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Study of antibacterial activity with stem extracts of *Achyranthes aspera, L.*

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Abstract

In the present study, the stem extracts of the plant *Achyranthes aspera* has been selected to investigate upon its antimicrobial activity against tooth bacteria and its antibacterial activities were compared with market products. The antimicrobial tests were conducted by using two different solvent based plant extracts i.e. aqueous and methanol stem extracts. Least bacterial colonies were found in nutrient agar plate streaked with tooth bacteria, after brushing with stem of *Achyranthes aspera* as compared to bacterial colonies obtained on nutrient agar plate, after brushing with sodium monofluorophosphate.

From the MIC test of aqueous and methanolic stem extracts against gram positive tooth bacteria, it was found that both aqueous and methanolic extracts were effective against garm positive tooth bacteria. It is also seen that methanolic stem extract is more effective than aqueous stem extract. In disc diffusion method , it was found that both aqueous and methanolic stem extracts showed inhibition zone at different concentrations against gram positive tooth bacteria and inhibition zone indicated that methanolic extract was more effective than aqueous extract of *A. aspera* dry stems. This investigation provides information, which could trigger further research in the direction of partial or full isolation and characterization of the constituents of stem of *Achyranthes aspera* in order to decipher the specific phyto chemical constituents responsible for the antimicrobial activity of the plant.

Keywords: Tooth bacteria, Achyranthus, Extracts, anti bacterial

1. Introduction

The literature survey of the folklore medicine revealed the use of *Achyranthes aspera* stem for the treatment of toothache but, little is known about the compound which is responsible for the antimicrobial effects. The natural phytochemicals could offer an effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for dental caries and other oral infections. Therefore the purpose of the present study was to identify and characterized the active principle involved in dry stem extract of *A. aspera* as a potential antimicrobial agent against dental isolates.

Ashwini and Arpana (2013) studied the antimicrobial activity of *Achyranthes aspera* using agar well diffusion and serial dilution method. The Dental pathogens *Bacillus pumilus, Bacillus thuringiensis and Pseudomonas aeruoginosa, Acinetobacter junii, Enterococcus faecalis* were isolated and identified from dental caries and dental abscess respectively. Results of this study revealed the strong antibacterial potential of isolated compound from *Achyranthes aspera* against dental pathogens.

Pandey et al (2013) studied various extracts of petroleum ether, methanol, ethanol, ethyl acetate and chloroform of medicinal plant *Achyranthes aspera* for their antibacterial activities against multi drug resistance organisms such as *Pseudomonas aeruginosa, Proteus mirabilis and Enterococcus faecalis*. The organic extracts of both the leaf and stem parts of the plants at a concentration of 5 mg/ml and their activities were measured by estimating zones of inhibition as produced by antibiotic sensitivity method on Mueller-Hinton agar. The results of this research support the use for further analysis in the treatment of infectious diseases such as urinary tract an infection caused by bacteria and has significant scope for antibacterial research.

Khan et al. (2010) reported that the ethanol and chloroform extracts of seeds of *Achyranthes aspera* shows mild to moderate antibiotic activity against *B. subtilis, E. coli* and *P. aeruginosa*. Prasad et al. (2009). studied the various extracts of the leaves and callus of the plant also shows antimicrobial activity.

Saravanan et al. (2008) reported the solvent leaf extracts were tested for antibacterial and antifungal activities against *E. coli, P. aeruginosa, P. vulgaris, S. aureus, Klebsiella species*. Misra et al. (1992) reported 17-pentatriacontanol as a chief constituent isolated from essential oil of the shoots of plant, the oil shows antifungal activity against *Asperigillus carneus* . Manjula et al. (2009) studied the extracts of *Achyranthes aspera* for antibacterial activity against various pathogenic strains such as *Escherichia coli, Pseudomonas aeruginosa, Citrobacter species,*

Bacillus subtilis and *Micrococcus species* using disk diffusion and well plate method. Phytochemical characterization of *Achyranthes aspera* extracts was done by thin layer chromatography (TLC) techniques and other phytochemical analysis. It was found that extracts of *Achyranthes aspera* shows the maximum inhibition of *E. coli* (17 mm) followed by *Pseudomonas species* (14 mm), *Citrobacter species* (12 mm), *Bacillus species* (12 mm) and *Micrococcus species* (12 mm). *Achyranthes aspera* shows predominant inhibition against gram negative bacteria at a higher concentration of 50µg/ml. In the well plate method the inhibition zone ranges from 7 to 19 mm against pathogenic strains thus by increasing the concentration of extracts.

