

ANTIBACTERIAL ACTIVITY OF THE MEDICINAL PLANT, *ACALYPHA FRUTICOSA* FORSSK.**Thambiraj, J.***

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ABSTRACT

The present study is to confirm the antibacterial efficacy of root extracts of the folklore medicinal plant species, *Acalypha fruticosa* by using three alcoholic solvents viz; petroleum ether, ethyl acetate and methanol were tested against ten human pathogenic bacteria viz., *Pseudomonas aeruginosa*, *P. stutzeri*, *Escherichia coli*, *Micrococcus* sp., *Lactobacillus* sp., *Serratia* sp., *Moraxella* sp., *Bacillus subtilis*, *B. thuriengensis*, and *Klebsiella pneumoniae* for assessing the antibacterial properties by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antibacterial activity against the tested pathogens. However, the methanol extract exhibited higher inhibition zone (21.83 mm) against the bacterium, *Bacillus subtilis*. This result supports the therapeutic importance of the species, *Acalypha fruticosa* in curing infectious diseases and encourages the extensive use of this species in health care practices.

Keywords: Folklore Medicinal plant, *Acalypha fruticosa*, Antibacterial activity, Microorganisms.

1. INTRODUCTION

Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds (Hancock *et al.*, 2012). The necessity to develop new drugs requires varied strategies, among them, the bioprospection of secondary metabolites produced by medicinal plants (Dionisi *et al.*, 2012). Plants have been an essential part of human society since the start of civilization. In Ayurvedic Medicine, there are numerous herbs which have been used historically for treating a large variety of ailments. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many herbs on earth which lies unexplored in the field of medicine or Science. Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal powers (Zaika, 1975). However several plants are used in India in the form of crude extracts. Infusions or plaster to treat common infections without scientific evidence of efficacy (Ahmad *et al.*, 1998). *Acalypha fruticosa* Forssk. belongs to the family, Euphorbiaceae is one such folklore plant used in traditional system of medicine in Coimbatore district of Tamil Nadu, India. It is Gregarious bushy shrub, occurring in the tropical regions of India (Matthew, 1995). This plant species has been used as a folk remedy for the

treatment of digestive troubles such as dyspepsia, colic and diarrhea and even to treat cholera. The root is used for gonorrhoea. Leaves and roots are used in siddha system of medicine for the treatment of skin diseases (Pullaiah, 2006). The plant is also used to cure cough, cold and headache. The boiled roots are used to cure cerebral malaria (Sahoo, 2001). The crushed leaves are used to relieve fever for children. A root decoction is drunk to treat convulsions, fever, colds and swellings of the scrotum and to treat whooping cough. Root decoction is taken to snake bites, fever and ulcer of venereal origin. However, no published works are available for the antimicrobial property of root part of this plant. Hence in the present study, an attempt has been made to focus the plant in this angle and hence to assess its therapeutic potency.

2. MATERIALS AND METHODS**2.1. Plant material**

Fresh root parts were collected from the population of *A. fruticosa* present in the Maruthamalai Hills of Coimbatore District and washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

2.2. Preparation of extracts

250g air-dried root powder was subjected to 250ml of methanol in soxhlet extraction for 8 hours (50-85°C). The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature (50-60°C) to yield crude residue, which was then stored in refrigerator. To obtain petroleum ether and ethyl

acetate extracts, the same method as used to obtain methanol extract was adopted.

2.3. Media used

Freshly prepared nutrient agar medium was used for the culture of bacteria.

2.4. Microorganisms

In vitro antibacterial activity was examined for the chemical extracts of root of the study plant, against ten bacterial species which include the gram positive strains viz., *Micrococcus* sp., *Lactobacillus* sp., *Bacillus subtilis*, *B. thuringiensis* and gram negative strains viz., *Pseudomonas aeruginosa*, *P. stutzeri*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia* sp. and *Moraxetta* sp. All these microorganisms were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. All the microorganisms were maintained at 4°C on nutrient agar slants until further use.

2.5. Antibacterial assay

The alcoholic extracts were tested for their effect against the growth of pathogenic bacteria by disc diffusion method (Bauer *et al.*, 1966). The microorganisms, bacteria tested was inoculated into

nutrient agar medium. After an incubation period of 24 hrs at a temperature of 35°C, three or four colonies isolated from this medium were inoculated into 4ml of nutrient broth and incubated for 2 hrs at 35°C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller- Hinton agar medium was streaked with these bacterial suspensions. Disks of 6mm diameter were impregnated with the extracts of petroleum ether, methanol and ethyl acetate separately. Tetracycline is used as positive control. After equilibrium at 4°C, the plates were incubated overnight at 37°C and the diameter of any resulting zones of inhibition was measured. Each experiment was repeated at least three times.

