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Biological Activities and Chemical Composition of *Cassia auriculata*

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ABSTRACT

The present study was designed to evaluate the antimicrobial, radical scavenging and chemical composition of aerial parts of *Cassia auriculata*. Three hundred grams of powdered material of *C. auriculata* extracted with methanol (CAMEt), C-18 silica gel based column chromatography was used to purify CAMEt using n-hexane, ethyl acetate and methanol fractions. Antimicrobial, DPPH radical scavenging effect of all the three fractions was determined and active fraction was characterized by GC-MS. The yield and colour of each fractions collected from column chromatography viz., fraction 1-3 (n-hexane: yellow) was 2.45 mg, (ethyl acetate: light orange) 1.78 mg and (methanol: light green) 5.25 mg, respectively. Fraction 3 recorded maximum zone of inhibition against *Pseudomonas aeruginosa*, *Aspergillus niger* and *Penicillium* sp. by fraction 3 antifungal and radical scavenging activity than compared to fractions. GC-MS results indicated 21 chemical constituents included alkanes, alcohol, esters and hydrocarbons. The major peak represented 1, 2-Benzenedicarboxylic acid and Di-(2-ethylhexyl) phthalate at 11.42% referred to NIST library. Further studies will be found in the mechanism of potential methanol fraction.

Key words: Chromatography, *Cassia auriculata*, DPPH, methanol, *Pseudomonas aeruginosa*

INTRODUCTION

Around the world, the ethno-medicinal plants still be an important beneficial aid for drug development for human kind (Nair *et al.*, 2008). The wide spread of multidrug resistant strains, which urge the discovery of new classes of compounds from plants (Lewis and Ausubel, 2006) to find out novel and non toxic therapeutic drug to overcome the side effects, caused the commercially available medicaments. A number of plants from different families of angiosperms have been recorded to show antimicrobial activity (Palombo and Semple, 2001). *Cassia auriculata* L. (Family: Caesalpinaceae) is an ethno botanically important shrub with attractive yellow flowers and commonly known as “Avaram” in Tamil. Historically, the aerial parts of the plant used to treat diabetes, conjunctivitis, rheumatism, astringent, anthelmintic, eye troubles, body odor, leprosy and liver disorders diseases (Anandan *et al.*, 2011; Hatapakki *et al.*, 2005). There are few reports available on antidiabetic, acute toxicity, hyperlipidemic, cardioprotective, antioxidant, antimicrobial and hepatoprotective activity (Devi *et al.*, 2006; Raj *et al.*, 2012). Chemical constituents such as protein, carbohydrate, alkaloids, flavonoids and tannin were reported from various parts of the plant (Purushotham *et al.*, 2014). There were no scientific data on aerial parts of *C. auriculata* and their antibacterial, antifungal and radical scavenging effect. Hence, the present study aimed to isolate and evaluate their biological effect of *C. auriculata* and characterized by GCMS.

MATERIALS AND METHODS

Plant material: Aerial parts of *Cassia auriculata* were collected from the district of Pudukkottai, Tamil Nadu, India during August-September 2012. The plant material was defined and authenticated by Dr. A. Kabeer, Scientist C, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. The specimen was deposited in the herbarium maintained at the Department of Biotechnology, Anna University, Tiruchirappalli (Voucher No.: AUBDT 0027/2012).

Extraction: *C. auriculata* aerial parts were shade dried, powdered and extracted with soxhlet apparatus using 80% methanol which is the widely used solvent for extraction at 55°C. The soluble part was concentrated over water bath maintained below 60°C and dried. The methanolic aerial parts extracts of *C. auriculata* (CAMEt) was used for further experiments.

