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ANTIBACTERIAL ACTIVITY OF *CASSIA AURICULATA* LEAVES

Gaurav M. Doshi^{1*}, Gayatri V. Aggarwal¹, Ojas Kumar Agrawal¹, Abhijeet R. Bhalerao²

1. VES College of Pharmacy, Hashu Advani Memorial Complex, Collectors Colony, Chembur (East), Mumbai – 400074. India
2. Principal K. M. Kundnani College of Pharmacy, Plot no. 23, Jote Joy Bldg. Rambhau Salgaonkar Marg, CuffeParade, Colaba, Mumbai 400 005. India

ABSTRACT

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For Correspondence:

Gaurav M. Doshi

VES College of Pharmacy, Hashu Advani Memorial Complex, Collectors Colony, Chembur (East), Mumbai – 400074. India

E-mail:

gaurav.pharmacology@gmail.com

The existence of traditional medicine depends on plant species diversity and related knowledge of their use as herbal medicine and its importance is correlated to herbal pharmaceutical industry which utilizes herbal raw materials to obtain wide range of herbal products. *Cassia auriculata* has enormous important amount of pharmacological actions hence present study was undertaken to evaluate its efficacy against gram positive and gram negative microorganisms. Antibacterial potential of methanolic extract of *Cassia auriculata* leaves was conducted using agar disc diffusion method. The microorganisms used include *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*. The maximum activity was observed against all microorganisms the minimum inhibitory concentration was determined depending on microorganisms. *Cassia auriculata* leaves were observed to have antibacterial activity used to combat against vast flora of different microorganisms which are omnipresent.

INTRODUCTION

India has 2.4% of world's area with 8% of global biodiversity. Across the country, the forests of India are estimated to harbor 90% medicinal plants diversity. The estimated numbers of plant species and those used for medicine purpose vary. Only One – fifth of all the plants found in India are used for medicinal purpose. The world average stands at 12.5% while India has 20% plant species of medicinal value. India has about 44% of flora, which is used medicinally. Although it is difficult to estimate the number of medicinal and aromatic plants present worldwide, the fact remains true that India with rich biodiversity ranks first in percent flora, which contains active medicinal ingredient [1].

With various microorganisms rising at instance and developing resistance to different antibiotics there is an utmost importance to develop new herbal antimicrobial compounds. Traditional medicine has served as a source of alternative medicine, new pharmaceuticals and healthcare products with wide era of healing potential. Since ancient times plants have been used to treat common infectious diseases [2].

Cassia auriculata Linn; commonly known as Tanner *Cassia* has great contribution in Ayurvedic medicine as **Avarai Panchaga Choornam** and main constituent of **Kalpa Herbal tea**. The annual production of this species in Madras is about 11,000 tons and imports

from Mysore and Hyderabad together is 12,000 tons. The leaves are 7.5-10cm long, rachis densely fulvous pubescent with an erect linear gland between each pair of leaflets, stipules foliaceous reflexed, very large, rotundate – reinform produced at the base on the side next the petiole into long sub acute point persistent. They are anthelmintic, good for ulcers, leprosy, skin diseases. Coffee made from the leaves is surely prescribed in giddiness due to heart disease. The leaves are made into tea in Ceylon called Matra which resembles ordinary tea in appearance when properly prepared. The infusion is aromatic and slightly purgative [3]. The plant has been reported to antibacterial and microbicidal activity [4,5].

The present study of the leaves focuses on the probable role of *Cassia auriculata* as antibacterial agent. Due to its ample flora of widespread actions, its existence towards four different microorganisms was evaluated.

MATERIALS AND METHODS

Plant collection and identification

Leaves of *Cassia auriculata* were collected from Nyeveli, Cuddalore district, Tamil-Nadu, India in month of August-September. The plant material was identified and authenticated at Agarkar Research Institute, Pune. A voucher specimen (No.3/386 /2006/Adm.7646) was deposited in the botany department of Agarkar Research Institute, Pune.

