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# *In vitro* antimicrobial activity of leaf and bark extract of *Cassia fistula* L.

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**Abstract**

Antimicrobial activity of ethanolic and aqueous extracts of leaves and bark of *Cassia fistula* L. was examined by disc diffusion method against selected pathogenic microorganisms. Although, both the extracts showed the promising antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Streptococcus faecalis, Bacillus subtilis, Enterobacter aerogenes, Pseudomonas aerogenosa, Aspergillus flavus, Aspergillus niger, and Candida albicans*. The maximum average zone of inhibition (34.0mm) and (32.5mm) was exhibited by bark and leaf extracts respectively at the concentration of 2.0 mg/ml against *Pseudomonas aeruginosa* and no zone of inhibition was formed by leaf extract and minimum (0.1mm) zone of inhibition was observed with bark extract against *Streptococcus faecalis*. Standard antibiotic Ketoconazole for fungi and streptomycin for bacteria were used for comparative zone of inhibition and MIC study. However these results revealed that the ethanolic and aqueous extract of *C. fistula* L. can be used as a potential antimicrobial source for various infections.

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## Introduction

Human beings are using the plant derived chemicals for curing the various ailments from a long time. These ancient indigenous practices were discovered by a series of "trial and error" which then could not be substantiated by proven scientific theories. However, these practices have produced results of proven efficacies (Chopra *et al*, 1956). In recent times herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nations (Fajimi *et al*, 2005). Approximately 62 – 80% of the world's population still relies on traditional medicines for the treatment of common illness (WHO, 2002; Zhang, 2004). In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce cost effective remedies that are affordable to the population (Pandey *et al*, 2011; Maharajan *et al*, 2012). The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources.

The genus *Cassia*, comprising 600 species widely distributed worldwide and is well known for its diverse biological and pharmacological properties. *Cassia fistula* L. belongs to Caesalpiniaceae family is widely used for its medicinal properties. In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculosis and its use in the treatment of rheumatism, haematemesis, pruritus, leucoderm and diabetes (Alam *et al*, 1990). This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections (Rajan *et al,* 2001). *C. fistula* flower and seed along with some other Indian medicinal plants were also studied and found good antimicrobial activity (Sangetha *et al*, 2008). Literature so far has revealed very little work on the antimicrobial activity of various plant parts against pathogenic bacteria as well fungi. Therefore, the present study has been undertaken to evaluate the antimicrobial potential of various parts of this. *Cassia fistula* L.

## Materials and methods

*Collection of plant materials, bacterial strains and growth conditions*

The plant material of *Cassia fistula* L. was collected in and around Khopoli, Raigad District, Maharashtra, India and was identified and authenticated by using Flora of Maharashtra (Almeida, 1996). The cultures - *Escherichia coli* NCIM 2065, *Staphylococcus aureus* NCIM 2079, *Salmonella typhi* NCIM 2501, *Streptococcus faecalis* NCIM 2603, *Bacillus subtilis* NCIM 2063, *Enterobacter aerogenes* NCIM 5139 *Pseudomonas aerogenosa* NCIM 5210 *Aspergillus flavus* NCIM 519, *Aspergillus niger* NCIM 520 *and Candida albicans* NCIM 3471 were obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. Cultures were checked for purity and biochemical test were carried out. The cultures were grown in liquid medium at 37˚C and maintained on agar slants at 2-8˚C.

*Selection of reference antibiotic*

Reference antibiotic Ketoconazole (Johnson and Johnson, USA), Streptomycin (Cipla, Mumbai) was obtained from Local Market in Mumbai. The purity of the antibiotic was 99.9%.

*Alcoholic and aqueous extraction of plant materials*

The plant material was dried in shade and powdered in a mechanical grinder. For aqueous extract 100 gm powder of *Cassia fistula* was soaked in 500 ml distilled water with periodic stirring for 72 hours and extract was filtered with Whatman filter paper. For alcoholic extraction the plant material initially defatted with petroleum - benzene at (60 - 80˚C) followed by 500 ml of ethanol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whattman filter paper (No. 1) and then concentrated in vaccum and dried at 45˚C, for ethanol elimination. The extract were kept in a sterile bottle under refrigerated condition about 2-8˚C for further analysis.

