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Research Article

Comparative Evaluation of Antimicrobial Properties of Leaf Extracts of *Achyranthes aspera* Plant and Chlorohexidine Against *Streptococcus mutans*, *Enterococcus faecalis* and Whole Salivary Samples of Children in Mixed Dentition Age Group

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ABSTRACT

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. There are many well established medicinal plants which have been evaluated and are known to have significant therapeutic properties. However, antimicrobial efficacy of many other lesser known plants is yet to be verified. In this study, the ethanolic extract of leaves, *Achyranthes aspera* is evaluated for antimicrobial activity against test samples of *Streptococcus mutans, Enterococcus faecalis* and human saliva samples. The salivary samples were collected from children in mixed dentition age group with moderate caries activity. Antibacterial assay was carried out by assessing the growth inhibition potential of plant extracts against tested samples using agar diffusion method. The results were comparatively evaluated with Chlorhexidine using it as a standard chemotherapeutic agent. The results depict that the plant extracts have marked activity against the tested samples showing significant zones of growth inhibition. Thus, this in vitro study supports use of ethanolic leaf extracts of *Achyranthes aspera* as a preventive and therapeutic remedy for dental caries and its sequelae.

Keywords: Antimicrobial activity; *Achyranthes aspera Linn*; Salivary microflora; *Streptococcus mutans*, *Enterococcus faecalis*.

INTRODUCTION

Dental caries is one of the most commonly occurring diseases in children. During the past decades the common consensus from many reports worldwide was that dental caries had declined significantly and was continuing to decline in populations due to 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. More than 40% of the children in India have shown dental caries in both primary and permanent teeth in the past 15 years[1]. 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[2].

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 such as *Streptococcus mutans* play a pivotal role in dental caries and its sequelae. Once the caries involves the pulp of the tooth, bacteria and their by products are the main cause of pulpitis and apical peridontitis[3]. Root canals of infected teeth have a complex microbial flora with the most prevalent species of *E. faecalis* (35%)[4]. The reduction or elimination of the bacterial infection seems to be the most relevant factor for the success of endodontic treatment of teeth with necrotic pulp. Chemotherapeutic and antimicrobial agents aiming at these predisposing factors in caries and its squeal 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[5]. The increased prevalence of strains with reduced susceptibility to antibiotics raises the spectrum of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies.

Herbal medicine has long been in great demand in the developed world of primary health care because of their availability, efficacy, safety and minimal documented side effects. It is a comprehensive system, which uses various remedies derived from plants and their extracts to treat disorders and to maintain good health[6]. Medicinal plants like *Tulsi, neem, turmeric, Aloe vera* and many others well known have been evaluated in several studies by many researchers worldwide and medicinal properties like anti-microbial, anti-fungal, anti-helminthic, anti-inflammatory,

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wound healing properties are found in the derived extracts of these plants[7]. These are presently very well established in the field of herbal medicine. However, there are many other plants which are less palpated for their medicinal properties.

Achyranthes aspera is one such plant which has very few studies reporting its medicinal properties. It is also known as *Aghada* (Marathi), *Latjeera* (Hindi) & Rough Chaff tree (English) languages. There are few 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[8].

Thus, we designed three pilot studies to evaluate ethanolic leaf extracts of *Achyranthes aspera* (Aghada) for its antimicrobial activity against salivary microflora, *Streptococcus mutans and Enterococcus faecalis* in context of above discussion. The observed properties of the leaf extract were compared with chlorhexidine mouth wash as it is a gold standard used in various oral conditions. Hence, the aim of this study was to evaluate and compare antimicrobial property of ethanolic leaf extract of *Achyranthes aspera* (Aghada) and chlorhexidine against salivary microflora in mixed dentition age group, microbial strains of *Streptococcus mutans* and *Enterococcus faecalis*.

MATERIALS AND METHODS

Material

Plant Material

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

Strains of Streptococcus mutans serotype c ATCC 25175.

Strains of Enterococcus faecalis serotype ATCC-29212.

Standard antimicrobial agent

Chlorhexidine mouthrinse- 0.2% Chlorhexidine gluconate Chlorhexidine irrigation solution – 2% Chlorhexidine gluconate

Patient Selection Criteria

In the present study, patients of 6-12 years of age, in the 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 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[9].

