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Comparative evaluation of *in vitro* antibacterial activity of several extracts of *Achyranthes aspera, Azolla pinnata, Cissus quadrangularis* and *Tinospora cordifolia*

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Abstract
Plants have provided anti- infective agents in the form of alkaloids, flavonoids, phenols, tannins, steroids, terpenoids, saponins and phytosterol which are highly effective against pathogenic microorganism. In the present study, various extracts namely ethyl acetate, methanol and benzene extracts of selected plant species i.e. *Achyranthes aspera, Azolla pinnata, Cissus quadrangularis* and *Tinospora cordifolia* were studied for its antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* The results showed that the plant extracts were specific in action against the growth of bacteria and showed zone of inhibition, but in benzene extract of selected plants there was not any kind of growth of tested microorganism was observed. The highest growth inhibition was found in the ethyl acetate extract of *T. cordifolia* (30±1.7mm) followed by *C. quadrangularis* (25±1.3mm), *A. aspera* (20±0.5mm) and *A. pinnata* (20±0.9 mm) against *S. aureus* similarly the methanol extract of *T. cordifolia* exhibited (25±0.87mm) more antibacterial activity against *S. aureus* followed by *A. aspera* (11±0.61mm), *C. quadrangularis* (10±0.5mm) and *A. pinnata* (10±0.14mm), whereas methanol and ethyl acetate extract of selected plants also exhibited good antibacterial activity against *P. aeruginosa* and *E. coli.* The results of the study indicates that these plants possess phyto-constituents having antibacterial activity thus can be utilized as a natural plant based antimicrobials.

Keywords: *Achyranthes aspera, Azolla pinnata, Cissus quadrangularis, Tinospora cordifolia,* antibacterial evaluation

1. Introduction
India has its long tradition and history of health care through herbal drugs and even today more than 76% of the total population is dependent on plants for their health care needs. India has 15 agro-climatic regions in which 2500 medicinal plants are dispersed across all biogeographic areas, serve as a regular source of medicine. The government of India has notified Good Manufacturing Practices (GMPs) under Drugs and Cosmetics Act, 1940. World Health Organization (WHO) has estimated that approximately 80% of the world population still relies on traditional medicines, which are mostly plant-based drugs. Leaves, flowers, stem, roots, seeds, fruit, bark and the whole plant can also be constituents of herbal medicines.
In India, the major cause of mortality, morbidity and economic loss is caused by an increased frequency of life threatening infectious diseases. The causative pathogenic organism develops resistant to many antibiotics because of indiscriminate use of antimicrobial drugs. The drugs which are already in use to treat infectious diseases are of concern because, drug safety remains an enormous global issue. There is a need to search and design new alternative drugs. Plant-based medicinal agents offer an alternative approach. Drugs from natural medicinal plants products have important chemical compounds with pharmacological and toxicological value may use as a substitute to control microbial infections. These herbal and natural products used in medicine have relatively lower incidences of adverse reactions as compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair *et al*., 2005) [1]. Plants have provided anti- infective agents in the form of alkaloids, flavonoids, phenols, tannins, steroids, terpenoids, saponins and phytosterol which are highly effective against pathogenic microbial. Therefore, researchers are more and more turning their attention to plant-based medicinal agents, looking for fresh leads

to develop better drugs against microbial infections. In the present study four plant species namely *A. aspera, A. pinnata, C. quadrangularis*, *T. cordifolia* are used.

A. 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. *A. pinnata* is a pteridophyte plant rich in protein used in mosquito and weed control and traditional medicine. Plant abundant in secondary metabolites such as flavonoids, tannins, terpenoids and alkaloids have significant biological activity against pathogens. *C. quadrangularis* 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. *T. cordifolia* is known by the common names heart-leaved moonseed, guduchi and giloy, is an herbaceous vine indigenous to the tropical areas of India, Myanmar and Sri Lanka. The chemical constituents show the presence of tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids which are used in medicinal purpose.

Bearing in mind the infectious diseases, resistant pathogenic organism and side effect of antibiotics an attempt was needed in order 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 four plants *A. aspera, A. pinnata, C. quadrangularis and T. cordifolia* on growth of *S. aureus, P. aeruginosa* and *E. coli.*

2. Material Method

2.1 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 *C. quadrangularis* (stem) was collected from various parks of Bikaner where it was cultivated as an ornamental plant whereas seed samples of *A. aspera* (seeds) and *T. cordifolia* (stem) were purchased from the shop of herbal medicine and were identified by a well-known taxonomist of Bikaner. The fresh sample of *Cissus* and *Tinospora* stem and seeds of *Achyranthes* was dried separately, ground and used for further analysis.

