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Brine Shrimp Toxicity of Leaf and Seed Extracts of *Cassia alata* Linn. and Their Antibacterial Potency

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Present study assayed the toxicity effect of ethanolic leaf and seed extract of *Cassia alata* and found promising activity. From the probit transformation of resulting mortality data we got LC_{50} values of 4.31 ppm ($\mu\text{g mL}^{-1}$) for seed and 5.29 ppm for leaf extract. Seed extract explored potent cytotoxicity similar to the standard gallic acid ($LC_{50} = 4.53$ ppm). From the antibacterial and MIC data it was evident that seed extract gave least activity against the tested bacteria whereas leaf extract deserved promising antibacterial activity.

Key words: *Cassia alata* Linn., *Artemia salina* Leach., toxicity, antibacterial activity

INTRODUCTION

The shrimp lethality assay was proposed by Michael *et al.*^[1] and later developed by Vanhaecke *et al.*^[2], Sleet and Brendel^[3]. It is based on the ability to kill laboratory-cultured *Artemia nauplii* brine shrimp. The assay is considered a useful tool for preliminary assessment of toxicity^[4] and it has been used for the detection of fungal toxins^[5], plant extract toxicity^[6], heavy metals^[7], cyanobacteria toxins^[8], pesticides^[9] and cytotoxicity testing of dental materials^[10]. For the assessment of cytotoxicity property of leaf and seed extracts of *Cassia alata* we used the conventional brine shrimp toxicity bioassay. The aim of the present study was to evaluate the medicinal property of the traditionally important plant grown in Bangladesh. In modern days scientists, all over the world, are engaged to explore bioactive properties of medicinal plants and also to explore single bioactive principles with the hope of adding new chemotherapeutic agents used against the most life threaten diseases like cancer, AIDS, SARS, infectious diseases etc. Biological studies on medicinal plants having their wide range of activities are going on throughout the world^[11-13]. Bangladesh is a rich cultural heritage of traditional medicine and proper scientific evaluation of the plants used in the practice of folk medicine would carry enormous potential and promise for "Health for All" by the 21st century. For isolate novel and effective bioactive agents, biological screening on medicinal plants grown in Bangladesh would play a vital role.

Cassia alata Linn. locally called "Dadmardan" is a large shrubby plant grows wild in all the districts of Bangladesh and also planted^[14,15]. Various parts of the plant are often used for boils, carbuncles, cut wound, foul ulcer, dysentery, ringworm, itch, eczema, helminthiasis and various intestinal troubles in rural areas^[14]. Some authors reported the antimicrobial activity of some segments of *Cassia alata* found in different country^[16-18]. Other workers reported diverse biological properties^[19-24]. So far we know that every segments of this plant available in Bangladesh are not critically evaluated for their biological property. So, the aim of the present study was to evaluate the cytotoxic property of leaf and seed extracts as well their antibacterial potency.

MATERIALS AND METHODS

Preparation of the extracts: Leaf and seed extracts of *Cassia alata* were collected from the month of January to March of 2002. The plant was taxonomically identified by Professor A. T. M. Naderuzzaman, Department of Botany,

Rajshahi University and voucher specimens have been deposited in the same department. The plant parts were then dried at room temperature avoiding sunlight and the dried parts were then powered by grinding machine and reserved in an airtight glass container. Dry powder of seed and leaf of *Cassia alata* were extracted with hot ethanol in soxhlet apparatus for 72 h. The ethanolic extracts were concentrated by rotary vacuum distillatory at 50°C under reduced pressure.

Cytotoxicity bioassay: Brine shrimp lethality bioassay^[25-28] was carried out to investigate the cytotoxicity of ethanol extracts of seed and leaf of *Cassia alata*. Here, *in vivo* lethality test were carried out using brine shrimp nauplii eggs (*Artemia salina* Lech.). Eggs 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 each extract was accurately measured and dissolved in 0.6 mL (600 μ L) of DMSO to get a concentration of 5 mg mL⁻¹. From the stock solutions 0.5, 1, 2, 5, 10, 20 and 40 μ L were placed in 7 different vials making the volume up to 5 mL by NaCl solution. The final concentration of the samples, in the vials became 0.5, 1, 2, 5, 10, 20 and 40 μ g mL⁻¹ (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 h 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^[29] for the determination of LC₅₀ values for the extracts.

