Int J Pharm Bio Sci 2013 July; 4(3): (B) 728 - 736

**Research Article** **Microbiology**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF SOME *CASSIA* SPECIES

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## ABSTRACT

Hexane, chloroform, ethyl acetate and methanol extracts of leaves of *Cassia alata, C. auriculata*, *C. fistula* and *C. tora* were screened for their antimicrobial activity against human pathogenic bacterial and fungal strains. Antimicrobial activity was carried out by disc diffusion method, determination of minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) against four strains of Gram positive bacteria, three strains of Gram negative bacteria and three species of fungi. The antimicrobial activity of various extracts of *Cassia* species showed varied levels of antimicrobial activity against the studied bacterial and fungal pathogens. The mean zone of inhibition produced by all the tested extracts ranged from 6.3±0.5 to 25.6±0.2 mm. The MIC, MBC and MFC values were between 62.5 and 1000 µg/mL. The ethyl acetate extract of *C. tora* showed good antimicrobial activity with the highest mean zone of inhibition (25.6±0.2 mm), lowest MIC (62.5 µg/mL) and MBC (125 µg/mL) values followed by ethyl acetate extract of *C. auriculata* (24.6±0.2 mm; MIC=62.5; MBC=125 µg/mL) against *S. aureus*.

**KEYWORDS:** **Antibacterial activity; Antifungal activity; *Cassia* sps; Solvent extracts**

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# INTRODUCTION

Infectious diseases caused by bacteria and fungi affect millions of people worldwide and continue the major cause of death in tropical countries like India and China[1,2]. The improved hygiene and development of new antimicrobial leads a remarkable progress in prevention, control and eradication of infectious diseases[3]. However, in the recent years multiple drug resistance has been developed in human pathogens due to the indiscriminate use of commonly available antibiotics in the treatment of infectious diseases. Moreover, infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death[4] and the current worldwide increase in antimicrobial resistance has serious public health and economic implications in the developing countries[5]. Due to the development of resistance to most of the available antimicrobial agents and the high costs of treatments consequent upon this resistance, has forced to search for new, safe, efficient and cost effective ways for the management of infectious diseases[6] especially from natural source. In that way, plant based antimicrobials will help to overcome the resistance problems as well as it will be more reliable than the synthetic products[7].

*Cassia* is a major genus of Caesalpiniaceae family contains about 600 species and widely distributed in tropical and subtropical countries. Several of them yield timber, dyes, fodder, vegetables and edible fruits and seeds are used as substitute for coffee[8]. About 45 species are found in India, of which, few have been introduced for ornamental[9]. In India, *Cassia* spp. are well known in Indian system of medicine and used for the treatment of a number of ailments including leprosy, itching, ringworm and skin diseases, eye disease, urinary disorders, asthma, diabetes and dysentery[10]. In the present investigation, various solvent extracts viz. hexane, chloroform, ethyl acetate and methanol of leaves of *C. alata, C. auriculata*, *C. fistula*, and *C. tora* were studied for its antimicrobial activity against human pathogenic bacteria viz. *Bacillus subtilis, B. pumilus, Micrococcus luteus, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli* and fungal strains viz. *Aspergillus niger*, *A. flavus* and *A. fumigatus*.

# MATERIALS AND METHODS

### *i) Collection of Plant materials*

Leaves of *Cassia alata, C. auriculata*, *C. fistula*, and *C. tora* were collected from Cuddalore district of Tamil Nadu. The voucher specimens were deposited at the Herbarium, Department of Botany, Annamalai University. The places of collection and its geographical locations are given in Table 1.

### *ii). Preparation of crude extracts*

The collected leaves were first washed with tap water and then surface sterilized in 10 per cent sodium hypochlorite to prevent the contamination of any microbes. The leaf samples were shade dried followed by oven drying (at 60 °C) and milled in an electrical blender. The powdered leaves (100 g) were extracted with hexane, chloroform, ethyl acetate and methanol in soxhlet apparatus. Each extract was extracted with respective solvent (300 ml × 3) for 72 hrs. The extracts were pooled and the solvents were evaporated using a rotary evaporator (Hei-VAP advantage HB/HL/G1, Heidolph, Germany) under reduced pressure at 40 °C. The crude extracts were kept at 4 °C until further assay.

