



## IN VITRO ANTIMICROBIAL ACTIVITY OF CASSIA AURICULATA L. : AN ETHNOMEDICINAL PLANT

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Crude extracts were prepared from the leaves, stem and pods of *Cassia auriculata* L. using different solvents viz. methanol, ethanol, aqueous, petroleum ether, acetone and screened for their antibacterial and antifungal activity against test microorganisms. The tested microorganisms were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*. Among the plant extracts the methanol, acetone and ethanol extracts of leaf showed higher antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Methanol and ethanol extracts of pods also exhibited higher antimicrobial activity against all test organisms which were most effective than the standard reference antibiotic (Streptomycin). In case of fungi acetone extract of leaf had significant antifungal activity.

**Keywords :** *Cassia auriculata* L., plant extracts, antimicrobial activity.

Plant based antimicrobials represents a vast untapped source for medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plant origin have enormous therapeutic potential ( Salau and Odeleye 2007). In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases (Mohanasundari *et al.* 2007). In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents (Towers *et al.* 2001).

Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents ( Werner *et al.* 1999, Samy and Ignacumuthu 2000). Different extracts from traditional medicinal plants have been tested. Many reports have shown the effectiveness of traditional herbs against microorganisms as a result, plants are one of the bedrocks for the modern medicine to attain new principles (Evans *et al.* 2002).

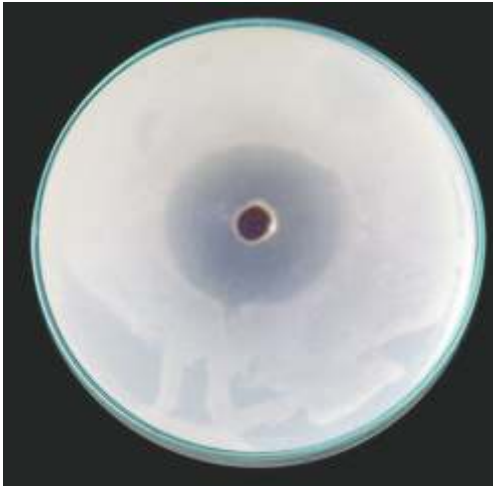
Biomolecules of plant origin appear to be

alternatives for the control of these antibiotic resistant human pathogens (Raghavendra *et al.* 2006). Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this study was to evaluate the potentiality of plant extracts of *Cassia auriculata* L. on standard microorganism strains as well as on the multi drug resistant bacteria.

### **MATERIAL AND METHODS**

**Plant collection :** Healthy, disease free leaves, stem and pods of *Cassia auriculata* L. were collected from different localities of Parbhani district and used for the preparation of extracts. The plant were identified and confirmed through available literature in the flora of Marathwada (Naik *et al.* 1998).

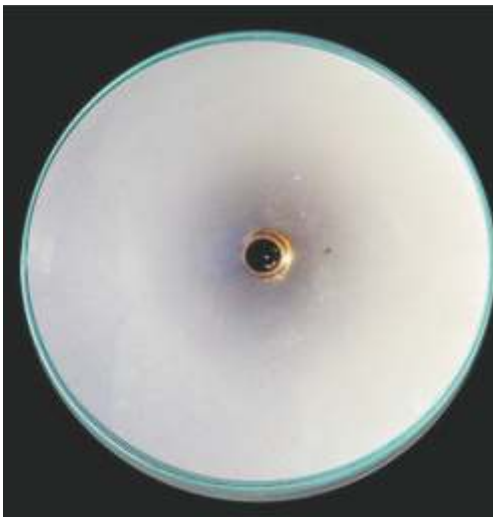
**Preparation of plant extracts :** The leaves, stem and pods were dried under shed at room temperature and then powdered with a grinder and stored in air tight container. A soxhlet apparatus was used for extraction of antimicrobial active compounds from the powder. 20gm of dried powder with thimble and then subjected to extraction with the methanol, ethanol, aqueous, petroleum ether and acetone



