

Evaluation of antibacterial potential of medicinal plant *Cassia sophera* against organisms causing urinary tract infection

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ABSTRACT

Urinary tract infection is one of the commonest encountered infections and there has been increase in the incidence of resistant organisms causing urinary tract infection (uropathogens), thereby, challenging the treatment of patients. This has led to search for newer therapeutic modalities including extracts obtained from medicinal plants. In this study, alcoholic leaf extract of medicinal plant *Cassia sophera* was analyzed for antibacterial potential against various uropathogenic bacteria by agar well diffusion method. Minimal inhibitory concentrations (MIC) of the alcoholic extract were determined by broth microdilution method. The alcoholic leaf extract of *C. sophera* effectively controlled the growth of most of the tested uropathogens. It showed maximum activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, with moderate activity against *Staphylococcus saprophyticus*, *Proteus mirabilis* and *Citrobacter freundii*. MIC values of the extract against tested uropathogens ranged from 3.05 to 49.0 µg/ml. The present study shows that extracts of *Cassia sophera* contain good antibacterial activity, and hence, in future it could be used to obtain novel therapeutic compounds for the treatment of patients suffering with urinary tract infections, especially by organisms resistant to currently available drugs. This is the first report of antibacterial activity of *Cassia sophera* extract against uropathogenic bacteria.

Keywords: *Cassia sophera*, antibacterial activity, uropathogens, agar well diffusion, MIC.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most frequently encountered bacterial infections in clinical practice throughout the world¹. Bacteria responsible for UTI, often originate from the resident gut flora and perineal flora of humans²⁻⁴. Under normal circumstances, these bacteria are cleared from the urinary system by effective innate mechanisms of protection. If, however, they overcome these mechanisms, they can colonize the lower urinary tract causing cystitis, and later by haematogenous spread it causes infection of upper urinary tract causing pyelonephritis⁵. Uncomplicated UTI occurs in patients with intact urinary tract, whereas, complicated UTI occurs in patients with structural and functional abnormalities of the urinary tract and those who suffer from chronic diseases like diabetes mellitus⁶. Amongst the bacteria responsible for causing UTI, *Escherichia coli* is the most predominant pathogen in both the community as well as hospital acquired UTIs accounting for 75% to 90% of uncomplicated UTI isolates⁷. *Proteus mirabilis* and *Pseudomonas aeruginosa* are more common in hospital associated UTIs.

Some less encountered organisms responsible for UTI are *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Citrobacter freundii*⁸.

Treatment of UTI depends upon the status of the patient. Especially, in case of pregnant females one has to recommend drug keeping its safety in mind. Also, the treatment of urinary tract infections is increasingly becoming difficult because of the multidrug resistance exhibited by the causative organisms. Isolates causing complicated UTI show greater drug resistance as compared to those isolated from uncomplicated UTI⁵. The increase in incidence of resistant organisms such as extended spectrum β -lactamase producing strains of *Escherichia coli* and *Proteus mirabilis* and methicillin resistant *Staphylococcus* species among clinical isolates over the past few years has resulted in limitation of currently available therapeutic options⁹⁻¹¹. This situation has forced the researchers to search for new antimicrobial substance from various sources including medicinal plants¹². Medicinal plants represent a rich source of antimicrobial agents¹³. The effects of plant extracts on bacteria have been studied by a large number of researchers in different parts of the world¹⁴.

Cassia sophera Linn. is a medicinal plant endemic in Indian sub-continent and Bangladesh and used as folk medicine for treatment of various ailments¹⁵. It belongs to family Caesalpiniaceae and commonly known as Senna sophera and Kasondi. It is an undershrub that grows to a height of 3 metres with yellow coloured flowers and oblong-lanceolate shaped leaves. It has been used for long as traditional medicine for the treatment of inflammatory diseases, psoriasis, cough, arthritis, snake bite and asthma^{16,17}. In Unani and ethnobotanical literature *Cassia sophera* has been described to be used for a wide therapeutic purposes as analgesic, sedative, repulsive of morbid humors (especially phlegm), blood purifier, carminative, diaphoretic, digestive and anticonvulsant agent especially for children¹⁸⁻²⁰. Its bark and seeds are found to be useful in diabetes²¹. The paste of leaves mixed with sandalwood or lime juice is used externally for treatment of ringworm. The leaves in addition possess purgative properties. Externally it is also used for washing syphilitic sores. The juice in honey is used for treatment of bronchitis, asthma and hiccups²². Also, infusion of leaves has been found to be given with sugar in treatment of jaundice and in sub acute stage of gonorrhoea²³.

