

**PHYTOCHEMICAL AND TOXICOLOGICAL STUDY OF
LEAF OF *CASSIA SOPHERA* LINN.**

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Summary

Cassia sophera is very popular among herbal practitioners in Bangladesh. In the present study the ethanolic leaf extract of *C. sophera* Linn. were used for phytochemical and cytotoxic screening. Phytochemical analysis of the leaf extract of *C. sophera* indicated the presence of alkaloid, steroid, tannin, and reducing sugar which may confer several possible pharmacological activities of the leaf extract of the plant. The crude extracts leaf extract of *C. sophera* were found to exhibit strong lethality against the brine shrimp nauplii in a dose dependent manner. At the concentration of 10 µg/ml and 34.65 µg/ml, the extract showed 50% mortality (LC₅₀) and 90% mortality (LC₉₀) respectively.

Key words: *C. sophera*, Phytochemistry, Toxicity, Brine shrimp, Lethal dose.

Introduction

The use of plants or plant parts for medicinal purposes is a main concern of herbal medicine. Using plant parts for healing purposes is an ancient form of medicine. Even in the records of ancient China, India, and Egypt use of plant part for medicinal purposes have been widely documented and all those practices were based on series of “trial and error”, which was not be verified by established scientific theories ^[1]. However these practices contributed a lot to the modern medicine, even sometimes results in proven efficacies compared to the conventional modern medicine ^[2]. The interest in using herbal remedies is increasing day by day because of their effectiveness, minimal side effects in clinical experience and relatively low cost whereas most available synthetic drugs have side effects and even too costly though they are relatively more effective than herbal remedies. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown ^[3]. In recent times, many countries make herbal medicines as an integral part of their Primary Health Care system ^[4].

C. sophora L. (Family- Caesalpiniaceae) is a medicinal plant available in the sub continent region (Bangladesh and India). It is widely used as folk medicine for the treatment of many diseases. According to the physicians of Unani medicine, three plants viz., *Cassia occidentalis* Linn., *C. sophora* Linn., and *C. sophora* Linn. var. *Purpurea* Roxb. are the varieties of ‘Kasondi’ ^[5,6,7]. ‘Kasondi’ is described in Unani literature to be repulsive of morbid humors (especially phlegm), resolvent, blood purifier, carminative, purgative, digestive, diaphoretic ^[5,6,8]. In ethno botanical literature, it is mentioned to be effective in the treatment of pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes, and convulsions of children ^[2,9,10,11]. Its leaves are used for the treatment of pain in many region of Bangladesh. Moreover this plant is used for treating antimicrobial infections, remedy of fever and also against fungal infections ^[12]. This existing information confirms that this plant may possess some important biological properties but still no scientific work has been done to give the proven explanation of the traditional use of this plant in herbal medicine.

In the present study *C. sophera* was investigated to determine its chemical composition and toxicity which may further help to find out the scientific basis of the traditional use of the plant in herbal medicine.

Materials and Methods

Plant material Collection and Extraction:

C. sophera leaves were collected from Jhikargacha, Dist-Jessore, Div.-Khulna, Bangladesh, in February, 2008. The plant was taxonomically identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (accession no. DACB- 32099) and a voucher specimen was also deposited there. Leaves were shed dried and powdered in a suitable blender. The powdered material was cold extracted in flat-bottomed glass container using ethanol for 15 days. The extract was filtered through Whatman filter paper and remaining portion of the extract was re-extracted for 7 days. The filtrate (ethanol extract) was evaporated by air. It rendered a greenish black type residue of 26 gm (yield 4.07 %).

Animals: *Artemia sauna* Leach (brine shrimp eggs form store).

Chemicals: All chemicals used were of analytical grade.

Procedure for identifying different chemical groups

Phytochemical screening was performed using standard procedures [12, 13, 14, 15]

Tests for Reducing Sugar:

➤ *Benedict's test*

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

➤ *Fehling's test (Standard Test)*

2ml of an aqueous extract of the plant material was added to 1ml of a mixture of equal volumes of Fehling's solutions A and B. Boiled for few minutes.

Test for Alkaloids

➤ *Mayer's test*

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added.

➤ *Dragendroff's test*

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added.

➤ *Wagner's test*

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

➤ *Hager's test*

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

Tests for Tannins

➤ *Ferric Chloride test*

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

➤ *Potassium dichromate test*

5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added.

➤ *Lead Acetate test*

1 ml of 10% Lead acetate solution was added to 5 ml of extract solutions. A yellow precipitate was formed.

Test for Flavonoids

Added a few drops of concentrated hydrochloric acid to a small amount of an alcoholic extract of the plant material.

Test for Saponins

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes.

Test for Gums

5 ml solution of the extract was taken and then molisch reagent and sulphuric acid were added.

Test for Steroids**➤ *Liebermann-Burchard test***

1 ml solution of chloroform extract was taken and then added 2 ml Liebermann-Burchard reagent.

➤ *Sulphuric acid test*

1ml solution of chloroform extract was taken and then 1ml Sulphuric acid was added.