Antibacterial screening: The ethanol extracts were examined for their antibacterial potency by disc diffusion method^[30,31]. This method is highly effective for rapidly growing microorganisms and the activities of the test compounds expressed by measuring the diameter of zone of inhibition (mm) around the disc. Generally the more susceptible the organism the larger is the zone of inhibition. Nine bacterial species (Gram-positive: *Sarcina lutea*, *Bacillus megaterium*, *Bacillus subtilis*, *Streptococcus β -haemolyticus* and *Staphylococcus aureus* and Gram-negative: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli* and *Salmonella typhi*) were selected for this investigation and all these strains were collected from the "Institute of Nutrition and Food Sciences" (INFS), Dhaka University, Bangladesh. The medium nutrient agar (DIFCO) was poured into sterile

petri dishes and the inoculum was adjusted to contain 10^5 to 10^7 bacteria per mL. The extracts were dissolved in ethanol to obtain a concentration of $10 \mu\text{g } \mu\text{L}^{-1}$. 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. Ciprofloxacin discs ($30 \mu\text{g disc}^{-1}$) were used as standard. The discs were then placed on the petri dishes seeded with the bacterial medium containing inoculum and allowed to diffusion at 4°C for 5-6 h. The petri dishes were then incubated at 37°C for 18 h and the zone of inhibitions observed were measured by scale.

Determination of minimum inhibitory concentration (MIC):

A current definition of the minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculum viability^[32]. The determination of the MIC involves a semi quantitative test procedure and done by serial dilution technique^[33] against the tested bacteria: *Streptococcus beta-haemolyticus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*. Dilution series were set up with 2, 4, 8, 16, 32, 64, 128, 256, 512 $\mu\text{g mL}^{-1}$ of nutrient broth medium (DIFCO). 100 μL (0.1 mL) of the standardized suspension of pathogenic bacteria ($10^7 \text{ cell mL}^{-1}$) were added and incubated at 30°C for 24 h. The lowest concentration which did not show any growth of the tested microorganism after macroscopic evaluation was determined as the MIC.

RESULTS

Brine shrimp toxicity: Results of brine shrimp lethality bioassay were estimated by probit software using mortality data. From these calculations it was revealed that seed extract gave maximum cytotoxicity with the lower LC_{50} value of 4.31 ppm (Table 1) compared with the standard gallic acid which gave the value at 4.35 ppm leaf extract also explored its promising cytotoxicity ($\text{LC}_{50} = 5.29 \text{ ppm}$). From the results it was clear that seed extract was more toxic to brine shrimp *Artemia salina*.

Antibacterial potency: Leaf and seed extracts of *Cassia alata* were inactive at lower concentration of $30 \mu\text{g disc}^{-1}$ against the tested bacterial species but the leaf extract was active at higher concentration of $200 \mu\text{g disc}^{-1}$ (Table 2). Seed extract did not exhibit any activity at this higher concentration against five tested bacteria *Bacillus megaterium*, *Streptococcus beta-haemolyticus*, *Salmonella typhi*, *E. coli* and *Pseudomonas aeruginosa* but displayed very smaller zones of 09-10 mm against other four tested bacteria. At the higher concentration the leaf extract gave zone of inhibitions of 10-14 mm against the tested bacteria species (Table 2) which indicated its intermediate antibacterial activity compared with the zone of inhibition of standard ciprofloxacin which gave the values at 24-28 mm.

