Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 3, 2011 ISSN - 0974-2441

Research Article

EVALUATION OF ANTIMICROBIAL EFFICACY OF THE FOLKLORE MEDICINAL PLANT, *ACACIA CAESIA* (L.) WILD

J. THAMBIRAJ AND S. PAULSAMY*

PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore-641 029, (Tamil Nadu), India.
Email: paulsami@yahoo.com

ABSTRACT

Stem extracts of the folklore plant species, *Acacia caesia* L. 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. thuriengensis*, 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. for assessing the antimicrobial properties by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antimicrobial activity against the tested pathogens. However, the ethyl acetate extracts exhibited higher inhibition zone (17.23 mm) against the bacterium, *Klebsiella pneumoniae* and the fungus, *Mucor rouxii* (30.77 mm). These results support the therapeutic importance of the species, *Acacia caesia* in curing infectious diseases and encourage the extensive use of this species in health care practices.

Key words: *Acacia caesia*, Antimicrobial activity, Disc diffusion, Pathogenic microorganisms.

INTRODUCTION

Traditional uses of plants have led to investigate their bioactive compounds through screening programmes, which have resulted in the detection of significant number of therapeutic properties. India, being a mega biodiversity nation with high species richness due to varied bioclimatic conditions and ecosystem diversity naturally has potential sources for medicinal plants.

Some of these plants produce valuable drugs, which have high export potential[1].

Traditionally different parts of several medicinal plants or their extracts are used in treatment of various diseases in India[2]. Among them, many species of

Acacia are found to have diverse photochemical compounds of medicinal properties [3-6]. *Acacia caesia* L. belongs to the family, Mimosaceae is one such folklore plant used in traditional system of medicine in Coimbatore district of Tamil Nadu, India. It is an armed woody shrub occurring throughout the tropical and sub-tropical regions of India [7]. This plant species has been used as a folk remedy for the treatment of skin diseases, asthma, bronchitis, scabies, cold, menstrual disorders and antiseptic also.

The leaves of this plant are used as vegetable and the powdered bark and pod are used as substitute for soap and their decoctions as lice killer [8].

Woody branches of this species are used as tooth brushes by tribal folk and the shrub is used as fuel wood. However, 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. caesia* 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 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.

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 study plant, against ten bacterial species which include the gram positive strains *viz*., *Micrococcus* sp., *Lactobacillus* sp., *Bacillus subtilis*, *B. thuriengensis* 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 [9]. 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 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.

Table 1: *In vitro* antibacterial activity of *Acacia caesia* extracts by disk diffusion method.

Control/ Extracts	Zone of inhibition (mm)									
	Gram positive bacteria				Gram negative bacteria					
	Bs	Bt	Ms	Ls	Kp	Ec	Ps	Pa	Ss	Ms
PC	24.77[a] ± 0.20	30.76[a] ± 0.56	22.63[a] ± 0.57	25.27[a] ± 0.64	12.33[a] ± 0.42	18.53[a] ± 0.61	12.97[a] ± 0.25	28.83[a] ± 0.67	32.63[a] ± 0.56	25.36[a] ±0.82
PE	12.87[b] ± 0.15	11.16[b] ± 0.57	-	-	9.16[b] ± 0.37	-	8.93[b] ± 0.40	-	-	6.97[b]± 0.25
EA	14.13[c] ± 0.61	12.13[b] ± 0.61	11.73[b] ± 0.56	8.77[b] ± 0.59	17.23[c] ± 0.58	12.16[b] ± 0.66	16.93[c] ± 0.40	12.87[b] ± 0.85	12.67[b] ± 0.36	10.03[c] ±0.45
ME	11.16[b] ± 0.47	16.63[c] ± 0.60	8.77[c] ± 0.56	8.77[b] ± 0.32	10.73[b] ± 0.75	8.06[c] ± 0.30	-	14.13[c] ± 0.61	7.93[a] ± 0.31	13.1[d] ±0.47

Means followed by different letter in columns are varied significantly at 5% level according to DMRT.
PC = Positive control (Tetracycline), PE = Petroleum ether, EA = Ethyl acetate, ME = Methanol.
Bs = *Bacillus subtilis*, Bt = *Bacillus thuriengensis*, 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.

Table 2*: In vitro* antifungal activity of *Acacia caesia* extracts by disk diffusion method.

Control/ Extracts	Zone of inhibition (mm)									
	An	Af	Ab	Fo	Fs	Mr	Aa	Ca	Cs	Rs
PC	27.67[a] ± 0.48	28.17[a] ± 0.67	26.73[a] ± 0.67	30.73[a] ± 0.67	23.73[a] ± 0.67	25.73[a] ± 0.67	27.67[a] ± 0.61	10.73[a] ± 0.67	15.77[a] ± 0.75	40.83[a] ± 0.85
PE	-	-	8.73[b] ± 0.66	-	9.73[b] ± 0.70	20.73[b] ± 0.67	15.77[b] ± 0.75	-	-	10.77[b] ± 0.75
EA	10.63[b] ± 0.53	12.77[b] ± 0.71	10.77[c] ± 0.71	12.67[b] ± 0.59	12.77[c] ± 0.75	30.77[c] ± 0.71	17.73[c] ± 0.70	7.67[b] ± 0.59	8.67[b] ± 0.65	14.77[c] ± 0.71
ME	7.73[c] ± 0.54	10.73[c] ± 0.70	11.63[c] ± 0.65	8.17[c] ± 0.38	10.76[d] ± 0.71	24.73[a] ± 0.67	18.67[c] ± 0.65	13.77[c] ± 0.75	8.03[b] ± 0.91	-

Means followed by different letter in columns are varied significantly 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.

