Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 4, 2011

Research Article

ANTIMICROBIAL SCREENING OF STEM EXTRACT OF THE FOLKLORE MEDICINAL PLANT, *ACALYPHA FRUTICOSA* FORSSK

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Received: 23 May 2011, Revised and Accepted: 3 July 2011

ABSTRACT

Antimicrobial activity of the folklore medicinal plant species, *Acalypha fruticosa* in the part of stem bark 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., *Servatia* sp., *Moraxetta* sp., *Bacillus subtilis*, *B. thuringiensis*, and *Klebsiella pneumoniae* and ten human pathogenic fungi *viz., Aspergillus niger, A. flavus, A. baumannii, Fusarium oxysporum, F. solani, Mucor rouxii, Alternaria alternata, Candida albicans, Cladosporium* sp. and *Rhizopus* sp. by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antimicrobial activity against the tested pathogens with the highest inhibition zone (32.67 mm) against the bacterium, *Bacillus subtilis* and the fungus, *Rhizopus* sp (30.87 mm)made by ethyl acetate extracts. Therefore, it is known that the stem extract of *A. fruticosa* can be used as a potential external antiseptic and may be incorporated into drug formulations, after confirming the results by pharmacognostical studies.

Keywords: Antimicrobial activity, Disc diffusion, Acalypha fruticosa.

INTRODUCTION

The herbal medicines are recognized as most reliable, and cost effective than any other system of medicinal practice. In addition, the use of higher plants and their preparation to treat infectious and non-infectious diseases is an age old practices and are the only method available in the past. Plants which constitute an active part of the ecosystem have been found to be useful to man both as sources of foods and medicine[1]. Hence in many parts of the world, medicinal plants have continued to be an integral part plant materials for curing diverse forms of ailments leads to human civilization, the scientific analysis of different natural sources for their possible medicinal potency is of the health care system and the people's culture. Though the use of natural sources like comparatively recent origin[2]. The emergence and spread of antibiotic resistant microorganisms also triggered this type of plant investigations[3]. Antibiotics are a class of antimicrobial agents. Antibiotics act by inhibition of cell wall synthesis, inhibition of folate metabolism and also binding to ribosomes to prevent translation and interference with nucleic acid synthesis[4]. Higher plants can serve both as potential antimicrobial crude drugs as well as source of new anti-infective agents[5]. The study species, *Acalypha fruticosa* Forssk. an erect woody shrub, 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 distributed up to 1800m above msl in southern Western Ghats[6]. This plant species has been used as a folk remedy for the treatment of dyspepsia, skin complaints, jaundice, cholera, sexually transmitted diseases, stomach problems, antipyretic and even as an antidote[7].The stem part of this species is used to heal wounds in animals and also used to treat toothache and the stem is used as fuel wood by tribal people. Despite these uses, no published works are available for the antimicrobial property of stem 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.

MATERIALS AND METHODS

Plant material

Fresh stem 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.

Preparation of extracts

250g air-dried stem powder was subjected to 250ml of methanol in soxhlet extraction for 8 hours (50-85°C). The extract was 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 was adopted as used to obtain methanol extract.

Media used

Freshly prepared nutrient agar medium and PDA medium were used for the culture of bacteria and fungi respectively.

Microorganisms

In vitro antimicrobial activity was examined for the chemical extracts of stem part of the species, *Acalypha fruticosa* 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, Servatia* sp. and *Moraxetta* sp. and fungal species *viz., Aspergillus niger, A. flavus, A. baumannii*, *Fusarium oxysporum, F. solani, Mucor rouxii, Alternaria alternata, Candida albicans, Cladosporium* sp. and *Rhizopus* 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 (for bacteria) and PDA slants (for fungi) until further use.