Antimicrobial Assay

The microbial inhibition assay was prepared using the agar 'well-diffusion' method. Adequate amount of agar was dispensed into sterile plates and allowed to solidify under aseptic conditions. The test samples of *Streptococcus mutans*, *Enterococcus faecalis* and saliva (0.1ml), were inoculated with a sterile spreader on the surface of agar plates. After the media was solidified; wells were made in the plates with the help of a cup-borer (5.0mm).

Antimicrobial analysis of Streptococcus mutans samples

The leaf extracts of *A. aspera* were diluted using ethanol in 200mg/1ml proportion. Sterile 5.0 mm diameter of well on agar plates with inoculated with *Streptococcus mutans* impregnated with the ethanolic leaf extract using 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. Chlorhexidine mouthrinse was used as positive control.

Antimicrobial analysis of Enterococcus faecalis samples

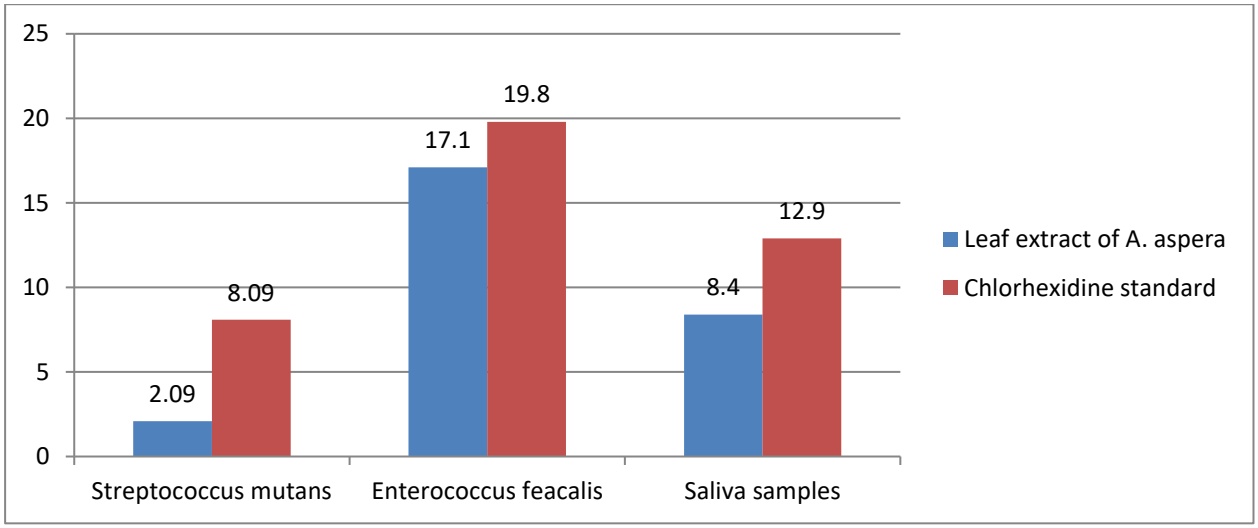
Similarly, agar plates with *Enterococcus faecalis*, were impregnated with the crude extract of leaves of *Achyranthes aspera* using 80 µl concentration and plates were incubated at 37 ± 0° C for 24 hours. After incubation, the plates were observed for zones of inhibition of growth and the diameters of these zones were measured in millimeters by using bacterial inhibition zone reading scale. All the tests were performed under sterile conditions. All the tests were performed under sterile conditions and 2% chlorhexidine irrigating solution was used as positive control.

Antimicrobial analysis of test saliva samples

The leaf extracts of *A. aspera* were diluted using ethanol in 200mg/1ml proportion. Sterile 5.0 mm diameter of well on agar plates with patients' saliva samples were impregnated with the ethanolic leaf extract using 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 mouthrinse was used as positive control[10].

RESULTS

The evaluation is performed using ethanolic extracts of leaves of *A. aspera* and it is compared with chlorohexidine formulations for *S. mutans*, *E. faecalis* and saliva test groups. The results depict that the plant extract shows significant antimicrobial activity against *Streptococcus mutans*, *Enterococcus faecalis* and salivary microflora at the tested concentrations. The mean values of zones of inhibition were 8.4 mm, 2.09 mm and 17.1 mm respectively for salivary microflora, *Streptococcus mutans* and *Enterococcus faecalis* groups. The results of the individual samples are depicted in the Graph No. 1 and Table no. 1 below show graphical representation of the values of zones of inhibition of the tested plant extracts and the standard. These results reveal that the plant shows a considerable antimicrobial activity and show a linear equation.



