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Evaluation of Antibacterial Activity of Flower Extracts of *Cassia auriculata*

S. Maneemegalai [1]* and T. Naveen[2]

[1]Department of Biochemistry, Bharathidasan University College for Women, Orathanadu – 614 625, Tamilnadu, India

[2] Department of Biochemistry, Prince Shri Venkateshwara Arts and Science College, Gowrivakkam, Chennai-600 073. Tamilnadu, India

*Coresponding Author: Email: maneedevi@yahoo.co.in

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Abstract

Since plants are used as therapeutic agents, the present study was conducted to evaluate the phytochemical profile and antibacterial activities of flower extracts of *Cassia auriculata*. Studies on the antibacterial activity of ethanol, methanol and aqueous extracts of dry flower and ethanol, methanol and acetone extracts of fresh flower of *Cassia auriculata* was conducted using agar disc diffusion method. The microorganisms used include *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi A, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio cholerae* and *Shigella dysentrae.* The maximum activity was observed against all organisms except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The minimum inhibitory concentration ranged between 12.5mg/mL and 75mg/mL depending on microorganism and various extract. Presence of phytochemicals such as terpenoids, tannins, flavonoids, saponin, cardiac glycosides and steroids were observed. *Cassia auriculata* was observed to have antibacterial activity and can be used for medicinal purposes.

Key words: Acetone extract, bacteria, *Cassia auriculata,* ethanol extract, methanol extract, phytochemicals.

Introduction

With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases. The healing potential of many plants have been utilized by Indian traditional medicines like Siddha, Ayurvedha and Unani. Being nontoxic and easily affordable, there has been a resurgence in the consumption and demand for medicinal plants (Jayashree and Maneemegalai, 2008).

Cassia auriculata L. commonly known as tanners cassia, also known as "avaram" in Tamil language is a shrub belongs to the Caesalpiniaceae family. The shrub is specially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits anthelminthic, seeds used to treat in eye troubles and root employed in skin diseases (Siva and Krishnamurthy,2005). It is also used for the treatment of ulcers, leprosy and liver disease (Kumar *et al*., 2002). The antidiabetic, hypolipidemic (Umadevi *et al*., 2006) and antioxidant (Kumaran and Joel, 2007) and hepatoprotective (Kumar *et al*., 2003) effect of *Cassia auriculata* have been reported. It was also observed that flower and leaf extract of *Cassia auriculata* shown to have antipyretic activity (Vedavathy and Rao, 1991).

The aim of the present study was to determine the antibacterial activity of various extracts of *Cassia auriculata* flowers which is having traditional claims for several diseases

Materials and Methods

Plant material

The flowers of *Cassia auriculata* were collected from Tiruchirappalli, Tamilnadu, India and authenticated by Prof. P. Jayaraman, Director of Plant Anatomy Research Centre (PARC) Chennai-600 045, following identification a voucher specimen of the plant was deposited in the herbarium of

Plant Anatomy Research Centre (PARC 2007/92). The flowers were examined carefully and old, infected, and fungus damaged flowers were removed. Extracts were prepared from dried and also from fresh flower. Healthy flowers were spread out and dried at room temperature for about ten days and ground into fine powder using electric blender.

Preparation of extract

Extract from dried flower

Methanol extract was prepared by taking 50 g of *Cassia auriculata* dried flower powder in a separate container, to this 200mL of methanol was added and kept for 24 h in a shaker. Filtered through eight layers of muslin cloth and extract was collected, the extraction process was repeated twice. The collected extracts were pooled. Ethanol extract was prepared like methanol extract. Water extract was prepared by taking 50 g of *Cassia auriculata* flower powder in a separate container, to this 200mL of water was added and boiled for 2 h in a mild heat and kept for 24 h. Then filtered and extract was collected. The extraction process was repeated twice. Then the collected filtrates were pooled (Akpulu *et al*., 1994).

Extract from fresh flower

50g of fresh flower of *Cassia auriculata* was ground with 200mL of acetone and kept in a shaker for 24 h and then filtered and the extract was collected. The procedure was repeated twice. Like the same way methanol and ethanol extracts were prepared using 100ml of solvents.

The various extracts of *Cassia auriculata* flower were concentrated using vacuum evaporator and dried at 60°C. The dried extract powder was used for the study and screened against selected bacterial strains.

