

# ANTIBACTERIAL EFFECT OF CASSIA FISTULA EXTRACT ON PATHOGENIC BACTERIA OF VETERINARY IMPORTANCE

T. Ranjith Vimalraj\*, S.Saravana kumar\*, S. Vadivel\*, S. Ramesh<sup>1</sup> and P. Thejomoorthy<sup>2</sup>

Department of Veterinary Pharmacology and Toxicology,  
Madras Veterinary College,  
Tamilnadu Veterinary and Animal Sciences University

## ABSTRACT

*Cassia fistula* (Family – Caesalpinaceae) (Tamil – Sarakondrai) is a deciduous and an ornamental tree native to India and Sri Lanka, its extracts have been shown to have antifungal, antineoplastic and anti-inflammatory activity. In the present study, the antibacterial activity aqueous and alcoholic extract of stem bark of *Cassia fistula* was tested. The bacterial isolates tested include: *Staphylococcus aureus* (MTCC 740), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443) and a few field isolates of *Staphylococcus aureus*, *Bacillus anthracis*, *Escherichia coli*, *Pasteurella multocida*, and *Salmonella typhi*. Disc diffusion and Minimum Inhibitory Concentration (MIC) studies were carried out to assess the antibacterial effect. Chloramphenicol was used as the reference standard. Aqueous extract of *Cassia fistula* in disc diffusion method showed significant activity against *Staphylococcus aureus* but not against other bacteria tested. Alcoholic extract showed greater inhibition against *Staphylococcus aureus* compared to aqueous extract. One of the field isolates of *Staphylococcus aureus* resistant to chloramphenicol too was susceptible to alcoholic extract of *Cassia fistula*. Zones of inhibition of alcoholic and aqueous extracts were in the range of 7.0-12.0 mm and 7.0-11.6 mm respectively. MIC values of the alcoholic extracts against *Staphylococcus aureus* were in the range of 0.78– 6.25 mg/ml.

**K.ey words:** *Cassia fistula*, Aqueous and Alcoholic Extract, Minimum Inhibitory Concentration (MIC), Disc Diffusion, Antibacterial

## INTRODUCTION

Antibacterial agents remain the mainstay in the treatment of bacterial infections. However in the last two decades, indiscriminate use of such agents has led to the development of drug resistance in many bacterial species. The emergence of drug resistant bacteria has become a medical catastrophe (Berkowitz, 1994). Thus there is a need to identify newer drugs for bacterial

diseases. Use of plant products for the control of human and animal diseases has certain advantages such as biodegradability, availability, low toxicity and cost effectiveness.

In the present work, the antibacterial activity of *Cassia fistula* was carried out with the following objectives:

1. To study the antibacterial activity of stem bark extracts of *Cassia fistula* against

---

\* Graduate Students, 1 Assistant Professor, Department of Veterinary Pharmacology And Toxicology,

2Associate Professor, Pharmacy , Department of Clinics, Madras Veterinary College.

pathogenic bacteria of veterinary importance using disc diffusion and minimum inhibitory concentration (MIC) studies.

2. To compare the efficacy with a commonly used antibiotic against standard isolates of pathogenic bacteria.

## MATERIALS AND METHODS

In this study, both aqueous and alcoholic bark extracts of *Cassia fistula* were prepared using standard procedure. For antibacterial testing, the following bacteria, which are pathogenic to animals, were used.

Reference isolates:

*Staphylococcus aureus* subsp aureus MTCC 740

*Bacillus subtilis* MTCC 441

*Escherichia coli* MTCC 443

Field isolates (from outbreaks):

*Staphylococcus aureus* - 4 isolates

*Bacillus anthracis* - 1 isolates

*Salmonella typhi* - 2 isolates

*Escherichia coli* - 5 isolates

*Pasteurella multocida* - 2 isolates

The isolates were obtained from VC &RI, Namakkal, VRC-BV, Madhavaram and Department of Veterinary Microbiology, MVC, Chennai and Veterinary College, Nagpur.