*Dilutions and inoculum preparations*

The dried plant extracts of *Cassia fistula* and antibiotics Ketoconazole and streptomycin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 0.5, 1.0, 1.5, 2.0 mg/ml. The inoculum of *E. coli, Staphylococcus aureus*, *Salmonella typhi, Streptococcus faecalis, Enterobacter aerogenes* and *Pseudomonas aerogenosa* were prepared in nutrient broth medium and *Aspergillus flavus, Aspergillus niger, and Candida albicans* were prepared in Sabouraud dextrose agar (Himedia) at 30 °C for 24 h and the stock culture was maintained at 4°C and subcultured as needed.

*Procedure for performing the Disc Diffusion test (Bayer et al., 1966)*

The required amount of Mueller-Hinton plates is prepared as per manufacturer instructions, (Himedia, Mumbai) and autoclaved at 121˚C for 15 minutes and allowed to cool under Laminar air flow. 25 ml of media was transferred into each sterile petri dishes and allowed to solidify. A readily prepared sterile cotton swab was dipped into the turbid culture suspension. The dried surface of Muller-Hinton agar plate was inoculated by streaking two more times rotating the plate approximately 60˚ each time. The lid was left aside for 3-5 minutes to dry the excess surface moisture content. The sterile discs (Himedia, Mumbai) were loaded with different concentrations of about 0.5, 1.0, 1.5 and 2.0 mg/ml of plant extract of *Cassia fistula* and antibiotic ketoconazole, streptomycin into each separate disc of about 100µl. The discs were placed on the medium suitably apart and the plate were incubated at 5˚C for 1 hour to allow good diffusion and then transferred to an incubator at 37˚C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone arou00nd the disc using zone reader (Hi media, Mumbai).

*Experimental Design*

The result was analysed statistically in factorial randomized design with 10 different microorganisms and 5 different inhibitory substances, the experiment was replicated thrice.

**Result and discussion**

Infectious diseases have increased dramatically in recent years. The treatment of these infectious diseases with chemicals has lagged behind due to development of resistance in the pathogens. Therefore, a search for new drugs is essential to overcome this problem. Many plants are now used to treat various infectious diseases. This study reports antimicrobial activity of *C. fistula* leaf and bark aqueous and ethanolic extract.

*Solvents, test organisms and concentrations*

The results shows that extract from the aerial parts of *Cassia fistula* posses antimicrobial activities against screening of the tested organism at different concentration such as 0.5, 1.0, 1.5 and 2.0 mg/ml respectively. The ethanolic and aqueous extract of *Cassia fistula* L. were subjected for antimicrobial activity against *Escherichia coli, Staphylococcus aureus*, *Salmonella typhi, Streptococcus faecalis, Enterobacter aerogenes* and *Pseudomonas aerogenosa*, *Aspergillus flavus, Aspergillus niger, and Candida albicans* (Table 1). The ethanolic and aqueous extracts posses good antimicrobial activity when compared with control drug Ketonazole and Streptomycin. At 2.0 mg/ml concentration *Cassia fistula* ethanolic extract of leaf and bark exhibits maximum zone of inhibition about 32.5mm and 34.0 mm and aqueous 22.0mm and 26.5 mm against *Pseudomonas aerogenosa*. The antibacterial activity of ethanolic extract was significantly more than that of streptomycin (30.2 mm). In case of *E. coli* both the plant alcoholic extract showed the zone of inhibition 24.9 mm, 25.7mm which is higher against control drug 24.0 mm. On the contrary aqueous extract exhibited slight lower diameter 21.4 and 23.9 mm. However, the activity was slightly more or lower than that of streptomycin. There was no inhibition of *Streptococcus faecalis* and lower inhibition 0.6 mm in *Salmonella typhi* at 2.0 mg/ml. The concentration *Staphylococcus aureus* exhibits 19.4 mm (leaf) and 19.9 mm (bark) zone of inhibition at 0.5 mg/ml. further the concentration raised to 2.0 mg/ml which shows the drastic increase in zone of inhibition about 27.5 mm and 27.9 mm respectively against control drug 26.9 mm. whereas, *Bacillus subtilis* and *Enterobacter aerogenes* exhibits the moderate zone

