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Original Article

Preliminary Phytochemical analysis and Antimicrobial activity of *Achyranthes aspera* Linn.

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Abstract

Medicinal plants are economically important major source for drug production. *Achyranthes aspera Linn* is one of the important medicinal plants having many theaurapetic uses as Odontalgic, Rheumatism, Bronchitis, skin disease and rabies. The aqueous, ethanol and methanol leaves and stem extracts of *Achyranthes aspera Linn*. were evaluated for antimicrobial activity against *Escherichia coli*. TLC observation of leaf and stem extracts showed the presence of secondary metabolites as flavonoid (Quercetin) and Glucoside (Saponin). The ethanol and methanol extract of the leaves and stem exhibited an elevated antimicrobial activity against *E.coli*. Thus results obtained in the present study suggest that the ethanol and methanol extracts of the leaf and stem revealed a significant scope to develop a novel broad spectrum of antimicrobial drug formulation.
Keywords*:* Antimicrobial activity, *Achyranthes aspera* Linn., Secondary metabolites.

Introduction

Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. They are rich source of bioactive compounds and thus serve as important raw materials for drug production. Now-a-days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Lakshmi Naidu *et al.,* 2006). This situation forced scientists to search for new antimicrobial substances. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Valsaraj *et al.,* 1997). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg, and Newman, 2001).

Numerous surveys on antimicrobial medicinal plants had been made in United States and in many countries throughout the world. Such study had demonstrated the wide occurrences of active compounds in higher plants (Hughes, 1952). *Achyranthes aspera Linn* belongs to the family Amaranthaceae. It is an annual, stiff erect herb, and found commonly as a weed throughout India and is one of the important medicinal plants having many theaurapetic uses as Odontalgic, Rheumatism, Bronchitis, skin disease and rabies (Girach, and Khan, 1992).The aqueous solution of the base achyranthine as well as the entire plant of *Achyranthes aspera* Linn showed antibacterial activity against *staphylococcus aureus, streptococcus heamolyticus* and *Bacillus typhosus* (Basu *et al,* 1957). While the alcoholic and aqueous extract of leaves showed antibacterial activity against *Staphylococcus aureus* and *E.coli* (George *et al.,* 1947). Leaf extracts were reported to posses thyroid stimulating and anti peroxidative properties (Tahiliani, and Kar, 2000). The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan diabetic rabbits (Akhtar, and Iqbal, 1991). It is reported to contain Alkaloids, flavonoids, saponins, steroids and terpenoids. Flavonoids have a number of nutritional functions and have been described as biological response modifiers; most act as a anti-oxidant and some have a anti-inflamatory properties. Flavonoids have been shown to prevent or slows the development of some cancers (Raj Narayana *et al.,* 2001*).* Saponins have long been known to have strong biological activity. Saponins can

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bind to cholesterol and thus interfere with cell growth and division. While drugs have side effects, many of them serious, saponins are safe (Chen *et al.*, 1999). The water soluble alkaloid achyranthine isolated from *Achyranthes aspera* possess anti-inflammatory activity (Gokhale *et al.*, 2002). In light of the above the present study was carried out to test the antimicrobial efficacy of the leaves extract of *Achyranthes aspera Linn*. with reference to microbes and the extraction of reported secondary metabolities.

Materials and Methods

Plant Material

The plant material of *Achyranthes aspera Linn* were freshly collected in and around Erode District and cleaned with distilled water. The authenticated stem and leaves of *Achyranthes aspera Linn* were allowed for shade dried at room temperature.

Preparation of Extracts

Achyranthes aspera. Linn (Amaranthaceace) dried leaves were ground to 5mm particle and macerated with 95% ethanol using magnetic stirrers for 12 hours at room temperature (Atak *et al.*,2000). After 12 hours, extract was filtered through Whatmann No:1 filter paper. Filtrate was concentrated to dryness in rotary evaporator. The concentrated extract was resuspended with 3ml extraction mixture.

Qualitative analysis of phytochemicals

A small quantity of the extract was treated with sodium hydroxide solution; Formation of yellow color indicates the presence of Flavonoids

Foam Test

The extracts were diluted with 20ml of distilled water and agitated in graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponins.

Haemolysis Test

About 2ml of blood was taken in two test tubes separately. To one of the test tubes, equal quantity of water was added. To the other test tube, an equal quantity of ethanolic extract dissolved in water was added. A clear red liquid was formed in the first tube, which indicates that red blood corpuscles were haemolysed. The extract in the second test tube also haemolysed. It indicates the presence of saponin (Kokate, 1991).

Culture media and inoculums preparation

Nutrient agar /broth were used as the media for the culturing of *E.coli*. A loop full of *E.coli* cultures was inoculated in the Nutrient broth at 37°C for 48 hrs.

Antibacterial Activity

Disc Diffusion Method

The discs were prepared from the whatman filter paper No.1 and were sterilized. The solution to be assayed are added at volume of 7.5 µl, 15 µl, 22.5 µl and 30µl for leaf and stem to discs of sterile filter paper usually 12.8mm in diameter (Casida, 1968). These discs were later saturated with extracts and dried in an oven at 50˚C for minutes for evaporation. Then the discs were transferred in to the culture plates with the help of sterilized forceps. Thus for the organism in one plate, two discs placed representing leaves and other two discs representing the stem extracts in different solvents at different concentrations. Then Plates were incubated at 37˚C for 18 hours. After incubation, inhibitory zones were observed. Based on the diameter of the zone of inhibition, antibacterial susceptibility was ranked (Heisey *et al.*, 1992).