Preparation of the plant extract

Cassia auriculata leaves were dried in an oven below 60°C for 2 hrs. They were finely powdered and extracted with 80 % aqueous methanol using Soxhlet apparatus at 55°C. The soluble part was concentrated over water bath maintained below 60°C and dried in a vacuum oven to obtain free flowing reddish brown powder. The extract obtained was termed as methanolic extract of *Cassia auriculata* (MECA).

Phytochemical screening

Phytochemical screening of plant extract was carried out qualitatively for the presence of terpenoids, tannin, flavonoids, saponin, cardiac glycosides and steroids [6].

Antibacterial assay

Microorganisms tested

The organisms used for this study include *Staphylococcus aureus*-ATCC 25923, *Bacillus subtilis*-ATCC 9372, *Salmonella typhi*-MTCC 531, *Escherichia coli*- MTCC 1687 were obtained from Department of Microbiology, Vivekanand Education Society's college of Arts, Science and Commerce, Mumbai, India.

In vitro determination of antibacterial activity

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiment was 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 [7]. Standard suspension of bacteria was inoculated on the surface of Muller-Hinton (Hymenia) 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) was 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 extract (200mg/mL, 150mg/mL, 100mg/mL, 50mg/mL, 25mg/mL and 12.5mg/mL) was 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. A positive control was equally set up by using solvents and test organisms without extract. The tube with

least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration [8].

Result and Discussion

Table No 1: Percent yield of the extract

Sr. No.	Name of Extract	Yield
1.	MECA extract	16.2 % w/w

Preliminary Phytochemical present in the extract

Percent yield of the extract

The extraction yield of dried powder of *Cassia auriculata* leaves for 80 % aqueous methanolic extract was as shown in Table-1.

MECA has shown presence of carbohydrates (reducing sugars), saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds as reported in Table-2.

Table No 2: Preliminary Phytochemical present in the extract

Sr. No.	Test / Reagent	Positive (+ ve) or Negative (-ve)
1.	For Carbohydrates- Molish's test	+ ve
2.	Reducing sugars -Fehling's test -Benedict's test	+ve
3.	Tests for Saponin glycosides- Foam test	+ve
4.	Flavonoids - Shinoda test	+ve
5.	Alkaloids -Dragendorff's test - Hager's test	+ve

	- Wagner's test - Mayer's test	
6.	Tests for Tannins and Phenolic compounds -5% fecl ₃ solution - Bromine water -Dilute iodine solution	+ve

In vitro antibacterial activity of aqueous methanolic extract of leaves

MECA was found to have maximum activity against all organisms. The present investigation confirmed the antimicrobial activity of leaves extract of *Cassia auriculata*. Antimicrobial activity of *Cassia auriculata* flower extract has been reported previously observed [9]. The extract of *Cassia auriculata* was found to have potent microbicidal activity against the *E.coli* in poultry[5]. In this study methanolic extract of leaves was found to have higher inhibitory activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*. The minimum inhibitory concentration ranged between

12.5mg/mL and 75mg/mL depending on microorganism and extract were reported in Table 3 and Table 4.

Table No 3: *In vitro antibacterial activity of aqueous methanolic extract of leaves (values are mean of three replicates).*

Microorganisms	Methanolic Leaves extract (2mg)	Tetracycline (30 µg)	DMSO:M (20µL)
Diameter of Zone of Inhibition (mm)			
<i>S. aureus</i>	17	26	NIL
<i>B.subtilis</i>	15	25	NIL
<i>S.typhi</i>	14	22	NIL
<i>E.coli</i>	15	22	NIL

Table 4. Minimum inhibitory concentration of *Cassia auriculata* leaves against microorganisms. (Values are mean of three replicates).

Microorganisms (mg/mL)	Methanolic extract of <i>Cassia auriculata</i> leaves (mg/mL)
<i>S.aureus</i>	50
<i>B.subtilis</i>	12.5
<i>S.typhi</i>	50
<i>E.coli</i>	12.5

Conclusion

The present studies suggested that *Cassia auriculata* leaves methanolic extract have profound antibacterial potential. The inhibitory effect of the extract justified the medicinal property of *Cassia auriculata* leaves and further study is required to investigate the active component which plays a vital role as antibacterial agent against wide range of microorganisms.

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