ISSN: 2320-9267

Indian Journal of Pharmaceutical and Biological Research (IJPBR)

Journal homepage: www.ijpbr.in

RESEARCH ARTICLE

Phytochemical and Elemental Screening on Different Extracts of Leaf, Flower, Stem and Seed of *Cassia Sophera* Linn: An Important Medicinal Plant of Bangladesh

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ABSTRACT

Qualitative analysis of *Cassia Sophera* Linn plant confirms various phytochemicals like alkaloids, flavonoids, terpenoids, saponins, steroids, carbohydrates, anthraquinone glycosides etc in different extracts of its leaves, flowers, stems, and seeds. Some minerals have also been identified in the leaves, flowers, stems, and seed part of the plant by Atomic absorption spectroscopic techniques.

Keywords: *Cassia sophera* Linn, Phytochemical screening, Plant species.

Indian J. Pharm. Biol. Res. (2020): https://doi.org/10.30750/ijpbr.8.4.3

INTRODUCTION

Plants are important sources of potentially useful substances for the development of new therapeutic agents. Various phytochemical compounds as secondary metabolites have been implicated in plants as the conferment of antibacterial activities.[1,2] Nowadays, there is a renewed interest in traditional medicine. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that 'green medicine' is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects.The medicinal action of plants is unique to a particular plant species, consistent with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct to other species.[3] Medicinal plants have provided modern medicine with numerous plant-derived therapeuticagents.[4]

In Bangladesh, about 500 plant species have been identified as medicinal plants because of their therapeutic properties. Approximately hundreds of traditional medicines have been developed in the form of Ayurvedic and Unani formulations in Bangladesh. About 400 herbal industries have been established in this country for producing Ayurvedic and Unani medicines and marketed herbal products of 500-crore taka worth annually.[5] Proper scientific evaluation of these plants' pharmacological properties, used in different formulations, would carry enormous potential and promise for the 21st century.

Cassia sophera Linn (Family Caesalpiniaceae), popularly known as Kasundi in Bangladesh, is found throughout the Indian subcontinent[6] and in most tropical countries are widely distributed throughout Asia, including Bangladesh, India, Mauritius, China, East Africa, South Africa, America, Mexico, WestIndies and Brazil.[7] Occasional weed in settled areas at low and medium altitudes from northern to central Luzon. Pantropic species of American origin. It is common in wastelands, on roadsides, and in the forests.[8]

There is currently an enormous surge of significance in the utilization, progress, and preservation of medicinal plants worldwide. The presence of these phytoconstituents

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How to cite this article: Aziz S, Sorowar MS, Al-Reza S. Phytochemical and Elemental Screening on Different Extracts of Leaf, Flower, Stem& Seed of *Cassia Sophera* Linn: An Important Medicinal Plant of Bangladesh. Indian J. Pharm. Biol. Res. 2020; 8 (4):11-16.

Source of support: Nil

Conflict of interest: None.

Received: 16/09/2020 **Revised:** 24/10/2020 **Accepted:** 20/11/2020 **Published:** 25/12/2020

makes the plant useful for treating different ailments and can provide useful drugs for human use.[9] Therefore; this study aims to evaluate the phytoconstituents and potential of plant extract against pathogens.

MATERIALS AND METHODS

Plant materials

Fully matured fresh flowers, leaves, seeds and stems of *Cassia sophera* Linn were collected from Andulbaria village of Jhenidah district, Bangladesh, in June 2019 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. = 43734) has been deposited. The plant samples were then grinded in a fine powder form and then stored in an air-tight container with marking for identification and kept in cool, dark, and dry place for future use.

Preparation of Extracts

Briefly, 100 g of each powdered plant material (leaf, flower, stem, and seed) is submerged in suitable solvents of increasing polarity as n-Hexane, chloroform, ethyl acetate, and methanol subsequently in an air-tight separating funnel for 5 days at room temperature with occasional shaking and stirring. The obtained extract was filtered by using Whatman No.1 filter paper. Each filtrate was concentrated under reduced pressure on a rotary evaporator till a viscous mass was obtained. Finally, the prepared extracts were stored at 4°C for further analysis.

