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Phytochemical Analysis, Anti Microbial and Anti Oxidant Activity of the Bark Extracts of *Acacia leucophloea* L.

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Abstract: *Acasia leucophloea* L. is a wild medicinal plant distributed through South and Southeast Asia. It is used against ailments like cough, inflammation, wounds, skin diseases, leukoderma, diarrhea, hemorrhages, dental caries, stomatitis and fever. In the present study, methanolic, ethanolic and aqueous extracts were obtained from the stem bark of *Acasia leucophloea* and phytochemical analysis was done to screen alkaloids, steroids, saponins, tri terpinoidal saponins, carbohydrates, flavanoids, polyphenols, tannins, glycosides, gums and mucilage. Antimicrobial activity was carried out against eight bacterial and three fungal species. Among the three extracts, Methanol extract exhibited a broad spectrum of antimicrobial activity and it was found to be effective against all the tested bacterial and fungal species viz, *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Micrococcus roseus, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Aspergillus nige,r candida tropicali and Sacharomyces cerevaceae.* Ethanol extract was found to be active against all but one (*Staphylococcus aureus*) of the above microbial strains. Water extract was active against all but two (*candida tropicali* and *Staphylococcus aureus*) of the above tested microorganisms. In case of antioxidant activity, Methanol extract showed highest antioxidant activity followed by ethanol and water extracts. Among methanol, ethanol and water extracts methanol extract showed high content of phytochemicals, highest antimicrobial and antioxidant activity. Our results supported the usage of Acasia leucophloea in folk and traditional medicine.

Key words: Methanol extract · Antimicrobial activity · Antioxidant activity · DPPH

INTRODUCTION

From ancient times plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being [1, 2]. With the rising prevalence of microorganisms developing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants [3]. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities [4]. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. Diseases that have been managed traditionally using medicinal plants include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections [5].

Acacia leucophloe L. is a thorny tree, distributed in South and Southeast Asia [6,7]. The common names for this species often refer to its light color, white-bark acacia (English), safed kikkar (Hindi), safed babul (Bengali), sarai, velvelam (Tamil). Other common names include pilang and besok (Indonesian). *A.leucophloea* is a medicinal plant employed in the Indian indigenous system of medicine [8,9]. This species has previously been found to contain octacosanol and (+)-pinkol [10] and betulinic acid-3-0-p-o-maltoside [11]. This genus pacifies vitiated kapha, cough, inflammation, wounds, skin diseases, leukoderma, diarrhea, hemorrhages, dental caries, stomatitis and fever. The present study reported the phytochemical analysis, antioxidant and antimicrobial activity of *Acacia leucophloe*.

METERIALS AND METHODS

Collection and Extraction of Plant Material: The bark of *Acacia leucophloe* was collected from Seshachalam forests of Chittoor district, Andhra Pradesh, India. Freshly collected stem barks of *Acacia leucophloe* were dried in

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shade and pulverized to a coarse powder and extracted with methanol, ethanol and water using the soxhlet apparatus. The filtrate obtained was evaporated to dryness at 50-65°C in a rotary vacuum evaporator to obtain a dark colored molten mass.

Phytochemical Analysis of Extract: The methods described by Harborne [12] with slight modifications were used to screen the presence of the active ingredients in the bark extracts.

Test For Steroids: 10 ml of the bark extract was evaporated to dry mass and dissolved in 0.5 ml of solvent. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds [12].

Test For Terpenoids: The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids [12].

Test For Tannins:

- 1 cm3 of freshly prepared 10% KOH was added to 1 cm3 of the extract. A dirty white precipitate indicated the presence of tannins [12].
- Powdered stem bark of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% $FeCl_3$ were then added. Production of greenish precipitate indicated the presence of tannins [12].

Test For Flavonoids: A small piece of magnesium ribbon was added to extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones [12].

Test For Alkaloids: The extract of the plant stem bark sample (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract [12].

Test For Saponins: Stem bark of the test plant was ground into powder form and 0.5 g of the powdered stem bark was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of Saponins [12].

Test For Glycosides: Coarsely powdered stem bark (1g) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3 – 5 min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides [12].

Test for Gums and Mucilage: about 10ml of various extracts were added separately to 25ml of absolute alcohol with constant stirring and then filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates [12].

