



Research Article

Studies On *In Vitro*

Anti-Microbial Activity

By Disc Diffusion

Method And FTIR

Analysis OF *Achyranthes*

***aspera* (L.)**

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Abstract

The antimicrobial activity of *Achyranthes aspera* against the bacteria and fungi plays a vital role in evaluation of the ethanomedicinal property of plants. FT-IR spectral analysis was carried out in ethanolic extract. The FT-IR analysis has revealed the presence of alcohols, alkanes, terminal alkyne, alkenes, halogen, ethers, aromatic compounds, aliphatic chloro compounds and aryldisulphides respectively. This provides and supports the therapeutical information of the plants. The medicinal values of *Achyranthes aspera* were investigated through agar well disc diffusion method. The aqueous and ethanolic extracts of plants to be tested were prepared at a concentration of 200µg/mL were dissolved in dimethyl sulphoxide (Dimethyl Sulphoxide (DMSO)). The zone of inhibition gives the degree antimicrobial property. It was compared with standard antimicrobial agents like Gentamycin for bacteria and Amphotericin for fungi. From this study, it can be concluded that *Achyranthes aspera* exhibits

antimicrobial activity against certain microorganisms.

Keywords: Antimicrobial activity, *Achyranthes aspera*, FTIR spectral analysis.

Introduction

Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effect. The medicinal use of plants is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology and chemistry. The World health Organization (WHO) has estimated that over 75% of the world's population still relies on plant derived medicines, usually obtained from traditional healers, for its basic health care needs. Herbal medicines are in great demand in the developed as well as developing countries for primary health-care because of their wide biological and medicinal activities, higher safety margins and lesser costs.

Achyranthes aspera is a species of plant in the Amaranthaceae family. It is distributed throughout the tropical world. It can be found in many places growing as an introduced species and a common weed. *Achyranthes aspera* L. (Latjeera) is an erect or procumbent, annual or perennial herb of about 1-2 meter in height, often with a woody base (Rawat *et al.*, 2008). These phytochemicals are known to possess antioxidant (Wong *et al.*, 2009) antibacterial (Nair *et al.*, 2005), antifungal (Khan and Wassilew, 1987), antidiabetic (Singh and Gupta, 2007), anti-inflammatory (Kumar *et al.*, 2008), hypolipidemic activity (Durkar *et al.* 2014) etc and due to these properties they are largely used for medicinal purpose.

Infectious diseases

Infectious diseases, also known as contagious diseases or transmissible diseases comprise clinically evident illness resulting from the infectious, presence and growth of pathogenic biological agents in an individual host organism. Infectious diseases are the world's leading cause of premature death, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. Hence the present study

to evaluate the antimicrobial activity and FT-IR analysis of *Achyranthes aspera* extracts against the pathogenic microbes.

MATERIALS AND METHODS

Collection, Identification and Authentication of plant materials

The plant species namely *Achyranthes aspera* L. plant was collected by in and around Koothanalur, Thiruvavur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's college, Tiruchirappalli (Voucher number of the specimen, AMTA 001) (Gamble, 1997).

Preparation of plant powder

The plant was air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used for further analysis.

Preparation of the aqueous extract

The plant material (Whole plant) was shade dried and coarsely powdered with electrical blender. 200g of *Achyranthes aspera* was mixed with 1200ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to preclinical screening.

Preparation of the Ethanol extract

Ethanolic extracts was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40°C – 50°C). The paste form of the extracts was put in an air tight container stored in refrigerator.

In vitro antimicrobial activity

The antimicrobial activity was performed by disc diffusion method of Beyar *et al.*, 1966..

Results And Discussion

FTIR analysis

The FT-IR spectrum was used to identify the

functional group of the active components based on the peak value in the region of infra red radiation. The ethanolic extracts of *Achyranthes aspera* was passed into the FT-IR and functional groups of the components were separated based on its peak ratio. The results showed the presence of alcohols, alkanes, terminal alkyne, alkenes, halogen, ethers, aromatic compounds, aliphatic chloro compounds and aryldisulphides respectively (Table 1 and Figure 1).