### *iii) Antimicrobial assay*

### *a) Microorganisms*

Anti bacterial activity was tested against four strains of Gram positive bacteria viz. *Bacillus subtilis* (MTCC 441), *B. pumilus* (MTTC 1640), *Micrococcus luteus* (MTCC 106), *Staphylococcus aureus* (MTCC 3160), three strains of Gram negative bacteria viz. *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 448) and three species of fungi viz. *Aspergillus niger* (MTCC

282), *A. flavus* (MTCC 277) and *A. fumigatus* (MTCC 343). The microbial strains were collected from Microbial Type Cell Culture, Chandigarh. India. The stock cultures were maintained on nutrient agar medium (for bacteria) and Sabouraud dextrose agar medium (for fungi) at 4°C.

***b) Disc diffusion assay***

Antimicrobial susceptibility test of the crude extracts were tested against the above mentioned Gram positive, Gram negative bacteria and fungi by disc diffusion method[11]. Petri plates were prepared with 20ml of sterile Muller Hinton Agar (Himedia, Mumbai) for bacteria and 20ml of Sabouraud dextrose agar (SDA) for fungi. The twenty four hours prepared test inoculums were swabbed on the top of the solidified media and allowed to dry for 10 minutes. Previously prepared extracts were impregnated with discs at concentrations of 1000, 500, 250 µg/ml and were placed aseptically on plates with appropriate controls. The loaded discs were placed on the surface of the medium and left for 30 minutes at room temperature. Negative control was prepared using 10 % DMSO. For bacteria, Ciprofloxacin (5µg/disc) and for fungi, Ketaconozole (10µg/disc) were used as positive controls. Finally, the inoculated plates were incubated at 37 °C for 24 h (for bacteria) and 35 °C for 48 h (for *Aspergillus*). The inhibition zones were observed including the diameter of the disc (6 mm).

***c) Determination of Minimum Inhibitory Concentration (MIC)***

Minimum inhibitory concentrations of the crude extracts were tested in Mueller Hinton broth for bacteria and Sabouraud dextrose broth for mycelial fungi to get the concentrations of 1000-15.2 µg/ml by the broth macro dilution method[12]. The culture tubes were incubated at 37°C for 24 h (bacteria) and at 35°C for 48 h (mycelial fungi).

***d) Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)***

The MBC and MFC of the crude extracts were determined[13] by plating 100 µl samples from each MIC assay with growth inhibition into freshly prepared Mueller Hinton agar (for bacteria) and Sabouraud dextrose agar (for mycelial fungi). The plates were incubated at 37°C for 24 h (bacteria) and at 35°C for 48 h (mycelial fungi).

***e) Statistical Analysis***

All the data of microbial activities were examined as mean ±SD. One-way analysis of variance (ANOVA) was carried out to determine the significant differences ($P < 0.05$) between the means. The analyses were carried out using SPSS package software, 11.5 (SPSS Inc., Chicago, IL).

## RESULTS

In the present investigation, different solvent extracts of four species of *Cassia* showed varied levels of antimicrobial activity (Tables 2 and 3) against the studied bacterial and fungal pathogens. The mean zone of inhibition produced by all the extracts ranged from 6.3±0.5 to 25.6±0.2 mm. The MIC, MBC and MFC values were between 62.5 and 1000 µg/mL. The ethyl acetate extract of *C. tora* showed good antimicrobial activity with the highest mean zone of inhibition (25.6±0.2 mm), lowest MIC (62.5 µg/mL) and MBC (125 µg/mL) values against *S. aureus* followed by ethyl acetate extract of *C. auriculata* (24.6±0.2 mm; MIC=62.5; MBC=125 µg/mL). Ethyl acetate extract of *C. fistula* also showed the highest mean zone of inhibition against *M. luteus* (24.3±0.2 mm; MIC=125; MBC=250 µg/mL) and *S. aureus* (24.3±0.2 mm; MIC=62.5; MBC=125 µg/mL). Whereas, ethyl acetate extract of *C. fistula* showed the highest antifungal activity against *A. flavus* (17.6±0.5 mm) and *A. fumigatus* (17.3±0.5 mm). The ethyl acetate extract of *C. tora* showed good antimicrobial activity against *B. subtilis* (24.6±0.5 mm; MIC=62.5; MBC=125 µg/mL), *M. luteus* (18.8±0.2 mm; MIC=125; MBC=250 µg/mL), *B. pumilus* (17.8±0.2 mm; MIC=125; MBC=250 µg/mL), *E. coli* (14.8±0.2 mm; MIC=250; MBC=500 µg/mL), *P. aeruginosa* (14±0.5 mm; MIC=250; MBC=500 µg/mL), *K. pneumoniae* (12.5±0.5 mm; MIC=250; MBC=500 µg/mL), *A. flavus* (16.1±0.7 mm; MIC=125; MBC=250 µg/mL) *A. niger* (14.6±0.7 mm;

