

ANTIBACTERIAL ACTIVITY OF *AMARANTHUS HYBRIDUS* LINN. ROOT EXTRACTS.

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ABSTRACT: The *in-vitro* antibacterial potential of *Amaranthus hybridus* Linn. root extracts in petroleum ether, ethylacetate, ethanol and water respectively was screened against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The ethyl acetate root extract showed highest antibacterial activity against test strains *Bacillus subtilis* and *Staphylococcus aureus* while alcoholic extract showed more inhibition against *Escherichia coli*.

Key words: antibacterial activity, *A. hybridus* Linn., human pathogenic microorganism

Plant-based medicaments have been man's prime therapeutic weapons to rescue him from the clutches of diseases (Rahman, et. al., 2001). Virtually all cultures worldwide have been relied historically, or continue to rely on medicinal plants for primary health care (Mantle, et. al., 2001). In recent years, multiple drug resistance in human pathogenic microorganism has been develops due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases (Mohanasundari et. al., 2007). To overcome the growing problem of antibiotic resistance plant based antibiotics are of great interest. There are reports about antimicrobial peptides isolated from amaranth seeds (Rivillas-Acevedo et. al., 2007, Broekaert et. al., 1992) and leaves (Cyrus et. al., 2008). But no study about the antibacterial activity of roots of *Amaranthus hybridus* Linn. (Amaranthaceae) of Indian origin. The present study aimed at evaluating the *in-vitro* antimicrobial activity of petroleum ether, ethyl acetate, ethanol and aqueous root extract of *Amaranthus hybridus* Linn. against some Gram-positive and Gram-negative bacterial strains. The roots were collected from the campus of Guru Jambheshwar University of Science and Technology, Hisar, Haryana in July 2008. Identify and authentication of plant was carried out at National Institute of Science Communication and Information Resources, New Delhi by Dr. H. B. Singh where a voucher specimen was lodged vide reference number, NISCAIR/RHMD/CONSULT/2008-09/1044/75.

For the preparation of extracts roots were shade dried for 15 days and grounded into powder by hammer mill. The selected plant part (300 gm) was successively extracted with petroleum ether, ethyl acetate, ethanol and distilled water using Soxhlet apparatus for 16 hours. The respective solvents were evaporated and concentrated. The dried weight of the each extract was used to determine the concentration in mg/ml. Extracts were stored in refrigerator and were suspended in DMSO (dimethyl sulfoxide) prior to use. Three pathogenic bacterial strains used in this study were *Escherichia coli* (NCIM 2065), *Bacillus subtilis* (NCIM 2901) and *Staphylococcus aureus* (NCIM 2106). The test cultures were obtained from National Chemical Laboratory, Pune, India. The stock bacterial cultures were maintained in nutrient agar slants at 4°C. Each of the microorganism was freshly cultured prior to susceptibility testing by transferring them into a separate sterile test tube containing nutrient broth and incubated overnight at 37°C. A microbial loop was used to remove a colony of each bacterium from pure culture and transferred into nutrient broth.

The agar-well diffusion method was used for the antibacterial study (Odunbaku et. al., 2008). The petriplates containing 25 ml of sterile nutrient agar were inoculated with standardized inocula (0.1×10^8 cell/ml) using sterile Pasteur pipette. Wells of 8 mm diameter were made at the centre of each plate and 0.2 ml of various root extract of concentration 1 mg/ml were dispensed aseptically into each well. The extracts were allowed to diffuse into medium for 1 hour at room temperature. This was then incubated for 24 hours at 37°C. After which the zones of growth inhibition were measured and recorded in millimeter.

The negative and positive control was set up in a similar manner except that the extract was replaced with sterile DMSO and commercial antibiotics (Streptomycin and Oxytetracycline) respectively (Table 1). The experiment was repeated thrice and the average values were recorded.

Table: 1 Antibacterial activity of *Amaranthus hybridus* Linn. Root extracts on different bacterial strains.

S.No.	Name of Organism	Diameter of zone of inhibition (mm)						
		Petroleum ether extract (1mg/ml)	Ethyl Acetate Extract (1mg/ml)	Ethanol Extract (1mg/ml)	Aqueous Extract (1mg/ml)	Streptomycin (1mcg/ml)	Oxytetracycline (2.5 mcg/ml)	DMSO
1.	<i>Staphylococcus aureus</i>	18	22	20	16	32	25	-
2.	<i>Bacillus subtilis</i>	19	24	22	12	28	28	-
3.	<i>Escherichia coli</i>	16	14	21	18	32	26	-

There is dependence on traditional medicine for a variety of ailments in a large part of the world population, especially in developing countries; the use of higher plants and preparations made from them to treat infections is a longstanding practice (Ahmad et. al., 1998). However, many species of plants containing substances of medicinal value have yet to be discovered (Nair et. al., 2005). The present study reveals the antibacterial potential of various extracts of root of *A. hybridus* Linn. All the extracts have shown inhibitory effect against the bacterial test strains (Table 1). The ethyl acetate extract possesses highest activity against *S. aureus* (22mm) and *B. subtilis* (24mm) and ethanolic extract showed more effectiveness against *E. coli* (21mm) as compared to other extracts. Furthermore, Gram-positive bacteria were found to have more susceptibility for organic solvent extracts as compared to Gram-negative bacterial species. Phytochemical elucidation of antibacterial principles especially the ethyl acetate fraction should be undertaken with the objective to isolate the active biochemical principles and develop novel antibacterial agents.

ACKNOWLEDGMENTS

Authors are thankful to Guru Jambheshwar University of Science and Technology, Hisar, Haryana for providing laboratory facilities. Thanks are also due to National Chemical Laboratory, Pune, India for supplying bacterial cultures.

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