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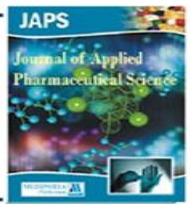

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Antimicrobial, cytotoxic and thrombolytic activity of *Cassia senna* leaves (family: Fabaceae)

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ABSTRACT

Cassia senna leaves belonging to the family Fabaceae have been investigated for the presence of its secondary metabolites and evaluation of biological activities of the crude extracts with special emphasis to the antimicrobial activity, cytotoxic activity and thrombolytic activity. The antimicrobial activities of n-hexane, chloroform, ethyl acetate & methanolic extracts of *C. senna* leaves were screened against five gram(+) bacteria, eight gram(-) bacteria and three fungi by 'disc diffusion method'. The methanol extract possesses no antimicrobial activity but chloroform and n-hexane fractions exhibited moderate to less activity against some organisms tested compared with the standard antibiotic Kanamycin. Brine shrimp lethality bio-assay was done using brine shrimp Nauplii and dimethyl sulfoxide as a solvent for the methanol extracts of *C. senna*. The LC_{50} value (1.5625) of methanol extract of the plant indicated that the cytotoxicity was very significant. The percentages found in thrombolytic tests are 41.46%, 53.22%, 33.33%, 4.08% and standard 92.85%. So, in comparison with standard, *C. senna* can be further use as mild thrombolytic agent.

Keywords: *Cassia senna,* antimicrobial activity, cytotoxic activity, thrombolytic activity, kanamycin, dimethyl sulfoxide.

INTRODUCTION

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar and Farrokhi et al., 2004). *Cassia* species have been of medical interest due to their good therapeutic value in folk medicine (Abu et. al., 2000). Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase according to Gennaro (2000) and Sweta et al., (2006). The plant sap can act against microorganisms by preventing the growth of microbial colony (Hammer et. al., 1999). Some of the plant extract also increase lethality of the cell due to their known cytotoxic effect. Brine shrimp lethality bioassay is performed for evaluating the level of toxicity according to the method of Persoone, 1980 and Goldstein et al., 1974. Keeping this fact in the consideration, the attempts were made to establish physiochemical standards of the plant *Cassia senna* (Common names- senna, Indian senna, English- sanay, hindi- sana ka pat) belonging to family Fabaceae.

The Fabaceae, also called Leguminosae or bean and pea family, is the third largest family of angiosperms after Orchidaceae (orchids) and Asteraceae (daisies, sunflowers), and second only to Poaceae (grasses) in terms of agricultural and economic importance (Ghani, 1990). This plant is widely found in India, Pakistan, Sri Lanka, Tropical Africa, Egypt, Sudan and Bangladesh. It is a small shrub grows up to 70 cm height with erect or spreading branches. Leaves are paripinnate, flowers are recemes, yellow, fruits are flat legumes, greenish brown or dark. Seeds are 5-7 per pods, dark brown and smooth (Hardman et al., 1996; Graves, 1990). In present study, *C. Senna* has been investigated for antimicrobial, cytotoxic and thrombolytic study.

MATERIALS AND METHODS

Preparation of extract

C. senna leaves were collected from Mymenshingh District of Bangladesh in May 2011 and were identified by the Bangladesh National Herbarium, Mirpur, and Dhaka. One voucher specimen was deposited in Bangladesh National Herbarium which accession number is 34186. After separating undesirable parts *C. senna* leaves were dried in open air under shed for approximately 15 days which was then ground to coarse powder with the help of suitable grinder form. About 850 gm of that powder material was then soaked with 4 litre of methanol in a sealed container for another 15 days. Then the mixture was filtered through whatman filter paper. After 10 to 15 days evaporation of methanol occurred and concentrated methanolic extract was acquired. It rendered a greenish black color. The greenish black color extract was designated as crude extract of methanol. 850 gm of powdered *C. senna* was taken and after evaporation it yields 16 gm of *C. senna*. So, percent yield is {(16/850) X 100%} =1.88%.

Preparation of mother solution

7gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This is the mother solution, which was partitioned off successively by three solvents of different polarity. In subsequent stages, each of the fractions were analyzed separately for the detection and identification of compounds having antimicrobial, cytotoxic and thrombolytic property. The mother solution was taken in a separating funnel. 100 ml of the n-hexane was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected and the process was repeated thrice. n-hexane fractions that were collected together evaporated in air until dried. To the mother solution left after washing with n-hexane, 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with ethyl acetate (100 ml).The ethyl acetate fractions were collected together and evaporated. The aqueous fraction was preserved for the next step. To the mother solution that left after washing with n-hexane and ethyl acetate, 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with Chloroform (100 ml X 3). The chloroform soluble fractions were collected together and evaporated. The aqueous methanolic fraction was preserved as aqueous fraction.