MIC=250; MBC=500 µg/mL) and *A. fumigatus* (14.3±0.7 mm; MIC=250; MBC=500 µg/mL) when compared to other solvent extracts. The control drugs, Ciproflaxacin (5 µg/disc) and Ketaconozole (10µg/disc) produced mean zone of inhibition ranged from 9.3±0.7 to 33.5±0.5 and there was no activity against blind control.

**Table 1**
***Place and geographical details of sample collection***

| Name of the species | Place of collection | Geographical location and Elevation | Herbarium No. |
|---|---|---|---|
| *C. alata* | Annamalai Nagar, Chidambaram Taluk | 11°2519N 079°2057E; 45M | AUBOT# 227 |
| *C. auriculata* | Mahimaipuram, Jayakondam Taluk | 11°2321N 079°2359E; 40M | AUBOT# 230 |
| *C. fistula* | Veeranathapuram, Kattumannar Koil Taluk | 11°2035N 079°2251E; 83M | AUBOT# 229 |
| *C. tora* | Srimushnam, Kattumannar Koil Taluk | 11°2346N 079°2438E; 35M | AUBOT# 228 |

## Table 2
## *Antimicrobial activity of different solvent extracts of some Cassia species*

| Name of the plant | Extract/ Standard Drug | Concentration (mg/disc) | Mean zone of inhibition [a] (mm)[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ***B.s*** | ***B.p*** | ***M.l*** | ***S.a*** | ***E.c*** | ***P.a*** | ***K.p*** | ***A.n*** | ***A.f*** | ***A.f*** |
| *C. alata* | Hexane | 250 | 6.3±0.5 | 6.8±0.7 | 6.3±0.5 | 7.8±0.7 | NA | NA | NA | 7.1±0.2 | 7.8±0.2 | 6.3±0.5 |
| | | 500 | 9.1±0 | 9±0.8 | 9.1±0.2 | 10±0.8 | 7.8±0.7 | 7.8±0.7 | 7.8±0.7 | 9.1±0.2 | 10.3±0.5 | 9.8±0.7 |
| | | 1000 | 11.1±0.7 | 10.5±0.8 | 10.8±0.7 | 11.3±0.2 | 9.8±0.7 | 10.1±0.2 | 9.8±0.7 | 11.8±0.7 | 12.5±0.8 | 11.8±0.7 |
| | Chloroform | 250 | 7.5±0.5 | NA | 7.1±0.2 | 8.1±0.2 | 7.8±0.7 | 6.3±0.5 | 8.1±0.2 | 7.8±0.7 | 8.1±0.2 | 7.5±0.8* |
| | | 500 | 10.1±0.7 | 9±0.8 | 8.8±0.2 | 10.3±0.2 | 9.3±0.2 | 9±0.8 | 9.3±0.2 | 9.6±0.2 | 10.8±0.2 | 9.1±0.2 |
| | | 1000 | 12.6±0.5* | 11.8±0.7* | 12.6±0.7 | 13.5±0.5* | 11.1±0.2 | 10.8±0.7 | 11.1±0.2 | 12.6±0.7 | 14.1±0.2* | 12.1±0.2 |
