

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

Thambiraj, J¹. and S. Paulsamy^{2*}

¹PG and Research Department of Botany, The Madura College (Autonomous), Madurai - 625 011.

²PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore - 641 029.

*E.mail: paulsami@yahoo.com

ABSTRACT

Leaf 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., *Moraxella* 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 (15.73 mm) against the bacterium, *Bacillus subtilis* and the fungus, *Mucor rouxii* (20.67 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.

Keywords: Medicinal plant, *Acacia caesia*, Antimicrobial activity, Microorganisms.

1. INTRODUCTION

Located in a tropical belt, India is endowed with a rich wealth of fauna and flora with innumerable medicinal plants. These plants have made a good contribution to the development of ancient Indian Materia Medica. One of the earliest treatises on Indian medicine, the Charaka Samhita (1000 B.C), records the use of over 340 drugs of vegetable origin (Pullaih, 2006). Most of these continue to be gathered from wild plants to meet the demand of the medical profession. India, in particular, has a big scope for the development of the pharmaceutical and phytochemical industry. Traditionally different parts of several medicinal plants or their extracts are used in treatment of various diseases in India (Balakrishnan *et al.*, 2006). Among them, many species of *Acacia* are found to have diverse photochemical compounds of medicinal properties (Lee *et al.*, 2000; Readle *et al.*, 2001; Seo *et al.*, 2002; Sathishkumar *et al.*, 2009). *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 (Krishnamurthy, 1993). 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 (Thamanna, 1990). 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 leaf 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 leaf 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.

2.2. Preparation of extracts

250g air-dried leaf 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 and PDA medium were used for the culture of bacteria and fungi respectively.

2.4. Microorganisms

In vitro antimicrobial activity was examined for the chemical extracts of leaf part 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. 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.

2.5. Antimicrobial assay

The alcoholic extracts were tested for their effect against the growth of pathogenic bacteria and fungi by disc diffusion method (Bauer *et al.*, 1966). 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°C, three or four colonies isolated from these media 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 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°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 leaf extracts of the study species, *Acacia caesia* generally showed inhibitory activity against the growth of *Bacillus thuringiensis* and *Lactobacillus* sp. However, towards *Pseudomonas aeruginosa*, *P. stutzeri*, *Escherichia coli*, *Micrococcus* sp., *Serratia* sp., *Moraxetta* sp., *Bacillus subtilis* and *Klebsiella*

pneumoniae, all these extracts showed activity with less pronounced manner (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 (Tambekar and Khante, 2010). Further, the ethyl acetate extract has determined to have highest inhibitory activity (15.73 mm diameter inhibitory zone) against the bacterium, *Bacillus subtilis* (gram positive) and (14.83 mm diameter inhibitory zone) against the bacterium, *B. thuringiensis* followed by the methanol extract against the bacterium, *Escherichia coli*, (gram negative) (12.43 mm diameter inhibitory zone). It indicates the presence of effective active principle compounds in the ethyl acetate and methanol extracts of leaf 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 *Bacillus subtilis* and *B. thuringiensis* 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 (Taylor *et al.*, 2001).

The antifungal activity of various alcoholic leaf extracts of the study species, *Acacia caesia* against the ten studied fungal species is given in Table 2. The results of the study report that the ethyl acetate extract has the highest inhibitory activity (20.67 mm diameter inhibitory zone) against the fungus, *Mucor rouxii*. The petroleum ether and methanol extracts were also found to be better with respect to inhibitory function against the two fungal species, *Mucor rouxii* and *Rhizopus* sp. (17.73 and 16.75 mm diameter inhibitory zone respectively).

This fact indicates the existence of strong antifungal activity of leaf 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 is nearly to the commercially available antibiotic tetracycline against the fungus, *Mucor rouxii* observed shows the superior healingness of leaf 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.

Table 1. Antibacterial activity of certain alcoholic leaf extracts of the species, *Acacia caesia*.

Plant extract	Diameter of zone inhibition (mm)									
	Gram positive bacteria					Gram negative bacteria				
	BS	BT	MC sp.	L sp.	KP	EC	PS	PA	S sp.	M sp
Standard*	8.53 ± 0.56	8.33 ± 0.35	22.43 ± 0.40	24.03 ± 0.45	15.43 ± 0.44	30.43 ± 0.40	12.73 ± 0.46	26.61 ± 0.54	14.93 ±0.31	8.87 ±0.42
Petroleum ether	-	12.76 ± 0.62	-	8.07 ±0.55	-	-	-	-	-	-
Ethyl acetate	15.73 ± 0.70	14.83 ± 0.80	-	8.03 ± 0.44	8.03 ± 0.15	12.17 ±0.35	7.76 ±0.70	-	-	-
Methanol	-	9.07 ± 0.21	9.07 ± 0.46	7.43 ±0.59	11.93 ± 0.40	12.43 ± 0.45	8.03 ±0.25	8.73 ± 0.67	9.03 ±0.25	12.07 ±0.40

BS - *Bacillus subtilis*; BT - *B. thuringiensis*; MC sp. - *Micrococcus* sp.; L sp. - *Lactobacillus* sp.; KP - *Klebsiella pneumoniae*; EC - *E. coli*; PS - *Pseudomonas stutzeri*; PA - *P. aeruginosa*; S sp. - *Serratia* sp.; M sp. - *Moraxetta* sp.

* Tetracycline

Table 2. Antifungal activity of certain alcoholic leaf extracts of the species, *Acacia caesia*.

Plant extract	Diameter of zone inhibition (mm)									
	AN	AF	AB	FO	FS	MR	AA	CA	C sp.	R sp.
Standard *	35.23 ± 0.59	38.17 ± 0.67	33.63 ± 0.65	32.73 ±0.67	25.73 ± 0.67	27.63 ±0.57	30.67 ± 0.59	16.03 ± 0.15	13.67 ± 0.65	36.67 ± 0.59
Petroleum ether	8.23 ± 0.49	-	-	-	8.13 ±0.70	17.73 ±0.67	-	-	-	-
Ethyl acetate	15.73 ±0.67	12.67 ± 0.61	10.77 ± 0.70	12.77 ±0.71	10.67 ± 0.65	20.67 ±0.61	10.73 ±0.67	10.76 ± 0.80	-	12.67 ±0.59
Methanol	14.77 ± 0.70	9.17 ± 0.38	10.83 ± 0.80	10.67 ± 0.65	-	15.70 ±0.66	8.67 ±0.65	8.27 ± 0.55	8.03 ±0.91	16.75 ±0.73

AN - *Aspergillus niger*; AF - *A. flavus*; AB - *A. baumannii*; FO - *Fusarium oxysporum*; FS - *F. solani*; MR - *Mucor rouxii*; AA - *Alternaria alternate*; CA - *Candida albicans*; C sp. - *Cladosporium* sp.; R sp. - *Rhizopus* sp.

* Tetracycline

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 leaf part of the study species, *A. caesia* against various ailments. Further, the alcoholic extracts of leaf part of this plant in general and ethyl acetate and methanol extracts 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.

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