Although *Cassia sophera* has been used as folk medicine but not much has been known about its antimicrobial potential against bacterial pathogens causing urinary tract infection. Hence, the present study was done to evaluate its antimicrobial activity against common uropathogenic bacteria causing UTI.

MATERIALS AND METHODS

Bacterial Isolates Tested

The uropathogenic bacteria included in our study were isolated from urine samples submitted in the Department of Microbiology. The tested bacterial species were *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*. The control bacterial species tested were *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) obtained from National Institute for Communicable Diseases (NICD), New Delhi, India. All the bacterial strains were grown on Blood agar or MacConkey agar plates at 37°C and maintained on nutrient and blood agar slants.

Collection of Plant materials

Fresh leaves were collected from a 6 months old parent plant of *Cassia sophera* (Figure 1).

Preparation of plant extract

The alcoholic extract of the plant was tested for antibacterial activity. To prepare alcoholic extract, fresh leaves (15 g) from parent plant were surface sterilized in 70% ethyl alcohol for 1 min followed by washing for 3 times with sterilized double distilled water (DDW). These were then grounded with a sterilized pestle and mortar in 150 ml of 95% ethanol and centrifuged at 5000 rpm for 15 min. The resultant supernatant was filtered and taken as the alcoholic extract which was immediately used for experimentation²⁴.

Antibacterial susceptibility testing

Antibacterial susceptibility was tested by agar well diffusion method²⁵. Mueller-Hinton agar (M 173; HiMedia, India) plates were used for determining the antibacterial activity.

The plates were lawn cultured with inoculum of bacterial suspension (equivalent to 0.5 McFarland standard) with the help of sterile swabs. Wells of 5mm diameter were made in each plate using a sterile borer. Plant extract (20µl) was poured in the wells using micropipette. Ethanol was used as negative control, whereas, antibacterial agent norfloxacin was used as positive control. The plates were kept upright for 5-10 min until the solution diffused into the medium and then incubated aerobically at 37°C for 24 hours. Later, the zone of inhibition was measured and recorded. All the experiments were performed in triplicate.

Determination of minimal inhibitory concentrations (MIC)

MICs of the alcoholic extract were determined by broth microdilution method performed according to Clinical and Laboratory Standards Institute (CLSI) approved standards M7-A7, with minor modifications as stated below²⁶. The leaf extract was dissolved in DMSO (Dimethyl sulphoxide), and further diluted 1:50 in RPMI-1640 medium (HiMedia, India), and the resulting solution was used for preparing a doubling dilution series. Doubling dilutions of the extract were prepared in RPMI-1640 broth supplemented with 0.3g/L L-glutamine (HiMedia, India), 0.165 mol/L of 3-[N-morpholino] propanesulfonic acid (MOPS) buffer (HiMedia, India) and 0.01% of DMSO (Qualigens Fine Chemicals, India). Microtitre plates were prepared containing 100µl of undiluted extracts in the first well, followed by doubling dilutions of the extract. Inoculum of each bacterial species having turbidity equivalent to 0.5 McFarland standard was added to the respective dilution wells including the first well. The final concentrations of the extracts ranged from $25 \times 10^3 \mu\text{g/ml}$ to $48 \times 10^{-3} \mu\text{g/ml}$. For each test there was a sterility control well containing alcoholic extract in RPMI-1640 broth plus DMSO and a growth control well containing bacterial suspension only. The microtitre plates were incubated in ambient air at $35 \pm 2^\circ\text{C}$ for 24 hours with their upper surface covered by sterile sealers. The lowest concentration of the extract that did not show any visible bacterial growth was considered MIC of the extract for that bacterial species. All the MIC experiments were performed in duplicate.