Toxicological study:

The test was performed as described by Meyer *et al.*, 1982 with slight modification ^[16]. 100 mg of dried ethanol extract of *C. sophera* leaves was taken in 10 ml volumetric flask and volume was adjusted by distilled water. A little amount of DMSO (Dimethyl Sulfoxide) was added to dissolve the extract completely. The final concentration of this solution was 10 µg/ml.

Brine shrimp eggs (*Artemia salina* Leach) were purchased from locality and hatched in artificial sea water (solution of NaCl 3.8%) at room temperature. The shrimps were allowed for two days to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to the lamp through the perforations in the dam and they were taken for bioassay. Twenty four clean test tubes were taken. Twelve of these were for the samples in six concentrations (two test tubes for each concentration) and twelve for control test. Sea water of 10ml was given to each of the test tubes. Then with the help of the micropipette the test solution were taken in test tubes to get final sample concentrations of 5, 10, 20, 40, 80, 160 µg/ml respectively.

For the control, same volumes of DMSO (as in the sample test tubes) were taken. Finally with the help of a Pasteur pipette 15 living shrimps were kept to each of the test tube. After 24 hrs, the test tubes were observed, the number of survived nauplii in each test tube was counted, and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration.

Results

Phytochemical study:

The crude extract of *C. sophera* was subjected for chemical group tests and found the presence of reducing sugar, alkaloids, steroids, and tannins but absence of flavonoids, saponins, and gums.

Table 1: Presence or absence of different chemical groups in crude ethanolic root extract of *Cassia sophera*.

Extract	Reducing Sugar	Alkaloid	Steroids	Flavonoids	Saponins	Tannins	Gums
Ethanol extract of <i>Cassia sophera</i>	+++	++	++	-	-	+++	-

+++ = Reaction intensity is high; ++ = Reaction intensity is medium; - = No reaction.

Brine Shrimp Lethality assay

In this bioassay, the crude extract of *C. sophera* leaves showed strong lethality against brine shrimp indicating the presence of biological active compound in the extract. Test sample showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus

log concentration on the graph produced an approximate linear correlation between them. From the graph (Fig.-1) the concentrations at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation and it was 10 $\mu\text{g}/\text{ml}$. The 90% mortality (LC_{90}) was obtained at 34.65 $\mu\text{g}/\text{ml}$. The table below shows the result obtained from brine shrimp lethality bioassay.

Table 2: Brine Shrimp lethality bioassay of ethanolic extract of *C. sophora* leaves.

Concentration ($\mu\text{g}/\text{ml}$)	160	80	40	20	10	5
Number of shrimps per test sample	30	30	30	30	30	30
Number of survivors	0	0	2	10	15	21
Number of deaths	30	30	27	21	15	9
Percentage mortality	100	100	93.33	66.67	50	30

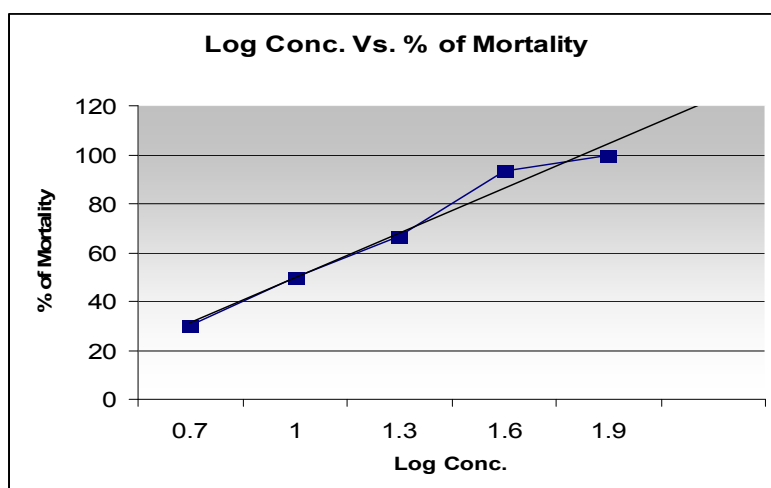


Fig. 1: The lethality of ethanolic extract of *C. sophora* in brine shrimp.

Discussion

The phytochemical screening of leaves of *C. sophera* indicates the presence of reducing sugar, alkaloids, steroids, and tannins. The result of phytochemical screening indicates that the leaf extract *C. Sophera* may have some important biological activities like anti-inflammatory or anti-oxidant activity. There are some reports on the role of tannins for the free radical scavenging effects ^[17]. Therefore this plant could be suitable for these purposes. There are few reports on the role of tannins in anti-nociceptive and anti-inflammatory activities ^[18]. Therefore the leaf extract of *C. sophera* may possess anti-inflammatory or analgesic activity due to their content of tannins ^[19]. So this phytochemical screening result suggest further researches to find out the possible biological properties of the leaf extract of *C. sophera*.

From the brine shrimp lethality bioassay it is observed that leaf extracts of *C. sophera* was considerably lethal to brine shrimp nauplii. This indicates that this plant part may contain potential bioactive compounds, which if properly and comprehensively studied, could provide many chemically interesting and biologically active drug candidates, including some with potential anti-tumor and anti-proliferative properties ^[20]. A detailed chemical study is also required to isolate the molecules that are responsible for these activities.

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