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Antibacterial and Antioxidant Activities in *Cassia auriculata*

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Abstract: The antibacterial properties of the *Cassia auriculata* were tested against ten human pathogens by using five different solvent namely, hexane, chloroform, ethyl acetate, acetone and methanol. The maximum antibacterial activity recorded in methanol extracts against *Vibrio cholorae* and *Staphylococcus aureus*. Minimum activity was noted in chloroform extracts against *Pseudomonas aeruginosa*, no inhibition zone present in chloroform extract against *E. coli*. Total anti oxidant level, total phenolic compounds and total flavonoid content was higher in ethyl acetate extract. In separation of compounds, ethyl acetate extract were more spots in (TLC) plate.

Key words: *Cassia auriculata* · Antibacterial activity · Ethyl acetate extract · Anti oxidant and thin layer chromatography

INTRODUCTION

Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. Medicinal values of many plants still remain unexplored for its enumerable activity of compounds responsible for later. Yet, plant materials remain important resources to combat serious diseases of the world. Pharmogonostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents [1]. Among, more than 2, 50,000 species of higher plants, only 5-10% are chemically investigated [2]. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [3]. Contrary to the synthetic drug, antimicrobials of plant origin are not associated with many side effects and an enormous therapeutic potential to heal many infectious diseases [4]. Plants are also known to contain enumerable biological active compounds [5] which posse's antibacterial properties [6]. Apart from being an antibacterial few medicinal plants also exhibit antiparasitic activity [7]. It was also investigated that *Cassia* leaf extract affected the conidial germination of fungi *Microsporum gypsum* [8]. The WHO (World Health Organization) considers phytotheraphy in its health programs suggested basic procedures for validation of drugs from plants origin in developing countries. Nowadays in medicinal plants are rarely used as antioxidants in traditional and modern medicines. Their claimed therapeutic properties could be due in part to their capacity for scavenging oxygen free radicals which may be involved in many diseases. For example, in the case of plants used to treat inflammatory diseases and gastric ulcers. In the present study, an attempt has been made to enrich the knowledge of antibacterial activity of *C. auriculata* plant extract against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Preparation of Plant Extract: The shade dried coarsely powdered leaves were subjected to cold extraction using hexane, chloroform, ethyl acetate, acetone and methanol, in the shaking condition. After one week the mixture was filtered through Whatman No 1 filter paper. The extract was used for antibacterial activity.

Bacterial Stains: The test organisms were supplied by the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, India. Two Gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and eight Gram-negative (*Escherchia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella*

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typhi, Salmonella paratyphi, Shigella boydii and *Vibrio cholera*) bacterial stains were used in the study. The organisms were sub-cultured on Mueller Hinton Agar medium, incubated at 37°C for 24 hrs and stored at 4°C in the refrigeration to maintain stock culture.

Antibacterial Assay: Antibacterial activity was carried out using disc-diffusion method [9]. Petri plates were prepared with 20 ml of sterile Muller Hinton Agar (HIMEDIA). The tested cultures were swabbed on top of the solidified media and allowed to dry for 10 min. The concentrated crude extract was dissolved in 40% DMSO (Dimethyl sulphoxide). The test was conducted at three different concentration of the crude extract (25, 50 and 75 µl/ disc) with three replicates. The loading disk were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Zone of inhibition was recorded in millimetres and the experiment was repeated three replicates.

Antioxidant Experiments: Known weight of Extracts was dissolved in respective solvents. To the known concentration of extract, 1ml of diluted ABTS was added and absorbance was measured at 734 nm after 5 min [10].

Estimation of Total Phenolic Acids: 250 µl of sample was added into the 25 ml of double distilled water and mixed well. Then 3 ml of 0.1M ferric chloride was added and mixed well. After 3 min, 3 µl of 0.008M potassium ferric cyanide was added and mixed well. After 10-15 min the reading was taken at 720 nm [11].

Estimation of Total Flavonoid: The estimation of total flavonoid was done by the method of [12]. 1ml of 2% methanolic aluminium chloride was added in to 1ml of ethanolic fraction. The absorbance was measured at 430 nm.

Thin Layer Chromatography

Preparation of TLC Plates: Desaga Brinkmann TLC apparatus was used for the PTLC. 25x 10 cm glass plates were washed with distilled water followed by smearing with acetone. After drying the plates were placed on the template in a row. The slurry of silica gel G prepared with glass distilled water in the ratio of 1:2 (w/v) was poured in the applicator. The glass plates were immediately coated with a layer of silica gel in 500 µm thickness. The coated plates were activated at 80°C for 3 h. Then the plates were stored in a plate chamber for further study. In that study chloroform and methanol (solvent) was used in 96:4 ratios.

Loading of Substances: The concentrated plant extract of 2.5 mg was loaded on the TLC plates just above 2 cm from the bottom using a capillary tube. The plates were reserved in a developing jar containing the solvent mixture. After, the solvent front reached approximately 18 cm height. The plates were removed and allowed at room temperature for 30 min. Then the plates were also observed under UV light (240 and 300 nm) and recorded the Rf value of fluorescence substances.

