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Original Article

Studies on Antibacterial Activity and Brine Shrimp Toxicity of Leaf Extract of *Cassia sophera*

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Abstract

Introduction: *Natural product plays an important role against microorganisms. Plants are unlimited source of natural products, which still form a major part of ingredients in almost all system of therapeutics. Cassia sophera Linn of Caesalpinaceae family is extensively used in the indigenous medicine as an antimicrobial agent.*

Methods: *The antibacterial activity and brine shrimp toxicity of ethanol extract of leaves of Cassia sophera were evaluated. The present study was carried out in Microbiology laboratory, Institute of Biological Sciences, Rajshahi University, Rajshahi from July/2006 to December/2007. Five Gram positive (Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Streptococcus β-haemolyticus, Staphylococcus aureus) and five Gram negative (Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Shigella dysenteriae) bacteria were tested using disc diffusion method. The harmful impact of the extract was also investigated on brine shrimp.*

Results: *The extract was inactive at the concentration of 30 µg/disc but exhibited improved activity at a concentration of 200 µg/disc against the tested pathogens which had relatively less effect than that of reference drug, Cephradine. In brine shrimp lethality bioassay test, it was observed that LC_{50} value of the extract was 8.62 ppm.*

Conclusion: *Ethanol extract of leaves of Cassia sophera may have some antibacterial as well as toxic effect on brine shrimp.*

Key words: *Cassia sophera Linn., Antibacterial activity, Caesalpinaceae.*

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Introduction

Microbial infection is a common health problem in Bangladesh. People of the rural areas use different parts of plant for the ailment of various bacterial infections. Medicinal plants continue to play an important role for the management of different microbial infections when over medication and long-term side effects of modern drugs have assumed alarming. In recent years, there has been a resurgence of scientific interest in the use of medicinal plants for the development of new pharmacotherapeutic agent for the management of diverse diseases, ranging from simple skin diseases to incurable cancer. The cheap, safe and effective medicinal agents may play as alternative source for cure of microbial infections particularly the resistant cases. The plant *Cassia sophera*, locally called "Chhoto Kalkasunda" belongs to family Caesalpinaceae is a shrub or undershrub, about 2.4-3 meter high, grows annually or perennially throughout the country. Flowering time of the plant: June-July and Fruiting time: Novemer-december.[1] Different parts of the plant are used for ringworm diseases, psoriasis, eczema, cough, bronchitis, diabetes, gonorrhea and in syphilitic sores. The leaves of the plant contain flavanol C glycoside and sennosides. Seeds contain tannic acid, mucilage, fatty oil emodin and a toxalbumin. Root bark contains anthraquinones, chrysophanol, physcion and β-sitosterol.[1,2] Some authors reported the antioxidant and antimicrobial activity of seeds[3] and leaves[4] of *Cassia sophera*. So far it is known from different sources that no antimicrobial work has been done with this plant in Bangladesh. On this perspective, the present study was carried out to investigate the antibacterial activity and brine shrimp toxicity on ethanol extract of leaves of *Cassia sophera* which are abundant in Bangladesh.

Materials and Methods

Cassia sophera leaves were collected from Rajshahi locality and taxonomically identified by an expert (Professor A T M Naderuzzaman (retired), Department of Botany, Rajshahi University). Adhering dirt's of the leaves were removed by washing, cut into small pieces and then dried at room temperature. The dried parts were then grinded to form powder. The dry powder was soaked in ethanol for 5 days in a glass container closed by glass cork with occasional shaking and stirring. The mixture was filtered through cotton-cloth and then concentrated by rotary evaporator at 50 °C under reduced pressure to obtain a semisolid mass. Antibacterial screening and brine shrimp toxicity study were carried out using this crude ethanol extract of *Cassia sophera.*

Ethanol extracts of *Cassia sophera* was examined for their antibacterial activity by disc diffusion method.[5] Ten bacterial species (*Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Streptococcus β-*

haemolyticus, Staphylococcus aureus and *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Shigella dysenteriae*) were selected for this investigation. The medium was (nutrient agar, DIFCO) poured into sterile petridishes and the inoculum was adjusted to contain 10^5 to 10^7 bacteria per ml. The extract was dissolved in ethanol to obtain a concentration of 10 μg/μl. The discs (6 mm in diameter) were prepared by sterile filter paper and dried in an oven to remove moisture. The solutions were applied on the dried filter paper discs by micropipette to obtain discs containing 30 μg and 200 μg of extracts in each disc. Cephradine discs (30 μg/disc) were used as standard. The discs were then placed on the petridishes seeded with the bacterial medium containing inoculum and allowed to diffusion at 4°C for 5-6 hours. The petridishes were then incubated at 37°C for 18 hours and the zone of inhibitions observed were measured.

