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RESEARCH PAPER

An *in vitro* evaluation of antibacterial activity of various extracts of *Achyranthes aspera* and *Cissus quadrangularis*

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Abstract : The antibacterial activity of aqueous and methanol extracts of *Achyranthes aspera* and *Cissus quadrangularis* was evaluated. *A. aspera* and *C. quadrangularis* plants collected from its natural habitat in and around Tirunelveli, Tamil Nadu, India were shadow dried at room temperature and pulverized into coarse powder. Aqueous extracts were prepared by extracting with distilled water at 100°C for 4h and methanol extracts were prepared by maceration process using methanol for 72h. The aqueous extract yield was 12.1% and 23.5% for *A. aspera* and *C. quadrangularis* respectively. Whereas, methanol extract yield was 6% and 4% for *A. aspera* and *C. quadrangularis,* respectively. The antibacterial activity of plant extracts against *Staphylococcus aureus* (MTCC-96) and *Escherichia coli* (MTCC-443) was evaluated by Agar well diffusion method. The diameters of zone of inhibition ranged from 8mm to 17mm with highest inhibition zone observed against *S. aureus* by methanol extract of *A. aspera* (17mm) followed by aqueous extract of *C. quadrangularis* (13mm) and methanol extract of *C. quadrangularis* (9mm). Whereas, zone of inhibition (8mm) against *Escherichia coli* was observed only with methanol extract of *C. quadrangularis*. The results necessitate further study to isolate the active principles from the plant extracts to exploit the potential antibacterial activity and to find novel pharmacological uses of these plants beyond their racial use.

Key Words : *Achyranthes aspera*, *Cissus quadrangularis*, Aqueous extract, Methanol extract, Antibacterial activity, ABST

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INTRODUCTION

Antimicrobial resistance is a global problem and has become matter of prime concern by World Health Organization. The situation is more alarming in countries like India where population of man, livestock and poultry has been growing rapidly and antimicrobials are misused to a great extent in an injudicious and unscientific manner. Since there is a serious threat of antimicrobial resistance to public health perspective, it requires action across all government sectors and society. A viable solution for replacing AMR is to utilize herbal antimicrobial agents.

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, pharmaceutical intermediates, nutraceuticals, food supplements, folk medicines and chemical entities for synthetic drugs. The medicinal value of these plants lies in some chemical substances that produce definite

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physiological actions on the human and animal body and these chemical substances are called phytochemicals (Ncube *et al*., 2008). Perhaps one of the greatest arguments against traditional medicine today is the lack of scientific proof of its efficacy. Most of the claims are made by traditional medicinal practitioner themselves and may have not been thoroughly investigated scientifically, for this reasons, therefore, it could be suggested that further investigation into this medicinal plant is needed (Sofowora, 1993).

Achyranthes aspera is a medicinal plant, used in traditional medicinal systems in India as anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases and in the treatment of irregular menstruation, fever, dysentery and asthma (Ved and Goraya, 2007; Upadhya *et al*., 2009 and Tandon, 2011). *Cissus quadrangularis* is an indigenous medicinal plant of India traditionally used as an anthelmintic, antidyspeptic, digestive tonic, analgesic and treatment for scurvy and asthma (Nadakarni, 1954; Chopra *et al*., 1954 and Singh *et al*., 1984). Western Ghats in southern region of Tamil Nadu is rich in medicinal plants and traditional healers possess rich knowledge about the medicinal plants available in this region. *Achyranthes aspera* and *Cissus quadrangularis* plants are used by the traditional healers for common ailments in animals. However, scanty information is available about antimicrobial activity of *Achyranthes aspera* and *Cissus quadrangularis* plants with specific reference to this region. Hence, the present study is taken upto evaluate antibacterial activity of various extracts of *Achyranthes aspera* and *Cissus quadrangularis*.

MATERIAL AND METHODS

Preparation of plant material :

Achyranthes aspera and *Cissus quadrangularis* plants (stem and leaves) were collected from its natural habitat in and around Tirunelveli, Tamil Nadu and were authenticated by Department of Botany, Rani Anna Government College for Women, Tirunelveli. The plants were shadow dried at room temperature and coarsely powdered using a mechanical grinder (Fig. A to F).

Preparation of plant extracts :

Aqueous extracts of *Achyranthes aspera* and *Cissus quadrangularis* were prepared as per the procedures of Uma *et al*. (2012). The powdered plant materials were extracted with distilled water at 100°C for 4h, centrifuged at 5,000g for 15min, and filtered using Whatman No.1 filter paper. The filtrate was vacuum evaporated and the yield was 12.1% and 23.5% for *A. aspera* and *C. quadrangularis,* respectively.

