

Original Research Article

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***In vitro* Screening of Antibacterial Potentials of *Achyranthes aspera*
Azolla pinnata and *Cissus quadrangularis***

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The present investigation was undertaken for *in vitro* screening of antibacterial activities of different extracts (ethyl acetate, methanol and benzene) of various plant parts of *Achyranthes aspera*, *Azolla pinnata*, *Cissus quadrangularis*. *In vitro* antibacterial efficacy of plants selected was assessed by well diffusion method against pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The ethyl acetate extract of *Cissus quadrangularis* exhibited highest zone of inhibition against *S. aureus* (25±1.3mm) followed by *Achyranthes aspera* (20.0±0.5 mm) and *Azolla pinnata* (20±0.9mm) whereas Methanolic extract of plants selected also showed good results against *P. aeruginosa*. None of activity is shown by Benzene extract against any test pathogen. Results of the present investigation indicate that these plants possess compounds with antibacterial properties and hence can be exploited for future natural plant based antimicrobials.

Introduction

Plants are capable of synthesizing variety of low molecular weight organic compounds, called secondary metabolites usually with very unique and complex structures. The role of secondary product has been rather ambiguous and earlier these were thought to be first waste materials. Now days, plant secondary metabolites are seen as tremendous source of pharmacological value for scientific and clinical research. Their biological activities have high therapeutic value, applicable in health care, drug development and synthesis of beneficial compounds. A number of plants have been screened for their antimicrobial properties especially due to the

presence of secondary metabolites. In the present scenario of emergence of multidrug resistance to human pathogenic infections, it has become very necessary to search for new antimicrobial substances from other sources such as plants. Plants with their wide variety of chemical constituent offer a promising source of new antimicrobial agent, with general as well as specific antimicrobial activity (Nair *et al.*, 2005; Luseba *et al.*, 2007; Yadav *et al.*, 2011).

Achyranthes aspera is an annual, stiff erect herb, and found commonly as a weed throughout India and prescribed in Ayurveda

as an alternative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases and in the treatment of irregular menstruation, fever, dysentery and asthma. The chemical constituents show the presence of tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids which are used in medicinal purpose. *Azolla pinnata* is a pteridophyte plant rich in protein used in mosquito and weed control and traditional medicine. Plant rich in secondary metabolites such as flavonoids, tannins, terpenoids and alkaloids have significant biological activity against pathogens (Bagavan *et al.*, 2008; Pakrashi *et al.*, 1977; Lakshmi *et al.*, 2006). *Cissus quadrangularis* is a succulent vine native to India. It is commonly known as asthisamharaka. The chemical constituents show the presence of tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids, cardiac glycosides and anthroquinones which are used in medicinal purpose.

Keeping in mind the infectious diseases, resistant pathogenic organism and side effect of antibiotics an attempt was needed to be done to determine the antimicrobial activity of plant based medicinal agents. Therefore, the aim of the current research focuses to investigate the effects of various extracts of three plants *Achyranthes aspera*, *Azolla pinnata* and *Cissus quadrangularis* on growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Materials and Methods

Collection of plant material

The *Azolla* had been cultivated at Livestock Feed Resource Management and Training Center, RAJUVAS, Bikaner, it was harvested, washed thoroughly, dried for 2 to 3 days under shed, grinded and packed in air tight bags whereas *Cissus quadrangularis* (stem) was collected from various parks of Bikaner

where it was cultivated as an ornamental plant whereas seed samples of *Achyranthes aspera* (seeds) were purchased from the shop of herbal medicine and were identified by a well known taxonomist of Bikaner. The fresh sample of *Cissus* stem and seeds of *Achyranthes* was dried separately, grinded and used for further analysis.

Preparation of extracts by solvent extraction

Crude plant extract was prepared by soxhlet extraction method. Five grams of powdered *Achyranthes aspera*, *Azolla pinnata*, and *Cissus quadrangularis* was filled in thimble directly, were placed in soxhlet apparatus, and extracted separately using methanol, benzene and ethyl acetate for 24 hrs or until the solvent in siphon tube of an extractor become colorless. The extracts were then concentrated in pre-weighted vials on a rotary evaporator below 50°C. Dried extract was weighted and reconstituted with known volume of solvent and were stored in vials at 4°C for further experimental studies.