| | Ethyl acetate | 250 | 8.5±0.8 | 7.5±0.8 | 8.1±0.2 | 8±0.8 | 7.3±0.2 | 7.6±0.2 | 7.3±0.2 | 7.6±0.2 | 8±0.8 | 7.6±0.7 |
| | | 500 | 11.8±0.7 | 10±0.5 | 11.5±0.5 | 9.3±0.7 | 10.5±0.5 | 10.8±0.2 | 9.1±0.7* | 11.3±0.5 | 12.1±0.2 | 10.6±0.7 |
| | | 1000 | 14.6±0.7* | 12.6±0.2 | 14.3±0.5* | 15.5±0.5* | 12.1±0.2* | 12.5±0.5 | 12.1±0.2 | 14.6±0.7 | 15.5±0.5 | 12.6±0.2 |
| | Methanol | 250 | 8.5±0.2 | 7.3±0.2 | 8.1±0.2 | 8.1±0.2 | 7.8±0.7 | 7.8±0.7 | 8.1±0.2 | NA | 8.5±0.5 | 8.8±0.7 |
| | | 500 | 10.8±0.7 | 10±0.7 | 11.8±0.7 | 10.8±0.7 | 9.1±0.2 | 9.1±0.2 | 9.5±0.5 | 9.5±0.5 | 10.5±10.5 | 11.8±0.7 |
| | | 1000 | 13.5±0.5* | 12.5±-.8 | 12.8±0.2 | 14.1±0.7 | 11.5±0.5 | 12.3±0.5* | 11.5±0.8 | 12.8±0.2 | 14.5±0.5* | 13.8±0.7 |
| | Ciproflaxacin | 5 µg/disc | 27.6±0.2 | 20.5±0.5 | 27±0.8 | 27.5±0.5 | 27.6±0.2 | 29.5±0.5 | 33.5±0.5 | NT | NT | NT |
| | Ketaconozole | 10µg/disc | NT | NT | NT | NT | NT | NT | NT | 11.1±0.7 | 9.3±0.7 | 11.1±0.7 |
| *C. auriculata* | Hexane | 250 | 8.3±0.5 | 7.1±0.2 | 6.3±0.5 | 8.1±0.2 | NA | 6.3±0.5 | NA | 7.1±0.2 | 8.1±0.7 | 8.1±0.2 |
| | | 500 | 9.5±0.8 | 9.5±0.5 | 9.5±0.5 | 10.6±0.2 | 8.5±0.2 | 8.5±0.5 | 8.1±0.2 | 11.5±0.8 | 10.3±0.5 | 10.5±0.5 |
| | | 1000 | 11.5±0.8 | 11.8±0.7 | 11.5±0.5 | 13.1±0.2 | 10.1±0.2 | 10.5±0.7 | 10.1±0.2 | 12.5±0.8* | 13.8±0.2* | 12.8±0.7* |
| | Chloroform | 250 | 8.1±0.7 | 8.3±0.2 | 7.5±0.5 | 8.5±0.5 | 7.8±0.7 | 6.3±0.5 | NA | 8.1±0.2 | 8.5±0.5 | 9.1±0.7 |
| | | 500 | 10.5±0.5 | 10.1±0.2 | 12.1±0.2 | 10.6±0.2 | 9.6±0.2 | 9.3±0.2 | 9.3±0.2 | 9.6±0.5 | 11.1±0.7* | 12.1±0.2 |
| | | 1000 | 13.3±0.5 | 12.8±0.7 | 14.5±0.5* | 14.6±0.5* | 11.5±0.5 | 11.1±0.2 | 11.1±0.2 | 14.1±0.2 | 14.5±0.5* | 14.1±0.2* |
| | Ethyl acetate | 250 | 10.1±0.7 | 8.5±0.5 | 8.5±0.5 | 12.3±0.5 | 7.3±0.2 | 7.6±0.2 | 7.3±0.2 | 7.6±0.2 | 7.6±0.2 | 10.5±0.5 |
| | | 500 | 13.3±0.5 | 12.3±0.2 | 11.5±0.5 | 17.5±0.5 | 10.8±0.2 | 9.1±0.7 | 8.8±0.7 | 11.3±0.5 | 12.1±0.2 | 13.3±0.5 |
