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Phytochemical Screening and Cytotoxicity Potential of Ethanolic Extracts of *Senna siamea* Leaves

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Abstract-The present study was carried out to evaluate the phytochemical properties and cytotoxicity potential for ethanolic extract of *Senna siamea* leaf. Identification of the chemical constituents of plant extract was determined following the standard procedures. In phytochemical screening, the crude extract was tested for the presence of different chemical groups like reducing sugar, tannins, saponins, steroids, flavonoids, gums, alkaloids and glycosides. Cytotoxicity potential was assessed by the brine shrimp lethality bioassay method. Phytochemical screening confirmed the presence of tannins, steroids and glycosides. Incase of cytotoxicity assay, the extract showed moderate cytotoxic activity having LC_{50} value of 68.633 µg/ml. The present study concludes that the leaf of *Senna siamea* may be a potential source of cytotoxic compounds. The next step would be to isolate the individual compounds responsible for the observed activity and the probable mechanism of action of it.

Keywords: *Senna siamea*, Phytochemical Screening, Cytotoxicity, Ethanolic extract.

INTRODUCTION

Medicinal plants possess therapeutic properties or exert beneficial pharmacological effects on the animal body. [1] Plants are the source of about 25% of prescribed drugs in the world. [2] In developing countries about 80% people rely on traditional plant based medicines for their primary health care needs. [3] There is abundant number of medicinal plants and only small amounts of them are investigated for its biological and pharmacological activities. The wide range of medicinal plant parts like flowers, leaves, barks, stems, fruits, roots extracts are used as powerful raw drug possessing a variety of pharmacological activities. Discovery of new pharmaceutical agents from medicinal plants can combat the drastic increase in infectious diseases in many countries especially in rural areas and it has been used as an economic reason as well. Now a days, there is widespread interest of drugs derived from plants which reflect its recognition of the validity of many traditional claims regarding the value of natural products in health care. [4] Therefore, in order to determine the potential use of medicinal plants, it is essential to intensify the study of medicinal plants that finds place in folklore. [4, 5]

Senna siamea Lamk is a non-nitrogen-fixing leguminous tree in the subfamily Caesalpinoideae of the family Leguminosae. Senna is an Arabian name and the herb was first brought into use by the Arabian physicians Serapion and Mesue. [6] It is commonly called Thailand shower, minjiri, or kassod and has many regional names. [7] It has been widely planted in many Southeast Asian countries including Bangladesh [8] and is naturalized in many locations. [9] The plant is a medium sized evergreen tree attaining 5 m height in and conditions. [7] It rarely exceeds 20 m height and 50 cm diameter at breast height. [10] Traditionally *Senna siamea* is used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, and is also used to reduce sugar level in the blood. Ethno medicinally *S. siamea* is used as laxative, blood cleaning agent, cure for digestive system and genitourinary disorders, herpes and rhinitis. [11] The leaves *of Senna siamea* are locally used as antimalaria drug especially when decocted. [12] In traditional medicine, the fruit is used to charm away intestinal worms and to prevent convulsion in children. [13]

There are insufficient studies on *Senna siamea* leaf extract and studies must be conducted to determine its activity as medicinal plants. The present study was designed to screen the ethanolic extract of *S. siamea* leaf for its cytotoxic potential and to report on its phytochemical properties. Because toxicity plays an important role in identification and isolation of new compounds from crude extracts. [14]

MATERIALS AND METHODS

Collection and Identification of Plant material: The *Senna siamea* leaf was collected from South-Western region of Bangladesh in 2007. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen was deposited having the accession no. 31391.

Preparation of the Plant material: The collected plant parts (leaves) were separated from undesirable materials or plants or plant parts. They were shade-dried for four weeks. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of Plant Extract: About 300 gm of powered leaf was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 80% ethanol. The container with its contents were sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate (ethanol extract) obtained was evaporated under vacuum with rotary evaporator.

Chemicals and Reagents: All the chemicals used are of analytical reagent grade. Mercuric iodide , potassium iodide, copper sulphate, sodium potassium tartarate, sodium hydroxide, cupric sulphate, sodium citrate, anhydrous sodium carbonate, naphthol, ferric chloride,

Lead acetate were obtained from Sigma Chemical Co. USA and concentrated hydrochloric acid, sulfuric acid were obtained from Merck, Germany.

Phytochemical screening: Testing of different chemical groups present in extract represent the preliminary phytochemical studies. To identify the chemical constituents of plant extract standard procedures were followed. The crude extract was qualitatively tested for the presence of chemical constituents using the following reagents and chemicals: reducing sugar with Benedict's solution, flavonoids with the use of Mg and HCl, tannins with ferric chloride, saponins with ability to produce stable foam, gums with molish reagent and sulphuric acid, steroids with Libermann Burchard reagent, alkaloids with Mayer's reagent and Glycosides with aqueous sodium hydroxide and observed color change in respective. [1, 15] In each test 10% (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test.

Determination of reducing sugar: 0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously.

Determination of tannins: 5 ml solution of the extract was taken in a test tube. Then 1 ml of 5%Ferric chloride solution was added.

Determination of flavonoids: A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of flavonoids.