Antimicrobial Activity

Test Organisms For Antimicrobial Activity: Four Gram positive bacterial strains (*Bacillus subtilis, Bacillus cereus Staphylococcus aureus and Micrococcus roseu*), four Gram negative bacterial strains (*Salmonella typhi, Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa*) and three fungal strains (*Aspergillus niger Candida tropicali and Sacharomyces cerevaceae*) were obtained from Institute of Microbial Technology (IMTECH),Chandigarh and Dept.of Virology, S.V.University, Tirupati. The strains were maintained and tested on nutrient agar for bacteria and potato dextrose (PDA) for fungi for the Antimicrobial tests.

Antimicrobial Activity: The agar disc diffusion method was used to determine the antimicrobial activity of the different plant extracts [13]. The discs (6 mm diameter) impregnated with different concentrations of the extracts were placed on the surface of the petri plates containing 20 ml of nutrient agar media for bacterial strains and potato dextrose agar media for fungal strains respectively, seeded with 100μl of microbial cultures (5 x 10^5 CFU/ml). The plates were incubated for 24 hrs at 35 ±2°C for bacteria and for 72 hrs for fungi at 30°C. The inhibition

zones formed around the discs were measured and expressed in millimeter. Three independent trials were conducted for each concentration and the average values calculated and given in Table 2. The microbial activity was confirmed by transferring a subculture from the clear zone of inhibition to a fresh broth media and observed for the growth of microbes.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration was determined, using a common broth micro dilution method in 96 well micro titer plates [13,14]. From the previously prepared different microbial suspensions, cultures (10^5 CFU/mL) were added to each well. Plates were incubated for 18 hr at 37°C and then were examined with Elisa reader (TECAN, Sunrise, China) at 620nm and the lowest concentration of each extract showing no growth was taken as its minimum inhibitory concentrations (MIC). All the samples were tested in triplicate to confirm the activity and the values were noted.

Antioxidant Activity: Evaluation of antioxidant activity was done by using 2, 2'-diphenly-1-picrylhydrazyl (DPPH) method described by Burits and Bucar [15]. Antioxidants react with DPPH and convert it to α, α -diphenyl.-β-.picryl hydrazine. One ml of plant extract was added to 4ml of 0.004% methanol solution of DPPH. After 20- 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated by using the following equation. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity.

% DPPH radical-scavenging = [(Absorbance of Control - Absorbance of test Sample)/(Absorbance of Control)] x 100

RESULTS AND DISCUSSION

Phytochemical Analysis: Preliminary phytochemical screening of the bark extracts of *Acasia leucophloea* L. showed positive results for the presence of secondary metabolites like Steroids, Saponins, Tri terpinoidal saponins, Alkaloids, Carbohydrates, Flavanoids, Tannins, Glycosides, Polyphenols and Gums and Mucilage. Triterpenes are completely absent in all the extracts. Saponins and Tri terpinoidal saponins were present only in aqueous extract with low quantity. Bioactive compounds like Steroids, Alkaloids, Carbohydrates, Tannins, Glycosides, polyphenols, Gums and Mucilage were present in high amounts in Methanol extract (Table 1).

Antimicrobial activity: The methanolic, ethanolic and aqueous extracts of the bark of *Acasia leucophloea* were tested against four Gram- positive, four Gram-negative and three fungal species (*Bacillus subtilis, Bacillus cereus and Staphylococcus aureus and Micrococcus roseus, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa, Asperillus niger and Candida tropicali and Sacharomyces cervaeae.* The results revealed that the extracts showed moderate to high antimicrobial activity against all the tested microbial strains. The antimicrobial activity was evaluated from the zone of inhibition. With increase in concentration of the bark extracts from 25 to 100 mg/ml, an apparent

Table 1: Phytochemical Screening of Methanolic, Ethanolic and Water Extract of *Acasia leucophloea Bark*

S.No	Secondary metabolites	Methanol	Ethanol	Water
1	Steroids	+++	++	++
2	Triterpenes	-	-	-
3	Saponins	-	-	+
4	Tri terpinoidal saponins	-	-	+
5	Alkaloids	++	+ +	+
6	Carbohydrates	+++	++	++
7	Flavonoids	-	+	+
8	Tannins	++ +	++	+++
9	Glycosides	++	++	+
10	Polyphenols	+++	++	++
11	Gums and mucilage	+++	++	+

+++ = Present in high quantity; ++ = Present in appreciable quantity; + = present in low quantity.