Antimicrobial assay

The antimicrobial assay was performed by using the disc diffusion method described by Evans (1996). All collected fractions of the leaves like n-hexane, ethyl acetate and chloroform were tested along with the methanol extracts of the whole plants for antimicrobial study by using standard disc diffusion method. In this study, 16 microorganisms were obtained from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. Standard Kanamycin (30 µg/disc) and blank sterile filter paper disc were used as positive and negative controls respectively Anderson et al., (1991) and Tripathi (2003). Nutrient agar medium (DIFCO) was used to prepare fresh cultures for testing the sensitivity of the organisms (Rundel et al., 1989). The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates which were previously inoculated with test bacteria. The discs were then incubated on the plate aerobically at 37ºC for 24 hours. The diameter of zone of inhibition around each disc was measured and recorded at the end of the incubation period.

Cytotoxic activity test

Brine shrimp lethality bioassay was used for probable cytotoxic action according to Persoone (1980). The eggs of Brine shrimp (Artemia salina Leach) were collected and hatched in a tank at a temperature around 37°C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. 4.0 mg of methanolic extracts of *C. senna* were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) to get stock solutions. By using the serial dilution method a series of solutions of different concentrations were prepared from the stock solution and the concentrations were as; 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml, 0.3906µg/ml, 0.1953µg/ml. With the help of a Pasteur pipette 10 living nauplii were put to each of the vials. After 24 hours the vials were observed and the number of nauplii survived in each vial was counted. From this, the percentage of lethality of Brine Shrimp nauplii was calculated for each concentration of the extract. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value (Meyer et al., 1982). This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period. Vincristine sulphate with DMSO were used as a positive control group whereas DMSO was used as negative control group according to Tyler and Brady (1988); McKey (1994) and Lewis et al., (2005). If the brine shrimps in negative control group show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds.

Thrombolytic test

100 mg extract was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The

suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. 100 µl of this aqueous preparation was added to each micro centrifuge tube containing clots to check thrombolytic activity. 5 micro centrifuge tubes were taken, sterilized, weighed and 5 ml blood was drawn from healthy volunteer. The blood was distributed in 5 different pre weighed (W_1) micro centrifuge tube, each tube containing 1ml of blood. The blood specimen was centrifuged at 2500 rpm for 5 minutes and incubated the blood for 45 minutes at 37ºC. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed (w_2) to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone). To each micro centrifuge tube containing pre-weighed clot, 100µl of aqueous extract of *C. senna* leaves was added separately. As a positive control, 100µl of streptokinase was added to clot of tube no.5 (Standard) and as a negative non thrombolytic control, 100µl water is added to clot of tube no.4 (Blank). All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed (w_3) to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis (Handin et al., 2005 and Wardlaw et al., 2003).

RESULTS AND DISCUSSION

Assay for antimicrobial activity of *C. senna*

The study showed that the methanol extract at a concentration of 300µg/disc has no zone of inhibition produced in case of 13 bacterial strains and 3 fungal strains where standard Kanamycin (30µg/disc) showed zone of inhibition of 32-39 mm (Table 1, fig. 1). The study was done for two times for the confirmation of no inhibitory effect. But, in case of n-hexane fraction of whole plant it shows a moderate antibacterial activity for two gram-positive bacteria like *Bacillus cereus* (12mm) & *Staphylococcus aureus* (14mm) & two gram-negative strains *Escherichia coli* (18mm) and *Vibrio mimicus* (16mm) where *Pseudomonas aeruginosa* (10mm) possess less effect in-contrast to standard Kanamycin. The experiments also revealed that n-hexane extract possess a very less antifungal activity for *Candida albicans* (8mm) & *Sacharomyces cerevacae* (7mm). The study also showed that the ethyl acetate fractions has no antifungal activity among the three fungi used in experiment. However, lower potentiality is seen as antibacterial agent against *Staphylococcus aureus*, *Escherichia coli*, *Shigella boydii* & *Vibrio mimicus*. Most likely the chloroform extracts also showed few potentiality against some microorganisms used in the experiment.

Assay for cytotoxicity of *C. Senna*

In the bioassay, the methanol extracts showed lethality indicating the biological activity of the compound present in the extract. Test samples showed different mortality rate at different concentrations. For the extract, the number of nauplii died and percent of mortality were counted. The LC_{50} value for the extract was obtained from the Table 2. Plot of percent of mortality versus log concentration on the graph paper produced an approximate linear correlation between them. From the graph (Figure 2) the concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred can be obtained by extrapolation.

