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Research Article

## Evaluation of antibacterial and antitubercular activities of *Cassia fistula* Linn root

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### ABSTRACT

The different extracts of *Cassia fistula* Linn root were evaluated for antibacterial and antitubercular activities against different Gram-positive, Gram-negative bacterial strains and *M. tuberculosis* H<sub>37</sub>Rv strain using ciprofloxacin, norfloxacin and isoniazid as standard drugs, respectively. Alcoholic extract showed significant activity against *Bacillus subtilis*, while this extract was found to show moderate activity against *Escherichia coli*. Alcoholic extract also showed good antitubercular activity. The presence of alkaloids and tannins in alcoholic extract could be attributed for the antibacterial and antitubercular activities.

**Keywords:** Alcohol extract; Antibacterial; Antitubercular; *Cassia fistula*; Root.

### INTRODUCTION

Tuberculosis is a highly infectious disease with about one third of the world's population including 40% from India estimated to be infected by it (Agarwal, 2004). However, this problem has become serious as *Mycobacterium tuberculosis* developed resistance against both the first line as well as second line drugs. Along with this, there is an emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* all over the world including India (Singh, 2007). Medicinal plants offer a great hope to fulfil these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties (Heinrich *et al.*, 2001).

*Cassia fistula* linn. belongs to the family *Fabaceae* (Basu 2005) a very common plant known for its medicinal properties is a semi-wild Indian Labernum known as the golden shower. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil (Prashanth, *et al.*, 2006).

Vernacular names of the plant *Cassia fistula* are in Kannada-Kakkigida, Hindi-Bandarlahi, Bharva, Suvarnaka, Telagu-Kondrakayi, English-Golden shower (Orwa *et al.* 2009). This plant is found to be used in Ayurvedic

system of medicine for its different pharmacological activities viz as, Seeds are used to treat skin diseases, fever, abdominal pain and leprosy. Fruits are also used in treatment of skin diseases, fever, abdominal pain and leprosy (Perry 1980; Markouk *et al.*, 2000). Roots are used to treat cardiac disorders, biliousness, rheumatic condition, haemorrhages, wounds, ulcers and boils, tubercular glands and various skin diseases (Alam. *et al.*, 1990, Asolkar.LV *et al.*, 1992). Literature survey revealed the presence of 7-Methylphyscion, Betulinic acid and  $\beta$ -Sitosterol, Rhamnetin-3-O-gentiobioside, Tannins, Phlobaphenes, Oxyanthraquinone (Anonymous, 2007, Rastogi, 2004, Chopra, 2006). However, *Cassia fistula* Linn root has not been investigated for antibacterial and antitubercular activities. Hence, this study was conducted to evaluate the potent bioactive constituents for antibacterial and antitubercular activities in *Cassia fistula* Linn.

### MATERIAL AND METHODS

The root of *Cassia fistula* Linn was collected from the local areas of Dharwad in Karnataka, and was authenticated by Dr. S. S. Hebbar, Department of Botany, Government Pre-university College Dharwad. A voucher specimen (No- SETCPD/ Pharmacog/ Herb/2013/14) has been deposited in the Herbarium of Department of Pharmacognosy, S.E.T's College of Pharmacy, Dharwad, Karnataka.

The *Cassia fistula* Linn root was shade dried and finely powdered to particle size (#) 40. About 300 g of dried powder was subjected to continuous hot soxhlet exhaustive extraction with petroleum ether, chloroform and ethanol (95%). Aqueous extract was also obtained by cold maceration of the drug (300 g) with 2% chloroform water. After the extraction, the extracts were

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filtered and concentrated under reduced pressure using a rota evaporator (Harborn 1984). The yield of petroleum ether, chloroform, ethanol and aqueous extracts was found to be 6.36 g (2.12 % w/w), 5.7 g (1.9 % w/w), 20.49 g (6.83 % w/w) and 21.03 g (7.01 % w/w), respectively. All the extracts were kept in a dessicator for the experiment.