Determination of saponins: About 0.1 gm of powdered plant material was boiled with 10 ml of water for 5 minutes & filtered. After cooling 5 ml of filtrate was diluted with water & shaken vigorously.

Determination of gums: 5 ml solution of the extract was taken and then molish reagent and sulphuric acid were added.

Determination of steroids: 1 ml solution of extract was taken and then added1 ml sulphuric acid. Red color indicates the presence of steroid.

Determination of alkaloids: 0.5 gm of the extract was stirred with 5 ml of 1% hydrochloric acid on a steam bath & filtered. 1 ml of the filtrate was treated with few drops of Mayer's reagent. White or creamy white precipitate considered as an indication for the presence of alkaloids.

Determination of Glycosides: A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1 ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.

Cytotoxicity Potential: Brine shrimp lethality bioassay was used to determine the cytotoxic activity of the plant extract. [16] It is a recent development in the assay procedure of bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal etc.) of the compounds. [17] The assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacterial toxins, pesticides and Cytotoxicity testing of dental materials.

The eggs of Brine Shrimp ware hatched in a tank at a temperature around 37^0C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). With the help of a pasteur pipette nauplii were exposed to different concentrations of the extracts.

Preparation of test groups: For the experiment, 300 mg of dried ethanol extract of leaf of *Senna siamea* was weighed, took in a volumetric flask to make 40 µg/µl concentration solutions and dissolved in 60 µl of DMSO. Thus 10 µg/µl, 20 µg/µl, 40 µg/µl, 80 µg/µl, 160 µg/µl, 320 µg/µl concentration solution was prepared.

The sample (80% ethanol extract) was initially dissolved in specific volumes of pure dimethyl sulfoxide (DMSO) to get stock solutions. Twenty clean test tubes were taken. Ten of these were for the samples in six concentrations (two test tubes for each concentration) and ten for control test. 4 ml of seawater was given to each of the tubes. Then with the help of the micropipette specific volumes of samples were transferred from the stock solutions to the test tubes to get final sample concentrations of 10, 20, 40, 80, 160 and 320 µg/ml. the concentration of DMSO in these test tubes should not exceed 40 µl/4ml of brine as because above this concentration toxicity due to DMSO may arise. In the vials taken for the control, same volumes of DMSO (as in the sample tubes) were taken. With the help of a Pasteur pipette 10 living shrimps were kept to each of the vials. [17]

Counting of nauplii: The test tubes were kept at room temperature for about 24 hours and then number of nauplii was counted and the results were noted. From this, the percentage of mortality of brine shrimp nauplii was calculated at each concentration for each sample. The median lethal concentration (LC_{50}) and 95% confidence interval was determined using probit analysis method as the measure of toxicity of the plant extract.

RESULTS AND DISCUSSION

Phytochemical screening: The crude extract was subjected for chemical group tests and identified various types of important chemical constituents. Results of different group tests are given in table 1. From the results it is observed that glycosides, steroids and tannins were present ethanolic extract of leaves of *Senna siamea* and other experimental chemical groups were absent. The observed result was consistent with the findings by Bukar *et al.,* 2009 who also confirmed the presence of tannins and steroids. [18]

Table 1: Results of phytochemical screening (chemical group tests)

Ethanolic extract	***Chemical Groups***							
	Reducing sugar	***Tanins***	***Flavonoids***	***Saponin***	***Gums***	***steroids***	***alkaloids***	***Glycosides***
Senna siamea leaf	-	+	-	-	-	+	-	+

(+) indicates Present, (-) indicates absent.

Table 2: Results of Brine shrimp lethality Bio-assay

Conc. (µg/ml)	Log(Conc.)	No. of nauplii taken	No. of dead nauplii	% mortality	Probit	LC_{50} (µg/ml)	95% Confidence interval
10	1	10	2	20	4.16	68.633	31.094-151.492
20	1.301	10	3	30	4.48		
40	1.602	10	4	40	4.75		
80	1.903	10	5	50	5.00		
160	2.204	10	6	60	5.25		
320	2.505	10	8	80	5.84		

Cytotoxicity: The brine shrimp test (BST) is considered as a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties. [19] In the present study, each of the test samples showed different mortality rates at different concentrations, the percentage mortality increased with an increase in concentration. The variation in results may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, glycosides, steroids) present in the crude extracts. At the conc. of 160 µg/ml and 320 µg/ml, brine shrimp nauplii died 60% and 80% respectively. The calculated LC_{50} value is of 68.633 µg/ml which indicates moderate cytotoxic effect of ethanolic extract of *Senna siamea leaf* (Table 2). The LC_{50} value of vincristine sulphate was 0.91 µg/ml.

CONCLUSION

In conclusion, it was observed from the present study that glycosides, steroids and tannins were present in the ethanolic extract of leaves of *Senna siamea* and other experimental chemical groups were absent. The extract had the potential for cytotoxicity activity where the percentage of mortality was increased with an increase in concentration. But further pharmacological studies are required to be undertaken to understand the possible underlying mechanisms of the observed activities as well as to isolate, identify and characterize the active phytochemicals responsible for these bioactivities.

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