Brine shrimp lethality bioassay test[6] is a convenient bioassay for active plant constituents. Eggs of *Artemia salina* Lech. were placed in one side of a small tank divided by a net containing 3.8 % NaCl solution for hatching. A light source was placed in other side of the tank to attract the nauplii. After two days of hatching period, the nauplii were ready for the experiment. Then 3 mg of the extract was accurately measured and dissolved in 0.6 ml (600 μl) of dimethyl sulfoxide (DMSO) to get a concentration of 5 mg/ml. From the stock solutions 2, 5,10, 20 and 40 μl were placed in 5 different vials making the volume up to 5 ml by NaCl solution. The final concentration of the samples, in the vials became 2, 5, 10, 20 and 40 μg/ml (ppm), respectively. Ten brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus seawater up to 5 ml was used. After 24 hours of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. Resulting data were transformed to the probit analysis[7] for determination of LC_{50} values for the extract.

Results

Table I showed that crude ethanol extract of the leaves of *Cassia sophera* was inactive against the tested bacteria at concentration of 30 μg/disc, whereas at concentration of 200 μg/disc, it showed improved activity against all the tested bacteria exhibiting their zones of inhibition 10-15 mm diameter. The maximum zone of inhibition (16 mm) was observed against *Shigella dysenteriae*. The standard cephradine was found to have pronounced effect (zone of inhibitions 23-28 mm) at the concentration of 30 μg/disc. In the brine shrimp lethality bioassay test, it was observed that LC_{50} value of the extract was 8.62 ppm whereas the standard ampicillin trihydrate showed its LC_{50} value 5.14 ppm (Table II).

Table I: Zone of Inhibition Against Test Organisms with Crude Leaf Extract of *Cassia sophera* and Reference Drug Cephradine

Test Organisms	Diameter of zone of inhibition (in mm)		
	Leaf extract		Cephradine
	30µg/disc	200µg/disc	30µg/disc
Gram positive:			
Bacillus subtilis	00	11	26
Bacillus megaterium	07	13	27
Sarcina lutea	00	11	23
Streptococcus β-haemolyticus	06	13	27
Staphylococcus aureus	07	12	26
Gram negative:			
Pseudomonas aeruginosa	00	10	24
Escherichia coli	05	10	27
Salmonella typhi	07	12	25
Klebsiella pneumonae	06	11	28
Shigella dysenteriae	08	16	26

Table II: LC_{50} Values of the Ethanol Extract of Leaves of *Cassia sophera* and Standard Ampicillin trihydrate

Extracts	LC_{50} (ppm)	95% Confidence Limit		Regression Equation	λ^2 (df)
		Lower	Upper		
Leaf	8.62	4.44	16.73	Y = 3.63 + 1.46 X	0.38 (2)
Ampicillin	5.14	2.57	10.28	Y = 4.12 + 1.23 X	0.15 (3)

Discussion

Medicinal plants are the rich sources of bioactive compounds and thus serve as important raw materials for drug production. Bacterial Pathogens are rapidly growing resistant to conventional drugs like methicillin and vancomycin resistant *Staphylococcus aureus*. Scientists are now engaged to explore alternative drug from plant source to explore new and potent antibacterial principles. In the continuation of new antibacterial drug discovery, we investigated ethanol extract of leaves of *Cassia sophera*, which is being used as a successive medicinal plant in different diseases by folklore practitioner in our locality. In the present investigation, we found greater antibacterial activity of the

crude ethanol extract of the leaves of *Cassia sophera* against the tested pathogens at the concentration of 200 μg/disc but relatively less than that of reference drug, Cephradine. Some workers reported the antibacterial[3,4] activity of the seed and leaf segments of this plant. They observed fairly good activity with the ethanol and methanol extracts against the tested pathogens that correlates our findings. Detailed study is required to isolate the bioactive antimicrobial constituents present in the extracts.

In the brine shrimp lethality study, extract of leaves of *Cassia sophera* was tested for their toxicity against brine shrimp nauplii and showed positive results indicating that these are biologically active. The mortality rates of brine shrimp were found to be increased with increasing concentration of the samples. There was no mortality in the control groups. The LC_{50} value of the extract of *Cassia sophera* was 8.62 ppm whereas the standard ampicillin trihydrate exhibited its LC_{50} value of 5.14 ppm. There are many reports on cytotoxic activities of the extracts of various plants growing in different parts of this region. It was revealed that crude ethanol extract of whole plant of *Commelina bengalensis* and its three organic solvent fractions demonstrated significant activity in the brine shrimp lethality bioassay test.[8] The LC_{50} values were 14.12, 10.00, 10.00 and 19.95 μg/ml for crude ethanol extract, n-hexane, carbon tetrachloride and chloroform soluble fractions respectively. The results correlate with the findings of the present study.

Conclusion

Phytochemical analysis, pharmacological and clinical tests are prerequisites for developing drugs from medicinal plants. The present findings provide a support for the use of the plant in traditional medicine and strongly suggests the necessity to use this as herbal source of antimicrobial agents.

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Contribution of the Authors

First author was the main researcher, second and third did the data analysis and computer composing. Last one was the supervisor of this study.

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