Partial purification: Column chromatography was performed on a classic 20×2 cm diameter glass column packed with 15 g of silica gel maize size 120 cm (Hi Media, Mumbai, India). The methanol solution of the extract (20 mL) was applied to the column by use of a pipette and the column was eluted sequentially with n-hexane (fraction1-F1), ethyl acetate (fraction2-F2) and methanol (fraction3-F3). The column was not allowed to go dry throughout the experiment maintained with respect solvent. Each fraction (F1, F2 and F3) collected and evaporated to dryness and the residue was dissolved in 5 mL respective solvent used for experiment.

Microbial strains: Bacterial strains *Aeromonas hydrophilla*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi* and *Shigella* sp. and fungal strains *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *C. tropicalis*, *Epidermophyton floccosum*, *Mucor* sp., *Penicillium* sp. and *Rhizopus* sp. were obtained from the Department of Medical Microbiology, Government Medical College and Hospital, Tiruchirappalli, Tamil Nadu, India.

Antibacterial activity: The antibacterial activity of all the three fractions was followed by the agar diffusion method (Bauer *et al.*, 1966). A loopful bacterium was taken from the stock culture and dissolved in 0.1 mL of saline. All the tests were conducted by placing impregnated 6 mm diameter disc in 100 µL of F1, F2 and F3 separately on the Mueller Hinton Agar surface previously inoculated with 10 mL of MHA liquid medium with ten bacterial strains. Respective solvent without the fractions served as negative control and 30 µg discs⁻¹ of tetracycline were used as positive control. At the end of the incubation, inhibition zones formed around the disc were measured. The study was performed in triplicates and then the mean values were presented.

Antifungal activity: Antifungal activity for F1, F2 and F3 was performed by using the disc diffusion method by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The fractions applied to 6 mm sterile discs in aliquots of 30 µL of solvent, were allowed to dry at room temperature and placed on agar plates seeded with microorganisms. Cultures were maintained on fungal agar plates and incubated at 37°C for 24 h. Zones of growth inhibition were measured in millimetres after incubation. All the fractions were tested separately and triplicate.

DPPH scavenging effect: The free-radical scavenging activity of F1, F2 and F3 was measured by the decrease in absorbance of methanolic solution of DPPH (Gurudeeban *et al.*, 2013). Briefly

10, 20, 30, 40 and 50 μL aliquot of the each fraction separately mixed with 450 Tris-HCL buffer (50 mm 0.1/1, pH 7.4) and 1.0 mL DPPH (2,2-diphenyl, 2-picrylhydrazyl). After, 30 min of the reaction period the resultant absorbance was recorded at 515 nm. α -tocopherol (30 μg) used as positive control. The control was performed in the absence of fractions and percentage inhibition.

GC-MS analysis: The optimized potential fractions were selected for GC-MS analysis. The residue of F1 diluted with appropriate volume of methanol was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl and 95% dimethyl polysiloxane) (30 nm $\times$ 0.25 mm ID $\times$ 0.25 μm df) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carrier's gas at a flow rate of 1 mL min $^{-1}$ and the injector were operated at 290 $^{\circ}\text{C}$ and the oven temperature was programmed as follows; 50 $^{\circ}\text{C}$ at 8 $^{\circ}\text{C}$ min $^{-1}$ to 200 $^{\circ}\text{C}$ (5 min) at 7 $^{\circ}\text{C}$ min $^{-1}$ to 290 $^{\circ}\text{C}$ (10 min). The identification of unknown phytoconstituent was done by the interpretation of a database of the National Institute Standard and Technology and Wiley8 library (Ausloos *et al.*, 1999).

RESULTS

The yield of fraction 1-3 (n-hexane: yellow) was 2.45 mg, (ethyl acetate: Light orange) 1.78 mg and (methanol: pale green) 5.25 mg, respectively. In the present study, the antimicrobial activity was screened for F1 (hexane), F2 (ethyl acetate) and F3 (methanol) of *Cassia auriculata* were collected from column chromatography against pathogenic bacteria (10) and fungal (9) strains. The maximum zone of inhibition was recorded against *Pseudomonas aeruginosa* (10 mm) and *Staphylococcus aureus* (9 mm) from F3 of *C. auriculata* and the moderate zone of inhibition was observed in F1 and minimum zone of inhibition in F2 than compared to control (Fig. 1). In the antifungal assay, the maximum zone of inhibition was observed against *Aspergillus niger* and *Penicillium* sp by F3 (Fig. 2).