RESULT AND DISSCUSION

The medicinal properties and pharmacological actions of *C. auriculata* and C. Quadrangular is well used in Indian traditional medicine. These plants are known to contain various active principle of therapeutic value and to possess biological activity against a number of diseases [13]. Aqueous extract of *Cassia tora* inhibited *S. aureus*, *P. aeruginosa* and *E. coli* at concentrations of 100µg/ml, 200µg/ml and 250µg/ml respectively but did not inhibit the growth of *B. subtilis* at any of the concentrations tested. Ethanol extract inhibited only *B. subtilis*, where as it was not effective against the other bacteria tested. Methanol extract was effective against two of the tested organisms i.e., *S. aureus* and *E. coli* both at the concentration of 64mg/ml. Pet ether did not inhibit the growth of any of the bacteria. [14]. In the present study, ethyl acetate, acetone and methanol extracts were showed significant zone of inhibition against *E. faecalis*, S. paratyphi and S. boydii but Other Gram-negative bacteria were less inhibited. The results indicated that the tested crude extracts showed antibacterial activity towards the Gram-positive bacteria. Among the five extracts tested at three different doses, the ethanol, acetone and ethyl acetate extracts at 75 µl/ disc dose were more potent in their antibacterial activity (Table 1).

The antioxidant activity, phenolic compounds and total flavonoid level was higher in ethyl acetate extract. In separation of compounds, ethyl acetate extract were more spots in Thin Layer Chromatography (TLC) plate (Fig. 1). The Resolution factor (Rf) values of respective spots were shown on Table 2. The antioxidant activity of the leaf extracts, were extracted by different organic solvents, including n-hexane, ethyl acetate and methanol. It was found that the methanol and ethyl acetate extracts gave an identical antioxidant activity that was markedly stronger than the n-hexane extract activity. Because the leaves produced a smaller amount of ethyl acetate extract (1.8 g), we were interested in studying the methanol

Table 1: Antimicrobial Activity of *C. auriculata*

		Hexane			Chloroform			Ethyl Acetate			Acetone			Methanol		
Microorganisms	Control	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Vibrio cholera	-	8	12	11	-	15	13	10	15	16	14	17	17	16	16	18
Enterococcus faecalis	-	10	-	-	-	-	-	12	13	13	13	13	13	13	13	17
Staphylococcus aureus	-	8	-	-	8	-	-	11	13	15	14	14	17	14	15	18
Salmonella typhi	-	10	-	8	10	-	-	-	12	-	-	12	12	-	11	12
Salmonella paratyphi	-	7	-	8	-	8	8	11	12	12	12	13	14	12	14	15
Shigella boydii	-	7	7	-	-	7	8	7	7	11	7	8	11	8	9	11
Proteus vulgaris	-	9	7	8	-	7	10	8	-	11	10	12	11	10	12	13
Pseudomonas aeruginosa	-	0	-	8	-	-	7	9	10	11	7	9	10	11	11	12
Escherichia coli	-	7	7	-	-	-	-	11	12	14	13	14	14	16	14	15
Klebsiella pneumonia	-	7	7	-	7	8	9	8	7	9	7	-	8	-	8	10

Zone of Inhibition mm/diameter,

A-25µl, B-50µl, C-75µl.

Table 2: R_f values of *C. auriculata* crude extract in different solvent extracts in Chloroform: Methanol

Number of Spots	Hexane	Chloroform	Ethyl Acetate	Acetone	Methanol
1	0.12	0.11	0.08	0.29	0.13
2	0.22	0.20	0.16	0.38	0.33
3	0.34	0.25	0.25	0.52	0.55
4	0.40	0.38	0.32	0.70	0.61
5	0.61	0.61	0.45	0.72	0.66
6	0.70	0.69	0.54	0.79	0.72
7	0.80	0.75	0.70	0.88	0.77
8	0.90	0.88	0.89	0.91	0.83
9	-	-	0.91	0.94	0.9
10	-	-	0.93	-	0.92
11	-	-	0.95	-	-

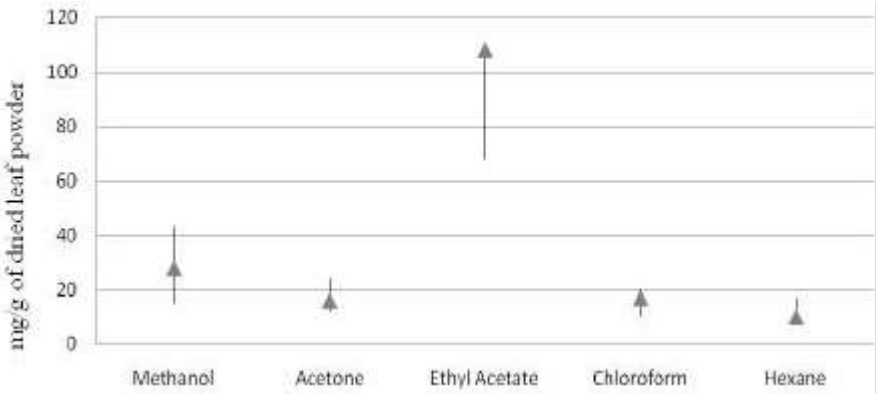

Fig. 1: Total antioxidant, Total phenolic compound and Total flavonoid content estimation (mg/g) of *Cassia auriculata* Dried Leaf Powder

extract (10.2 g) [15]. In the results of different solvent extracts in *piper betel* the mean value of methanol extract is 13.6 mm. Ethanol extract is 13.8mm. Acetone extract is 13.1mm. The mean value of different solvent extracts of *c. auriculata* in methanol 14mm, ethanol 14.3mm and acetone 13.6mm. The results of solvent extracts disclose that the microbial activity of above two herbs are more in solvent extracts than in aqueous extracts [16].

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