| | | 1000 | 22.5±0.5* | 15.5±0.5* | 17.8±0.7 | 24.6±0.2* | 13.8±0.7 | 12.1±0.2* | 11.8±0.5 | 14.6±0.7 | 15.5±0.5* | 16.5±0.5 |
| | Methanol | 250 | 11.8±0.7 | 7.6±0.2 | 7.6±0.2 | 12.3±0.5 | 7.8±0.7 | 7.8±0.7 | 7.8±0.7 | 7.8±0.7 | 8.5±0.5 | 9.5±0.5 |
| | | 500 | 13.8±0.7 | 10.5±0.2 | 10.3±0.2 | 16±0.8 | 9.1±0.2 | 9.1±0.2 | 9.5±0.5 | 9.5±0.5 | 10.1±0.2 | 12.5±0.5 |
| | | 1000 | 20.3±0.5* | 14.1±0.7 | 14.1±0.7* | 21±0.8* | 11.5±0.5 | 12.3±0.5 | 11±0.5 | 12.5±0.5* | 14.5±0.5 | 14.5±0.5* |
| | Ciproflaxacin | 5 µg/disc | 27.6±0.2 | 30.5±0.5 | 27±0.8 | 27.5±0.5 | 27.6±0.2 | 29.5±0.5 | 33.5±0.5 | NT | NT | NT |
| | Ketaconozole | 10µg/disc | NT | NT | NT | NT | NT | NT | NT | 11.1±0.7 | 9.3±0.7 | 11.1±0.7 |
| *C. fistula* | Hexane | 250 | 8.6±0.5 | 7.1±0.2 | NA | 8.1±0.2 | NA | NA | 6.3±0.5 | 7.5±0.5 | 7.1±0.2 | NA |
| | | 500 | 9.2±0.7 | 9.5±0.5 | 9.1±0.2 | 10.3±0.2 | 8.1±0.2 | 7.8±0.7 | 8.1±0.2 | 9.1±0.7 | 10.6±0.5 | 9.8±0.7 |
| | | 1000 | 11.1±0.8 | 12.1±0.2 | 10.1±0.2 | 13.1±0.2 | 10.1±0.2 | 9.8±0.7 | 10.1±0.2 | 12.8±0.7 | 13.8±0.2 | 12.5±0.8 |
| | Chloroform | 250 | 7.5±0.5 | 8.3±0.2 | 7.1±0.2 | 8.1±0.2 | 7.8±0.7 | NA | 7.8±0.7 | 8.1±0.2 | 8.5±0.5 | 7.8±0.7 |
| | | 500 | 8.3±0.2 | 10.1±0.2 | 9.1±0.2 | 10.3±0.2 | 9.3±0.2 | 9±0.8 | 9.3±0.2 | 9.6±0.2* | 10±0.8 | 9.3±0.2 |
| | | 1000 | 11.8±0.5 | 12.8±0.7* | 11.5±0.5 | 14.3±0.5 | 11.1±0.2 | 10.8±0.7 | 11.1±0.2 | 14.1±0.2* | 14.5±0.5* | 14.1±0.2* |
| | Ethyl acetate | 250 | 9.5±0.5 | 8.5±0.5 | 8.1±0.2 | 9.3±0.2 | 7.3±0.2 | 7.3±0.2 | 7.6±0.2 | 7.6±0.2 | 8±0.8 | 7.6±0.2 |
| | | 500 | 13.5±0.5 | 12.3±0.2 | 11.1±0.2 | 15.1±0.2 | 9.5±0.8 | 8.5±0.5 | 9.1±0.7 | 12.6±0.5 | 13.6±0.5 | 13.3±0.5 |
| | | 1000 | 20.2±0.2* | 14.1±0.2* | 24.3±0.2* | 24.3±0.2 | 13.8±0.7 | 11.8±0.7* | 12.1±0.2 | 15.8±0.2 | 17.6±0.5 | 17.3±0.5 |
| | Methanol | 250 | 8.3±0.2 | 8.1±0.2 | 8.1±0.2 | 8.1±0.2 | 7.8±0.7 | 7.8±0.7 | 7.8±0.7 | 7.8±0.7 | 8.1±0.2 | 7.8±0.7 |

