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Original Article

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

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Abstract

Antibacterial activity and toxicity of ethanol extract of *Cassia grandis* leaves to Brine Shrimp was evaluated. Five Gram-positive and 4 Gram-negative bacteria, namely *Sarcina lutea*, *Bacillus megaterium*, *Bacillus subtilis*, *Streptococcus ß-haemolyticus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* were tested using disc diffusion method. The extract was inactive at the concentration of 30 µg/disc but exhibited moderate to good activity at concentration of 200 µg/disc against the tested bacteria. In Brine Bhrimp lethality bioassay test, it was observed that LC_{50} value of the extract was 10.68 µg/ml. From these findings, it is indicative that *C. grandis* may be useful against microbial diseases.

Key words: Antibacterial activity, Brine shrimp, *Cassia grandis*

Introduction

Microbial infection is a common health problem in Bangladesh. People of the rural areas use different parts of plants for ailment of various bacterial infections.[1] Medicinal plants continue to play an important role for the management of different microbial infections when overmedication and long-term side effect(s) of modern drugs have assumed alarming range. In recent years, there has been a resurgence of scientific interest in the use of medicinal plants for the development of new pharmacotherapeutic agents. The usage of herbal agents for the management of diverse diseases ranging from simple skin diseases to incurable cancer have been investigated. Effective, safe and cheap medicinal agents may appear as potential alternatives for controlling microbial infections particularly the resistant cases.

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Cassia grandis, belonging to family Leguminosae, is a large sized tree, usually planted in the roadside and also in the garden. This is a spreading tree with fairly smooth gray bark. The leaves fall in the cold weather and the flowers appear in the February to April (sometimes when the tree is almost leafless). This is a very beautiful and ornamental tree when in full bloom.[2] Some authors reported the antifungal activity of bark and leaves of *C. grandis*.[3] So far it is known, no antibacterial work has been done with this plant. On this perspective, the present work was objected to carry out scientific investigations on antibacterial activity and toxicity of leaf extract of *Cassia grandis* to Brine Shrimp.

Methods

Preparation of the extracts

Fresh leaves of *Cassia grandis* were collected from Rajshahi locality and taxonomically identified by an expert. Adhering dirts of the leaves were removed by washing and were cut into small pieces. The plant parts were then dried at room temperature avoiding sunlight. The dried parts were then

milled to form powder. The dry powder was soaked in ethanol for 5 days in a closed glass container 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 ethanol extract of *C. grandis*.

Antibacterial screening

The ethanol extracts were examined for their antibacterial potency by disc diffusion method[4] against nine bacterial species (5 Gram-positive and 4 Gram-negative). The bacterial speces were collected from Microbiology Laboratory, Department of Pharmacy, Rajshahi University and Department of Microbiology, Rajshahi Medical College, Rajshahi. The medium was (Nutrient agar, DIFCO, UK) 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 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 inoculum over the medium and allowed to diffuse at 4°C for 5-6 hours. The petridishes were then incubated at 37°C for 18 hours and the zones of inhibitions observed were measured.

Brine Shrimp lethality bioassay test

Brine Shrimp lethality bioassay test[5] 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. In other side of the tank, a light source was placed in order 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. The resulting data were transformed to the probit analysis[6] for the determination of LC_{50} values for the extracts.

Results

The crude ethanol extract of the leaves of *Cassia grandis* was found inactive against the tested bacteria at concentration of 30 µg/disc, whereas at concentration of 200 µg/disc, it showed moderate to good activity against all the tested bacteria exhibiting their zones of inhibition of 10-15 mm in diameter. The maximum zone of inhibition (15 mm) was observed against *Pseudomonas aeruginosa*. The standard Cephradine was found to have pronounced effect (zone of inhibitions 25-29 mm) at the concentration of 30 µg/disc. (Table I)

Table I. Comparison of *in vitro* antibacterial activity shown by zone of inhibition of crude leaf extract of *Cassia grandis* and standard Cephradine (C)

Test organisms	Diameter of zone of inhibition in mm of-		
	Leaf extract discs		Cephradine
	30µg/disc	200µg/disc	30µg/disc
Sarcina lutea	00	13	26
Bacillus megaterium	00	11	25
Bacillus subtilis	00	10	28
Streptococcus ß-haemolyticus	00	12	27
Staphylococcus aureus	00	10	25
Pseudomonas aeruginosa	00	15	27
Escherichia coli	00	11	29
Salmonella typhi	00	10	26
Klebsiella pneumoniae	00	12	29

In the Brine Shrimp lethality bioassay test, it was observed that LC_{50} value of the extract was 10.68 ppm whereas the standard Ampicillin trihydrate showed its LC_{50} value 5.14 ppm. (Table II)

Table II: Results of crude ethanol extract of leaves of *Cassia grandis* on Brine Shrimp nauplii

dose (µgm)	Log dose	Number tested	Killed	% killed	Corr %	Emp probit	Expt probit	Wrk probit	Weight	Final probit
2	0.3010268	10	1	10	10	3.72	3.698228	3.73	3.02	3.727573
5	0.6989628	10	3	30	30	4.48	4.428875	4.48	5.58	4.423418
10	0.9999897	10	5	50	50	5	4.981587	4.99	6.34	4.949806
20	1.301017	10	6	60	60	5.25	5.5343	5.22	5.81	5.476192
40	1.602043	10	9	90	90	6.28	6.087013	6.21	4.39	6.002579

Discussion

Infection-causing-bacteria are rapidly becoming resistant to conventional drugs for example Methicillin- and Vancomycin-resistant *Staphylococcus aureus* (MRSA/ VRSA). Scientists are now working to explore alternative drugs from plant sources to explore new and potent antibacterial principles.[7] In the continuation of new antibacterial drug discovery, ethanol extract of leaves of *Cassia grandis* was investigated, which is being used as a successive medicinal plant in different diseases by folklore practitioner in our locality. In the present investigation, moderate to good antibacterial activity of the crude ethanol extract of the leaves of *C. grandis* were found against the tested pathogens at the concentration of 200 µg/disc.

Previously, some workers[3] reported the antifungal activity of the bark and leaf segments of the plant, but no antibacterial study has been found with the plant part. Probably, this is the first-time report on antibacterial activity regarding this plant. A detailed study is required to detect and isolate the active antimicrobial constituents present in the extract.

In the Brine Shrimp lethality study, extract of leaves of *C. grandis* 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 concentrations of the samples. There was no mortality in the control groups. The LC_{50} value of the extract of *C. grandis* was 10.68 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 g/ml for the crude ethanol extract, n-hexane, carbon tetrachloride and chloroform soluble fractions respectively. These results correlate the findings of the present study.

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[Conflict of Interest: none declared]