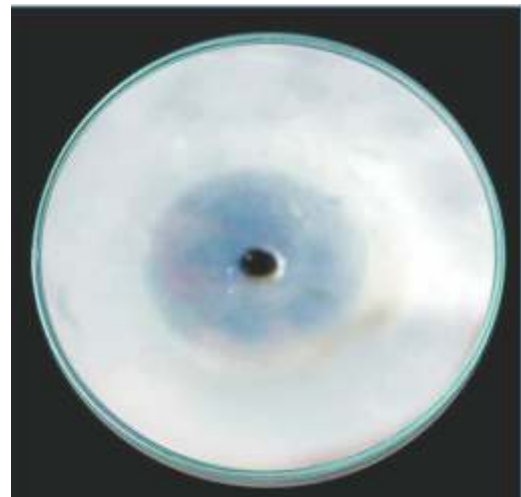
*Escherichia coli*+*Cassia auriculata* leaves in methanol



*Escherichia coli*+*Cassia auriculata* leaves in acetone



*Bacillus subtilis* + *Cassia auriculata* leaves in acetone



*Cassia auriculata*+ control leaves in acetone



*Candida albicans*+*Cassia auriculata* leaves in acetone



*Staphylococcus aerus*+*Cassia auriculata* leaves in acetone

TABLE 1. *In Vitro* Antimicrobial Activity of *Cassia auriculata* L.

Diameter of zone of inhibition in mm of different extracts (2mg/ml) (Mean $\pm$ SD)																		
Methanol			Ethanol			Aqueous			Petroleum ether			Acetone			Standard reference antibiotic (Streptomycine)			
Leaf	Stem	Pod	Leaf	Stem	Pod	Leaf	Stem	Pod	Leaf	Stem	Pod	Leaf	Stem	Pod	Leaf	Stem	Pod	
23 $\pm$ 0.12	12.3 $\pm$ 1.25	19 $\pm$ 2.50	16 $\pm$ 3.21	9 $\pm$ 0.00	20 $\pm$ 0.17	-	7 $\pm$ 1.24	7 $\pm$ 1.24	9 $\pm$ 2.21	11.0 $\pm$ 1.3	12 $\pm$ 0.00	17 $\pm$ 1.12	4 $\pm$ 1.25	14 $\pm$ 1.15	10 $\pm$ 1.12	15 $\pm$ 1.11	20 $\pm$ 2.08	
13 $\pm$ 0.45	11.3 $\pm$ 0.25	20 $\pm$ 1.46	12 $\pm$ 1.23	-	18 $\pm$ 1.14	-	4 $\pm$ 0.45	4 $\pm$ 0.45	4 $\pm$ 1.24	-	16 $\pm$ 1.57	16 $\pm$ 0.41	3 $\pm$ 3.12	14 $\pm$ 0.57	12 $\pm$ 0.45	12 $\pm$ 0.45	15 $\pm$ 2.51	
2 $\pm$ 0.00	12.2 $\pm$ 1.2	18 $\pm$ 3.02	10 $\pm$ 0.15	12 $\pm$ 0.17	20 $\pm$ 3.21	-	2.6 $\pm$ 2.25	16 $\pm$ 2.25	3 $\pm$ 0.12	8.3 $\pm$ 0.12	15 $\pm$ 1.73	16 $\pm$ 1.45	2 $\pm$ 1.52	14 $\pm$ 1.52	15 $\pm$ 0.35	18 $\pm$ 0.10	16 $\pm$ 1	
13 $\pm$ 3.05	13 $\pm$ 3.05	18 $\pm$ 1.45	10 $\pm$ 0.00	-	16 $\pm$ 2.09	-	2.6 $\pm$ 1.45	16 $\pm$ 1.45	4 $\pm$ 3.12	--	14 $\pm$ 2.51	16 $\pm$ 1.74	4.3 $\pm$ 0.45	15 $\pm$ 1	9 $\pm$ 0.15	14 $\pm$ 1.13	16 $\pm$ 0.00	
12 $\pm$ 3.25	12 $\pm$ 3.25	18 $\pm$ 0.25	10 $\pm$ 1.05	14 $\pm$ 1.15	15 $\pm$ 1.17	-	3 $\pm$ 1.73	3 $\pm$ 1.73	6 $\pm$ 0.00	8.3 $\pm$ 1.31	15 $\pm$ 1.73	17 $\pm$ 1.2	3 $\pm$ 0.12	15 $\pm$ 2	13 $\pm$ 0.12	13 $\pm$ 0.41	18 $\pm$ 1	
10 $\pm$ 0.25	12.6 $\pm$ 1.24	10 $\pm$ 0.00	16 $\pm$ 0.57	9.3 $\pm$ 2.05	18 $\pm$ 0.25	-	3 $\pm$ 0.17	3 $\pm$ 0.17	6 $\pm$ 0.14	-	10 $\pm$ 1.52	17 $\pm$ 0.00	4 $\pm$ 0.45	12 $\pm$ 2	12 $\pm$ 0.15	8.6 $\pm$ 0.35	20 $\pm$ 3.21	