In vitro thrombolytic effect of *C. senna*

Atherothrombotic diseases occur as serious impacts of the thrombus formed in blood vessels. Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels. But these drugs are not above limitations and can lead to serious and sometimes fatal consequences. The present study was carried out to investigate the thrombolytic activity of the *C. senna* leaves extract. An in vitro thrombolytic method was used to investigate the thrombolytic activity of leaves extract in blood sample from healthy human volunteers, along with streptokinase as a positive control and water as a negative control. The comparison of positive control with negative clearly demonstrated that clot dissolution does not occur when water was added to the clot. On the basis of the result obtained in this present study we can say that the *C. senna* leaves extract have mild thrombolytic activity compared to negative control (water).

Data analysis: Experiment

No of sample	W_1 (gm)	W_2 (gm)	W_3 (gm)
Sample-1	6.15g	6.56g	6.39g
Sample-2	6.16g	6.78g	6.45g
Sample-3	6.20g	6.50g	6.40g
Standard	6.29g	6.57g	6.31g
Blank	6.18g	6.67g	6.65g

% of clot lysis = (wt of released clot /clot wt) × 100

$= (W_2 – W_3 / W_2 – W_1) \times 100$

The percentages found in thrombolytic tests were- sample1 (methanol extract): 41.46%, sample 2 (n-hexane)**:** 53.22%, sample 3 (ethyl acetate)**:** 33.33%, blank**:** 4.08% and standard (streptokinase)**:** 92.85%. So, in comparison with standard, *C. senna* can be further use as a mild thrombolytic agent (fig. 3).

CONCLUSION

In the antimicrobial study, methanol extracts of leaves did not show any antibacterial or antifungal properties. However, the n-hexane, chloroform & ethyl acetate fraction have moderate to less antibacterial functions against some strains along with a less potent antifungal activity. The thrombolytic activity of standard was found 92.85% and the methanol, n-hexane and ethyl acetate extracts of *C. senna* were found 41.46%, 53.22%, and 33.33% respectively, which indicates mild thrombolytic activity. So, in comparison with standard, *C. senna* can be further use as mild thrombolytic agent. As apparent from our results of brine shrimp lethality bioassay it can be revealed that the methanol extracts of *C. senna* have cytotoxic activity. In summary, pharmacological evaluation of *C. senna* leaves extract shows activities like antimicrobial, cytotoxic and thrombolytic. However, further studies are necessary to elucidate the mechanism behind these effects. This report may serve as an important issue to use this plant as a new source of medication.

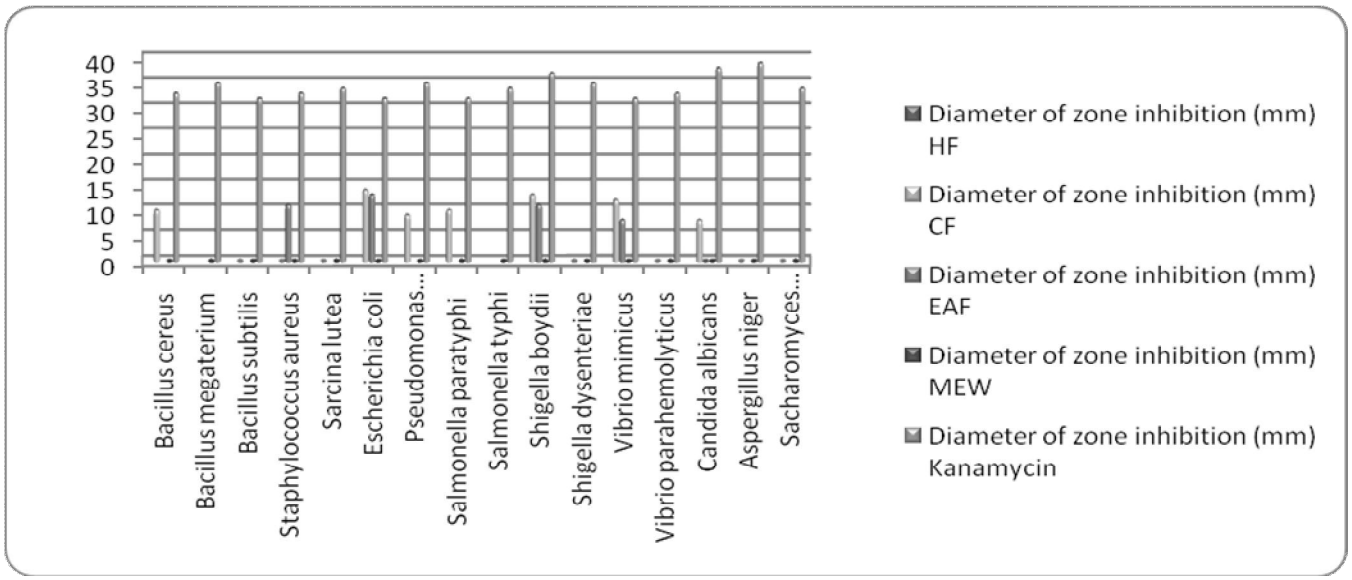

Fig1: Antimicrobial Screening Test.