Spectral differences are the objective fraction of componential differences. By using, FT-IR spectrum, we can confirm the functional constituents presence in the given extracts, identify the medicinal materials from the adulterate and even evaluate the qualities (Liu *et al.*, 2005). Many researchers applied the FT-IR spectrum as a tool for distinguishing closely associated plants and other organisms (Durates *et al.*, 2008).

Antimicrobial activity

The antimicrobial activities of aqueous and ethanolic extracts of *Achyranthes aspera* were studied against two pathogenic bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) and two fungal strains (*Aspergillus niger* and *Aspergillus flavus*). Antibacterial and antifungal potential of aqueous and ethanolic extracts were assessed in terms of zone of inhibition of bacterial and fungal growth. The results of the antibacterial and antifungal are presented in Table 2, and Table 3,.

The test organisms used in the study are associated with various forms of human infections. *E.coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and the immunodeficient patients (Douguz *et al.*, 2008). Whole plant extract of *Achyranthes aspera* also shows high activity against *E.coli* with zone of inhibition of 21mm in aqueous extract but poor in ethanolic extract (16mm) as the zone of inhibition 17mm or more considered as high antimicrobial activity and *Staphylococcus aureus* with zone of inhibition of 20mm in ethanolic extract but poor in aqueous extract (17mm) as the zone of inhibition 18mm more considered as high antimicrobial activity (Veeramuthu *et al.*, 2006).

Plate 1 and Plate 2 showed that the antibacterial and antifungal activity of aqueous and ethanolic extract of *Achyranthes aspera*.

In the present study, aqueous and ethanolic extracts obtained from *Achyranthes aspera* plant

shows the significant activity against most of the tested bacterial and fungal strains. The present study support the traditional usage of plant material *Achyranthes aspera* which possess compounds with antibacterial and antifungal potential that can be used as antimicrobial agents as new drugs for the therapy of infectious diseases caused by pathogens. The results were compared with standard antibiotic drugs gentamycin for bacteria and amphotericin for fungi.

Conclusion

The spectral analysis indicated that the specific functional groups. FT-IR spectroscopy technique

showed the presence of functional groups which can be identified and further screened for different kinds of biological activities depending on their therapeutic uses. The antimicrobial activity of aqueous and ethanolic extract of *Achyranthes aspera* was evaluated and determined by using disc diffusion method. The antimicrobial activities of aqueous and ethanolic extract (200µg/ml) were tested against *E. coli*, *S. aureus* and *A. niger*, *A. flavus*. The results showed that the *Achyranthes aspera* was found to be more effective. On the basis of the results obtained in the present study, it is concluded that the whole plant of *Achyranthes aspera* has potent antimicrobial activity.

Table 1: FT-IR spectral peak values and functional groups obtained for the whole plant extract in ethanol of *Achyranthes aspera*

Extracts prepared in	Peak values	Functional groups	Interpretation
Ethanol	3425.43	-OH group	Alcohols
	2927.07	C - H stretching	Alkanes
	2103.04	C = C stretching	Terminal alkyne
	1631.68	C = C group	Alkenes
	1490.61	C - F stretching	Halogen
	1401.60	C - H stretching	Ethers
	1127.27	C - O group	Ethers
	1054.73	C - O group	Ethers
	933.94	C - H stretching	Aromatic compounds
	899.81	C - O group	Ethers
	828.69	C - Cl stretching	Halogen
	719.01	C - Cl stretching	Aliphatic chloro compounds
	438.14	S - S stretching	Aryl disulfides

Figure 1: FT-IR spectral peak values and functional groups obtained for the whole plant extract in ethanol of *Achyranthes aspera*