Screening of plant extracts for antimicrobial activity

Antibacterial activities of different extracts were studied by the well diffusion method.

Test organisms

The pure cultures of bacteria maintained in the nutrient broth medium. The test organisms used are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Preparation of inoculums

Stock cultures were maintained at 4°C in nutrient broth. Active cultures for experiments were prepared by transferring a

loopful of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated without agitation for 24 h at 37°C.

Preparation of media

Media was prepared by dissolving 0.5% Peptone, 0.3% beef extract/yeast extract, 1.5% agar, 0.5% NaCl and dissolved in 100 ml distilled water and autoclaved at 121°C for 15 min.

Antibacterial susceptibility test

Standard well diffusion method was carried out to screen the antibacterial activity. *In vitro* antibacterial activity was screened by using nutrient agar media. The nutrient agar plates were prepared by pouring 10 ml to 15 ml of molten liquid media into sterile Petri plates. The plates were allowed to solidify for a few minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 10 min. wells were prepared on agar plates and 100µl extract and solvent in control well was inoculated and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones formed around the wells were measured with transparent ruler in millimeter.

Results and Discussion

In the present investigation, *in vitro* antibacterial activity of the crude extracts of three plants was qualitatively assessed on the basis of the inhibition zone. The inhibition effect on growth of *S. aureus*, *P. aeruginosa* and *E. coli* by three extracts of *A. aspera*, *A. pinnata*, and *C. quadrangularis* (Table 1, Fig. 1, 2 and 3.), the results showed that the plant extracts were specific in action against the growth of bacteria. The zone of inhibition of solvents (control) was negligible. Against *S. aureus* in the zone of inhibition was

20±0.5mm, 20±0.9mm and 25±1.3mm by ethyl acetate extract of *A. aspera*, *A. pinnata*, and *C. quadrangularis* respectively. On comparison of mean values the *C. quadrangularis* exhibited maximum antibacterial activity in ethyl acetate extract. In methanol extract of *A. aspera*, *A. pinnata*, and *C. quadrangularis* the zone of inhibition against *S. aureus* was 11±0.61mm, 10±0.14mm and 10±0.5mm respectively. On comparison of mean values the methanol extract of *A. aspera* exhibited maximum antibacterial activity followed by methanol extract of *A. pinnata*. The minimum zone of inhibition was observed in methanol extract of *C. quadrangularis*. In benzene extract of selected three plants diminutive growth of *S. aureus* was observed.

The zone of inhibition against *P. aeruginosa* was 11±0.52mm, 11±0.3mm and 13±0.5mm in ethyl acetate extract of *A. aspera*, *A. pinnata* and *C. quadrangularis* respectively. On comparison of mean value it was observed that the highest antibacterial activity was showed by ethyl acetate extract of *C. quadrangularis* followed by *A. pinnata*. In methanol extract of *A. aspera*, *A. pinnata*, and *C. quadrangularis* the zone of inhibition against *P. aeruginosa* was 12±0.7mm, 10±0.1mm, and 16±0.4mm respectively. *P. aeruginosa* was most sensitive for the methanol extract of *C. quadrangularis* followed by methanol extract of *A. aspera*. The antibacterial activity against *P. aeruginosa* by methanol extract of *A. pinnata* was found minimum. A little growth of *P. aeruginosa* was reported in benzene extract of all selected three plants. Against *E. coli* the zone of inhibition was 13±0.7mm, 15±0.5mm and 11±0.6mm by ethyl acetate extract of *A. aspera*, *A. pinnata*, and *C. quadrangularis* respectively. *E. coli* was more sensitive to the ethyl acetate extract of *A. pinnata* followed by *A. aspera* and *C. quadrangularis*.