Phytochemical Screening

Reagent Preparation for the Detection of Different Class of Compounds

a. 1% picric acid: 1ml of picric acid dissolved in 99 ml distilled water.
b. Dragendroff's reagent: It is used for the detection of alkaloids. 0.17 g Bismuth nitrate dissolved in 2 mL acetic acid solution and added 8 ml of distilled water. (Solution A). 4 g of potassium iodide dissolved in 10 mL acetic acid solution and added 20 mL of distilled water (Solution B). Solution A and B were mixed and made 100 mL with distilled water.
c. Mayer's reagent: It is used for the detection of alkaloids. Solution (A) was made to dissolve 0.68g mercuric chloride in 30mL of distilled water. Solution (B) was made to dissolve 2.5g of potassium iodide in 10 mL of distilled water. Solution A & B were mixed and adjusted the volume to 100 mL with distilled water.
d. Molisch's reagent: 10 g of α-napthol was dissolved in 100 mL of 95% alcohol. It is used for the detection of carbohydrates.
e. Fehling's solution: It is used for the detection of reducing sugar. 3.4650 g copper sulphate was dissolved in distilled water and made the volume up to 50 mL (Solution A). 17.30 g of potassium sodium tartarate and 5 g of sodium hydroxide was dissolved in distilled water and made volume up to 50 mL (Solution B) with water. Two solutions were mixed in equal volume to prior use.

Test for Qualitative Estimation of Bioactive Compounds from different solvent extracts of leaf, flower stem and seed of Cassia sophera L.:

The extracts of different parts of the plant *Cassia sophera*L. (20 mg) were subjected to qualitative analysis to detect the presence of different classes of chemical constituents in the plant.

1. The extracts of leaves, flowers, stems and seeds of *Cassia sophera* L.were subjected to qualitative analysis to detect the presence of different classes of chemical constituents in the plant.
2. **Test for Alkaloids**: n-hexane, choloform, Ethylacetate and methnol extract of leaf, flower, stem and seed part of the plant *Cassia sophera* L.were warmed separately with 2% H_2SO_4 for two minutes. It was filtered and few drops of the following reagents were added and indicated the presence of alkaloids.
   a. Dragendroff's reagent: A red precipitation indicated the positive.
   b. Mayer's reagent: A creamy white color indicated the positive.
   c. Picric acid (1%)- A yellow precipitation indicated the positive.
3. **Test for Flavonoids**: A small quantity of the extract was heated with 10 mL of ethyl acetate in boiling water for 3 minutes. The mixture was filtered and the filtrates were used for the following test.
   a. The filtrate was shaken with 1 mL of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed in the ammonia layer indicated the presence of flavonoid.
   b. The filtrate was shaken with 1mL of 1% ammonium chloride solution and observed light yellow color. It indicates the presence of flavonoids.
4. **Test for Carbohydrates**: The extracts were shaken vigorously with water and then filtered. A few drops of Molisch's reagents was added to the aqueous filtrate, followed by vigorous shaking again. Concentrated H_2SO_4 (1 mL) was carefully added to form a layer below the aqueous solution. A brown ring at the

interface indicated the positive test.

5. **Test for Saponins**: A small quantity of different extracts was diluted with 4 mL of distilled water. The mixture was shaken vigorously and then observed on standing for stable brake indicated the positive test.
6. **Test for Steroids**: 2 mL of acetic anhydride and 2 mL H_2SO_4 were added to the extracts. The color changed from violet to blue or green indicated the presence of steroids.
7. **Test for Anthraquinone glycosides (Borntragers's test):** To the extracts, dil. H_2SO_4 was added and boiled. Then it was filtered and cooled. To the cold filtrate, 3 mL of benzene was added and mixed. The benzene layer was separated, and the ammonia (2mL)solution was added to it and rose pink to red in the ammonical layer, which indicated a positive test.
8. **Test for Cardiac Glycosides: (Legal's test):** To each extracts, 1mL of pyridine and 1mL of sodium nitroprusside solution were added and observed. A deep red color was observed for positive test.
9. **Test for Terpenoids (Salkowski test):** Each extract was mixed with 2 mL of chloroform and then concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish-brown coloration in the interface indicated a positive result for the presence of terpenoids.
10. **Test for Gum and Mucilages:** Each extract was dissolved in 10 mL of distilled water and 25 mL of absolute alcohol was added into it with constant stirring. White or cloudy precipitate indicated the presence of gum and mucilages.
11. **Test for Proteins and Amino acids:** Each extract was dissolved in 10 mL of distilled water and . The filtrate was subjected to test the presence of proteins and amino acids.