of inhibition in range of 3.3 mm to 15.3 mm and 3.4mm to 7.8mm. With *Aspergillus flavus* and *Aspergillus niger,* the plant extract showed the lower to moderate zone of inhibition with the increase in concentration. 11.3 to 23.0 mm in and 9.4 to 21.1 mm. The leaf extract against *Candida albicans* expressed minimum 7.9mm, bark extract 6.2mm and 20.0mm and 16.3mm maximum zone of inhibition. The aqueous leaf and bark extract also showed the increase in the zone of inhibition with the increase in concentration up to 2.0 mg/ml from 7.1 to 17.5mm and 5.7mm to 15.1mm which is slightly lower than the ethanolic extract. From the above results *Cassia fistula* shows good antimicrobial activity against *Pseudomonas aerogenosa* and *Staphylococcus aureus* which exhibits maximum zone of inhibition when compared with control drug streptomycin which show less activity when compared with plant extracts.

**Table 1.** Interaction effect of various microorganism and different inhibitory substances on zone of inhibition.

| Microorganism | Concentration mg/ml | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Plant material | | | | Antibiotic* |
| | | Leaf (Ethanol) | Leaf (Aqueous) | Bark (Ethanol) | Bark (Aqueous) | |
| *Escherichia coli* | 0.5 | 10.12 | 8.4 | 13.3 | 9.9 | 16.2 |
| *Staphylococcus aureus* | 0.5 | 19.4 | 17.2 | 19.9 | 19.1 | 17.1 |
| *Salmonella typhi* | 0.5 | 0 | 0 | 0 | 0 | 30.2 |
| *Streptococcus faecalis* | 0.5 | 0 | 0 | 0 | 0 | 18.1 |
| *Bacillus subtilis* | 0.5 | 3.3 | 0 | 4.4 | 0 | 35.2 |
| *Enterobacter aerogenes* | 0.5 | 3.4 | 2.0 | 3.9 | 3.0 | 22.8 |
| *Pseudomonas aerogenosa* | 0.5 | 22.1 | 11.1 | 27.4 | 14.0 | 26.1 |
| *Aspergillus flavus* | 0.5 | 11.3 | 11.1 | 12.4 | 9.2 | 44.3 |
| *Aspergillus niger* | 0.5 | 12.7 | 11.6 | 9.4 | 7.8 | 44.9 |
| *Candida albicans* | 0.5 | 7.9 | 7.1 | 6.2 | 5.7 | 20.4 |
| | **SE** | **1.85** | **CD** | **5.49** | | |
| *Escherichia coli* | 1.0 | 15.9 | 13.7 | 16.7 | 14.2 | 18.0 |
| *Staphylococcus aureus* | 1.0 | 21 | 19 | 22.0 | 20.9 | 20.4 |
| *Salmonella typhi* | 1.0 | 0 | 0 | 0 | 0 | 34.0 |
| *Streptococcus faecalis* | 1.0 | 0 | 0 | 0 | 0 | 19.8 |
| *Bacillus subtilis* | 1.0 | 5.9 | 0 | 7.3 | 0.5 | 39.1 |
| *Enterobacter aerogenes* | 1.0 | 4.0 | 3.1 | 4.8 | 4.0 | 25.1 |
| *Pseudomonas aerogenosa* | 1.0 | 24.9 | 15.5 | 29.0 | 18.1 | 28.0 |
| *Aspergillus flavus* | 1.0 | 15.2 | 14.6 | 16.2 | 11.9 | 46.5 |
| *Aspergillus niger* | 1.0 | 14.8 | 13.8 | 11.7 | 9.5 | 48.6 |
| *Candida albicans* | 1.0 | 11.7 | 11.1 | 9.8 | 8.9 | 26.3 |
| | **SE** | **1.97** | **CD** | **5.85** | | |
| *Escherichia coli* | 1.5 | 19.4 | 17.0 | 20.1 | 19.6 | 20.2 |
| *Staphylococcus aureus* | 1.5 | 24.0 | 21.5 | 24.1 | 22.3 | 23.5 |
| *Salmonella typhi* | 1.5 | 0 | 0 | 0.3 | 0 | 35.2 |
| *Streptococcus faecalis* | 1.5 | 0 | 0 | 0 | 0 | 22.0 |
| *Bacillus subtilis* | 1.5 | 9.0 | 0.5 | 11.9 | 1.3 | 42.2 |
| *Enterobacter aerogenes* | 1.5 | 5.8 | 4.0 | 6.2 | 5.3 | 28.0 |
| *Pseudomonas aerogenosa* | 1.5 | 28.8 | 18.6 | 31.2 | 22.4 | 29.9 |
| *Aspergillus flavus* | 1.5 | 18.1 | 17.0 | 19.9 | 14.0 | 49.1 |
| *Aspergillus niger* | 1.5 | 17.0 | 16.2 | 14.4 | 12.8 | 52.8 |
| *Candida albicans* | 1.5 | 15.3 | 13.9 | 13.0 | 12.0 | 29.8 |
| | **SE** | **2.25** | **CD** | **6.68** | | |
| *Escherichia coli* | 2.0 | 24.9 | 21.4 | 25.7 | 23.9 | 24.0 |
| *Staphylococcus aureus* | 2.0 | 27.5 | 23.0 | 27.9 | 24.7 | 26.9 |
| *Salmonella typhi* | 2.0 | 0.5 | 0 | 0.6 | 0 | 37.3 |
| *Streptococcus faecalis* | 2.0 | 0 | 0 | 0.1 | 0 | 23.7 |
| *Bacillus subtilis* | 2.0 | 13.8 | 1.1 | 15.3 | 2.8 | 46.0 |
| *Enterobacter aerogenes* | 2.0 | 6.0 | 5.0 | 7.8 | 6.5 | 30.4 |
| *Pseudomonas aerogenosa* | 2.0 | 32.5 | 22.0 | 34.0 | 26.5 | 30.2 |
| *Aspergillus flavus* | 2.0 | 21.4 | 19.5 | 23.0 | 17.2 | 52.8 |
| *Aspergillus niger* | 2.0 | 21.1 | 19.8 | 17.2 | 15.3 | 54.9 |
| *Candida albicans* | 2.0 | 20.0 | 17.5 | 16.3 | 15.1 | 37.0 |
| | **SE** | **2.73** | **CD** | **8.10** | | |