Minimum inhibitory concentration: MIC was determined by visual observation of the bacterial growth in the test tubes containing extracts and inoculum of the five

Table 1: The results of cytotoxic effect of the ethanolic extracts of seed and leaf of *Cassia alata* and reference standard Gallic acid

Test samples	LC_{50} (ppm)	95% confidence limit (ppm)		Regression equation	λ^2 (df)
		Lower	Upper		
Ethanol extract of seed	4.31	2.75	6.75	$Y = 3.68 + 2.08 X$	0.26 (2)
Ethanol extract of leaf	5.29	2.50	11.18	$Y = 4.07 + 1.29 X$	0.13 (2)
Gallic acid (Reference standard)	4.53	3.33	6.15	$Y = 3.93 + 1.62 X$	1.25 (2)

df = Degree of freedom λ^2 = Chi-squared value

Table 2: *In vitro* antibacterial activity of leaf and seed extracts (crude) of *Cassia alata* and standard ciprofloxacin (CF)

Test organisms	Diameter of zone of inhibition (mm)				Ciprofloxacin $30 (\mu\text{g disc}^{-1})$
	Crude ethanol extracts of <i>Cassia alata</i>				
	Leaf ($\mu\text{g disc}^{-1}$)		Seed ($\mu\text{g disc}^{-1}$)		
	30	200	30	200	
Gram positive					
<i>Sarcina lutea</i>	00	12	00	10	24
<i>Bacillus megaterium</i>	00	13	00	00	25
<i>Bacillus subtilis</i>	00	11	00	09	25
<i>Streptococcus beta-haemolyticus</i>	00	13	00	00	25
<i>Staphylococcus aureus</i>	00	11	00	09	26
Gram negative					
<i>Salmonella typhi</i>	00	10	00	00	26
<i>Escherichia coli</i>	00	11	00	00	27
<i>Pseudomonas aeruginosa</i>	00	14	00	00	26
<i>Klebsiella pneumoniae</i>	00	14	00	10	28

Table 3: Minimum inhibitory concentration (MIC) values of leaf and seed extracts (crude) of *Cassia alata* and standard ciprofloxacin (CF).

Test organisms	Minimum inhibitory concentration ($\mu\text{g mL}^{-1}$)		
	Leaf extract of <i>Cassia alata</i>		Ciprofloxacin
	Ethanol extract	Seed extract of <i>Cassia alata</i>	
<i>Streptococcus \beta</i> -haemolyticus	128	512	4
<i>Staphylococcus aureus</i>	128	512	4
<i>Pseudomonas aeruginosa</i>	128	512	2
<i>Salmonella typhi</i>	256	512	4
<i>Klebsiella pneumoniae</i>	256	512	2

pathogenic bacteria. Against the tested species (*Streptococcus \beta*-haemolyticus, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*) ethanol extract of leaf gave the values at 128, 128, 128, 256 and 256 $\mu\text{g mL}^{-1}$, respectively where as seed extract gave MIC values at the same of 512 $\mu\text{g mL}^{-1}$ (Table 3). Standard ciprofloxacin displayed very low MIC values at 2-4 $\mu\text{g mL}^{-1}$ against the species.

DISCUSSION

Cancer is a big challenge to the world as suitable remedy is very costly and even impossible in some cases. On the other hand the conventional chemotherapeutic agents are growing resistance^[34]. Microbial infections are also creating health hazards for the multi-drug resistance bacteria. Scientists are now engaged to find potent remedy from cancer and other infectious diseases through the discovery of new and effective chemotherapeutic agents from plant, microbes and other suitable sources. In the continuation of this search we have studied the cytotoxic and antibacterial effects of two segments (Leaf and Seed) of *Cassia alata* grown in Bangladesh. Potent cytotoxicity have been found for both the ethanol extracts of leaf and seed but seed extract gave comparatively higher toxic effects against *Artemia*. Brine shrimp lethality bioassay is a primary assay for to detect cytotoxic property of plant extract and for this, further studies are required to establish the cytotoxicity of the plant extracts against human cancer cell lines but we can predict that these segments (Leaf and Seed) will give better results on cancer cell lines.

Previously many scientists have got promising antibacterial properties of plant extracts against resistance organisms^[35-37]. So, in this study interest goes to antibacterial assay and have got mentionable activity for the leaf extract. Though it was investigated taking crude extract only so, further studies taking single components from these extracts may explore potent antibacterial as well as cytotoxic properties.

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