Phytochemical screening

Phytochemical screening of plant extract was carried out qualitatively for the presence of terpenoids, tannin, flavonoids, saponin, cardiac glycosides and steroids (Faraz *et al*., 2003; Harborne, 1998; Edeogo *et al*., 2005).

Antibacterial assay

Microorganisms tested

The organisms used for this study include *Staphylococcus aureus*-ATCC 25923, *Enterococcus faecalis*-ATCC 29212, *Bacillus subtilis*-ATCC 9372, *Salmonella typhi*-MTCC 531, *Salmonella paratyphi* A-MTCC 735, *Escherichia coli*- MTCC 1687, *Proteus mirabilis*- MTCC 425, *Pseudomonas aeruginosa*-ATCC 27853, *Klebsiella pneumoniae*- MTCC 618, *Vibrio cholerae*- Clinical isolate and *Shigella dysentrae*-Clinical isolate were obtained from Department of Microbiology, Prince Shri Venkateshwara Arts and Science College, Gowrivakkam, Chennai, India.

In vitro determination of antibacterial activity

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of colonies from the stock culture to peptone water and incubated for 4h at 37°C. Antibacterial activity was determined by agar disc diffusion method (Bauer *et al*., 1966) . Standard suspension of bacteria was inoculated on the surface of Muller-Hinton (Himedia) agar plates. Dimethyl Sulphoxide and Methanol (1:1) was used to dissolve the plant extract. Sterilized filter paper discs (5mm) containing 20µL of each extract (100mg/mL) were arranged on the surface of the inoculated plates and incubated at 37°C for 18-24h. Along with this 30µg tetracycline disc (Himedia standard) was studied for antimicrobial activity as a positive control whereas the solvent used for preparing extract was used as a negative control. At the end of incubation, inhibition zones formed around the disc were measured with Himedia zone
scale. The study was performed in triplicate and the mean values were presented.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration values were determined by broth dilution assay. Varying concentrations of the extracts (200mg/mL, 150mg/mL, 100mg/mL, 50mg/mL, 25mg/mL and 12.5mg/mL) were prepared. 0.1mL of each concentration was added to each 9mL of nutrient broth containing 0.1mL of standardized test organism of bacterial cells. The tubes were incubated at 37°C for 24h. Positive controls were equally set up by using solvents and test organisms without extracts. The tube with least concentration of extract without growth after incubation was taken and recorded as the

minimum inhibitory concentration (Atata *et al*., 2003).

Result and Discussion

The present study carried out on the plant extract revealed the possession of medicinal activities. The % yield of various extracts of *Cassia auriculata* were shown in Table 1 and phytochemicals present in *Cassia auriculata* were represented in Table 2.

Table 1. % yield of various extracts of Cassia *auriculata*.

Extract	Dry Flower (%)	Fresh Flower (%)
Methanol	14	16
Ethanol	15.5	17
Acetone	-	15
Aqueous	12	-

Terpenoids and cardiac glycosides were present in all extracts of *Cassia auriculata*. Tannins were present in ethanol and acetone extract but absent in methanol and aqueous extract. Flavonoids and steroids were present in ethanol, methanol and acetone extracts where as saponin was found to be present in ethanol, methanol and aqueous extracts of *Cassia auriculata*. Presence of phytochemicals saponins, tannins (Balasooriya *et al*., 1982; Sabu and Subburaju,2002) in *Cassia auriculata* have been reported. Antibacterial activity of phytochemicals flavonoids (Tsuchiya *et al*., 1996), saponin(Soetan *et al*., 2006) and cardiac glycosides (Ebana *et al*., 1991) isolated from plant materials have been studied. The antibacterial activity of *Cassia auriculata* flower suggests that the extract contains the

effective active phytochemicals responsible for the elimination of microorganisms.

Table 2.Qualitative analysis of the phytochemicals in the flower extract of *Cassia auriculata.*

	EE	ME	AE	AqE
Terpenoids	+	+	+	+
Tannins	+	−	+	−
Flavonoids	+	+	+	WP
Saponin	+	+	WP	+
Cardiac glycoside	+	+	+	+
Steroids	+	+	+	−

EE = Ethanol extract, ME= Methanol extract, AE = Aqueous extract, AqE = Aqueous extract, WP = Weakly positive.

Invitro antibacterial activity of ethanol, methanol extracts of dry and fresh flowers, aqueous extract of dry and acetone extracts of fresh flowers of *Cassia auriculata* were observed in Table 3.