3. Materials and Methods

Plant materials

Plant materials of *Achyranthes aspera* is used in this study which belongs to family Amaranthaceae. Plant materials of *Achyranthes aspera* were collected from the botanical garden of Department of Botany, Khallikote Autonomous College, Berhampur of Ganjam district, Odisha and its peripheries with the help of local people.

Plant samples collected are true representatives of the plant material to get the accurate results of the study. The plant materials of same age group were collected during the month of August 2017.

Preparation of plant extract

For this study maceration extraction process was selected. 20 gm of shade dried, powdered stems were soaked separately in 100 ml of distilled water and in 100 ml of methanol for 72 hours. Each mixture was stirred periodically using sterile glass rod. At the end of 72 hours, each extract was filtered through Whatman filter paper (No: 1). Filtered extracts were evaporated on a rotary evaporator and reduced to 30 ml of each extract. Collected stem extracts were stored at -4^0c in an air tight bottle for further use.

At the time of use, dried plant extracts were dissolved in their same corresponding solvents. For this experiment, concentration of 200mg/ml of aqueous and methanolic stem extracts were required. So the extracts were diluted as per their requirement.

Chemicals

Peptone, yeast extract, beef extract, sodium chloride, agar were purchased from Hi-Media Company. Methanol and other chemicals used were purchased from Sigma Company. Chloramphenicol and Erythromycin were acquired from a local pharmacy.

Culture media

Nutrient broth medium and nutrient agar medium were used throughout the experiment both for culturing and maintaining the test bacteria for bioassay study.

Collection of Tooth Bacterial Samples:

Before brushing, bacteria from the tooth are isolated with the help of a ear bud and one nutrient agar plate was streaked with it by streak plate technique. This procedure is repeated after brushing with sodium monofluorophosphate. Then after 8-10 hours this procedure is again repeated after brushing the tooth with stem of *Achyranthes aspera.* All the 3 inoculated nutrient agar plates were incubated for 24-48 hours at 37^0 c in an inverted position.

Antibacterial activity assay

The antibacterial activity was evaluated by paper disc-diffusion method. Minimum inhibitory concentration (MIC) values were also studied for microorganisms. The MIC was identified as the lowest concentration of the chemical agent, which resulted in confirmed inhibition of the growth of the tested microorganism, after 24 hr of optimal incubation conditions (Wiegand *et al.*, 2008). Antibiotics activity assay method was the zone diameter of inhibition is measured to determine the inhibition capacity of plant extract (Wiegand *et al.*, 2008).

Paper disc diffusion method

The antibacterial activity of *A. Aspera* stem methanolic and aqueous extracts were analysed separately by using disc diffusion assay. Commercial Chloramphenicol (30 mcg/disc) and Erythromycin (15 mcg/disc) antibiotic disc were used as standard drugs. Sterile antibiotic discs were used for the present investigation. The extract of *A. aspera* was incorporate to the sterile paper discs, individually with 200, 100, 50, 25 mg./ml respectively using a micropipette. Activity of the above mentioned extracts was tested separately, using disc diffusion method.

MIC determination of Plant drugs against bacteria:

The methanolic and aqueous extracts of *A. aspera* were incorporated to the sterile test tubes containing broth for serial dilution individually using micropipette. This can be done in a serials of 12 test tubes, which, were incubated at appropriate culture conditions and examined by turbidity. Each extract was assayed in triplicate.

4. Results :

The present study was designed to compare antibacterial activities of plant extracts of *Achyranthes aspera* with market products against tooth bacteria. Large amount of bacterial colonies were seen in the nutrient agar plate streaked with

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bacteria before brushing, comparatively small amount of colonies were seen in the nutrient agar plate streaked with bacteria after brushing with sodium monofluorophosphate, and, minimum amount of bacterial colonies were obtained from nutrient agar plate streaked with bacteria, after brushing with stem of *Achyranthes aspera* ,after incubation of all the three plates for 24-48 hours at 37^0 c in an inverted position.Gram staining of bacteria isolated from the nutrient agar plate streaked with tooth bacteria, after brushing with *A.aspera* stem, showed that the pure culture of tooth bacteria were gram positive bacteria. The aqueous and methanol stem extracts of the plant were tested against particular identified gram positive tooth bacteria. The aqueous and methanol stem extracts of the plant were tested against particular identified gram positive tooth bacteria.