Table 2 Contd…

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 500 | 10.8±0.7 | 10.3±0.2 | 12.1±0.2 | 15.3±0.2 | 8.8±0.7 | 9.1±0.2 | 9.1±0.2 | 9.5±0.5 | 10.5±0.5 | 10.1±0.2 |
| | | 1000 | 17.8±0.2 | 13.5±0.5 | 16.1±0.2 | 20.3±0.2 | 12.3±0.5 | 12.6±0.5 | 12.6±0.5 | 13.8±0.7 | 14.8±0.2 | 12.5±0.5 |
| | Ciproflaxacin | 5 µg/disc | 27.6±0.2 | 30.5±0.5 | 27±0.8 | 27.5±0.5 | 27.6±0.2 | 29.5±0.5 | 33.5±0.5 | NT | NT | NT |
| | Ketaconozole | 10µg/disc | NT | NT | NT | NT | NT | NT | NT | 11.1±0.7 | 9.3±0.7 | 11.1±0.7 |
| *C. tora* | Hexane | 250 | 8.3±0.5 | 7.1±0.2 | 7.8±0.2 | 9.1±0.7 | 6.3±0.5 | NA | 6.3±0.5 | 7.1±0.2 | 8.1±0.7 | 8.3±0.2 |
| | | 500 | 10.1±0.7 | 9.6±0.2 | 10.8±0.2 | 11±0.2 | 8.5±0.5 | 8.1±0.2 | 8.5±0.7 | 10.6±0.2 | 11.8±0.7 | 10.1±0.2 |
| | | 1000 | 12.5±0.5 | 11.8±0.2* | 12.8±0.7 | 13.6±0.2* | 10.5±0.5 | 10.1±0.2* | 10.5±0.5 | 12.5±0.5 | 13.6±0.2 | 12.1±0.2 |
| | Chloroform | 250 | 8.1±0.7 | 10.3±0.2 | 7.5±0.5 | 10.6±0.2 | 7.8±0.7 | 8.1±0.2 | 8.1±0.2 | 8.1±0.2 | 9.3±0.7 | 7.8±0.7 |
| | | 500 | 10.5±0.5 | 11.8±0.7 | 10.8±0.2 | 12.6±0.2 | 10±0.8 | 9.6±0.2 | 10±0.8 | 10±0.8 | 11.1±0.7 | 9.5±0.5 |
| | | 1000 | 13.3±0.5 | 14.6±0.2 | 13.8±0.2 | 15.8±0.2 | 13.8±0.2 | 11.5±0.5 | 12.3±0.7 | 13.3±0.7 | 14.5±0.5 | 12.5±0.5 |
| | Ethyl acetate | 250 | 12.8±0.7 | 10.8±0.2 | 12.1±0.2 | 15.6±0.2 | 11.8±0.7 | 8±0.8 | 7.6±0.2 | 7.3±0.2 | 8±0.8 | 8±0.5 |
| | | 500 | 17.6±0.2 | 13.8±0.7 | 14±0.5 | 21.5±0.5 | 11.1±0.7 | 10.5±0.5 | 11±0.5 | 11.6±0.5 | 12.8±0.7 | 11±0.5 |
| | | 1000 | 24.4±0.5* | 17.8±0.2 | 18.8±0.2* | 25.6±0.2* | 14.8±0.2 | 14±0.5 | 12.5±0.5* | 14.6±0.7 | 16.1±0.7* | 14.3±0.7* |
| | Methanol | 250 | 12.5±0.5 | 10.8±0.7 | 8.1±0.2 | 11.8±0.2 | 8.1±0.2 | 8.1±0.2 | 8.1±0.2 | 7.6±0.2 | 9.3±0.7 | 8.8±0.7 |
| | | 500 | 16.6±0.2* | 11±0.8 | 12.1±0.2 | 15.6±0.2 | 12.1±0.2 | 9.5±0.5 | 10.6±0.*2 | 11.3±0.2* | 11.3±0.7 | 11.8±0.7 |
| | | 1000 | 20.6±0.2* | 15.8±0.2 | 17.7±0.2* | 21.8±0.7 | 13.6±0.2 | 12.6±0.5 | 12.5±0.5 | 12.8±0.2 | 15.1±0.5* | 14.1±0.7* |
| | Ciproflaxacin | 5 µg/disc | 27.6±0.2 | 30.5±0.5 | 27±0.8 | 27.5±0.5 | 27.6±0.2 | 29.5±0.5 | 33.5±0.5 | NT | NT | NT |
| | Ketaconozole | 10µg/disc | NT | NT | NT | NT | NT | NT | NT | 11.1±0.7 | 9.3±0.7 | 11.1±0.7 |