The free radical scavenging effect of five different concentration of F1, F2 and F3 increased with an increase in their concentration (Table 1). DPPH is a stable radical generator and has the capability to capture free radicals is due to the delocalization of the unpaired electron all over the

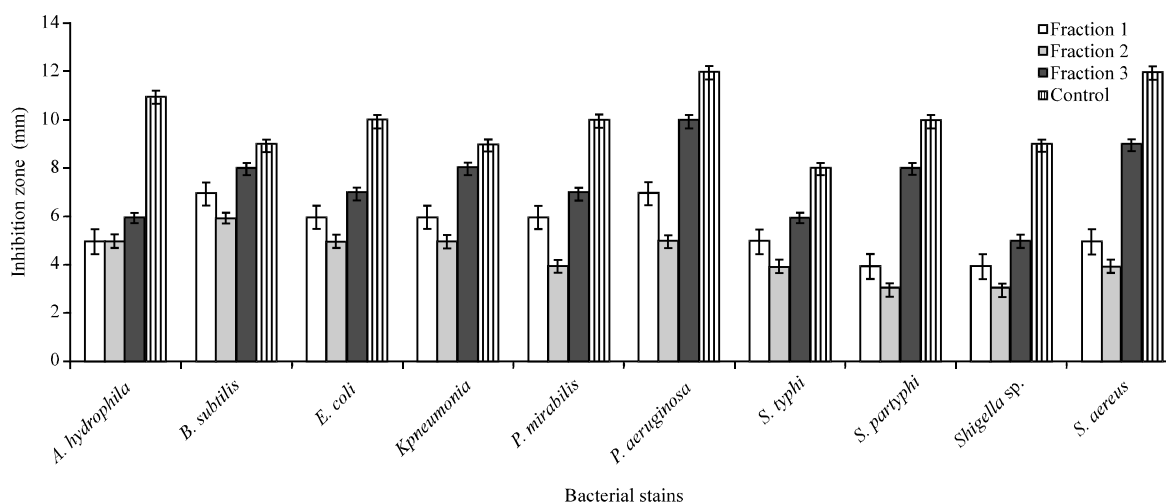


Fig. 1: Antibacterial effect of three different fractions of *C. auriculata*

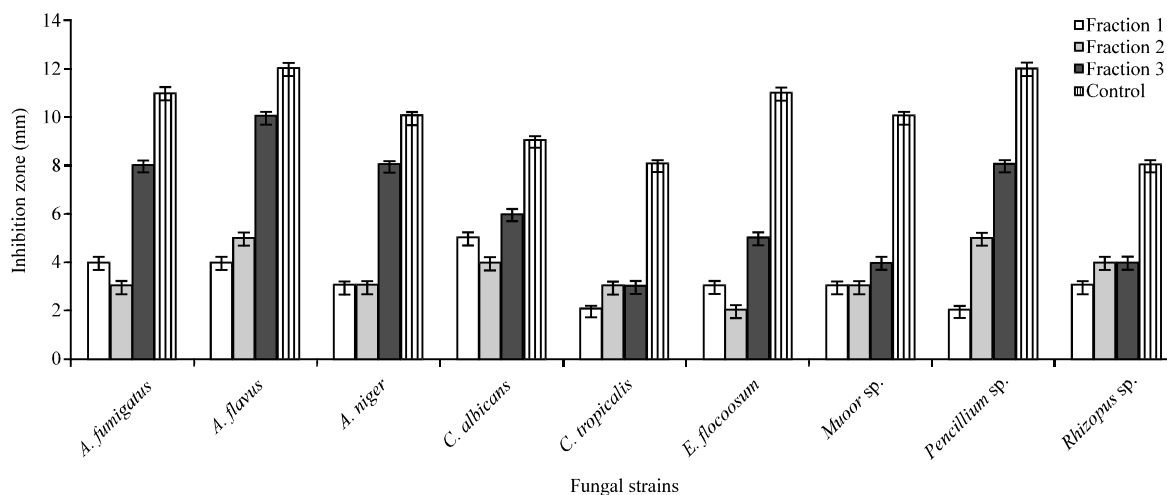
Fig. 2: Antifungal effect of three different fractions of *C. auriculata*