Statistical analysis

All the experiments of antimicrobial susceptibility testing were performed in triplicate. The results were expressed as the mean \pm standard error (SE). Data were statistically analyzed by using one way analysis of variance (ANOVA) followed by Tukey's multiple analysis test using SPSS Software, Chicago, III, version 10. P values were calculated by one-sample T-test and $P < 0.05$ was taken as statistically significant.

RESULTS

Antibacterial activities of alcoholic leaf extract of *C. sophera* against the tested uropathogenic bacterial species are shown in Table 1. The Negative control (Ethanol) showed the zone of inhibition in the range of 7.33 ± 0.33 to 8.67 ± 0.33 mm, whereas, the positive control (Norfloxacin) showed the zone of inhibition in the range of 9.33 ± 0.33 to 13.00 ± 0.58 mm. The alcoholic extract of *C. sophera* showed significant activity ($P < 0.05$) against most of the tested uropathogens with maximum inhibition shown against *Escherichia coli* ($P = 0.003$), *Klebsiella pneumoniae* ($P = 0.007$) and *Pseudomonas aeruginosa* ($P = 0.009$) and moderately inhibiting the growth of *Proteus mirabilis* ($P = 0.015$), *Staphylococcus saprophyticus* ($P = 0.017$) and *Citrobacter freundii* ($P = 0.024$). However, the extract did not show any activity against *Enterococcus faecalis*. The minimal inhibitory concentrations (MIC) of the alcoholic extract against these tested uropathogens ranged from 3.05 to 49.0 µg/ml (Figure 2).

Table 1: Antibacterial activity of alcoholic extract of *Cassia sophera* against uropathogenic bacteria

Bacteria tested	Zone of inhibition (mm) ± SE		
	Alcoholic leaf extract ^Δ	Ethanol [†]	Norfloxacin [£]
<i>Staphylococcus saprophyticus</i>	12.67±0.33 ^d	7.67±0.33 ^c	11.33±0.33 ^{cd}
<i>Enterococcus faecalis</i>	0.00±0.00 ^f	7.33±0.33 ^d	9.33±0.33 ^f
<i>Escherichia coli</i>	15.33±0.33 ^a	7.67±0.33 ^c	11.67±0.33 ^c
<i>Klebsiella pneumoniae</i>	14.67±0.33 ^b	7.33±0.33 ^d	10.67±0.33 ^d
<i>Proteus mirabilis</i>	12.67±0.33 ^d	7.33±0.33 ^d	11.67±0.33 ^c
<i>Pseudomonas aeruginosa</i>	13.33±0.33 ^{cd}	7.33±0.33 ^d	10.33±0.33 ^e
<i>Citrobacter freundii</i>	12.33±0.33 ^e	7.67±0.33 ^c	11.67±0.33 ^c
<i>S. aureus</i> ATCC 25923	14.67±0.33 ^b	8.67±0.33 ^a	13.00±0.58 ^a
<i>E. coli</i> ATCC 25922	14.33±0.33 ^{bc}	8.67±0.33 ^a	12.67±0.33 ^b
<i>P. aeruginosa</i> ATCC 27853	13.67±0.33 ^c	8.33±0.33 ^b	11.33±0.33 ^{cd}