3. RESULTS AND DISCUSSION

The antibacterial activity of the all the alcoholic root extracts of the study species, *Acalypha fruticosa* generally showed inhibitory activity against the growth of *Bacillus subtilis*, *B. thuringiensis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. However, towards *Micrococcus* sp., *Lactobacillus* sp., *Escherichia coli*, *Pseudomonas stutzeri*, *Moraxetta* sp. and *Serratia* sp., and all these extracts showed activity with less pronounced manner (Table 1).

Table 1. Antibacterial activity of certain alcoholic root extracts of the species, *Acalypha fruticosa*.

Plant extract	Diameter of zone inhibition (mm)									
	Gram positive bacteria				Gram negative bacteria					
	BS	BT	Ms	Ls	KP	EC	PS	PA	Ss	Mos
Standard *	30.83 ± 0.80	30.73 ± 0.67	20.67 ± 0.59	23.63 ± 0.60	12.13 ± 0.71	25.67 ± 0.61	13.73 ± 0.67	21.73 ± 0.70	14.23 ± 0.49	20.83 ± 0.80
Petroleum ether	9.67 ± 0.75	-	-	-	8.13 ± 0.71	-	-	7.32 ± 0.49	-	-
Ethyl acetate	16.13 ± 0.38	15.16 ± 0.38	9.73 ± 0.67	10.73 ± 0.67	8.77 ± 0.71	8.73 ± 0.75	9.77 ± 0.75	11.73 ± 0.67	7.77 ± 0.71	8.77 ± 0.71
Methanol	21.83 ± 0.60	20.63 ± 0.60	8.06 ± 0.31	8.63 ± 0.60	10.16 ± 0.47	9.67 ± 0.58	9.76 ± 0.86	7.67 ± 0.61	-	-

BS - *Bacillus subtilis*; BT - *B. thuringiensis*; Ms - *Micrococcus* sp.; Ls - *Lactobacillus* sp.; KP - *Klebsiella pneumoniae*; EC - *Escherichia coli*; PS - *Pseudomonas stutzeri*; PA - *P. aeruginosa*; Ss - *Serratia* sp.; Mos - *Moraxetta* sp.* Tetracycline

It is explained that the different phytochemicals like alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids and phenols extracted by different solvents may be responsible for their antibacterial effects (Ananda kumar *et al.*, 2009). Further, the methanol extract has determined to have highest inhibitory activity (21.83 mm diameter

inhibitory zone) against the bacterium, *Bacillus subtilis* and (20.63 mm diameter inhibitory zone) against the bacterium, *Bacillus thuringiensis* (gram positive) and ethyl acetate extract also showed highest inhibitory activity (16.13 mm diameter inhibitory zone) against the bacterium, *Bacillus subtilis* and (15.16 mm diameter inhibitory zone)

against the bacterium, *Bacillus thuringiensis*. It indicates the presence of effective active principle compounds in the methanol and ethyl acetate extracts of root part of *A. fruticosa* to suppress both gram negative and gram positive bacteria. It has been observed further that the methanol extracts showed significantly higher inhibitory activity against the colonial growth of *Bacillus subtilis* and *B. thuringiensis*, than that of the commercially available antibiotic, tetracycline. This fact shows the higher therapeutic potential of methanol extract of the study species. The ethyl acetate and petroleum ether extracts have comparatively less activity against most of the tested pathogens. It may be attributed to the presence of respective active compounds with insufficient quantities in this crude extract (Taylor *et al.*, 2001).

This fact indicates the existence of strong antibacterial activity of rootpart of the study species, *A. fruticosa* and hence its effective healing property against the infectious diseases. The variation in antibacterial activity across the extracts studied may be due to the polarity of the solvents used. Significantly higher inhibitory activity of methanol extract is nearly to the commercially available antibiotic tetracycline against the bacteria, *Bacillus subtilis* and *B. thuringiensis* observed shows the superior healingness of root part of *A. fruticosa*. Proper isolation and purification of active compounds by using methanol solvent would ensure the therapeutic value of this folklore medicinal plant when it will be used commercially.

The overall study on antibacterial activity reports that the study species contains adequate variety of active compounds to reduce or check the growth of microbial colonies. It confirms the therapeutic value and hence the traditional usage of the root part of the study species, *A. fruticosa* against various ailments. Further, the alcoholic extracts of root part of this plant in general and methanol extracts and ethyl acetate in particular are suggested for the therapy of infectious diseases caused by pathogens and further studies are recommended to purify the active compounds for the formulation of new drugs, while go for commercialization.

ACKNOWLEDGEMENT

The authors are gratefully acknowledging the authorities of Tamil Nadu State Council for Science and Technology, Chennai for their financial assistance to carryout the work.

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