



Studies on antibacterial potential and phytochemical screening of different extract of *Achyranthes aspera*

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Abstract: There is an alarming increase in the problem of resistance towards antibiotics amongst most of the pathogenic bacterial strains in recent years. This has drawn the attention of researchers around the world to search for novel and eco-friendly antibacterial compounds. Several biological sources have been explored in this respect but medicinal plants have taken a centre stage out of all. Plants have been known as a reservoir of number of bioactive compounds specially the antibacterial ones since time immemorial. Therefore, the present investigation was undertaken to analyze the antibacterial potential of the medicinal plant *Achyranthes aspera*. This study revealed that highest antibacterial activity was observed in the methanolic extract of stem against almost all test Bacteria. It showed maximum activity against *E.coli* (30 mm), followed by *S. aureus* (28 mm), *Enterococcus sp.* (25mm), *Salmonella typhi* (20 mm) and least activity was recorded in same extract against *K.pneumoniae* (6 mm). Four phytochemicals were screened in various solvent extracts. They are alkaloid, flavonoids, saponins and tannins.

Key words: Medicinal Plants; Antibacterial activity; *Achyranthes aspera*

Introduction

Plants have been used in traditional medicine for several thousand years. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of immense therapeutic value (Pandey *et al.*, 2014). The medicinally active plant compounds are usually their secondary metabolites like terpenoids, quinones, flavonoids, tannins, resins and saponins etc. are responsible for protecting the plants from microorganisms, insects and other natural pests. In the recent past, there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally.

Achyranthes aspera. Linn. belonging to the family Amaranthaceae, is an annual, stiff erect or procumbent, annual or perennial herb, 1-2m in height, often with a woody base, commonly found as a weed of waysides, on roadsides (Jain *et al.*, 2006; Zafar, 2009). *Achyranthes aspera* Linn. is a well-known plant drug in Ayurvedic, Unani, Siddha, Allopathic, Homeopathic, Naturopathic and Home Remedies (Dhale *et al.*, 2013). It is found and distributed throughout the tropical and subtropical regions. This wild tropical plant is

known by different names such as Chirchita (Hindi), Apamarga (Sanskrit), Aghedi (Gujarati), Apang (Bengali), Nayurivi (Tamil), Kalalat (Malyalam) and Agadha (Marathi) in India (Dwivedi *et al.*, 2008).

The plant shows many pharmacological activities like, anti-allergic (Tyler, *et al.*, 1994), hepatoprotective (Bafna and Mishra, 2004), cardiovascular (Han, *et al.*, 2003), nephroprotective, antidiabetic, antiparasitic (Banerji, *et al.*, 1970), hypoglycemic, analgesic and antipyretic (Gokhale, *et al.*, 2002) and antimicrobial (Beulah *et al.*, 2011). It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles and snake bites (Singleton, 1999) and the juice of the plant is used in the treatment of diarrhea, dysentery, rheumatic pains, itches and skin eruptions (Londonkar *et al.*, 2011). It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids, flavonoids have shown to prevent the development of some cancers (Narayana *et al.*, 2001) and mostly act as an anti-oxidant and anti-inflammatory agents. Besides the above mentioned biological activities, the different extracts of *A. aspera* are also known to exhibit antibacterial activity. Exploring this potential has become a significant area of research in recent

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years due to the increasing resistance problem among the pathogenic bacterial strains. Studies on antibacterial compounds in the extracts of *A. aspera* would lead to the emergence of novel compounds with new mechanism of action against pathogenic bacteria. Thus, it is clear from the literature review that *A. aspera* possess various medicinal properties which could be exploited to derive novel treatment strategies for a number of diseases. Further research is necessary to identify and characterize the active compounds responsible for these medicinal properties. In the present investigation, the antibacterial activity of *A. aspera* was screened out against various pathogenic bacteria.

Materials and Methods

Plant sample collection

Plant samples were collected from Dumna Nature Reserve, Jabalpur (M.P.) and were carefully stored in sterile polythene bags. They were air dried and used for present study.