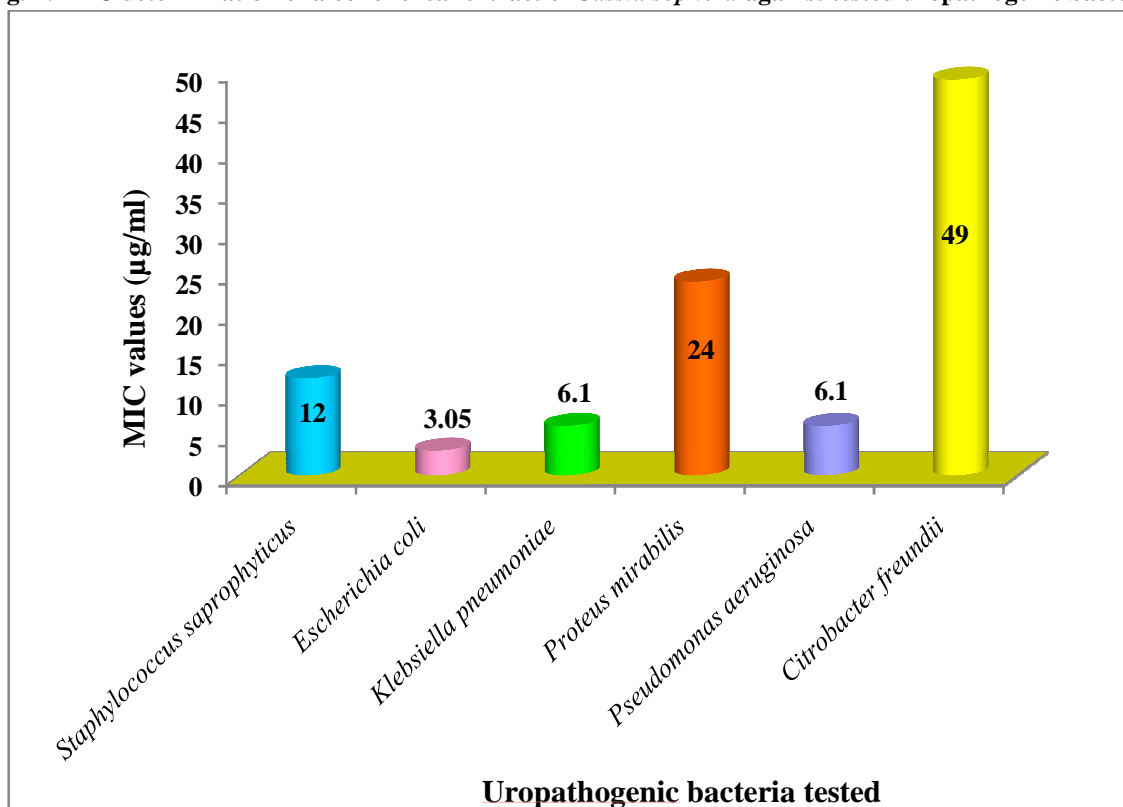
Δ = concentration of plant extract used in the test (2 mg / 20 μl). † = 20 μl of 95% ethanol used as negative control.

£ = 500 μg / 20 μl of norfloxacin used as positive control. Diameter of zone of inhibition is a mean of triplicates ± SE (mm).

Differences were assessed statistically using one way ANOVA followed by Tukey's test. P<0.05 was considered as significant.

The mean represented by same letter is not significantly different within the column.

Fig. 1: Leaves of *Cassia sophera* included in our study

Fig. 2: MIC determination of alcoholic leaf extract of *Cassia sophera* against tested uropathogenic bacteria

DISCUSSION

The alcoholic leaf extract of *Cassia sophera* gave excellent activity against most of the frequently encountered isolates of uropathogens causing community and hospital acquired urinary tract infections. It effectively controlled the growth of *Escherichia coli*, which is the commonest pathogen of UTI. It also inhibited the growth of *Proteus mirabilis* and *Pseudomonas aeruginosa* which are common isolates in hospital acquired UTIs. *Staphylococcus saprophyticus* a common causative agent of UTI in young sexually active females was also found to be inhibited by this plant extract.

In one study good activity of alcoholic leaf extract of *Cassia sophera* was shown against *Escherichia coli* and *Klebsiella pneumoniae*¹⁷. In another study workers showed good activity of alcoholic extract of this plant against *Escherichia coli* and *Pseudomonas aeruginosa*²¹. Both the studies support our present research findings.

Since, in the present study we have tested most of the common causative agents of UTI, we were able to detect a wide spectrum of antibacterial activity of this plant extract against common uropathogens. It was found that alcoholic extract of *C. sophera* effectively controlled the growth of most of the tested uropathogens except *Enterococcus faecalis* which was found to be totally resistant to it. Hence, it may be used in future to obtain novel therapeutic compounds in the treatment of UTIs caused by organisms which show resistance to the currently available antimicrobial agents.

CONCLUSION

Extracts from medicinal plants are increasingly being tested and tried as an alternative mode of therapy in various infectious diseases. The present study explores the antimicrobial potential of extract of *Cassia sophera* against uropathogenic organisms and hence this can be used as an alternative mode of treatment of patients suffering with uncomplicated as well as complicated UTI, thereby, preventing the development of adverse sequelae associated with it.

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