Table 1: DPPH radical scavenging activity (%) of fractions

Concentration ($\mu\text{g mL}^{-1}$)	Scavenging fraction (%)		
	1	2	3
10	59.44 $\pm$ 1.01	62.44 $\pm$ 1.65	65.72 $\pm$ 1.87
20	59.21 $\pm$ 1.11	61.44 $\pm$ 2.23	69.77 $\pm$ 1.33
30	63.73 $\pm$ 2.10	66.76 $\pm$ 1.13	74.28 $\pm$ 1.22
40	70.11 $\pm$ 1.76*	72.55 $\pm$ 2.03*	83.76 $\pm$ 1.41*
50	82.98 $\pm$ 2.66*	78.49 $\pm$ 1.43*	87.66 $\pm$ 1.66*
α -tocopherol (30)	84.33 $\pm$ 0.21		

Values represent Mean $\pm$ SD of 3 replicates, * p <0.05 vs. α -tocopherol

molecules. The violet colour of DPPH fades into the yellow colour of its reduced congener (DPPH-H) with a high shift in the visible spectra (520-330 nm). Among these, the F3 was showed a high percentage of DPPH radical scavenging activity than compared to F1 and F2.

Among the three, the optimized F3 were analyzed by GC-MS. It revealed the presence of 21 compounds and the peak ranges from 1.01-11.42%. The retention time, percentage of peak area, molecular formula and molecular weight of each compound was showed in Table 2. GC-MS chromatogram was showed in the Fig. 3. The identified compounds were broadly divided in to acid, alcohol, alkenes, esters and hydrocarbons. The major peak area showed the presence of 1,2-Benzenedicarboxylic acid and Di-(2-ethylhexyl) phthalate at 11.42% referred with NIST library.

DISCUSSION

The selection of plant samples and extraction solvent played a key role in chemical composition isolation and its biological activities determination. In this concern, the most widely used methanol has been found for extraction of polyphenols with extraordinary penetration ability and safe for human consumption (Metivier *et al.*, 1980). Hence the effective CAMEt has been extracted from aerial parts of *C. auriculata*. Using Colum chromatograph, the three active fractions were collected and showed their antibacterial and antifungal activity which might be due to presence of anti biologically active compound (Singh and Mishra, 2010).