Processing of plant materials

The dried sample was then grinded to fine powder with the help of the mortar and pestle. The powdered samples were stored in a clean glass container until needed for analysis.

Solvent extraction-

1 gm powder was dissolved in 10 ml of different solvents separately; extract was kept for 24 hrs. at room temperature. The mixture was then filtered with the help of Whatmann No.1 filter paper. The filtrate was used for antibacterial activity and phytochemical screening using various bioassays

Test Organisms used for antibacterial activity assay.

Four Bacterial species were used in screening for antibacterial activities which were procured from Fungal Biotechnology and Invertebrate Pathology Laboratory, Department of Biological Sciences, Rani Durgavati University, Jabalpur (M.P.)

Antibacterial Activity via Agar Well Assay

In this Assay Nutrient Agar Media (Peptone:5 gm; Beef extract: 3 gm; NaCl: 5 gm; Agar: 20 gm; Distilled Water: 1000 ml) plates. Plates were prepared and 25 µl of bacterial suspension was applied on each of these prepared plates under laminar air flow by spread plate method. Wells of 4-5 mm diameter were prepared on the plates with the help of sterilized cork borer. The filtrates were obtained after solvent extraction was filled in the wells with the help of micropipette (100 µl). These plates were then kept in bacteriological incubator at 37 °C for 24 hrs. After incubation the activity was recorded in terms of zone of inhibition in mm (Shukla et al., 2016)

Phytochemical Screening

Test for alkaloids

Hager's test – extracts were dissolved individually in dilute HCl and filtered. Filtrate was treated with Hager's reagent. Formation of yellow coloured precipitate indicated the presence of alkaloids.

Test for flavonoids

Alkaline Reagent test–In the test solution having extract few drops of NaOH solution was added. Formation of intense yellow colour which turns to colourless by addition of few drop of dilute acetic acid indicated the presence of flavonoids.

Test for Tannins

Ferric chloride test: To the test solution having extract few drops of ferric chloride solution was added. An intense green, purple, blue or black colour indicated the presence of tannin.

Test for Saponins

Froth test: - In the test solution having extract, 2-3 ml of distilled water was added. The mixture was shaken vigorously. Formation of foam indicated the presence of saponins.

Test for Regins

In a dry test tube 1ml of extract was taken and one drop of concentrated Sulphuric Acid was added to it. Formation of purple colour changes into violet indicating the presence of regins.

Results and Discussion

The antibacterial activity of different solvent extracts of *Achyranthes aspera* was recorded in terms of zone of inhibition as presented in Table 1. According to the above data.

The highest antibacterial activity was observed in the methanolic extract of stem against almost all test Bacteria. It showed maximum activity (Fig.1) against *E.coli* (30 mm), followed by *S. aureus* (28 mm), *Enterococcus sp.*(25mm), *Salmonella typhi* (20 mm) and least activity was recorded in same extract against *K.pneumoniae* (6 mm). On the otherhand Methanolic extract of root showed moderate activities against *K.pneumoniae* (10 mm), *S. aureus* (8 mm), *Enterococcus sp.*(2 mm) *E.coli* (5 mm) while no activity was recorded against *S.typhi*. Butanolic extract of root also showed good activities against *K.pneumoniae* (16mm), *S. aureus* (15mm) and moderate activities against *S.typhi* (7mm), *Enterococcus sp.*(5 mm). Butanolic extract of stem showed no activities against any tested bacterial strains. Ethanolic extract of root showed moderate activities against all bacterial strains but ethanolic extract of stem showed no activities against all bacterial strains except *E.coli* (10mm). Similarly chloroform extract of root and stem showed no activities against any bacterial strains except chloroform extract of root against

Enterococcus sp. (10mm). Such behavior of the antibacterial action was also showed by Alam (2009). Variation in antibacterial activities of

different parts of *A. aspera* in different extracts were also reported by Beulah et al., (2011).