Each 100 grams of dried stem bark powder was soaked in 500 ml of distilled water and 500 ml of 95% v/v ethanol with periodic stirring for 76 hours and filtered using Whatman No.1 filter paper. The filtrates were evaporated at 55° C in hot air oven. The yield of aqueous and alcoholic extract with respect to original dry plant material was about 2% w/w and 4% w/w respectively. The extracts were obtained as dark brown amorphous crystals.

To test the antibacterial activity of the extracts, pure cultures of bacterial isolates were

maintained. To standardize the inoculum density for a susceptibility test, a barium sulphate standard equivalent to a 0.5 Mc Farland standard was used. The disc diffusion method using Mueller Hinton agar as described in National Committee on Clinical Laboratory Standards (NCCLS, 2003) was used to qualitatively assess the antibacterial activity. The activity was compared with chloramphenicol at the concentration of 30 mcg/disc. For those isolates against which the extracts showed antibacterial activity, the minimal inhibitory concentration by dilution technique was found out using macro dilution method (Jones *et al.*, 1985) wherein the minimum inhibitory concentration of the extract required to inhibit bacterial growth in broth culture was determined.

## RESULTS AND DISCUSSION

Both aqueous and alcoholic extracts of *Cassia fistula* exhibited antibacterial activity against *Staphylococcus aureus*. However, the activity was significantly lower than that of chloramphenicol. There was no inhibition of *B. anthracis* and *B. subtilis*. None of the Gram negative bacteria tested viz., *E.coli*, *Pasteurella multocida*, *Salmonella tyhimurium* were inhibited by the extracts. The MIC of the alcoholic extract against one standard isolate and four field isolates of *S. aureus* tested were in the range of 0.78-6.25 mg per ml (Table 2).

The zones of inhibition for *Staphylococcus aureus* to alcoholic and aqueous extracts were in the range of 7.0-12.0 mm and 7.0-11.6 mm. For one of the field isolates of *Staphylococcus aureus* (CNS-2), the antibacterial activity of alcoholic extract was significantly more than that of chloramphenicol (11.5 mm) (Table 1).

The results of the study indicate the antibacterial activity of extracts of *Cassia fistula* against *S. aureus*. The activity is narrow in spectrum as there was no activity against some other Gram positive

and Gram negative bacteria tested. Ethanolic extracts of *T. cordifolia*, *Vitex negundo* and *Acorus calamus* too exhibited antibacterial activity only against Gram positive bacteria viz. *S. aureus* and *B. subtilis* (Damodaran, 2004). The results are important since *S. aureus* is an important pathogen in man and animals, where resistance to other drugs is frequently reported. Methicillin resistant *Staphylococcus aureus* are widely distributed among hospitals and are increasingly isolated from community-acquired infections (Chambers and Sande, 1996). Abbas Ali *et al.*, (2003) reported strong antibacterial activity of the three lectins CSL-1, CSL-2 and CSL-3 purified from the seeds of *Cassia fistula*. The stem barks of *Cassia fistula* have been shown to contain lupeol, betasitosterol and hexacotanol (Gupta *et al*, 1989). It will be worthwhile to isolate the active fractions for further testing and also to use this plant along with other plants for preparing a broad -spectrum drug.

#### REFERENCE

- Abbas Ali, M., M. Abu Sayed and Nurul Absar (2003). Antibacterial activity and cytotoxicity of three lectins purified from *Cassia fistula* Linn seeds. *J.Med.Sci*, **3**(3): 240-244.
- Berkowitz, F.E.,(1994). Antibiotic resistance in bacteria. *South. Med. J.*, **88**: 797-804.
- Chambers, H.F and Merle A. Sande (1996). Antimicrobial agents-General considerations. In: Goodman and Gilman's

the pharmacological basis of therapeutics, 9<sup>th</sup> edition.(Eds). Joel G. Hardman and Lee E. Limbird. McGraw-Hill, Medical Publishing Division, New York.