Results and Discussion

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Table-1 presents the extraction values of dried leaves and stem in different solvents. The values were high in ethanol extract for both leaf (7.9 mg) and stem (7 mg). Among other solvents used for study, Petroleum ether has shown extract value as 6.4 mg for leaves and for Benzene extract value as 4.2 mg for stem. Water when used as extract solvent showed only 3.0 mg for leaves and 2.8 for stem (Fig 1). The traditional healers or practitioners make use of water primarily as a solvent, but our studies showed that ethanol, methanol extracts of these plants were certainly much better than water. This may be due to the better solubility of the active components in organic solvent (Boer *et al.*, 2005).

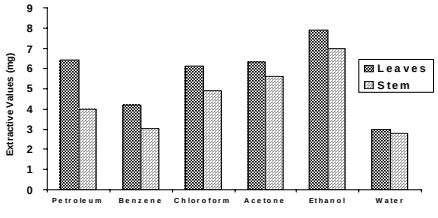

Fig.1: Extraction value of *A. aspera Linn* Leaves and Stem

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Detection of Flavonoids

As the solvent system Benzene: methanol: acetic acid (90: 16: 8) rises, it carries the flavonoids in the spotted extracts on TLC plate to the various length based on their solubility. Two specific spots were observed both for leaf and stem extracts. The leaf extract showed Rf values 0.62 and stem extract showed Rf values as 0.31 (Table 2).

Detection of Saponins

As the solvent system Benzene: methanol: acetic acid (90: 16: 8) rises, it carries the saponins in the spotted extracts on TLC plate to the various length based on their solubility. Two specific spots were observed both for leaf and stem extracts. The leaf extract showed Rf values 0.59 and stem extract showed Rf values as 0.57 (Table 3).

Tables -1: Extraction Value of *A. aspera Linn* Leaves and Stem

S.No	Solvents	Average value of extraction (mg / 50g)	
		Leaf	Stem
1	Petroleum ether	6.4	4.01
2	Benzene	4.2	3.01
3	Chloroform	6.1	4.9
4	Acetone	6.3	5.6
5	Ethanol	7.9	7.0
6	Water	3.0	2.8

Table- 2: Rf Values of standard Quercetin and Extracts obtained by TLC

S.No	Sample	Rf Value
1	Plant extract – leaf	
	Spot – 1	0.59
	Spot – 2	0.62
2	Plant extract – Stem	
	Spot – 1	0.40
	Spot – 2	0.31
3	Standard quercetin	0.52

Table -3: Rf Values of Standard Saponin and Extracts obtained by TLC

S.No	Sample	Rf Value
1	Plant extract – leaf	
	Spot – 1	0.42
	Spot – 2	0.59
2	Plant extract – Stem	
	Spot – 1	0.41
	Spot – 2	0.51
3	Standard saponin	0.48

Antimicrobial Activity

In the present study the antimicrobial activity of plant *Achyranthes aspera. Linn* extracts of leaf and stem from various solvents like Ethanol, Benzene, Chloroform, Acetone, Petroleum ether and Water were evaluated against *E.coli*. Among these ethanolic extracts of both the leaves and stem extracts showed a measurable zone of inhibition of about 25 mm and 20 mm respectively. The leaves extracts of Benzene showed a measurable zone of inhibition of about 18 mm whereas the stem extract showed 18 mm as a remarkable zone of inhibition than any other extract of stem. The Chloroform extracts of leaves and stem is about 19 mm and 14 mm respectively. Both the leaves and stem extracts of Acetone exhibited the same zone of inhibition as 21 mm. Where as the Petroleum ether extracts of leaves and stem is of about 23 mm and 19 mm zone of inhibition. But our studies revealed that for a water extract a minimum zone of inhibition is about 7 mm and 8 mm for leaf and stem extracts respectively (Table 4).The results of present study suggest that the plant extracts possess bioactive compounds with antimicrobial activity against many pathogens. Thus results obtained in the present study suggest that the alcohol extracts of the leaf and stem revealed a significant scope to develop a novel broad spectrum of antimicrobial drug formulation (Cragg, and Newman, 2001). Hence these active extracts can be used to carry out further pharmacological evaluation.

Table -4: Antimicrobial Activity on Leaf and Stem Extracts of *A. aspera Linn* against *E.coli*

Sl.No.	Solvents	Concentration of leaf extract (µl)				Concentration of Stem extract (µl)			
		7.5	15.0	22.5	30.0	7.5	15.0	22.5	30.0
		Zone of inhibition (mm)							
1.	Ethanol	Nil	15	20	25	Nil	10	17	20
2.	Benzene	Nil	10	15	18	Nil	12	16	18
3.	Chloroform	Nil	12	17	19	Nil	10	13	16
4.	Acetone	Nil	14	18	21	Nil	12	14	17
5.	Petroleum ether	Nil	12	19	23	Nil	10	16	19
6.	Water	Nil	Nil	Nil	7	Nil	Nil	Nil	8

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