Table 1: Antibacterial activities of the different parts of *Achyranthes aspera* in different organic solvents

S. No.	Test Bacteria	Zone of inhibition (in mm)								Positive control Streptomycin (40µg/ml)	Negative control (100 µl solvents)
		B		M		E		CH			
		Rt	Sm	Rt	Sm	Rt	Sm	Rt	Sm		
1	<i>K. pneumoniae</i>	16mm	-	10mm	6 mm	8mm	-	-	-	15 mm	-
2	<i>S. aureus</i>	15mm	-	8mm	28 mm	10mm	-	-	-	10 mm	-
3	<i>Enterococcus sp.</i>	5mm	-	2mm	25 mm	4mm	-	10mm	-	12 mm	-
4	<i>S. typhi</i>	7mm	-	-	20 mm	5mm	-	-	-	6 mm	-
5	<i>E. coli</i>	-	-	5 mm	30 mm	2mm	10 mm	-	-	20 mm	-

M= Methanol, B=Butanol, E=Ethanol, Ch=Chloroform, Rt= Root, Sm= stem

The inhibitory activities of all the extracts reported in Table 1 are comparable with standard antibiotic streptomycin. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional practitioners make use of water primarily as a solvent, but our studies showed that even organic solvents were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvent and flavonoids are least stable in water which is the primary polyphenolic compound in plants (Boer et al., 2005). Literature survey revealed that chemical

constituents like flavonoids, triterpenoids, polyphenolic compounds and steroids are responsible for antioxidant and antibacterial activity and these chemical constituents were reported in the methanolic extract of aerial parts of *Achyranthes aspera* (Chakraborty et al., 2002; Tahiliani et al., 2000). The preliminary Phytochemical screening of the extracts showed the presence of phenolic compounds, flavonoids, Alkaloids, Steroids, Tannins etc. These compounds may be responsible for antibacterial activity and may serve as a substitute for Synthetic drugs.

Table 2: Phytochemical screening of stem and roots of *Achyranthes aspera*

Phytochemicals	Stem extracts				Root extracts			
	M	B	E	Ch	M	B	E	Ch
Alkaloids	+	-	+	+	+	++	+	+
Flavonoids	+	++	-	-	++	-	+	++
Tannins	+	-	+	+	+	+	++	+
Saponins	+	-	-	++	+	+	+	+

M= Methanol, B=Butanol, E=Ethanol, Ch=Chloroform, ++: High Quantity, +: Medium Quantity, Absent

These Phytochemical compounds are the measure compounds which impart medicine value of the plant. Results obtained from screening of phytochemicals in stem and roots of *Achyranthes aspera* are presented in table 2 (Fig.2). Four phytochemicals were screened in various solvent extracts. They are alkaloid, flavonoids, saponins, tannins.

Data presented in Table 2, Methanolic, extract of stem was found to contain medium quantity of alkaloids, flavonoids, tannins and saponins. Butanolic extract of Stem was found to contain high quantity of flavonoids but devoid of alkaloids, tannins and saponins. Ethanolic extract of stem was found to contain medium quantity of alkaloids and tannins but devoid of flavonoids and saponins. Similarly chloroform extract of stem was found to contain high quantity of saponins and medium quantity of alkaloids and tannins but devoid of flavonoids.

Methanolic extract of root was found to contain high quantity of flavonoids and medium quantity

of alkaloids, tannins and saponins. Butanolic extract of root was found to contain high quantity of alkaloids and medium quantity of tannins and saponins but devoid of flavonoids. Ethanolic extract of root was found to contain high quantity of tannins and medium quantity of alkanoids, flavonoids and saponins. Similarly chloroform extract of root was found to contain high quantity of flavonoids and medium quantity of alkaloids, tannins and saponins (Table-2). According to Tiwari et al., (2011) the factors affecting the choice of solvent are; quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process.