Table.1 Mean inhibitory (mm) values by the various crude extract of three medicinal plants against tested microorganism

Bacterial organism	<i>A. aspera</i>			<i>A. pinnata</i>			<i>C. quadrangularis</i>		
	EA	M	B	EA	M	B	EA	M	B
<i>S. aureus</i>	20±0.5	11±0.61	-	20±0.9	10±0.14	-	25±1.3	10±0.5	--
<i>P. aeruginosa</i>	11±0.52	12±0.7	-	11±0.3	10±0.1	-	13±0.5	16±0.4	--
<i>E. coli</i>	13±0.7	12±0.8	-	15±0.5	11±0.1	-	11±0.6	13±0.5	--

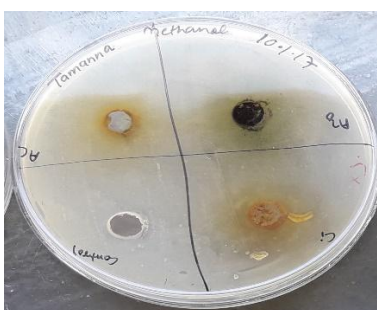
*EA= Ethyl acetate, M=Methanol, B= Benzene

Results are mean of triplicate

Fig.1 Antibacterial activity against *S. aureus* by various extract of plant *C. quadrangularis* (Ci), *A. aspera* (Ac) and *A. pinnata* (Az)



Ci, Ac and Az in ethyl acetate extract



Ci, Ac and Az in methanol extract



Ci, Ac and Az in benzene extract

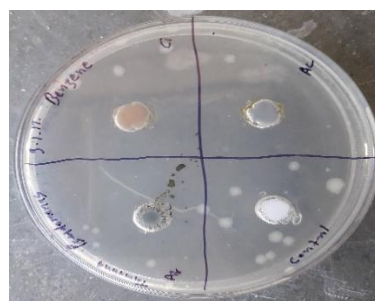
Fig.2 Antibacterial activity against *P. aeruginosa* by various extract of plant *C. quadrangularis* (Ci), *A. aspera* (Ac) and *A. pinnata* (Az)



Ci, Ac and Az in ethyl acetate extract



Ci, Ac and Az in methanol extract



Ci, Ac and Az in benzene extract

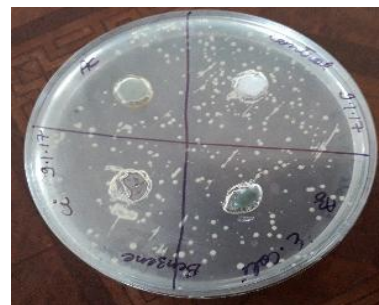
Fig.3 Antibacterial activity against *E. coli* by various extract of plant *C. quadrangularis* (Ci), *A. aspera* (Ac) and *A. pinnata* (Az)



Ci, Ac and Az in ethyl acetate extract



Ci, Ac and Az in methanol extract



Ci, Ac and Az in benzene extract

The minimum antibacterial activity was reported by ethyl acetate extract of *C. quadrangularis* against *E. coli*. In methanol extract of *A. aspera*, *A. pinnata*, and *C. quadrangularis* the zone of inhibition against *E. coli* was 12 ± 0.8 mm, 11 ± 0.1 mm and 13 ± 0.5 mm respectively. The highest zone of inhibition against *E. coli* was showed by methanol extract of *C. quadrangularis* followed by *A. aspera* and *A. pinnata*. In benzene extract of all selected three plants there were little bacterial colonies of *E. coli* was observed.

In conclusion the plants parts antibacterial effectiveness on the tested bacterial isolates resulted within 24h of incubation in all the crude extract screening. From the result obtained it can be concluded that ethyl acetate and methanol extracts of *Cissus* as well as all the plants have a marked antimicrobial activity against all microorganism tested. The ethyl acetate extracts of the plants displayed extensively a competitive inhibitory potency with the more effective methanol and benzene extracts of the plants selected on the tested isolates. The result of this study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel

drugs for the treatment of various diseases and hence can be exploited for future natural plant based antimicrobial agents.

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