The data on mean zone of inhibition due to different concentration of extract on various microorganisms are present in Table 1 which indicated that the zone of inhibition of microorganism was differed significantly.

At 0.5 mg/ml concentration maximum zone of inhibition was recorded for *Pseudomonas aerogenosa* (20.14 mm) which was significantly higher than other microorganism zone of inhibition except for *Staphylococcus aureus* and *Aspergillus flavus* where it was at par with them. Least zone of inhibition was recorded with *Streptococcus faecalis*. Similar trend of observation was recorded with concentration of 1.0, 1.5 and 2.0 mg/ml.

Various inhibitory substances influenced the zone of inhibition significantly at different concentration. At concentration of 0.5 mg/ml maximum zone of inhibition was recoreded with antibiotics which was significantly higher than plant extract. It was followed by bark (ethanol) extract which was significantly higher than the aqueous extract of leaf and bark but at par with ethanol extract of leaf. Trend of observation was similar for concentration of 1.0, 1.5 and 2.0 mg/ml.

*Statistical analysis*

Interaction effect of various microorganism and different inhibitory substances was found to be significant. At concentration of 0.5mg/ml maximum zone of inhibition was recorded for *Aspergillus niger* with antibiotic, which was significantly higher than other treatments. Among plant extract maximum zone of inhibition was recorded for *Pseudomonas aerogenosa* with ethanol extract of bark. Minimum or no zone of inhibition was recorded for *Salmonella typhi* and *Streptococcus faecalis* with extract of leaf and bark (both aqueous and ethanol). Observations were similar for the other higher concentration(Table 2).

