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ANTIBACTERIAL POTENTIAL OF *CASSIA AURICULATA* ROOTS

Gaurav M. Doshi [1]*, Gayatri.V.Aggarwal[1] , Preeja.G. Pillai

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

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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

ABSTRACT

A significant number of modern pharmaceutical drugs are thus based on or derived from medicinal plants which serve as potential source of therapeutic aids in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaing proper health. *Cassia auriculata* termed as avaram has numerous pharmacological actions hence present study was undertaken to evaluate its efficacy against gram positive and gram negative microorganisms. Antibacterial activity of methanolic extract of *Cassia auriculata* roots 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* was observed to have profound antibacterial potential against vast flora of microorganisms.

INTRODUCTION

About 25% of modern medicines are descended from plants first used traditionally according to WHO. Many others are synthetic analogues built on prototype compounds isolated from plants. Almost, 70 % modern medicines in India are derived from natural products[9]. In the dual role as a source of healthcare and income, medicinal plants make an important contribution to the larger development process[1]. The healing potential of many plants have been utilized by Indian traditional medicines like Siddha, Ayurvedha and Unani[2].Microrganisms are widely spread around the ecological environment and have attained maximum resistance towards majority of antibiotic compounds. Hence, its high probable time to use different medicinal plants to check its utility against different microorganisms.

Cassia is a genus of Fabaceae (subfamily Caesalpiniaceae). *Cassia* species are used as food plants by the larve Lepidoptera species including *Endoclita malabaricus*[3]. Its great contribution in Ayurveda is as main constituent of Avarai Panchaga Choornam and Kalpa Herbal tea[4]. Dr. P .S. Mootooswamy informs us that the root of the plant in Tanjore is used in decoction as an alternative as well as medicinal –oil prepared from the bark in Tamil called as *averai – yennai*[5]. The root of the plant in Tanjore is used in decoction as alternative as well as medicinal oil prepared from the bark in Tamil called as *averai – yennai*. The roots are alexeteric, useful in thirst, urinary discharges, cures tumors, skin diseases, and asthma causes flatulence. They are astringent, cooling alterative, depurative and used in diabetes [6]. The plant has been reported to posses' antibacterial and microbicidal activity[7, 8].

The present studies of the roots enlighten the probable role of *Cassia auriculata* as antibacterial agent due to resistance developed against different existing antibiotics.

MATERIALS AND METHODS

Plant collection and identification

Roots 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 roots were dried in an oven below 60^{o}C for 2 hrs. They were finely powdered and extracted with 80 % aqueous methanol using Soxhlet apparatus at 55^{o}C. The soluble part was concentrated over water bath maintained below 60^{o}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 [9].

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[10]. 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 [11].

Result and Discussion

Percent yield of the extract

The percentage yield of the extract of *Cassia auriculata* roots was found to be 15.2% w/w.

Preliminary Phytochemical present in the extract

MECA has shown presence of carbohydrates (reducing sugars), saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds as reported in Table-1.

Table No 1:

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 - Wagner's test - Mayer's test	+ve
6.	Tests for Tannins and Phenolic compounds -5% $fecl_3$ solution - Bromine water -Dilute iodine solution	+ve

Table No 2: In vitro antibacterial activity of *Cassia auriculata* roots (values are mean of three replicates).

Microorganisms	***Cassia auriculata* roots extract (2mg)**	**Tetracycline (30 µg)**	**DMSO:M (20µL)**
Diameter of Zone of Inhibition (mm)			
S. aureus	15	25	NIL
B.subtilis	12	24	NIL
S.typhi	15	24	NIL
E.coli	15	22	NIL

The roots extract was found to have maximum activity against all organisms with significant zone of inhibition towards *Staphylococcus aureus.* The extract of *Cassia auriculata* was found to have potent microbicidal activity against the *E.coli* in poultry[6]. In the present study methanolic extract of roots 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.

Table 3. Minimum inhibitory concentration of *Cassia auriculata* roots against microorganisms. (Values are mean of three replicates).

Microorganisms **(mg/mL)**	**Methanolic extract of *Cassia auriculata* roots** **(mg/mL)**
S.aureus	25
B.subtilis	12.5
S.typhi	25
E.coli	12.5

Conclusion

The present studies suggested that *Cassia auriculata* roots methanolic extract have significant antibacterial potential. The inhibitory effect of the extract justified the medicinal use of *Cassia auriculata* roots and further study is required to find out the active component which is of utmost medicinal value against wide range of microorganisms.

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