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IN VITRO ANTIBACTERIAL ACTIVITY OF *AMARANTHUS SPINOSUS* ROOT EXTRACTS

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ABSTRACT

Ethanol and aqueous extracts of *Amaranthus spinosus* (roots) were investigated for their antibacterial activity against ten bacterial strains including Gram-positive and Gram-negative bacteria using the agar-well diffusion method. The 2 extracts tested, the ethanol extract presented the best results while the aqueous extract showed moderate inhibition of the microbial growth. Each extract is unique against different microorganisms. It is concluded that the plants studied may be a source of antibacterial agents.

Keywords: *Amaranthus spinosus*, Antibacterial activity, Agar well diffusion method.

INTRODUCTION

Amaranthus spinosus is an annual weed that is widely distributed in the humid zone of the tropics. It is used in tropical and subtropical countries for human nutrition both as vegetables and grains and also as animal feed (Berghofer and Schoenlechner, 2002; Miralles *et al.*, 1988). The weed has been reported to have some pharmacological properties (Ayethan *et al.*, 1996). Extracts of the leaf had also been used in the treatment of menstrual disorders in women (Ayethan *et al.*, 1996). The plant is used as a sudorific and febrifuge and is recommended for eruptive fevers. The leaves are considered a good emollient, lactogogue and a specific treatment for colic (Ayethan *et al.*, 1996). Externally, the bruised leaves are applied locally to treat eczema. Furthermore, it is used to treat several ailments such as malaria, hepatic disorders, jaundice, and scanty urine and to cure wounds (Berghofer and Schoenlechner, 2002; Samy *et al.*, 1999; Srivastava *et al.*, 1998). The leaves and roots are applied as poultice to relief bruises, abscesses, burns, menorrhagia, gonorrhoea and inflammatory swelling. *Amaranthus spinosus* is also used as antiinflammatory, antimalarial, antibacterial, antidiuretic and antiviral agents. The plant has several active constituents like alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, betalains, B-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins and carotenoids. It also contains amaranthoside, a lignin glycoside, amaricin, a coumaroyl adenosine along with stigmasterol glycoside, betaine such as glycinebetaine and trigonelline (Azhar-ul-Haq *et al.*, 2006; Blunden *et al.*, 1999).

Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result some natural products have been approved as new

antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance (Cragg et al., 1997; Recio, 1989). *A. spinosus* plant contains lots of substances having medicinal value, which are yet to be explored. The in vitro antibacterial study was designed to investigate the antibacterial spectrum of the crude extracts by observing the growth response. The rationale for this experiment is based on the fact that bacteria are responsible for many infectious diseases, and if the test materials inhibit bacterial growth then they may be used in those particular diseases.

MATERIALS AND METHODS

Plant Materials

Fresh material of plant in the flowering stage was collected in Bangalore in May 2011. The taxonomic identification of the plant was confirmed by Dr. S. Sundara Rajan, Center for Vrikshayurveda a division of Center for Advance Studies in Biosciences (Voucher specimen number AS-202).

Extraction

Freshly collected roots of *Amaranthus spinosus* were shade-dried and then powdered using a mechanical grinder. One hundred gram of pulverized plant material were taken in five hundred capacity thimble of Soxhlet apparatus and refluxed with ethanol and water separately until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a faint colour. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland). A portion of the residue was used for the antibacterial assay.

Bacterial Culture

The bacterial strains used in this study were clinical isolates. The isolates were identified by a standard method (Cowan and Steel, 1993). The organisms were maintained on nutrient agar slope at 4 ^{o}C and sub-cultured into nutrient broth by a picking-off technique (Aneja, 2003) for 24 hrs before use.

Bacterial Susceptibility Testing

In vitro antibacterial activity of the crude extracts was studied against Gram-negative and Gram-positive bacteria by the agar well diffusion method (Nair *et al.*, 2005). Nutrient agar (Hi Media, India) was used as the bacteriological medium. The extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100 mg/ml. Pure DMSO was taken as a negative control and 50 mg/ml ciprofloxacin as the positive control. 100 µl of inoculum was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculum. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter. 100 µl of test and control compound was introduced in the well. The same procedure was used for all the strains. The plates were incubated aerobically at 35 ^{o}C and examined after 24 hours (Ali-Shtayeh *et al.*, 1998; Collins, 1998). The diameter of the zone of inhibition produced by each agent were measured with a ruler and compared with those produced by the commercial antibiotic ciprofloxacin.

RESULTS AND DISCUSSION

In the present study *A. spinosus* root, extracted in ethanol and aqueous were investigated for their antibacterial potentiality against 10 clinically important bacterial strains. The two extracts showed varying results against the bacterial strains. Extracts was active against Gram-positive bacteria, whereas it is moderate against Gram-negative organisms. The aqueous extract were inactive against two bacteria (*Serratia marcescens* and *Proteus mirabilis*) studied. Details of the result are shown in table1.

The extracts were most active against the Gram-positive bacteria in comparison to Gram-negative bacteria, tested at the same

concentration. This observation supports the earlier reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria (Rabe and Van Staden, 1997; Vlietinck *et al.*, 1995).

It is therefore theorized that Gram-positive bacteria are more susceptible than Gram-negative bacteria due to the differences in their cell wall structure. Gram-negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics (Tortora *et al.*, 2001). However, the results from this study reveals that the crude extract of *Amaranthus spinosus* contain certain constituents with significant antibacterial property.

From our investigation, the results obtained confirm the therapeutic potency of *Amaranthus spinosus* used in traditional medicine. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation. The results of the present study supports the folkloric usage of the studied plant and suggests that the plant extract possess certain constituents with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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Table 1: Antibacterial activity of *Amaranthus spinosus* root extract against bacterial strains

	Pseudomonas aeruginosa	*Klebsiella pneumonia*	*Salmonella typhi*	*Escherichia coli*	*Serratia marcescens*	*Proteus mirabilis*	*Staphylococcus aureus*	*Staphylococcus citreus*	*Bacillus polymyxa*	*Bacillus cereus*
ETHANOL EXTRACT	9.33±0.67	7.53±0.24	10.00±0.58	10.67±0.88	6.40±0.12	5.40±0.64	11.13±0.24	12.07±0.29	13.67±0.33	12.27±0.18
AQUEOUS EXTRACT	5.73±0.87	5.33±0.33	6.23±0.96	8.67±0.67	0.00±0.00	0.00±0.00	9.87±0.47	10.80±0.12	11.60±0.35	10.93±0.41
STANDARD	14.67±0.67	12.60±0.12	15.33±0.67	18.20±0.12	10.20±0.20	8.20±0.23	24.00±0.12	25.53±0.29	28.27±0.37	27.00±0.58