1. Study of Minimum Inhibitory Concentration Test (MIC test)

SL NO.	Different plant extracts of*A.aspera*	Concentration of plant extract (mg/ml)	Optical density at 610 nm
1	Aqueous	0	0.8
		3.12	0.75
		6.25	0.72
		12.5	0.65
		25	0.63
		50	0.61
		100	0.29
		200	-
2	Methanol	0	0.7
		3.12	0.28
		6.25	0.26
		12.5	0.24
		25	0.21
		50	0.08
		100	-
		200	-

Table No.1: MIC test of aqueous and methanol extracts of plant *A. aspera* against gram positive tooth bacteria**.**

The MIC test of plant stem exact (aqueous) against gram positive tooth bacteria is obtained at concentration 100mg/ml at which bacteria shows minimum activity. Similarly, the MIC of plant *Achyranthes aspera* stem exact (methanol) against the bacteria is obtained at concentration 50mg/ml at which gram positive tooth bacteria shows minimum activity. Both aqueous and methanol extracts were effective against the gram positive tooth bacteria. From the result , it was clearly seen that the methanol extract provides better result than the aqueous extract.

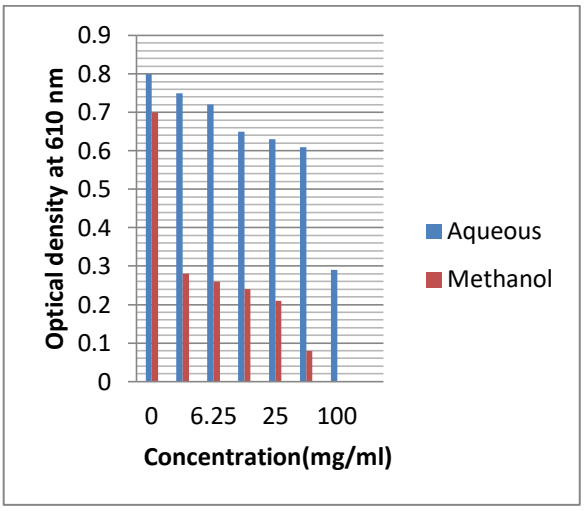

Fig No.2: Value of O.D. at 610 nm of aqueous and methanolic stem extracts of *Achyranthes aspera* against gram positive tooth bacteria.

2. Study of inhibition zone (Disc diffusion method)

Aqueous and methanol stem extracts of the plant *Achyranthes aspera* were tested against the gram positive tooth bacteria for disc diffusion method.

The maximum zone of inhibition obtained is 7 mm at concentration 200mg/ml for the methanol extract and 4 mm at same concentration i.e., 200mg/ml for the aqueous extract. The zone of inhibition in mm decreases constantly with decrease in concentration for both aqueous and methanol stem extracts of the plant *Achyranthes aspera*, and, the minimum zone of inhibition in mm is obtained finally at concentration 25mg/ml for both methanol and aqueous stem extract i.e., 3mm and 1mm respectively The methanol extract of the plant *Achyranthes aspera* shows better zone of inhibition than aqueous extract against gram positive tooth bacteria.

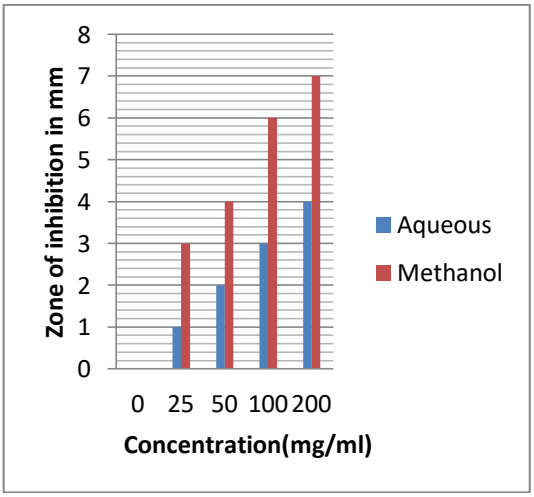

Fig No.3: Inhibition zone in mm of aqueous

and methanolic extracts of *Achyranthes aspera* dry stems against gram positive tooth bacteria

Table No.1: Antimicrobial zone of inhibition for antibiotics obtained**.**

Standard	Zoneof Inhibition (mm)
Chloramphenicol(10mcg/disc)	17
Erythromycin(15 mcg/disc)	15

Commercial Chloramphenicol (30 mcg/disc) and Erythromycin (15 mcg/disc) antibiotic disc were used as standard drugs against tooth bacteria for disc diffusion method.

