



## NOTE

### Antimicrobial Activity of Sequential Extracts from Leaves of *Cassia nodosa* Bunch.

D. SINGH, R.A. SHARMA\*, B. SHARMA, S.K. SHARMA, D. SHARMA, P. CHANDRAWAT and S. SHARMA

Department of Botany, University of Rajasthan, Jaipur-302 055, India

\*Corresponding author: E-mail: Sharma\_ra2007@yahoo.co.in

(Received: 19 June 2010;

Accepted: 19 November 2010)

AJC-9325

The antimicrobial activity of the sequential extracts (petroleum ether, benzene, acetone, chloroform, ethanol, water etc.) of the leaves of *C. nodosa* carried out against certain bacteria viz., *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and fungi viz., *Aspergillus flavus*, *A. niger*, *Fusarium moniliformae* and *Rhizoctonia bataticola* using disc diffusion technique. Present study showed that most of the extracts were effective against all the test microorganisms. The minimum inhibitory concentrations of the extracts of chloroform, benzene, acetone and ethanol were found to be  $2 \times 10^4$  mg/mL, while the petroleum ether and water showed no inhibition. Present investigation provides scientific basis for the use of the plant extracts in the treatment of fungal and bacterial diseases. It is concluded that the active principles possessing antimicrobial activity may be extracted from the leaves of *Cassia nodosa* by various organic solvents.

**Key Words:** *Cassia nodosa*, Antimicrobial activity, Petroleum ether, Benzene, Chloroform, Acetone, Ethanol, Water, Bacteria, Fungi.

*Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. Different classes of natural products, possessing potent physiological and pharmacological activities<sup>1</sup> have been isolated from *Cassia* species and they include anthracene derivatives, flavonoids and polysaccharides. Some of these compounds have been shown to possess considerable antimicrobial activity<sup>2</sup>. *Cassia* species are well known in folk medicine for their laxative and purgative activities<sup>3</sup>. The leaves and pods are normally used. *Cassia nodosa* is one of the beautiful fast growing ornamental exotic plant with pink flowers found at the roadsides and gardens in Jaipur. It is widespread in world's tropical and sub-tropical parts. Phytochemically *Cassia nodosa* have been studied for fixed oils of the seeds<sup>4</sup>, galactomann and nodoside a new anthraquinone glycoside from the flowers<sup>5,6</sup> from bark<sup>7</sup> and stilbenetr<sup>8</sup>. The phytochemical and cytotoxic screening of the plant have been carried out earlier and the four crude extracts showed strong cytotoxic activity. The extracts were found to be positive for carbohydrate, anthracene derivatives, cardiac and saponin glycosides as well as alkaloid<sup>9</sup>. The aim of the present study is to investigate the antimicrobial activity of the various extracts of the leaves of *Cassia nodosa* in different solvents.

The leaves of *Cassia nodosa* were collected from Central Park, Jaipur. The plant was identified at the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

**Extraction procedure:** The leaves (50 g) were air dried, each of the experimental material was Soxhlet extracted suc-

cessively with petroleum ether (60-80 °C), benzene, chloroform, acetone, alcohol and water 36-48 h. Each of the resultant extract was filtered, dried *in vacuo* and weighed to calculate the extractive value (%) on dry weight basis. Later, following the established protocols<sup>10</sup> each of the test sample was used against test microorganisms.

**Test microorganisms:** Standard strains of *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* obtain from microbiology Lab. SMS medical College, Jaipur and *Aspergillus flavus*, *A. niger*, *Fusarium moniliformae* and *Rhizoctonia bataticola* obtain from seed Pathology Lab, Department of Botany, University of Rajasthan, Jaipur.

**Antimicrobial screening:** The disc diffusion method was used to determine the antimicrobial activities of the crude extract of petroleum ether, benzene, acetone, chloroform, ethanol and water using standard procedure<sup>9,10</sup> of 6 mm disc were prepared from Whatmann's filter paper No. 1. Solutions of varying concentrations ranging from  $1.0 \times 10^4$  -  $5.0 \times 10^4$  mg/mL/disc were prepared. They were also prepared using the pure extracting solvent for each extract. Nutrient agar was prepared, sterilized and used as the growth medium for the culture of microorganisms, 20 mL of the sterilized medium was poured into each sterilized petri dish, covered and allowed to solidify. The treated discs were air dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of compounds from the test disc into the agar plate and later incubated at

TABLE-1  
ANTIMICROBIAL ACTIVITIES OF *Cassia nodosa* LEAVES

Test microorganism	Diameter of inhibition zone (mm)					
	Petroleum ether	Benzene	Acetone	Chloroform	Ethanol	Water
Bacteria						
<i>Staphylococcus aureus</i>	07	13	14	17	16	06
<i>Salmonella typhi</i>	04	10	12	12	13	00
<i>Pseudomonas aeruginosa</i>	07	13	24	21	10	07
<i>Escherichia coli</i>	04	11	20	12	16	06
Fungi						
<i>Aspergillus niger</i>	00	18	20	16	18	00
<i>Aspergillus flavus</i>	00	15	18	23	21	00
<i>Fusarium moniliformae</i>	03	11	12	15	17	00
<i>Rhizoctonia bataticola</i>	00	10	09	10	11	00