Antimicrobial assay

The alcoholic extracts were tested for their effect against the growth of pathogenic bacteria and fungi by disc diffusion method [8]. Both the organisms, bacteria and fungi tested were inoculated into nutrient agar and PDA media respectively. After an incubation period of 24 hrs at a temperature of 35 ^{0}C, three or four colonies isolated from these media were inoculated into 4ml of nutrient broth and incubated for 2 hrs at 35 ^{0} C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller-Hinton agar medium and PDA medium were streaked separately with these microbial suspensions of bacteria and fungi respectively. 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 ^{0} C, the plates were incubated overnight at 37 ^{0} C and the diameter of any resulting zones of inhibition was measured. Each experiment was repeated at least three times.

RESULTS AND DISCUSSION

The results of the antimicrobial study report that all the three alcoholic extracts of the stem part of *A. fruticosa* generally showed

significant activity against the growth of the colonies of ten bacteria tested (*Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Micrococcus* sp., *Lactobacillus* sp., *Servatia* sp., *Moraxetta* sp., *Bacillus subtilis*, *B. thuringiensis* and *Klebsiella pneumoniae*). Among the three extracts, the ethyl acetate extract has determined to have highest inhibitory activity (32.67mm diameter inhibitory zone) against the bacterium, *Bacillus subtilis* (gram positive) followed by the methanol extract against the same bacterium, (12.17 mm diameter inhibitory zone). It is explained that the different phytochemicals like steroids, cardiac glycosides, anthraquinone, flavonoids and phenolics extracted by using different solvents may be responsible for their antibacterial effects[9]. Plants contain chemical substances that take part in the metabolic activities thereby helping to fight the bacterial infections[10]. Amongst the gram-positive and gram-negative bacteria, the former bacterial strains were determined to be more susceptible to the extracts when compared to the later. This may be explained that the variation in structure of cell wall components between these two groups may cause this response[11]. In addition, it has been explained that the ability of tannin compounds to cause the bacterial colonies to disintegrate, probably results from their interference with the bacterial cell wall; thereby inhibiting the microbial growth [12,13]. It has been observed further that the ethyl acetate extracts showed significantly higher inhibitory activity against the colonial growth of *Bacillus subtilis* than that of the commercially available antibiotic, the tetracycline. This fact shows the higher therapeutic potential of ethyl acetate extract of the study species, *A. fruticosa*. The petroleum ether extract has comparatively less activity against the most of the tested bacteria. It may be attributed to the absence of respective active principle compounds or present with insufficient quantities in this crude extract[14] (Table-1).

Table 1: Antibacterial activity of various solvent extracts of *A. fruticosa*

Control/ Extracts	Diameter of inhibition zone (mm)									
	Gram positive bacteria				Gram negative bacteria					
	Bs	**Bt**	**Ms**	**Ls**	**Kp**	**Ec**	**Ps**	**Pa**	**Ss**	**Ms**
PC	28.06^{a} ±0.21	30.03^{a} ± 0.35	26.03^{a} ± 0.25	25.16^{a} ± 0.96	12.03^{a}± 0.35	22.37^{a}± 0.90	12.07^{a}± 0.20	28.03^{a}± 0.15	14.13^{a} ±0.42	23.37^{a} ± 0.72
PE	-	7.96^{b} ± 0.35	-	-	7.87^{b} ± 0.85	8.82^{b} ± 0.91	-	-	7.33^{b} ± 0.76	-
EA	32.67^{b}± 0.61	14.97^{c} ± 0.65	10.67^{b} ± 0.70	12.03^{b} ± 0.65	13.03^{a}± 0.35	10.37^{b}± 0.81	12.13^{a}± 0.32	12.16^{b}± 0.37	16.77^{a}± 0.71	11.03^{b} ± 0.35
ME	12.17^{c}± 0.38	-	-	9.16^{b} ± 0.47	7.97^{b} ± 0.35	-	-	-	-	8.83^{b} ± 0.91

Means in column followed by different letter are significant to each other at 5% level according to DMRT.

PC = Positive control (Tetracycline), PE = Petroleum ether, EA = Ethyl acetate, ME = Methanol.
Bs = *Bacillus subtilis*, Bt = *Bacillus thuringiensis*, Ms = *Micrococcus* sp., Ls = *Lactobacillus* sp., Kp = *Klebsiella pneumoniae,* Ec = *Escherichia coli,*Ps = *Pseudomonas stutzeri,* Pa = *Pseudomonas aeruginosa,* Ss = *Servatia* sp., and Ms = *Moraxetta* sp.