2.2 Preparation of Extracts by Solvent extraction

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

2.3 Screening of Plant Extracts for Antibacterial Activity

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

2.4 Test Organisms

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

a) 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.

b) 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 100ml distilled water and autoclaved at 121 °C for 15min.

c) 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 10ml to 15ml 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 10min. 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 24h. At the end of incubation, inhibition zones formed around the wells were measured with a transparent ruler in millimeter.

3. Result and Discussion

In the present investigation, *in vitro* antibacterial activity of the crude extracts of four plants were 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 four plants *A. aspera, A. pinnata, C. quadrangularis* and *T. cordifolia* (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) were negligible.

Against *S. aureus* in the zone of inhibition was 20±0.5mm, 20±0.9mm, 25±1.3mm and 30±1.7mm by ethyl acetate extract of *A. aspera, A. pinnata, C. quadrangularis* and *T. cordifolia* respectively. On comparison of mean values the *T. cordifolia* exhibited maximum antibacterial activity in ethyl acetate extract. Similarly, ethyl acetate extract of *C. quadrangularis* showed more inhibitory zone then *A. aspera* and *A. pinnata*. In methanol extract of *A. aspera, A. pinnata, C. quadrangularis* and *T. cordifolia* the zone of inhibition against *S. aureus* was 11±0.61mm, 10±0.14mm, 10±0.5mm and 25±0.87mm respectively. On comparison of mean values the methanol extract of *T. cordifolia* exhibited maximum antibacterial activity followed by methanol extract of *A. aspera* and *A. pinnata.* The minimum zone of inhibition was observed in methanol extract of *C. quadrangularis.* In benzene extract of selected four plants diminutive growth of S. *aureus* was observed.

The zone of inhibition against *P. aeruginosa* was 11±0.52mm, 11±0.3mm, 13±0.5mm and 18±0.7mm in ethyl acetate extract of *A. aspera, A. pinnata, C. quadrangularis* and *T. cordifolia* respectively. On comparison of mean value it was observed that the highest antibacterial activity was showed by ethyl acetate extract of *T. cordifolia* followed by *C. quadrangularis.* The antibacterial activity against *P. aeruginosa* by ethyl acetate extract of *A. aspera* and *A. pinnata* was comparable with each other. In methanol extract of *A. aspera, A. pinnata, C. quadrangularis* and *T. cordifolia* the zone of inhibition against *P. aeruginosa* was 12±0.7mm, 10±0.1mm, 16±0.4mm and 10±0.2mm respectively. *P.*

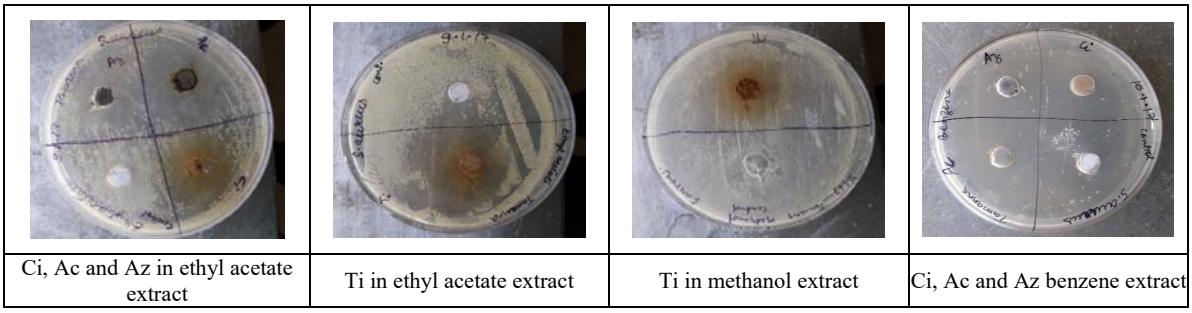
aeruginosa was most sensitive for the methanol extract of *C. quadrangularis* followed by methanol extract of *A. aspera*. The antibacterial activity against *P. aeruginosa* by methanol extract of *A. pinnata* and *T. cordifolia* was comparable with each other. A little growth of *P. aeruginosa* was reported in benzene extract of all selected four plant.