- Damodaran, A. (2004) *in vitro* and *in vivo* antibacterial activity of medicinal plants. M.V.Sc thesis submitted to Tamilnadu Veterinary and Animal Sciences University, Chennai.
- Gupta, V., M. Agarwal, I.A.De Gracia, R.De Tello and Gupta (1989). Isolation and characterization of two flavonoids and a xanthine glycoside from the stem bark of *Cassia fistula* Linn. *I.J.Chem.*, **28**: 282-284.
- Jones, R.N., A.L.Barry, T.L.Gavan and J.A. Washington (1985). Susceptibility tests: microdilution and macrodilution broth procedures. In: *Manual of clinical microbiology*, 4<sup>th</sup> edition. (Eds). E.H. Lennette, A.Balows, W.J. Hausler and J.H.Shadomy. American society for microbiology, Washington, pp.972-977.

#### ACKNOWLEDGEMENT

The authors wish to thank TNSCST for funding the student project and the Dean, MVC for providing the necessary facilities

**Table 1. Antimicrobial activity of *Cassia fistula* against the bacterial strains tested based on disc diffusion method**

Bacteria	Isolate no.	Disc diffusion method				
		Ethanol extract		Aqueous extract		Chloramphenicol
		Inhibition	Zone of inhibition (in mm)	Inhibition	Zone of inhibition (in mm)	Zone of inhibition (in mm)
<i>Staphylococcus aureus</i> subsp aureus	MTCC 740	+	11	+	11.6	32
<i>Bacillus subtilis</i>	MTCC 441	-	-	-	-	53
<i>Escherichia coli</i>	MTCC 443	-	-	-	-	36.5
<i>Staphylococcus aureus</i>	CPS-1	+	12	+	7	40
<i>Staphylococcus aureus</i>	CPS-2	+	7	+	7.5	29
<i>Staphylococcus aureus</i>	CNS-2	+	11.9	+	7.7	11.5
<i>Bacillus anthracis</i>	1	-	-	-	-	--
<i>Escherichia coli</i>	N <sub>3</sub>	-	-	-	-	22
<i>Salmonella typhi</i>	S <sub>1</sub>	-	-	-	-	35
<i>Salmonella typhi</i>	S <sub>2</sub>	-	-	-	-	32
<i>Pasteurella multocida</i>	P <sub>1</sub>	-	-	-	-	12.5
<i>Pasteurella multocida</i>	P <sub>2</sub>	-	-	-	-	13.5

**Table 2. MIC values of alcoholic and aqueous extracts of *Cassia fistula* against bacteria tested**

Bacteria	Isolate no.	Minimum Inhibitory Concentration			
		Alcoholic extract		Aqueous extract	
		Inhibition	Inhibitory concentration	Inhibition	Inhibitory concentration
<i>Staphylococcus aureus</i> subsp aureus	MTCC 740	+	6.25 mg/ml	-	-
<i>Bacillus subtilis</i>	MTCC 441	-	-	-	-
<i>Escherichia coli</i>	MTCC 443	-	-	-	-
<i>Staphylococcus aureus</i>	CPS-1	+	6.25 mg/ml	-	-
<i>Staphylococcus aureus</i>	CPS-2	+	0.78 mg/ml	-	-
<i>Staphylococcus aureus</i>	CNS-1	+	6.25 mg/ml	-	-
<i>Staphylococcus aureus</i>	CNS-2	+	6.25 mg/ml	-	-
<i>Bacillus anthracis</i>	1	-	-	-	-
<i>Escherichia coli</i>	N <sub>9</sub>	-	-	-	-
<i>Escherichia coli</i>	N <sub>3</sub>	-	-	-	-
<i>Salmonella typhi</i>	S <sub>1</sub>	-	-	-	-
<i>Salmonella typhi</i>	S <sub>2</sub>	-	-	-	-
<i>Pasteurella multocida</i>	P <sub>1</sub>	-	-	-	-
<i>Pasteurella multocida</i>	P <sub>2</sub>	-	-	-	-