a. **Biuret test:** 2 mL filtrate was treated with one drop of 2% copper sulfate solution and then 1mL of ethanol (95%) was added to it followed by excess potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.
b. **Ninhydrin test:** Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.

Determination of Elements

Major minerals/elements serve as structural components of tissues and function in cellular and basal metabolism, water and acid-base balance, clotting of blood and formation of bones and teeth etc.[10-14] Atomic absorption spectrophotometer (AAS) was used to determine elements in the dry powdered leaves, flowers, seeds, and stem of *Cassia Sophera.* Definite amount (10 g) of leaf, seed and stem samples were taken and kept in a furnace at 400˚C for 8 hours. Then there was a formation of ash. The ash samples were transferred into a mixture of HNO_3 and $HClO_4$ (2:1) which was prepared earlier in a Kjeldahl flask and left overnight. Before starting digestion, ice bath was available for cooling Kjeldahl flask. Then it was placed on heating mantle set at low temperature. Once boiling was initiated, red-orange fumes of NO_2 driven off. Gentle heating was continued until HNO_3 and H_2O were driven off. At this point effervescent reaction occurred between organic material and $HClO_4$. The flask was then put on heating mantle at room temperature and let digestion proceed with occasional heating from mantle. The reaction between organic material and HClO4 must not go too fast because charring would occur. If charring occurred, immediately need to place the flask in ice bath to stop digestion. Then I mL of HNO_3 will be added and resume gentle boiling. After reaction of test portion with $HClO_4$ was completed (identified by cessation of effervescent reaction between organic material and $HClO_4$), high heat was applied for Ca for 2 minutes. Then the flask was removed from heating mantle and left it to cool. Each digest sample was transfer to 50 mL volumetric flask and dilute with H_2O. Then each digest sample from leaves, flowers, seeds and stems of *Cassia Sophera*was ready for elemental analysis in ICP-MS spectrometry.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of the qualitative analysis of different extracts from leaves, flowers, stems and seeds of *Cassia Sophera*are presented in Table 1 and Table 2. Phytochemicals are non-nutritive plant chemicals that have disease preventive properties.[15] The investigation of n-Hexane, chloroform, ethyl acetate and methanol extracts of *Cassia sophera* L. showed differences in their phytoconstituents. Methanol and ethyl acetate extracts yielded better results. Hexane and chloroform extracts showed moderate results. This reveals that solubility of each constituent in each solvent is different.

According to the Table 1, alkaloids are mostly found on methanolic extracts, and terpenoids are commonly found in non-polar extracts (n-hexane and chloroform) of *Cassia sophera* L. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities.[16-17] Moreover, cardiac glycosides are commonly used to treat congestive heart failure and cardiac arrhythmia.[18] Terpenoids are aromatic compounds found in plant species, which is responsible for flavour and fragrance.

Table 1: Results of screening of various extracts from leaves and flowers of *Cassia Sophera* L.

Test for	*LH*	*LC*	*LEA*	*LM*	*FH*	*FC*	*FEA*	*FM*
Alkaloids								
a)Dragendroff;s test	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive
b)Mayer;s test	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive
c)Picric acid test	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive
Flavonoids	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Carbohydrates	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive
Saponins	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Positive
Steroids & tannins	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Anthraquinone glycosides	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive
Cardiacglycosides	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive
Gum and Mucilages	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Positive
Terpenoids	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Proteins and Amino acids	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive

LH:Leaf n-Hexane Extract, LC: leaf Choloform Extract, LEA: Leaf Ethyl Acatate Extract, LM:Leaf methanol extract FH: Flower n- hexane Extract, F:Flower Chloroform, FEA: Flower Ethyl Acetate Extract, FM:Flower methanol extract.