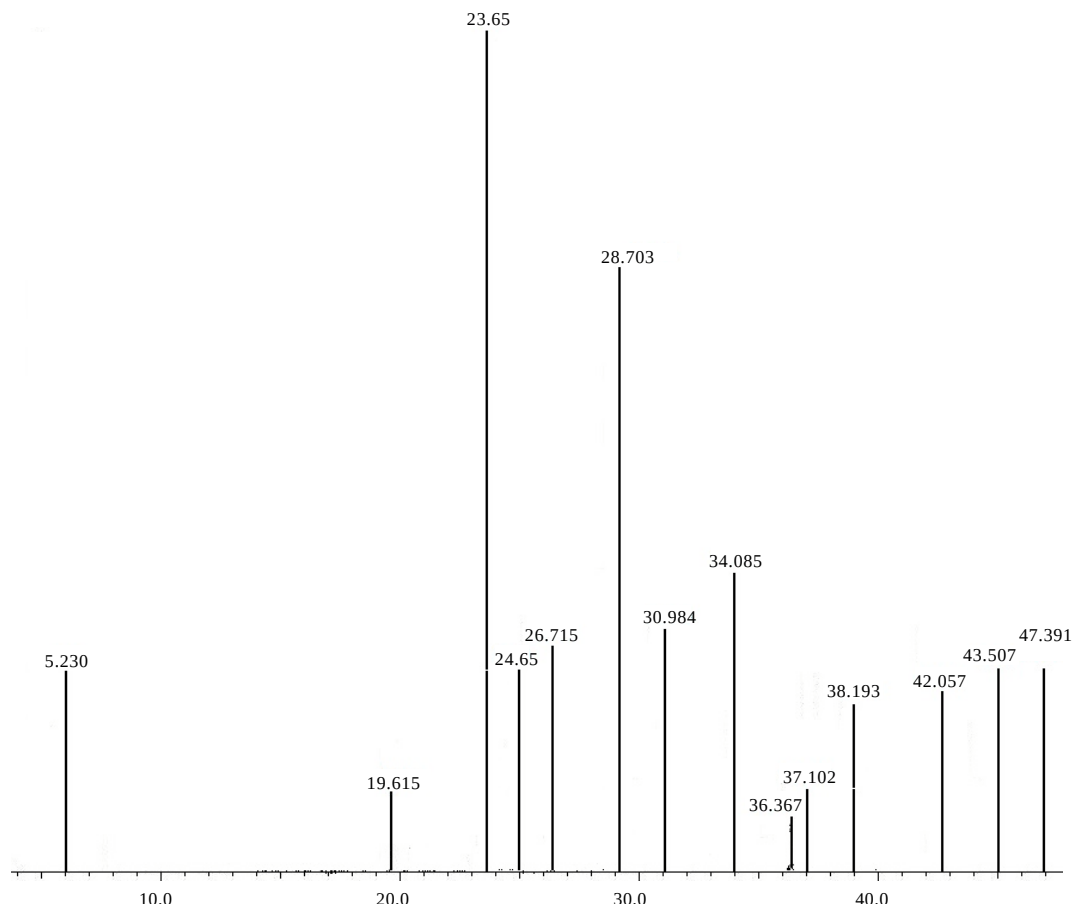


Fig. 3: GC-MS chromatogram of methanolic fraction of *C. auriculata*

It is well known the *E. coli* bacteria are inhabit in human intestine. Those are normally harmless but certain harmful strains bind to epithelial wall causes release of toxin that adversely affects the intestinal (Tortora *et al.*, 1998). Also fractions exhibited antibacterial activity to *E. coli*. *Aspergillus flavus* released aflatoxin which contaminating the food leads to cirrhosis of liver and cancerous growth which are prevalent in India and Africa (Tortora *et al.*, 1998), our results showed the F3 significant antifungal activity against *Aspergillus* sp. It revealed the microbial activity examined in the ethanolic (F3) of the study plant could be economically significant. Purushotham *et al.* (2014) reported there was a strong correlation was found between the phenolic content and antioxidant activity of the (0.2 mL) of ethanolic extracts of root (766.7%), leaf (679.3%) and flower (644.9%). DPPH scavenging activities of fractions of *C. auriculata* might be attributed to phenolic compounds redox properties, which allow them to act as potential free radical scavengers (Vladimir-Knezevic *et al.*, 2011). 90.66% of DPPH radical scavenging was found in F3. Diethyl phthalate is a suitable solvent for many organic molecules, cosmetics and fragrances also used as plasticizers, detergent bases and aerosol sprays industries (WHO, 2008).