#### Evaluation of antibacterial activity

The MIC determination of different extracts was carried out simultaneously in comparison with ciprofloxacin, norfloxacin against Gram-positive (*Staphylococcus aureus*, *Staphylococcus faecalis*, *Bacillus subtilis*) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*) by broth microdilution method (Jalwa et al., 2012, Villanova 1985). Serial dilutions of the all extracts and reference drugs were prepared in Mueller-Hinton broth. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions were done to obtain final concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL. The tubes were inoculated with  $10^5$  cfu mL<sup>-1</sup> (colony forming unit/mL) and incubated at 37°C for 18h. The MIC was the lowest concentration of the extracts that yield no visible growth on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and DMSO had no effect on the microorganisms in the concentrations studied. The MIC values are given in µg/ml. Ciprofloxacin and norfloxacin were used as standard drugs. The preliminary results of antibacterial activities are depicted in Table1.

#### Evaluation of antitubercular activity

MIC values were determined for the different extracts against *M. tuberculosis* strain H<sub>37</sub>Rv using the Microplate Alamar Blue assay (MABA) using isoniazid as the standard drug (Franzblau. et al.,1998). The 96 wells plate received 100 µL of Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate with drug concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. Then, 25 µL of freshly prepared 1:1 mixture of almar blue reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC was defined as the lowest drug concentration, which prevented colour change from blue to pink. The results of antitubercular activity are depicted in Table 2.

#### RESULTS AND DISCUSSION

The different extracts of *Cassia fistula* Linn root were screened for antibacterial activity against Gram-positive bacteria: *Staphylococcus aureus*, *Streptococcus faecalis* and *Bacillus subtilis*. Gram-negative bacteria; *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* the results are represented in Table 1. Alcoholic extract of *Cassia fistula* Linn root has showed significant activity at MIC of 1.6 µg ml<sup>-1</sup> against *Bacillus subtilis*. Similarly this extract showed moderate activity at MIC of 3.125 µg ml<sup>-1</sup> against *Escherichia coli* as compared to standard drugs ciprofloxacin and norfloxacin.

**Table 1: In vitro antibacterial activity of different extract of *Cassia fistula* Linn root**

Compound	MIC values (µg ml <sup>-1</sup> )					
	Gram-positive organisms <sup>a</sup>			Gram-negative organisms <sup>b</sup>		
	Sa	Sf	Bs	Kp	Ec	Pa
Aqueous Extract	100	25	25	100	50	100
Alcohol Extract	25	6.25	1.6	12.5	3.125	12.5
Chloroform Extract	100	50	100	>100	50	>100
Pet-ether Extract	100	50	25	100	50	100
CIP <sup>c</sup>	<5	<5	≤1	≤1	≤1	>5
NOR <sup>d</sup>	<5	<5	≤1	≤1	≤1	>5

The screening organisms: <sup>a</sup>Gram-positive bacteria: *Staphylococcus aureus* ATCC 11632 (Sa), *Streptococcus faecalis* ATCC 14506 (Sf), *Bacillus subtilis* ATCC 60511 (Bs); <sup>b</sup>Gram-negative bacteria: *Klebsiella pneumoniae* ATCC 10031 (Kp), *Escherichia coli* ATCC 10536 (Ec) *Pseudomonas aeruginosa* ATCC 10145 (Pa); Reference drugs: <sup>c</sup>Ciprofloxacin, <sup>d</sup>Norfloxacin.

**Table 2: In vitro antitubercular activity of different extract of *Cassia fistula* Linn root**

Compound	MIC values (µg ml <sup>-1</sup> ) <i>M. tuberculosis</i> H <sub>37</sub> Rv
Aqueous Extract	100
Alcohol Extract	12.5
Chloroform Extract	100
Pet-ether Extract	100
Isoniazid	0.25

Similarly different extracts of *Cassia fistula* Linn root were screened for antitubercular activity against *M.tuberculosis* strain H<sub>37</sub>Rv using the Microplate Alamar Blue assay (MABA) and results are represented in Table 2. The alcoholic extract showed good activity at 12.5 µg ml<sup>-1</sup> as compared to standard drug isoniazid.

Phytochemical investigation of alcoholic extract of *Cassia fistula* Linn root revealed the presence of alkaloids and tannins. Hence, the presence of alkaloids and tannins in alcoholic extract could be attributed for observed significant antibacterial and antitubercular activities (Navneet, K et al.,2009, Oladosu, P et al.,2007). However; research work is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principles for the claimed antibacterial and antitubercular activities.

## CONCLUSION

The present study confirms antibacterial and antitubercular activities of alcoholic extract of *Cassia fistula* Linn root. However; research work is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principles for the claimed antibacterial and antitubercular activities. The present result may form the basis for selection of plant species for further investigation in potent bioactive compounds for antibacterial and antitubercular activities.

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