Table 2: Results of screening of various extracts from Stems and Seeds of *Cassia Sophera* L.

Test for	*St H*	*St C*	*St EA*	*St M*	*SH*	*SC*	*SEA*	*SM*
Alkaloids								
a)Dragendroff;s test	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive
b)Mayer;s test	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive
c)Picric acid test	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive
Flavonoids	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Carbohydrates	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive
Saponins	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Positive
Steroids & tannins	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Anthraquinone glycosides	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive
Cardiacglycosides	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive
Gum and Mucilages	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Positive
Terpenoids	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Proteins and Amino acids	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive

StH: Stem n-Hexane Extract, StC: Stem Choloform Extract, StEA: Stem Ethyl Acatate Extract, StM: Stem methanol extract , SH: Seed n-hexane Extract, SC : Seed Choloroform Extract, SEA: Seed Ethyl Acetate extract, SM: Seed Methanol Extract

Plant terpenoids play vital role in the herbal remedies.[19] Terpenoids are secondary metabolites present in plants, and have bioactivities like antibacterial, antiparasite, antiviral, anticancer and anti-inflammatory.[20]

Ethyl acetate and methanol on the other hand, could have extracted more polyphenols such as flavonoids better than another solvent, which explains why the ethyl acetate and methanol extract of *Cassia sophera* L. tested positive for flavonoid. Flavonoids belong to the group of polyphenolic compounds and are typically known for health-promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties.[21] They exist widely in the plant kingdom and displayed positive correlation between increased consumption of flavonoids and reduced risk of cardiovascular and cancer diseases.[22]

The presence of steroids was found in n-hexane and methanol, while tannins and saponins in methanolic extract of *Cassia sophera* L. Steroids derived from plants are known to have cardiotonic effect and also possess antibacterial

Table 3: Minerals/elements detection (calculated on dry matter basis) by AAS for leaves, flowers stem and seeds of *Cassia Sophera.*

Sl No.	*Component as element*	*Leaf*	*Flower*	*Stem*	*Seed*
1	Sodium (Na)	Present	Present	Present	Present
2	Potassium(K)	Present	Present	Present	Present
3	Calcium (Ca)	Present	Present	Present	Present
4	Magnesium(Mg)	Present	Present	Present	Present
5	Zinc (Zn)	Present	Present	Present	Present
6	Iron(Fe)	Present	Absent	Present	Absent
7	Manganese(Mn)	Present	Present	Present	Present
8	Aluminium (Al)	Present	Absent	Present	Absent
9	Cupper (Cu)	Present	Absent	Present	Absent
10	Nickel(Ni)	Present	Absent	Present	Absent
11	Chromium (Cr)	Present	Present	Present	Absent
12	Lead (Pb)	Present	Present	Present	Present
13	Cadmium(Cd)	Present	Present	Present	Present

and insecticidal properties.[23] Similarly, tannins possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.[24] Saponin was present in Cassia sophera L. extracts, which are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties, etc their haemolytic effect on red blood cells.[25]

There was a tremendous legacy of folklore uses of different parts of *Cassia Sophera* in medicine. There is an increasing interest in the importance of dietary minerals at present for theprevention of several diseases. Together with other essential nutrients, the trace elements are necessary for growth, normal physiological functioning, and maintaining of life. They must be supplied by food, since the body can not synthesize them. So it is necessary to find out which elements are present in the selected plant. The results of elemental detection in the leaves, flowers, stems, and *Cassia Sophera* are presented in Table 3. Results indicated the presence of Na, K. Fe, Ni, S and Cl_2 in both the leaves and flowers.

CONCLUSIONS

The plant studied here can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent. We therefore, suggest further the isolation, purification, and characterization and of the bioactive compounds of the leaf, flower, stem and seed part of *Cassia Sophera* with a view to obtain useful chemotherapeutic agents.

ACKNOWLEDGMENT

We are thankful to the Director, BCSIR Laboratories, Dhaka, for providing necessary facilities to carry out this research work.

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