Table 2: Chemical composition of methanolic fraction of *C. auriculata* by GC-MS

Compound name	Retention time	Peak area (%)	Molecular formula	Molecular weight
Mome Inositol	20.583	1.01	C ₇ H ₁₄ O ₆	194
α -mannofuranoside, methyl	20.583	1.01	C ₇ H ₁₄ O ₆	194
Neophytadiene 2, 6, 10-Trimethyl, 14-Ethylene-14-Pentadecne	23.631	4.96	C ₂₀ H ₃₈	278
Hexadecane	23.631	4.96	C ₂₀ H ₄₀ O	296
9-Eicosyne	24.650	1.74	C ₂₀ H ₃₈	278
Cyclopentene, 3-heptyl-	24.650	1.74	C ₁₂ H ₂₂	166
Pentadecane	26.915	2.32	C ₁₅ H ₃₀ O ₂	242
Palmitic acid	26.915	2.32	C ₁₆ H ₃₂ O ₂	256
1, 2-Benzenedicarboxylic acid	28.703	11.42	C ₈ H ₆ NO ₆	211
Di-(2-ethylhexyl) phthalate	28.703	11.42	C ₂₄ H ₃₈ O ₄	390
9, 12, 15-Octadecatrienoic acid	30.650	2.36	C ₁₉ H ₃₂ O ₂	292
Ethyl Linoleolate	30.650	2.36	C ₂₀ H ₃₆ O ₂	308
11, 14, 17-Eicosatrienoic acid	30.650	2.36	C ₂₁ H ₃₆ O ₂	320
Pentadecanoic acid	30.984	2.26	C ₁₅ H ₃₀ O ₂	242
Octadecanoic acid	30.984	2.26	C ₁₈ H ₃₆ O ₂	284
Ethyl linoleolate	34.266	1.26	C ₂₀ H ₃₆ O ₂	308
Lycopersen	37.102	1.56	C ₄₀ H ₆₆	547
2, 6, 10, 15, 19, 23-Hexamethyl-2, 6, 10, 14, 18, 22-Tetracosahexaene	37.102	1.56	C ₃₀ H ₅₀	410
8, 11-Eicosadienoic acid, methyl ester	38.638	1.01	C ₂₁ H ₃₈ O ₂	322
Ethyl linoleolate	38.759	1.25	C ₂₀ H ₃₆ O ₂	308
Dotriacontane	42.057	2.71	C ₃₂ H ₆₆	451
Tetracontane	42.057	2.71	C ₄₀ H ₈₂	563
Tetrapentacosan	47.391	2.05	C ₅₄ H ₁₁₀	759
Dotriacontane	47.391	2.05	C ₃₂ H ₆₆	451

A number of metabolites and its derivatives were determined by GC-MS from various parts of the plant. For example, the bis (2-ethylhexyl) phthalate, were successfully identified from the root of medicinally valuable plant *Arbutus unedo* (Dib *et al.*, 2013). Hence in the present study, the fraction 3 has Di-(2-ethylhexyl) phthalate with highest peak area 11.42% then compared to others. During the samples processing and drying, may be some volatiles active compounds destroyed or evaporated from the samples. Earlier reports showed the presence of higher alkanes in the *Excoecaria agallocha* using GC-MS (Satyavani *et al.*, 2012). In accordance with that, hexadecane and pentadecane were observed in peak area of 4.96 and 2.32%. Palmitic acid is used primarily to produce soaps, cosmetics and release agents. Sodium palmitate is inexpensive and adds texture to process foods and natural additive in organic products. Rats fed a diet of 20% palmitic acid and 80% carbohydrate for extended periods showed alterations in central nervous system control of insulin secretion and suppression of the body's natural appetite-suppressing signals (Ghorpade *et al.*, 2002). However, the GC-MS chromatogram showed the palmitic acid at 2.32%.

CONCLUSION

The potential antibiotic and radical scavenger of F3 might be the action of Di-(2-ethylhexyl) phthalate and 1, 2-Benzenedicarboxylic acid were confirmed by GCMS.

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