Against *E. coli* the zone of inhibition was 13±0.7mm, 15±0.5mm, 11±0.6mm and 14±0.7mm by ethyl acetate extract of *A. aspera, A. pinnata, C. quadrangularis* and *T. cordifolia* respectively. *E. coli* was more sensitive to the ethyl acetate extract of *A. pinnata* followed by *T. cordifolia, A. aspera* and *C. quadrangularis.* The minimum antibacterial activity was reported by ethyl acetate extract of *C. quadrangularis* against *E. coli*. In methanol extract of *A. aspera, A. pinnata, C. quadrangularis* and *T. cordifolia* the zone of inhibition against *E. coli* was 12±0.8mm, 11±0.1mm, 13±0.5mm and 11±0.7mm respectively. The highest zone of inhibition against *E. coli* was showed by methanol extract of *C. quadrangularis* followed by *A. aspera, A. pinnata* and *T. cordifolia.* In benzene extract of all selected four plants there were little bacterial colonies of *E. coli* was observed.

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

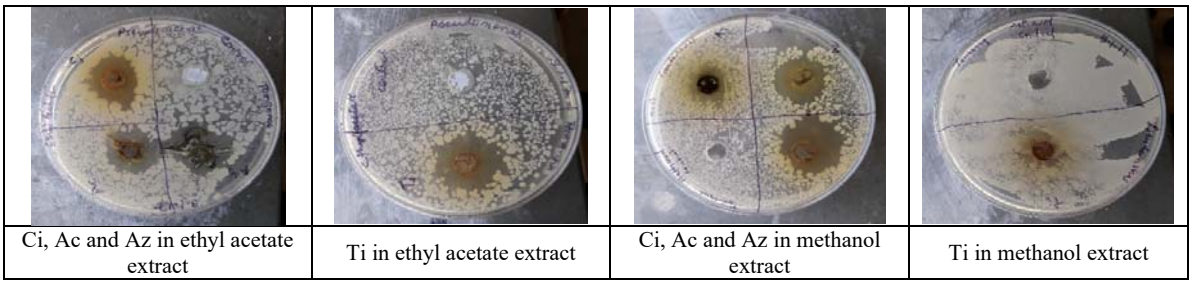
Bacterial organism	*A. aspera*			*A. pinnata*			*C. quadrangularis*			*T. cordifolia*		
	EA	**M**	**B**	**EA**	**M**	**B**	**EA**	**M**	**B**	**EA**	**M**	**B**
S. aureus	20±0.5	11±0.61	-	20±0.9	10±0.14	-	25±1.3	10±0.5	--	30±1.7	25±0.87	-
P. aeruginosa	11±0.52	12±0.7	-	11±0.3	10±0.1	-	13±0.5	16±0.4	--	18±0.7	10±0.2	-
E. coli	13±0.7	12±0.8	-	15±0.5	11±0.1	-	11±0.6	13±0.5	--	14±0.7	11±0.7	-

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



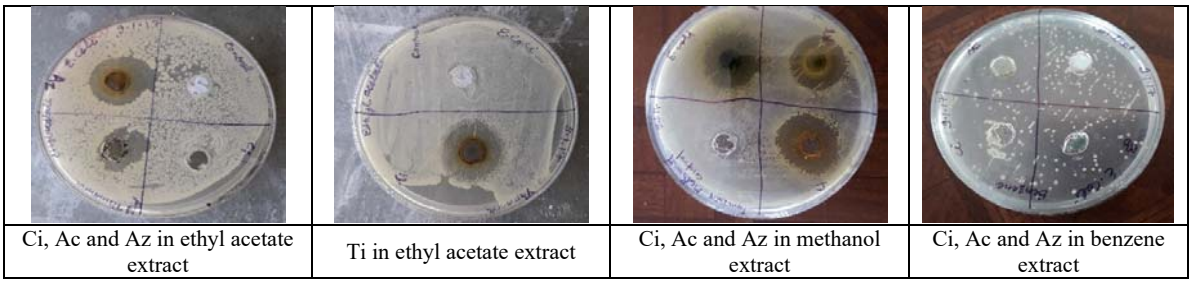
Ci, Ac and Az in ethyl acetate extract	Ti in ethyl acetate extract	Ti in methanol extract	Ci, Ac and Az benzene extract

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



Ci, Ac and Az in ethyl acetate extract	Ti in ethyl acetate extract	Ci, Ac and Az in methanol extract	Ti in methanol extract

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



Ci, Ac and Az in ethyl acetate extract	Ti 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), *A. Pinnata* (Az) and *T. cordifolia* (Ti)

4. Conclusion

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 all plant have a marked antibacterial activity against all the 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.

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