**Table 2.** Zone of inhibition due to different concentration of extract on various microorganisms by disc diffusion method.

| Treatments | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Concentration mg/ml | | | |
| | 0.5 | 1.0 | 1.5 | 2.0 |
| Microorganism (M) | | | | |
| M1 *Escherichia coli* | 11.58 | 15.7 | 19.26 | 23.98 |
| M2 *Staphylococcus aureus* | 18.54 | 20.66 | 23.08 | 26.00 |
| M3*Salmonella typhi* | 6.04 | 6.80 | 7.10 | 7.68 |
| M4 *Streptococcus faecalis* | 3.62 | 3.96 | 4.40 | 4.76 |
| M5 *Bacillus subtilis* | 8.58 | 10.56 | 12.98 | 15.8 |
| M6 *Enterobacter aerogenes* | 7.02 | 8.20 | 9.86 | 11.14 |
| M7 *Pseudomonas aerogenosa* | 20.14 | 23.10 | 26.18 | 29.04 |
| M8 *Aspergillus flavus* | 17.66 | 20.88 | 23.62 | 26.78 |
| M9 *Aspergillus niger* | 17.28 | 19.68 | 22.64 | 25.66 |
| M10 *Candida albicans* | 9.46 | 13.56 | 16.80 | 21.18 |
| *SE* | 0.87 | 0.76 | 0.95 | 1.17 |
| *CD* | 2.58 | 2.25 | 2.82 | 3.47 |
| Inhibitory substances | | | | |
| I1Leaf (Ethanol) | 9.02 | 11.34 | 13.74 | 16.77 |
| I2Leaf (Aqueous) | 6.85 | 9.08 | 10.87 | 12.93 |
| I3 Bark (Ethanol) | 9.69 | 11.75 | 14.11 | 16.79 |
| I4 Bark (Aqueous) | 6.87 | 8.80 | 10.97 | 13.20 |
| I5 Antibiotic* | 27.53 | 30.58 | 33.27 | 36.32 |
| *SE* | 1.03 | 1.10 | 1.22 | 1.35 |
| *CD* | 3.05 | 3.26 | 3.62 | 4.00 |
| *Interaction effect* (MxI) | | | | |
| *SE* | 1.85 | 1.97 | 2.25 | 2.73 |
| *CD* | 5.49 | 5.85 | 6.68 | 8.10 |

**Discussion**

The methanolic extract of *C. fistula* leaves exhibited more effects on *S. paratyphi, B. cereus, B. subtilis, B. megaterium* and *E. coli* while *P. aeruginosa, K. nemoniae, E. facalis*, *M. luteus, S. aureus, S. epidermidis,* and *S. typhi* were less affected (Yogesh and Mohan, 2006). Hence, alcoholic extract of *C. fistula* leaves show greater zone of inhibition for *S. aureus* and *P. aeruginosa* (Muthusamy *et al*., 2006). Similarly, *Cassia fistula* chloroform extract was found to be highly susceptible, against *K. pneumoniae*. Acetone extract has shown 0.0156 mg/ml MIC value against *K. pneumoniae, E. coli, B. cereus* and *S. pneumoniae*. *Cassia fistula* aqueous extract has shown MIC value i.e. 0.0975 mg/ml in *K. pneumoniae* and 0.048 mg/ml in *E. coli, B. cereus*, *M. luteus* and *L. acidophilus*. Besides this, antibiotics such as tetracycline, amphicillin and ciprofloxacin has shown higher MIC values which were obtained in a range of 0.892-3.57mg/ml (Upadhyay *et al*, 2011). AbbasAli *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).

**Conclusion**

The results are important since *Staphylococcus 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).

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. The existing antibiotics become resistant to the infectious diseases and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanism of activities for new and re-emerging infectious disease. The drugs derived from herbs have the possibility of using in medicine because of its good antimicrobial activity as well as less toxic effects over control drugs. A novel drug should not contain any toxic side effects, therefore the present study aimed to focus the antibacterial effects of above mentioned pathogens.

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