RESULTS AND DISCUSSION

The results of the antimicrobial study report that all the three alcoholic extracts of the stem part of *A. caesia* generally showed significant activity against the growth of the colonies ten bacteria tested (*Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, , Micrococcus* sp., *Lactobacillus* sp., *Servatia* sp., *Moraxetta* sp., *Bacillus subtilis*, *B. thuriengensis* and *Klebsiella pneumoniae*) (Table 1).

It is explained that the different phytochemicals like steroids, cardiac glycosides, anthraquinone, flavonoids and phenolics extracted by different solvents may be responsible for their antibacterial effects [10]. Further, the ethyl acetate extract has determined to have highest inhibitory activity (17.23 mm diameter inhibitory zone) against the bacterium, *Klebsiella pneumoniae* (gram negative) followed by the methanol extract against the bacterium, *Bacillus thuriengensis* (gram positive) (16.63 mm diameter inhibitory zone). It indicates the presence of effective active principle compounds in the ethyl acetate extract of stem part of *A. caesia* to suppress both gram negative and gram positive bacteria. It has been observed further that the ethyl acetate extracts showed significantly higher inhibitory activity against the colonial growth of *Klebsiella pneumoniae* and *Pseudomonas stutzeri* than that of the commercially available antibiotic, tetracycline.

This fact shows the higher therapeutic potential of ethyl acetate extract of the study species. The petroleum ether extract has 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 [11].

The antifungal activity of various extracts of stem part of the species, *A. caesia* against the ten studied fungal species showed the following results: As shown in antibacterial activity, the ethyl acetate extract has the highest inhibitory activity (30.77 mm diameter inhibitory zone) against the fungus, *Mucor rouxii* followed by methanol extract (24.73 mm diameter inhibitory zone) and petroleum ether extract (20.73 mm diameter inhibitory zone) against the same fungus ((Table 2). This fact indicats the existence of strong antifungal activity of stem part of the study species, *A. caesia* and hence its effective healing property against the infectious diseases. The variation in antifungal activity across the extracts studied may be due to the polarity of the solvents used. Significantly higher inhibitory activity of ethyl acetate extract than the commercially available antibiotic tetracycline against the fungus, *Mucor rouxii* observed shows the superior healingness of stem part of *A. caesia.* 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 overall study on antimicrobial 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 stem part of the study species, *A. caesia* against various ailments. Further, the alcoholic extracts of stem part of this plant in general and ethyl acetate extract 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.

REFERENCES

1. Bupesh GM, Amutha C, Nandagopal S, Ganesh kumar A, Suresh kumar P, Saravana Murali K. Antibacterial activity of *Mentha piperita* from leaf extracts. Acta agriculture slovenica 2007; 89: 73-79.

2. Balakrishnan N, Bhaskar VH, Jayakar B, Sangeswaran B. Antibacterial activity of *Mimosa pudica*, *Aegle marmels* and *Sida cordiffolia*. Pharmacogysonya mag 2006; 2: 198-199.
3. Lee TH, Qiu F, Walle GR, Chou CH. Three new flavanol galloyglycosides from leaves of *Acacia confuse.* J Nat Prod 2000; 10: 125.
4. Readel K, Seigier D, Hwang K, Keesy J, Sellheimer S. Tannins from mimosid legumes of Texas and Mexico. Econ Bot 2001; 55 (2): 212-222.
5. Seo Y, Hoch J, Abdel-Kader M, Malone S, Derveld I, Adams H, Werkhoven M CM, Wisse JH, Mamber SW, Dlton JM, Kingston D G I. Bioactive saponins from *Acacia tenuifolia* from the Suriname Rainforest. J Nat Pro 2002; 65: 170.
6. Sathishkumar P, Paulsamy S, Anandakumar AM, Senthilkumar P. Effect of habitat variation on the content of certain secondary metabolites of medicinal importance in the leaves of the plant, *Acacia caesia* Wild. Journal of Advances in Plant Sciences 2009; 22: (11) 451-453.
7. Krishnamurthy T. Minor Forest Products of India Oxford & IBH Publishing Co. Ltd. New Delhi 1993.
8. Thammanna, Narayana RK. Medicinal Plants of Tirumala. 1st ed. Department of Gardens, Tirumala Tirupahti Devasthanams, Tirupathi 1990.
9. Bauer RW, Kirby M D K, Sherris JC, Turek M. Antibiotic susceptibility testing by standard single disc diffusion method. American Journal of Clinical Pathology 1966; 45: 493-496.
10. Tambekar DH, Khante BS. Evaluation of antibacterial properties of ethnomedicinal herbs used by Korkus in Melghat of India against Enteric pathogens. International Journal of Pharma and Bio Sciences 2010 V1 (1) (http: // www.ijpbs.net).
11. Taylor JL, Rabe ST, McGraw LJ, Jager AK, van Staden J. Towards the Scientific Validation of traditional medicinal plants, Plant Growth Regul 2001; 34: 23-37.