The antifungal activity of various extracts of stem part of the species, *A. fruticosa* against the ten studied fungal species showed the following results: As shown in antibacterial activity, the ethyl acetate extract has the highest inhibitory activity (30.87 mm diameter inhibitory zone) against the fungus, *Rhizopus* sp. followed by methanol extract (14.83 mm diameter inhibitory zone) against the fungus, *F. solani,* and petroleum ether extract (12.87 mm diameter inhibitory zone) against the fungus, *Cladosporium* sp. (Table 2). This fact indicates the existence of strong antifungal activity of stem part of the study species, *A. fruticosa* and hence its effective healing property against the infectious diseases caused by fungal species. Presence of high amount of different flavonoids which has the antifungal property in the study species, *A. fruticosa* may be the possible reason for this fact[6]. The variation in antifungal activity across the extracts studied may be due to the polarity of the solvents used and hence the ingredients present. Significantly higher inhibitory activity of ethyl acetate extract than the commercially available antibiotic tetracycline against the fungus, *Rhizopus* sp. showed the superior healingness of the stem part of the species, *A. fruticosa.* Therefore proper isolation and purification of active compounds by using ethyl acetate solvent would ensure the therapeutic value of this folklore medicinal plant when it will be used commercially.

The present investigation on antimicrobial activity reports that the study species, *A. fruticosa* contains adequate variety of active principle compounds to reduce or check the growth of microbial colonies. It confirms the therapeutic value and hence the traditional usage of the stem part of the study species, *A. fruticosa* against various ailments. Further, these findings may lead support to the traditional use of *A. fruticosa* in the treatment of microbial infections. Further studies are recommended to purify the active compounds for the formulation of new drugs, while go for commercialization.

Table 2: Antifungal activity of various solvent extracts of *A. fruticosa*

Control/ Extracts	Zone of inhibition (mm)									
	An	**Af**	**Ab**	**Fo**	**Fs**	**Mr**	**Aa**	**Ca**	**Cs**	**Rs**
PC	48.83^{a} ± 0.65	42.76^{a}± 0.75	40.73^{a} ± 0.70	43.67^{a} ±0.65	30.77^{a} ± 0.86	41.73^{a} ±0.70	43.83^{a} ±0.85	15.77^{a}± 0.86	36.76^{a}± 0.80	35.87^{a}± 0.90
PE	-	8.76^{b} ± 0.80	10.87^{b} ± 0.85	-	8.76^{b} ± 0.71	8.63^{b} ±0.60	-	-	12.87^{b}± 0.90	9.73^{b} ±0.70
EA	26.87^{b} ± 0.73	18.87^{c} ± 0.85	20.73^{c} ± 0.75	20.73^{b} ±0.70	25.83^{c} ± 0.90	18.67^{c}± 0.65	15.77^{b}± 0.75	10.63^{b}± 0.71	30.03^{c}± 0.35	30.87^{c}± 0.96
ME	-	9.77^{b} ± 0.86	11.87^{c} ± 0.85	9.73^{c} ±0.81	14.83^{d} ± 0.80	12.73^{d}± 0.66	8.83^{c} ± 0.91	7.87^{c} ± 0.85	14.13^{b}± 0.42	12.83^{b}± 0.85

Means in column followed by different letter are significant to each other at 5% level according to DMRT.

PC = Positive control (Tetracycline), PE = Petroleum ether, EA = Ethyl acetate, ME = Methanol.
An = *Aspergillus niger,* Af = *Aspergillus flavus,* Ab = *Aspergillus baumanii,* Fo = *Fusarium oxysporum,* Fs = *Fusarium solani,* Mr = *Mucor rouxii,* Aa = *Alternaria alternate,* Ca = *Candida albicans,* Cs = *Cladosporium* sp., and Rs = *Rhizopus* sp.

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