Methanol extracts of both the plants were prepared by following the methods described by Gawande and Goel (2015). The powdered plant materials were subjected to simple maceration process by using methanol for 72h with orbital shaking at room temperature. The extracts were filtered through Whatman No.1 filter paper and the solvents were evaporated with the help of the rotary evaporator under reduced pressure at 45°C. The concentrated extracts were kept at 4°C in a refrigerator until further use. The percentage yield of *A. aspera* and *C. quadrangularis* methanol extract was 6% and 4%, respectively.

Finally, the extracted substances were resuspended in the respective solvents at a concentration of 100 mg/ml prior they were tested for the antibacterial activity.

Test organisms :

Test organisms *Staphylococcus aureus* (MTCC-96) and *Escherichia coli* (MTCC-443) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh-160036, India and used for the study. Stock culture of *Staphylococcus aureus* and *Escherichia coli* were prepared in sterile nutrient broth and maintained at 4°C. Active cultures for experiments were prepared by transferring a loop full of cultures from the stock to test tubes containing sterile nutrient broth for bacteria and were incubated for 24h at 37°C.

Evaluation of antibacterial activity :

Agar well diffusion method was used to evaluate the antibacterial activity of plant extracts at higher concentration as described by Chidambara Murthy *et al*. (2003). The sterile Muller Hinton agar plate was inoculated by spreading a standard inoculum of $1.5x10^8$ cfu/ml which matched 0.5 McFarland standard over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer, and the wells were loaded with 50µL of extract solution at the concentration of 100mg/mL. The same quantity of respective solvents (methanol and distilled water) alone served as a negative control. Filter paper discs loaded with antibiotic (Doxycycline and Ceftriaxone) were used as positive control (standard) for comparative

Fig. A: ***Achyranthes aspera***

Fig. D: ***Cissus quadrangularis*** **– dried plant**

Fig. B: ***Achyranthes aspera*** **– dried plant**

Fig. E: ***Achyranthes aspera*** **– plant powder**

Fig. C : ***Cissus quadrangularis***

Fig. F: ***Cissus quadrangularis*** **– plant powder**

purpose. Then, agar plates were incubated at 37°C for 18h and the zone of inhibition around the wells was measured from the edge of the well to the inner margin of the surrounding bacterial growth.

Agar disc diffusion method was used to evaluate the antibacterial activity of plant extracts at lower concentration as described by Pandey *et al*. (2013) and Mostafa *et al*. (2018). The sterile discs were soaked separately with 10µL of each of the extract prepared in water and methanol solvents at different appropriate concentrations so as to get the concentration of 3000µg, 2000µg, 1000µg, 500µg, 250µg, 100µg per disc and then dried at 37^{0}C in an incubator. These discs were placed on Mueller-Hinton agar plates, previously swabbed with the reference bacterial organisms *Staphylococcus aureus* (MTCC-96) and *Escherichia coli* (MTCC-443)

of $1.5x10^8$ cfu/ml which matched 0.5 McFarland standard. In one disc, the respective solvent (methanol and distilled water) was added as negative control to determine possible inhibitory activity of the solvent. Filter paper discs loaded with antibiotic (Doxycycline) was used as positive control (standard) for comparative purpose. This preparation was incubated for 18h at 37°C and the zone of inhibition around the disc was measured.

RESULTS AND DISCUSSION

The results of evaluation of antibacterial activity of both aqueous and methanol extracts of *Achyranthes aspera* and *Cissus quadrangularis* by agar well diffusion method were shown in the Table 1 and Fig. 1 to 3. The diameters of zone of growth inhibition ranged from 10mm to 19mm with highest inhibition zone values observed against *Staphylococcus aureus* (19mm) by methanol extract of *A. aspera* followed by aqueous extract of *C. quadrangularis* (15mm), methanol extract of *C. quadrangularis* (11mm). Whereas, zone of inhibition (10mm) against *Escherichia coli* was observed only with methanol extract of *C. quadrangularis*.

The evaluation of antimicrobial activity of both plant extracts at lower concentrations (3000µg, 2000µg, 1000µg, 500µg, 250µg, 100µg per disc) by Agar disc-diffusion method revealed no zone of inhibition.