***[a]- Diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]- Mean of three assays; ± - Standard deviation; NT- Not tested; NA – No activity; B.s - Bacillus subtilis; B.p – B. pumilus; M.l - Micrococcus luteus; S.a - Staphylococcus aureus; E.c -Escherichia coli; S.a - Pseudomonas aeruginosa; K.p - Klebsiella pneumoniae; A.n - Aspergillus niger; A.f - A. flavus; A.f - A. fumigatus; *–Significant at P< 0.05 level.***

**Table 3**
***MIC, MBC and MFC values of different solvent extracts of some Cassia species***

| Name of the plant | Extract | (µg/ml) | Microorganisms | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *B.s* | *B.p* | *M.l* | *S.a* | *E.c* | *P.a* | *K.p* | *A.n* | *A.f* | *A.f* |
| *C. alata* | Hexane | MIC | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| | | MBC/MFC | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| | Chloroform | MIC | 250 | 500 | 500 | 500 | 250 | 500 | 500 | 500 | 500 | 500 |
| | | MBC/MFC | 500 | 1000 | 1000 | 1000 | 500 | 1000 | 1000 | 1000 | 1000 | 1000 |
| | Ethyl acetate | MIC | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 |
| | | MBC/MFC | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| | Methanol | MIC | 250 | 500 | 500 | 250 | 250 | 500 | 500 | 250 | 250 | 250 |
| | | MBC/MFC | 500 | 1000 | 1000 | 500 | 500 | 1000 | 1000 | 500 | 500 | 500 |
| *C. auriculata* | Hexane | MIC | 500 | 500 | 500 | 250 | 500 | 500 | 500 | 500 | 250 | 500 |
| | | MBC/MFC | 1000 | 1000 | 1000 | 500 | 1000 | 1000 | 1000 | 1000 | 500 | 1000 |
| | Chloroform | MIC | 250 | 250 | 250 | 250 | 250 | 500 | 500 | 250 | 250 | 250 |
| | | MBC/MFC | 500 | 500 | 500 | 500 | 500 | 1000 | 1000 | 500 | 500 | 500 |
| | Ethyl acetate | MIC | 62.5 | 125 | 125 | 62.5 | 250 | 250 | 250 | 250 | 250 | 125 |
| | | MBC/MFC | 125 | 250 | 250 | 125 | 500 | 500 | 500 | 500 | 500 | 250 |
| | Methanol | MIC | 125 | 250 | 250 | 125 | 250 | 250 | 250 | 250 | 250 | 250 |
| | | MBC/MFC | 250 | 500 | 500 | 250 | 500 | 500 | 500 | 500 | 500 | 500 |
| *C. fistula* | Hexane | MIC | 500 | 500 | 500 | 250 | 500 | 500 | 500 | 250 | 250 | 500 |
| | | MBC/MFC | 1000 | 1000 | 1000 | 500 | 1000 | 1000 | 1000 | 500 | 500 | 1000 |
| | Chloroform | MIC | 250 | 250 | 500 | 250 | 500 | 500 | 500 | 250 | 250 | 250 |
| | | MBC/MFC | 500 | 500 | 1000 | 500 | 1000 | 1000 | 1000 | 500 | 500 | 500 |
| | Ethyl acetate | MIC | 125 | 125 | 125 | 62.5 | 250 | 250 | 250 | 250 | 250 | 250 |
| | | MBC/MFC | 250 | 125 | 250 | 125 | 500 | 500 | 500 | 500 | 500 | 500 |
| | Methanol | MIC | 125 | 250 | 250 | 125 | 500 | 500 | 500 | 250 | 250 | 250 |
| | | MBC/MFC | 250 | 500 | 500 | 250 | 1000 | 1000 | 1000 | 500 | 500 | 500 |
| *C. tora* | Hexane | MIC | 250 | 500 | 250 | 250 | 250 | 500 | 500 | 250 | 250 | 500 |
| | | MBC/MFC | 500 | 1000 | 500 | 500 | 500 | 1000 | 1000 | 500 | 500 | 1000 |
| | Chloroform | MIC | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 |
| | | MBC/MFC | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| | Ethyl acetate | MIC | 62.5 | 125 | 125 | 62.5 | 250 | 250 | 250 | 250 | 125 | 250 |
| | | MBC/MFC | 125 | 250 | 250 | 125 | 500 | 500 | 500 | 500 | 250 | 500 |
| | Methanol | MIC | 125 | 125 | 125 | 62.5 | 250 | 250 | 250 | 250 | 125 | 250 |
| | | MBC/MFC | 250 | 250 | 250 | 125 | 500 | 500 | 500 | 500 | 250 | 500 |

***MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration; MFC – Minimum Fungicidal Concentration; B.s - Bacillus subtilis; B.p – B. pumilus; M.l - Micrococcus luteus; S.a - Staphylococcus aureus; E.c -Escherichia coli; S.a - Pseudomonas aeruginosa; K.p - Klebsiella pneumoniae; A.n - Aspergillus niger; A.f - A. flavus; A.f - A. fumigatus***

# DISCUSSION

The hexane, chloroform, ethyl acetate and methanol extracts of *Cassia* species showed broad spectrum of antimicrobial activity against all the microorganisms tested. In the present study, Gram positive bacteria was more susceptible than Gram negative and fungal pathogens. The differences in the antimicrobial activity of crude extracts may be due to the amount of antimicrobial agent present in the extract[14]. The present study showed the highest antimicrobial activity with lower MIC values and the same trend was observed by Kannathasan *et al.*[15] The methanol extract of *V. peduncularis* showed the highest mean zone of inhibition (22.670 ±0.667 mm) and the lowest MIC (62.5 µg /mL) and MBC values (125.0 µg /mL) against *S. aureus* followed by *M. luteus* (21.670 ±0.667 mm; MIC (62.5 µg /mL). Bakht *et al.*[16] investigated antibacterial activity of different solvent extracts of *Nicotiana tabacum* extracts at different concentrations. Ethyl acetate extract was more effective to control *B. cereus* and *E. carotovora* followed by butanol extract against *S. aureus* and *A. tumefaciens*. However, Abrimi *et al.*[17] studied the antimicrobial activity of chloroform, acetone and methanol extracts of *Enicostemma littorale* leaf, stem and root against various Gram positive, Gram negative and fungal pathogens. The chloroform extract of *E. littorale* showed the highest antibacterial activity (20 mm) against *B. subtilis* with the MIC values of >8.5 mg/mL. This value is much higher than that of the present study. But, all the extracts of *E. littorale* did not inhibit the growth of the studied fungal pathogens, *A. fumigatus* and *A.flavus*. Moreover, varying degree of sensitivity of test microorganisms may be due to inherent tolerance of microorganism[18]. In the present

study, the ethyl acetate extract of *C. tora* was identified for its potential antimicrobial activity against all the microorganisms tested. Based on this preliminary study, isolation and identification of antimicrobial molecule from ethyl acetate extract of *C. tora* is in progress.

## CONCLUSION

The ethyl acetate extract of *C. tora* had a potential antimicrobial activity against all the microorganisms tested. Based on this preliminary study, isolation and identification of antimicrobial molecule from ethyl acetate extract of *C. tora* is in progress.

## ACKNOWLEDGMENT

We thank Dr. R. Panneerselvam, Professor and Head, Department of Botany and Dean, Faculty of Science, Annamalai University for providing laboratory facilities. One of the authors, S.A. Elakkia is grateful to Department of Science and Technology, Govt. of India for the award of INSPIRE fellowship (Award No. IF110319) to carry out this work.

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