Graph 1: Average zone of inhibition (mm) at 80µl concentration of the ethanolic leaf extracts of *Achyranthes aspera* and respective formulations of chlorhexidine against *S. mutans, E. faecalis* and whole saliva samples.

Table 1: Zone of inhibition (mm) at 80µl concentration of the ethanolic leaf extracts of *Achyranthes aspera* and respective formulations of chlorhexidine against *Streptococcus mutans, Enterococcus faecalis* and whole salivary samples.

Serial No.	S. mutans samples		E. faecalis samples		Salivary samples	
	Ethanolic leaf extracts of A. aspera (200mg/1ml)	0.2% Chlorhexidine mouth rinse	Ethanolic leaf extracts of A. aspera (200mg/1ml)	2% Chlorhexidine irrigating solution	Ethanolic leaf extract of A. aspera (Pure form)	0.2% Chlorhexidine mouth rinse
1	2	8.3	15	21	15	12.5
2	2.4	8	16	23	1	13.5
3	2.1	8	20	17	8	11
4	2	8.1	15	20	8	13
5	2.1	8.4	18	19	9	13.2
6	1.9	8	20	20	3	14
7	2	8.1	21	19	11	15
8	2	7.9	18	20	10	13.2
9	2.3	8.1	14	18	13	11.5
10	2.1	8	14	21	6	12.5

DISCUSSION

In developing countries, herbal medicine is still the mainstay of about 75–80% of the world population for primary health care because of cultural acceptability, better compatibility with the human body and lesser side effects[6]. 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[6-8,10]. In this study, we have evaluated a seasonal plant like *Aghada*, which has very few studies reporting its antimicrobial properties against oral microflora. Thus, to investigate this seasonally available plant, we evaluated the ethanolic leaf extracts of *A. aspera* (aghada) and compared its microbial growth inhibition potential measured in the form of zones of inhibition by agar diffusion method against saliva samples of caries active group of children, *Streptococcus mutans* and *Enterococcus faecalis* test groups. 0.2 % chlorhexidine mouth wash was used as a standard to compare salivary and *S. mutans* test groups. The results of *E. faecalis* test group were compared with those of 2% Chlorhexidine irrigating solution for their growth inhibition potential.

The antimicrobial analysis was carried out using agar diffusion method. For the microbial growth inhibition analysis the ethanolic extracts used for saliva samples and *S. mutans* test groups were diluted in 200mg/ml concentration using ethanol as diluting media and 0.2 % chlorhexidine mouth rinse was used as a standard control. The average zone of inhibition observed against salivary samples by plant extracts was 8.4 mm and that by 0.2% chlorohexidine mouth rinse was 12.9 mm. The leaf extracts showed an average zone of inhibition of 2.09 mm against *Streptococcus mutans* sample group which was significantly less as compared to that of 0.2% chlorohexidine mouth rinse which showed an average zone of inhibition of 8.09 mm. In this study considering the higher concentration of standard antimicrobial agent i.e. 2% irrigating solution of chlorhexidine, crude ethanolic leaf extracts of *A. aspera* were used to evaluate its microbial growth inhibition potential against *E. faecalis*.

Achyranthes aspera showed significant antimicrobial activity against *Enterococcus faecalis* with average zone of inhibition of 17.1 mm and it was comparable to that of 2% chlorhexidine irrigating solution with which showed 19.8 mm average values of zone of inhibition.

From this study, it was evident that the ethanolic leaf extracts of *A. aspera* show significant antimicrobial activity in the tested samples. The appearance of significant zones of inhibition indicates that an active molecule must be present in the ethanolic leaf extracts of the plant and further studies with higher concentrations of the plant extracts, using different dilution media and larger sample size need to be carried out. 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 and used in prevention and treatment of oral diseases such as caries and its sequelae.

CONCLUSION

This study shows that along with the well documented medicinal plants like *Tulasi, Aloe vera, Neem, Turmeric etc*, seasonal and less palpated plants like *Achyranthes aspera (Aghada)* also exhibit significant antimicrobial activity against *Streptococcus mutans*, *Enterococcus faecalis* and salivary microflora. The ethanolic leaf extract obtained from *Achyranthes aspera* plant is an effective anti-microbial agent against the tested microorganisms and has comparable antimicrobial activity to that of chlorohexidine formulations, thus supporting its folklore application as an antimicrobial agent even in the oral cavity.

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