Table 3. In vitro antibacterial activity of ethanol, methanol and aqueous extracts of dry flowers and ethanol, methanol and acetone extracts of fresh flowers of *Cassia auriculata*. (values are mean of three replicates).

Organisms	Dry flower			Fresh flower			TC	DMSO:M
	EE	ME	AqE	EE	ME	AE		
	(2mg)			(2mg)			(30 µg)	(20µL)
	Zone of Inhibition (mm)							
S. aureus	15	16	18	13	13	16	25	Nil

E. faecalis	17	18	18	16	17	18	20	Nil
B.subtilis	12	12	13	9	13	13	24	Nil
S.typhi	17	16	13	16	14	19	24	Nil
S. paratyphi A	16	15	14	14	14	17	28	Nil
E.coli	11	15	18	9	7	18	22	Nil
P.mirabilis	14	13	14	15	14	14	16	Nil
P. aeruginosa	7	7	7	9	6	7	14	Nil
K. pneumonia	6	8	7	7	7	6	15	Nil
V. cholerae	17	17	9	15	17	18	24	Nil
S. dysentrae	13	18	15	17	13	19	20	Nil

EE= ethanol extract, ME = Methanol extract, AqE = Aqueous extract, AE = Acetone extract, TC = tetracycline, DMSO:M = Dimethyl sulphoxide: Methanol.

The extracts were found to have maximum activity against all organisms except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Acetone extracts of fresh flower was found to have maximum activity compared to ethanol and methanol extracts against all microorganisms except *Proteus mirabilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Aqueous extract of dry flower of *Cassia auriculata* was shown to have minimum activity against *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Vibrio cholerae*. The present investigation confirmed the antimicrobial activity of flower extract of *Cassia auriculata*. Perumalsamy and Ignacimuthu(2000) reported that the leaf extracts of *Cassia auriculata* exhibited significant broad spectrum activity against *Bacillus subtilis* and *Staphylococcus aureus*. Antimicrobial activity of *Cassia auriculata* flower extract has been observed by Narayanan *et al*.,(2007). The extract of *Cassia auriculata* was found to have potent microbicidal activity against the *E.coli* in poultry (Prakash,2006). In this study ethanol and methanol extract of dry and fresh flower, aqueous extract of dry flower, acetone extract of fresh flower was found to have higher inhibitory activities against *Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus mirabilis, Salmonella typhi, Salmonella paratyphi A, Vibrio cholerae* and

Shigella dysentrae when compared to *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

The minimum inhibitory concentration ranged between 12.5mg/mL and 75mg/mL depending on microorganism and various extracts were shown in Table 4. The result of minimum inhibitory concentration suggests that ethanol and aqueous extract of fresh flower of *Cassia auriculata* could possibly act as a bactericidal agent to these microorganism. Except *Staphylococcus aureus*, *Enterococcus faecalis, Salmonella typhi* and *Salmonella paratyphi* A all other microorganisms were inhibited by the lowest concentration (12.5mg/mL) of ethanol and methanol extract of dry flower.

Table 4. Minimum inhibitory concentration of ethanol, methanol extracts of dry flower and ethanol, methanol and acetone extracts of fresh flowers of *Cassia auriculata* against microorganisms. (values are mean of three replicates).

Organisms	Dry flower (mg/mL)		Fresh flower (mg/mL)		
	EE	ME	EE	ME	AE
S. aureus	75	50	12.5	50	12.5
E. faecalis	25	75	12.5	75	12.5
B.subtilis	12.5	12.5	25	12.5	12.5
S.typhi	50	50	12.5	75	12.5
S. paratyphi A	50	50	12.5	75	12.5
E.coli	12.5	12.5	12.5	75	12.5
P.mirabilis	12.5	12.5	12.5	Nil	12.5
P. aeruginosa	12.5	12.5	12.5	50	12.5
K. pneumonia	12.5	12.5	12.5	50	12.5
V. cholerae	12.5	12.5	12.5	12.5	12.5
S. dysentrae	12.5	12.5	12.5	12.5	12.5

EE= ethanol extract, ME = Methanol extract, AE = Acetone extract.

Conclusion

The present study exhibited the antibacterial effect of various extracts of *Cassia auriculata*. The inhibitory effect of the extract justified the medicinal use of *Cassia auriculata* and further study is required to find out the active component of medicinal value.

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