solvents separately. The collected extracts were concentrated by heating till the boiling point so as unwanted solvents either evaporated or removed and the negative control of the solvents get reduced. The remaining plant extracts used for antimicrobial assays.

### Test Microorganisms

Authentic pure cultures of human pathogenic bacteria like *Staphylococcus aureus* (SRTCC1073), *Bacillus subtilis* (SRTCC1091) and two are gram negative viz. *Pseudomonas aeruginosa* (SRTCC708), *Escherichia coli* (SRTCC3260). Two species of fungi viz. *Aspergillus niger* (SRTCC1073), *Candida albicans* (SRTCC3971). These were obtained from the School of Life Sciences, S.R.T.M. University, Nanded (M.S.).

### Preparation Of Test Organism Suspension

The test organisms were maintained on slants of medium containing nutrient agar (2.5 gm/ 10ml) and sub cultured once a week. The slants were incubated at 37°C for 24 hrs and stored under refrigeration. The inoculum was 1x 10<sup>8</sup> cells/ml (Chessbrogh 2000) in each case.

### ANTIMICROBIAL ACTIVITY ASSAY:

The *in vitro* antimicrobial activity of leaves, stem and pod extracts of *Cassia auriculata* L. was determined by the agar-well diffusion method (Perez *et al.* 1990). The plant extracts were dissolved in distilled water at concentration 2 mg/ ml. The standard antimicrobial solution containing 50 $\mu$ g/ml

streptomycin was used as reference antibiotic. Each plate was inoculated with 20 $\mu$ l microbial suspension having concentration 1x 10<sup>8</sup> cells/ml. 0.1 ml extract was added to each well. The plates containing bacteria were incubated at 37°C for 24 hrs and those containing fungi were incubated at 25°C for 7 days. Positive antimicrobial activity was based on growth inhibition zone and compared with standard drug (Perez *et al.* 1996). The diameter of zone of inhibition surroundings each of the well was recorded.

### Statistical analysis:

Data was subjected to statistical analysis using standard deviation followed by student's T-test to compare the mean percentage of growth inhibition with respective controls.

### RESULTS AND DISCUSSION

The results of the antimicrobial activity of different extracts of leaf, stem and pods of *Cassia auriculata* L. are presented in Table 1. The results revealed that nearly, all the crude extracts showed antibacterial and antifungal activity. Aqueous extracts of leaf did not show antimicrobial activity against test organisms.

In the present work methanol, acetone and ethanolic extracts of leaf showed higher antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the reference antibiotic Streptomycin. Methanolic and ethanolic extracts of pods showed significant

antibacterial activity which was higher than the reference antibiotic to both Gram positive and Gram negative bacteria. The results are consistent with previous reports on related plants regarding Gram positive bacteria (Cowan 1999, Chavan *et al.* 2010). Senthilkumar and Reetha (2009) have reported higher antibacterial activity in the methanolic extracts of leaf. Girish and Satish (2008) reported that methanol extract of leaves had significant antimicrobial activity. Usually most of the Gram negative bacteria are more resistant than Gram positive bacteria (Tomas-Barberan *et al.* 1988), but in this present investigation methanol, acetone and ethanol extracts of leaf and pod showed maximum inhibition for Gram negative bacteria also. In case of fungi acetone extract of leaf had significant activity in test organisms which was found higher than the reference antibiotic.

On the basis of the results obtained in this present investigation we conclude that the methanol, acetone and ethanol extracts of leaves and pods had significant *In vitro* antimicrobial activity. The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non polar and they were extracted only through the organic solvents medium (Britto 2001, Mohanasundari *et al.* 2007). The present observation suggests that the organic solvents extraction was suitable to verify the antimicrobial properties of medicinal plants and they are supported by many investigators (Girish and Satish 2008, Natarajan *et al.* 2005, Krishna *et al.* 1997).

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