Discussions

Plants contribute in diverse way to the survival of human communities. Plants have been used to heal different diseases since a long time. The plant-derived drugs or their modified products may be the answer to this problem. Ancient literatures suggest that almost all plants have medicinal values. Crude plant extracts have been used in traditional medicine since long. But scientific validation, detail phytochemical analysis and study of possible side effect is given importance only since last few years. Herbal remedies are viewed as health aid in many countries.

Oral infections are one of the most common diseases worldwide, which can be responsible for dental caries and periodontal diseases. Over 700 bacterial taxa have been found in the oral cavity, however they are not all present in the same mouth (Aas et al., 2005). Oral cavity pathogens include *Streptococcus mutans, lactobacilli, Streptococcus salivarius, Halobacterium sp., Veilonella sp.* etc.

It is well-known, that chemical agents such as fluoride and chlorhexidine, which have been used to prevent dental caries for several decades, were associated with some side effects such as staining of teeth and fluorosis. Thus, there is no perfect antimicrobial agent to prevent dental caries until now (Gold ., 2008). The use of natural products has been one of the most successful strategies for the discovery of new drugs (Gold , 2008). Natural products have been used for thousands of years in folk medicine and they are believed to be the new source of antimicrobial agents (Xavier and Vijyalakshmi., 2007).

The relationship between the high incidence of oral diseases and microorganisms is well known. Because of the increased bacterial resistance to antibiotics, toxic and harmful effects of few common antibacterial agents, there is a continuous need for alternative therapies which are affordable, not toxic and effective, such as plants (Palombo , 2011; Rishton , 2008).

Plant extracts are able to restrict the growth of bacteria due to the presence of active principles in them. Plants are important source of potentially useful structures for the development of chemotherapeutic agents. The first step towards these goals is the in vitro antimicrobial activity assay (Tona et al., 1998). The interference of active constituents of plants usually affects growth and metabolism of microorganisms in a negative manner (Aboaba et al., 2006).

According to earlier reports, *Achyranthes aspera* plant contains secondary metabolites which are proved to have significant antimicrobial activities. Plant extracts of *A. Aspera* Linn. was reported to be more effective on fungal and bacterial species than *O. basilicum* (Sutha et al., 2015).A study proved that stem and root extracts of plant *A. aspera* possesses a significant antibacterial activity against *S. mutans*, which is the causative organism playing a major role in the pathogenesis of dental caries (Yadav et al., 2016). Results of a study revealed the strong antibacterial potential of isolated compound, identified as 3', 4', 5, 7-tetrahydroxy flavonol from ethanolic extract of *Achyranthes aspera* stem against dental pathogens (Ashwini and Arpana., 2013). In a study, the methanolic extract and aqueous extracts of leaf, stem, inflorescence and roots of *Achyranthes aspera* were screened. Preliminary phytoc hemicals screening of methanol extract and aqueous extracts showed the presence of flavonoids, saponins, proteins and glycosides (Pandey et al., 2014).

In the present study, least bacterial colonies were found in nutrient agar plate streaked with tooth bacteria, after brushing with stem of *Achyranthes aspera* as compared to bacterial colonies obtained on nutrient agar plate, after brushing with sodium monofluorophosphate. Gram staining of bacteria isolated from the nutrient agar plate streaked with tooth bacteria, after brushing with *A.aspera* stem, showed that the pure culture of tooth bacteria were gram positive bacteria. From the MIC test of aqueous and methanolic stem exacts against gram positive tooth bacteria, it was found that both aqueous and methanolic extracts were effective against gram positive tooth bacteria. It is also seen that methanolic stem extract is more effective than aqueous stem extract. In disc diffusion method it was found that both aqueous and methanolic stem extracts showed inhibition zone at different concentrations against the gram positive tooth bacteria and inhibition zone indicated that methanolic extract was more effective than aqueous extract of *A. aspera* dry stems.

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