the plant Leaf, stem and root extracts of *Achyranthes aspera* and 0.2% Chlorhexidine against salivary microflora.

Serial No.	<i>Achyranthes aspera</i> etahnolic extracts			0.2% Chlorhexidine
	Leaves	Stem	Root	
1	15	4	6	13
2	1	1	4	13
3	8	6	8	11
4	8	4	5	13
5	9	5	7	13
6	3	3	6	15

7	11	7	9	15
8	10	8	10	13
9	13	2	4	11
10	6	2	3	11



DISCUSSION

In the developing countries herbal medicine is still the mainstay of about 75–80% of the world population for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects [3]. As discussed earlier, there are numerous conventional studies performed to analyze the antimicrobial potential of many well known plants by Indian as well as researchers worldwide. Thus, in this study we have evaluated a seasonal plant like *Aghada*, which has been in less focus for palpating their medicinal properties. Thus, to unfold the bliss of this seasonally available plant, we comparatively evaluated *A. aspera* (aghada) with Chlorhexidine mouth wash for their antimicrobial activity against salivary microflora.

From this study, it was evident that all the different plant extracts of *A. aspera* show significant antimicrobial activity in the tested samples. Out of which leaf extracts of the plant showed maximum antimicrobial activity against salivary microflora which was comparable with that of chlorhexidine. The significant zones of inhibition indicates that an active molecule must be present in the plant and further studies need to be carried out in order to confirm and isolate the active ingredients of *A. aspera*. The demonstration of antimicrobial activity by various extracts provides the scientific basis for the use of this plant as preventive and therapeutic measure in traditional treatment of oral diseases. It may have fewer side effects as it falls in the category of natural medicine. The effective plant extracts can be formulated in the form of a dentifrices, mouth washes, gum paints or as an intracanal medicament where an antimicrobial agent is required. The various extracts of *A. aspera* have been conclusive in demonstrating antimicrobial action.

This study has evaluated the antimicrobial activity of *A. aspera* using 80µl concentration of the plant extracts. Further studies with a range of higher concentrations may show increased zones of inhibition by the plant extracts thereby increasing its antimicrobial potential. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the preventive and therapeutic

needs in dentistry. The effect of this plant on more pathogenic organisms, evaluation of further higher concentrations for toxicological investigations and further purification however needs to be carried out.

CONCLUSION

This study indicates that along with the well known and documented medicinal plants from the main stream line, seasonal and less palpatated plants like *Achyranthes aspera* also show significant medicinal properties. The crude ethanol leaf extract obtained from *Achyranthes aspera* plant is found to be an effective anti-microbial agent against the salivary micro flora having a comparable activity with that of chlorohexidine mouthwash. The study also confirmed the antimicrobial potentials of the plant in its stem and root extracts, thus supporting its folklore application as a preventive remedy for various microbial diseases of hard and soft tissues in the oral cavity.

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