Table 2: *Invitro* antimicrobial activity of bark extracts of *Acasia leucophloea*

		Zone of Inhibition (mm)								
		Methanol extract(mg/ml)			Ethanol extract(mg/ml)			Aqueous extract (mg/ml)		
S.No	Micro organisms	100	50	25	100	50	25	100	50	25
1	*Bacillus subtilis*	15	11	7	13	9	7	11	6	–
2	*Bacillus cereus*	14	12	8	10	6	–	8	–	–
3	*Staphylococcus aureus*	16	11	7	7	–	–	9	–	–
4	*Micrococcus roseus*	11	7	–	12	9	–	10	–	–
5	*Salmonella typhi*	13	9	7	11	6	–	13	9	–
6	*Klebsiella pneumoniae*	10	–	–	14	12	9	10	6	–
7	*Escherichia coli,*	17	13	9	12	9	6	16	12	8
8	*Pseudomonas aeruginosa*	13	7	–	10	7	–	9	–	–
9	*Aspergillus niger*	15	12	6	16	11	7	11	8	6
10	*candida tropicali*	9	–	–	12	7	9	–	–	–
11	*Sacharomyces cerevaceae*	16	12	7	11	6	–	–	–	–

Table 3: Antioxidant activity of bark extracts of *Acasia leucophloea*

Extracts of *Acasia leucophloea*	Concentration of extract in PPM	% of DPPH free radical Scavenging activity
Methanol	50	61%
	100	66%
	150	70%
	200	74%
	400	81%
Ethanol	50	52%
	100	58%
	150	63%
	200	71%
	400	75%
Water	50	58%
	100	64%
	150	67%
	200	69%
	400	70%

increase in antimicrobial activity was observed in all the extracts. Among various solvent extracts studied, Methanol extract at a concentration of 100 mg/ml showed the highest degree of inhibition followed by ethanol and water extracts (Table 2). The decreasing order of inhibition of the eight bacterial strains in terms of their zones of inhibition with Methanolic extract is as follows, *Escherichia coli* (17mm), *Staphylococcus aureus* (16mm), *Bacillus subtilis* (15mm), *Bacillus cereus* (14mm), *Salmonella typhi* (13mm), *Pseudomonas aeruginosa* (13mm), *Micrococcus roseu* (11mm) *and Klebsiella pneumonia* (10mm). Among three fungal strains, the decreasing order of zone of inhibition by Methanolic extract is *Sacharomyces cerevaceae* (16mm), *Aspergillus niger* (15mm) *and candida tropicali* (9mm).

The composition and concentration of secondary metabolites determines the antimicrobial efficacy of plants. The higher levels of steroids, polyphenols, tannins, alkaloids, gums and mucilages noted in bark extracts of *Acasia leucophloea* could be responsible for the medicinal properties of this plant. Similar reports of antimicrobial activity was observed with extracts of *Azaderecta indica*, *Curcuma longa*, *Oscimum spp*, *Morinda citrifolia* L, *Zingeber officinale, Cassia auriculata* etc, [8,16 - 20].

Antioxidant Activity: Methanol, ethanol and water extracts of *Acasia leucophloea* bark were checked for antioxidant activity by using DPPH method. With increase in bark concentration from 50 to 400 ppm, the antioxidant activity was increased in all the extracts. Methanolic extract showed highest antioxidant activity followed by aqueous and ethanol extracts. The highest antioxidant activity showed by Methanolic, aqueous and ethanolic extracts at 400 ppm was 81, 75 and 70% respectively (Table 3). The presence of secondary metabolites like polyphenols, steroids, alkaloids etc, in higher amounts in methanolic extracts (Table 1) could be attributed to the highest antioxidant activity shown by this extract.

CONCLUSION

Among the three extracts of *Acacia leucophloea*, bioactive compounds like Steroids, Alkaloids, Carbohydrates, Tannins, Glycosides, Polyphenols, Gums and Mucilage were present in high amounts in Methanol extract and it was found to be active against all the tested microorganisms like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Micrococcus roseus, Klebsiella pneumonia*, *Sacharomyces cerevaceae*, *Aspergillus niger and candida tropicali,* indicating the broad spectrum of antimicrobial activity of Methanolic extract. With regard to antioxidant activity, Methanolic extract showed highest antioxidant activity followed by aqueous and ethanolic extracts. Our results authenticate the usage of *Acacia leucophloea* by local people for treating skin diseases and against some bacterial and fungal infections. Our studies provide the basis for further isolation and evaluation of major active principles present in the plant material and test their efficiency against various infections.

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