Literature survey has revealed that tannins promote wound healing activity through several mechanisms that include chelation of free radicals; antioxidant, antimicrobial and astringent property (Amarowicz et al., 2002). In all, more phytochemicals were found present in the root

than in the stems. This suggests that the root extracts offer a wider array of phytochemicals than the stem.

Figure numbers

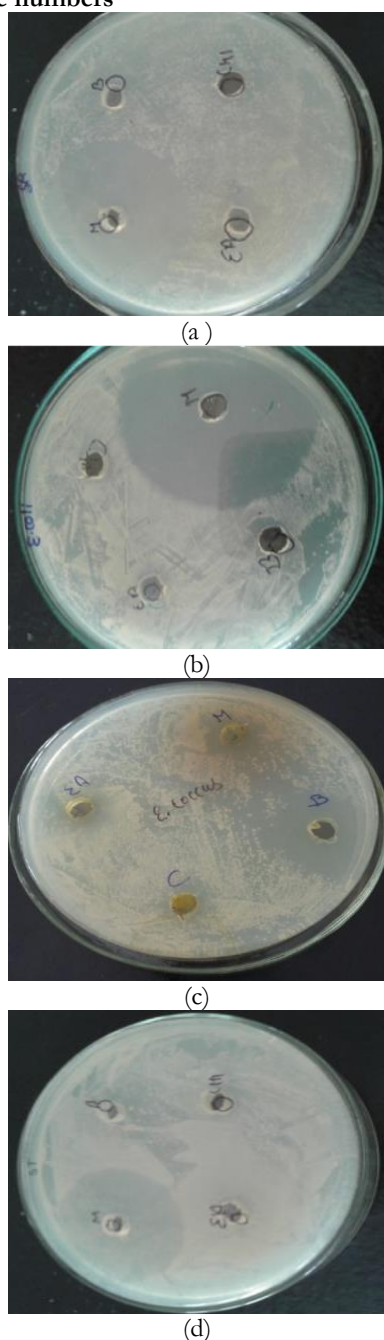


Fig. 1: Antibacterial activity of different extract of *Achyranthes aspera*

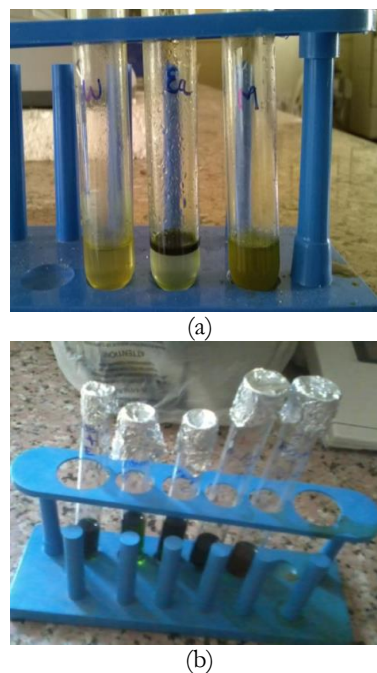


Fig. 2: Phytochemical screening of stem and roots of *Achyranthes aspera*

Conclusion

The present work deals with the study of different parts of *Achyranthes aspera*. Its antibacterial activity screening was done to support the traditional use of plants and suggests that the plant extracts possess compounds having antibacterial properties. It may be used as antibacterial agents in new drugs therapy of infectious diseases caused by human pathogenic bacteria. *A. aspera* extracts inhibit the growth of various pathogenic bacteria. This activity may be due to various phytoconstituents including flavonoids, triterpenoids, alkaloids and natural phenolic compounds which are classified as active antimicrobial compounds. The phytochemical screening of *Achyranthes aspera* was done in methanol, chloroform, ethanol and butanol extract which showed the presence of alkaloids, flavonoids, Saponins and Tannins. It was reported that *Achyranthes aspera* possesses high antibacterial activity. The most active extracts can be subjected to isolation of the active compound and carry out further pharmacological evaluation. This will surely complement to the previously known therapeutic values and improve the popularization of this plant.

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