TABLE-2  
ANTIMICROBIAL ACTIVITIES OF DIFFERENT CONCENTRATION OF *Cassia nodosa* ( $\mu\text{g/mL}$ )

Text organisms	Diameter of inhibition zone (mm)																																		
	Petroleum ether					Benzene					Chloroform					Acetone					Ethanol					Water									
	$1 \times 10^4$	$2 \times 10^4$	$3 \times 10^4$	$4 \times 10^4$	$5 \times 10^4$	$1 \times 10^4$	$2 \times 10^4$	$3 \times 10^4$	$4 \times 10^4$	$5 \times 10^4$	$1 \times 10^4$	$2 \times 10^4$	$3 \times 10^4$	$4 \times 10^4$	$5 \times 10^4$	$1 \times 10^4$	$2 \times 10^4$	$3 \times 10^4$	$4 \times 10^4$	$5 \times 10^4$	$1 \times 10^4$	$2 \times 10^4$	$3 \times 10^4$	$4 \times 10^4$	$5 \times 10^4$	$1 \times 10^4$	$2 \times 10^4$	$3 \times 10^4$	$4 \times 10^4$	$5 \times 10^4$					
Bacteria																																			
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	±	+	+	-	±	+	+	+	-	±	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-
Fungi																																			
<i>Aspergillus niger</i>	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-
<i>Aspergillus flavus</i>	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-
<i>Fusarium moniliformae</i>	-	-	-	-	-	-	±	+	+	-	-	+	+	+	-	-	+	+	+	+	-	-	±	+	+	-	-	-	-	-	-	-	-	-	-
<i>Rhizoctonia bataticola</i>	-	-	-	-	-	-	±	+	+	-	-	+	+	+	-	-	+	+	+	+	-	-	±	+	+	-	-	-	-	-	-	-	-	-	-

37 °C for 24 h in case bacteria and 48 h for fungi, after which the zone of inhibition could be easily observed. Five replicates of each text extract were examined and the mean values were then referred.

Table-1 shows the results of the antimicrobial activities against the test microorganisms. The zone of inhibition were measured in mm of the diametrical sections of the respective sequential extracts. Table-2 shows the results of minimum inhibitory concentrations. The results demonstrated that the chloroform, benzene, acetone and ethanol had very high growth inhibitory effects on all microorganisms. The MIC values for the chloroform on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus flavus* and *A. niger* was found to be  $2 \times 10^4$   $\mu\text{g/mL}$ , while for *Salmonella typhi*, *Fusarium moniliformae* and *Rhizoctonia bataticola*, the MIC value was  $3 \times 10^4$   $\mu\text{g/mL}$ . For acetone, the MIC value was observed to be  $2 \times 10^4$   $\mu\text{g/mL}$ . Against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus*, *A. niger* and while for *Salmonella typhi*, *Fusarium moniliformae* and *Rhizoctonia bataticola*, the MIC value was  $3 \times 10^4$   $\mu\text{g/mL}$ . The MIC for the benzene and ethanol was found to be  $2 \times 10^4$   $\mu\text{g/mL}$  for all the test microorganisms, except for *Salmonella typhi* and *Rhizoctonia bataticola* which was  $3 \times 10^4$   $\mu\text{g/mL}$ .

The petroleum ether and water extracts did not show any inhibition against the test microorganisms. The results of the antimicrobial activity of the various sequential extracts were in agreement with the uses of the extract of the leaves of *Cassia nodosa* in traditional medicine for the treatment of bacterial and fungal diseases. The leaves of the plant appeared to be a potential source of broad spectrum antibiotics. Studies are in progress to purify and characterize the active principles in the leaves.

## REFERENCES

- S.C. Jain, R.A. Sharma, R.Jain, F. Cappasso and N. Mecudo, *J. Ethnopharmacol.*, **58**, 135 (1997).
- R.G. Ayo, J.O. Amupitan and Y. Zhao, *Afr. J. Biotechnol.*, **6**, 1276 (2007).
- A.A. Eluojoba, A.T. Abere and S.A. Adelusi, *Nigerian J. Nat. Prod. Med.*, **3**, 51 (1999).
- S.A. Rizvi, P.C. Gupta and R.K. Kaul, *Planta Med.*, **19**, 221 (1968).
- S.A. Rizvi, P.C. Gupta and R.K. Kaul, *Planta Med.*, **16**, 317 (1971).
- S.A. Rizvi, P.C. Gupta and R.K. Kaul, *Planta Med.*, **20**, 24 (1971).
- N.K. Yadava, B.K. Singh and A. Kumar, *Asian J. Chem.*, **10**, 204 (1998).
- P. Suhag, M. Rani and S.B. Kalithar, *Sciences*, **21**, 1026 (1999).
- S.V. Agarkar and D.R. Jadge, *Asian J. Chem.*, **11**, 295 (1999).
- J.C. Gould and J.H. Bowie, *Ednib. Med. J.*, **59**, 178 (1952).