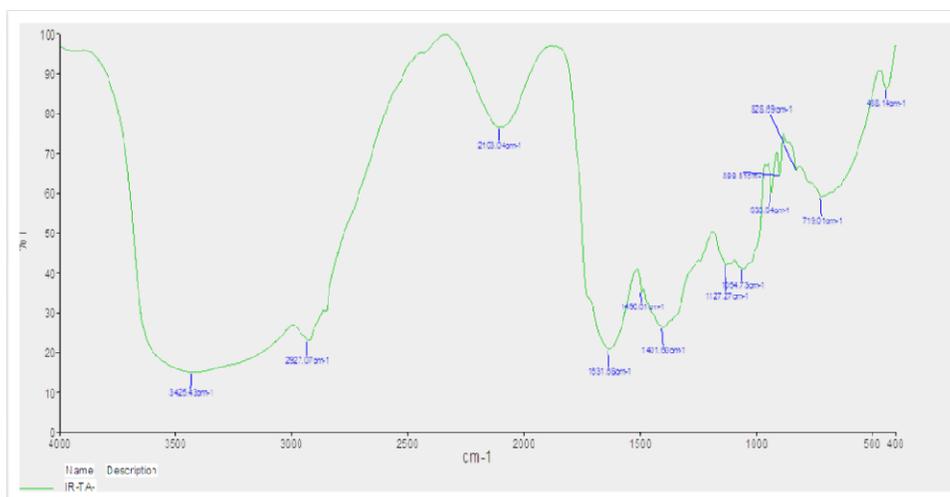
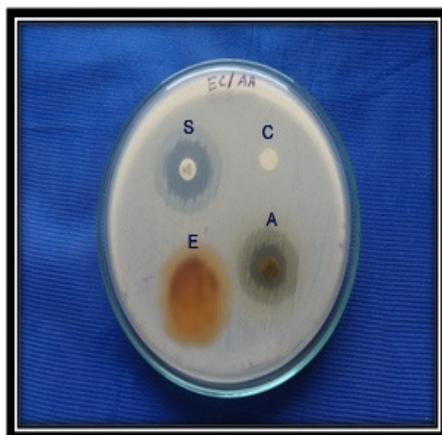
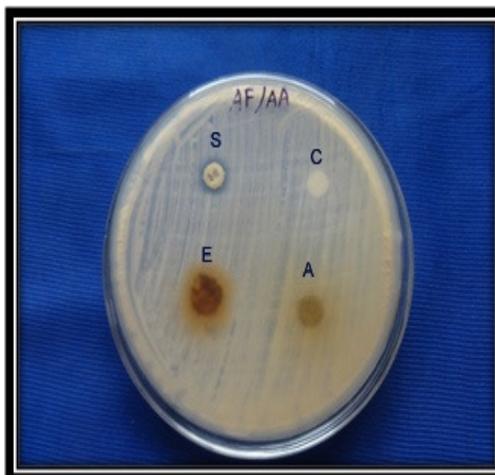


Table 2: *In vitro* antibacterial activity of aqueous extract of *Achyranthes aspera*

S.No	Microorganisms	Zone of inhibition (mm in diameter)		
		Gentamycin (30µg/ml)	Aqueous extract (200µg/ml)	Ethanol extract (200µg/ml)
1	<i>Escherichia coli</i>	23	21	16
2	<i>Staphylococcus aureus</i>	19	17	20

Table 3: *In vitro* antifungal activity of ethanolic extract of *Achyranthes aspera*

S.No	Microorganisms	Zone of inhibition (mm in diameter)		
		Amphotericin (20µg/ml)	Aqueous extract (200µg/ml)	Ethanol extract (200µg/ml)
1	<i>Aspergillus niger</i>	15	20	17
2	<i>Aspergillus flavus</i>	13	14	18

Plate 1: Antibacterial activity of aqueous and ethanolic extracts of *Achyranthes aspera**Escherichia coli**Staphylococcus aureus***Plate 2: Antifungal activity of aqueous and ethanolic extracts of *Achyranthes aspera****Aspergillus niger**Aspergillus flavus*

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