The results revealed that methanol extract of *A. aspera* exhibited significant antibacterial activity against *Staphylococcus aureus* at the concentration of 100mg/mL (5mg per well), which is comparable with that of antibacterial activity of positive control Ceftriaxone. These findings are in accordance with Ramesh *et al*. (2011) who also demonstrated that methanol extracts of leaves of *Achyranthes aspera* obtained by infusion, which has showed a strong inhibitory activity against the Gram-positive bacteria *Staphlyococcus aureus*. But, in the present study the methanol extract did not show any antibacterial activity against *Escherichia coli*. Further, aqueous extract did not exhibit antibacterial activity against both the test organisms *Staphylococcus aureus* and *Escherichia coli.* Pandey *et al*. (2013) also reported that different organic solvent extracts (Petroleum ether, methanol, ethanol, ethyl acetate and chloroform) of *A. aspera* demonstrated significant antibacterial activity against *Pseudomonas aeruginosa, Proteus mirabilis* and *Enterococcus faecalis*.

In the present study, Gram-negative bacteria *Escherichia coli* exhibited resistance to both the plant extracts except methanol extract of *Cissus quadrangularis* which demonstrated antibacterial activity against *Escherichia coli.* In contrast to this, Chidambara Murthy *et al*. (2003) reported that ethyl acetate extract and methanol extract of both fresh and dry stems of *Cissus quadrangularis* exhibited antimicrobial activity against Gram-positive bacteria, including *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Streptococcus species*, but, no activity against *Escherichia coli.* However, Srivastava *et al*. (2013) reported that both ethanol and chloroform extracts of *Cissus quadrangularis* exhibited antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

The investigation of antibacterial activity of *Achyranthes aspera* and *Cissus quadrangularis* revealed that methanol extracts of both the plants and aqueous extract of *Cissus quadrangularis* exhibited antibacterial activity. The outcome of the study encourages taking up further study to isolate and characterize the active principles from the *Achyranthes aspera* and *Cissus quadrangularis* plant extracts to exploit the potential antibacterial activity and to find novel pharmacological uses of these plants beyond their racial use.

Table 1 : Antibacterial activity of aqueous and methanol extracts of *Achyranthes aspera* and *Cissus quadrangularis* by Agar well diffusion method

Test organism	Zone of inhibition (mm)							
	Achyranthes aspera		*Cissus quadrangularis*		Positive control		Negative control	
	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	Doxycycline	Ceftriaxone	Methanol	Water
Staphylococcus aureus (MTCC-96)	19	Nil	11	15	26	21	2	Nil
Escherichia coli (MTCC-443)	Nil	Nil	10	Nil	17	28	2	Nil

Antibacterial activity of *Achyranthes aspera* (Aa) and *Cissus quadrangularis* (Cq) @ the concentration of 100mg/mL (5mg per well)

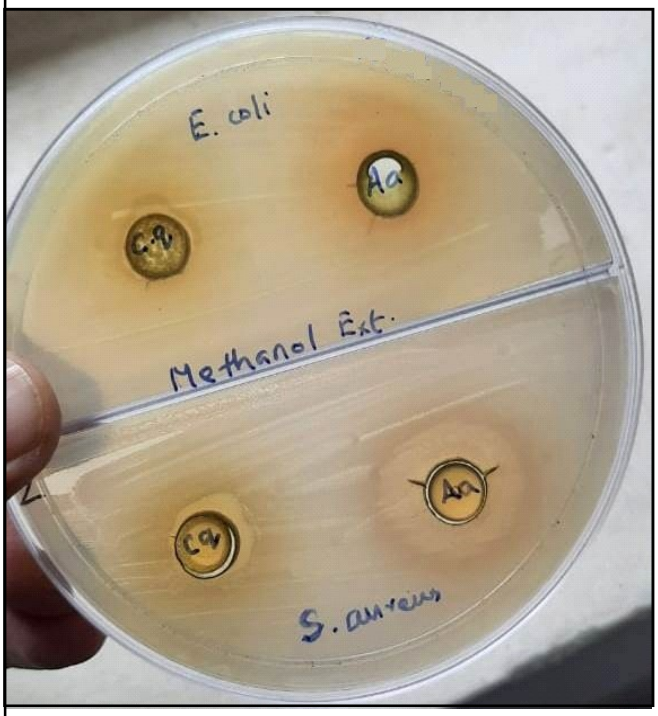

Fig. 1: Methanol extract against *S. aureus* and *Escherichia coli*

Fig. 2 : Aqueous extract against *S. aureus* and *Escherichia coli*

Fig.3: ControlPositive control: Doxycycline (DC) and Ceftriaxone(CTR)Negative control: Distilled water (DW) and Methanol (M)

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