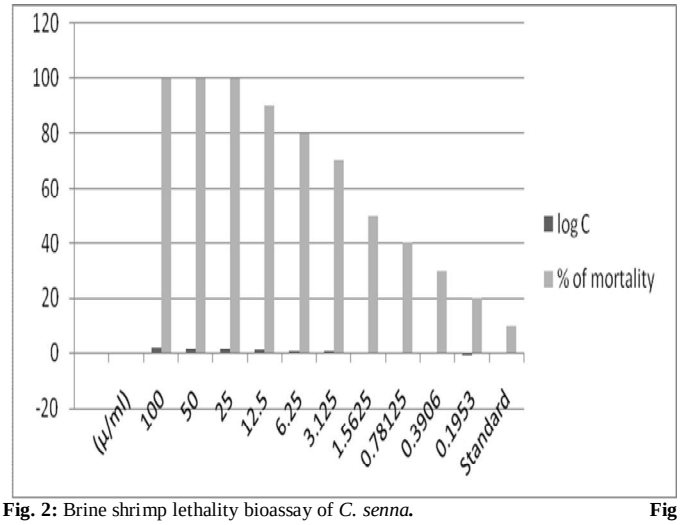

Fig. 2: Brine shrimp lethality bioassay of *C. senna.*

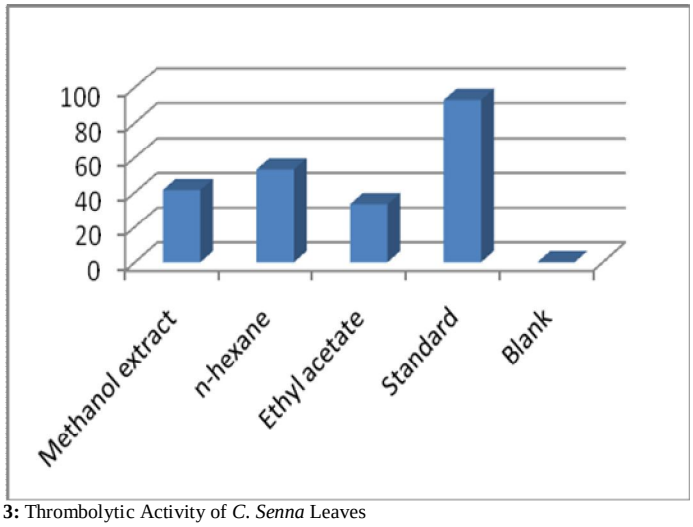

Fig 3: Thrombolytic Activity of *C. Senna* Leaves

Table 1: Antimicrobial activity of different fractions of *C. senna*.

Organism type	Test organism	Diameter of zone inhibition (mm)				
		HF	CF	EAF	MEW	Kanamycin
Gram (+) bacteria	Bacillus cereus			12	10	
	Bacillus megaterium					35
	Bacillus subtilis					32
	Staphylococcus aureus			14	11	
	Sarcina lutea					34
Gram (-) bacteria	Escherichia coli		16	14	13	32
	Pseudomonas aeruginosa		10	09		35
	Salmonella paratyphi			10		32
	Salmonella typhi					34
	Shigella boydii			13	11	32
	Shigella dysenteriae					35
	Vibrio mimicus				8	32
	Vibrio parahemolyticus		-	-	-	33
Fungi	Candida albicans		8	8		38
	Aspergillus niger					39
	Sacharomyces cerevacae		7			34

MEW = Methanol extracts of Whole Plant leaves, CF = Chloroform fraction, HF = Hexane fraction, EAF = Ethyl Acetate fraction

Table 2: Result of Brine shrimp Lethality Bio-assay of *C. senna.*

Concentration of Sample C(µg/ml)	Log Conc. (log C)	No. of nauplii	No. of alive	Percentage(%) of mortality	LC_{50}(µ/ml)
100	2	10	00	100	
50	1.6989	10	00	100	
25	1.3979	10	00	100	
12.5	1.0969	10	01	90	
6.25	0.7958	10	02	80	1.5625
3.125	0.4948	10	03	70	
1.5625	0.1938	10	05	50	
0.78125	-0.1072	10	06	40	
0.3906	-0.4082	10	07	30	
0.1953	-0.7092	10	08	20	
Standard		10	09	10	

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