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# PHYTOCHEMICAL SCREENING, PROXIMATE AND ELEMENTAL ANALYSIS OF STEM BARKS OF *Cassia fistula* Linn

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## ABSTRACT

Different parts of *Cassia fistula* Linn are utilized in ayurveda medicine and homemade cures for common illnesses. This research work was aimed at screening the phytochemicals in different solvent extracts, proximate composition and analysis the quantity of different essential elements of the stem barks of *C. fistula* Linn using standard methods. From phytochemical screening, different types of natural compounds such as steroids, terpenoids, flavonoids, phenolic compounds, coumarines, quinones, saponins, tannins and carbohydrates were confirmed in the extracts. The result of the analysis revealed that steroids, terpenoids and flavonoids were present in all the extracts but alkaloids were not found in any of the extracts. Proximate analysis was carried out on the barks of *C. fistula* which showed that it has 11.37% moisture content, 84.99% dry matter and 3.64% ash content. Meanwhile, using atomic absorption spectroscopic technique, 14 elements in total were identified after analyzing the micro and macro elements. From the analysis, it was observed that the plant showed higher concentration of Ca, K, Mg and lower concentration of Cd, Pb, Cr and As. The most significant part of this analysis was that the concentration of essential elements in the plant is high and the concentration of toxic heavy elements is very low within the tolerance limit, which signifies that plant is safe for the medicinal use.

**KEYWORDS:** Phytochemical Screening, Proximate composition, Elemental analysis, Stem bark, *Cassia fistula* Linn.

## INTRODUCTION

Plants offer a wide range of possible applications, particularly in traditional medicine and pharmaceuticals. Due to the paucity and expensive expense of orthodox treatment, a sizable segment of the global population relies on traditional medicine.[1] Medicinal plants have developed a variety of therapeutic chemicals that have been made available to modern medicine.[2] Numerous plants contain a range of phytopharmaceuticals, which have significant uses in human, veterinary and agricultural medicine. The discovery of novel drug leads for the treatment and prevention of diseases heavily relies on natural products.[3]

*Cassia fistula* Linn from Fabacea family is a flowering plant and is considered as an herbal medicine for the treatment of various diseases.[4] It is known as Golden shower tree which has therapeutic importance in healthcare since pre-historic times. This plant is around 30-50 feet long. The leaves are evergreen, deciduous, 15-50 cm long and pinnate with three to eight pairs of leaflets. The flowers are showy, fragrant, bright yellow and petals are widely spaced. This plant and its extracts are used as a pest controlling agent.[5] Different pharmacological response including antitussive, antibacterial, antifungal, anti-oxidant, anti-inflammatory, cathartic activity, wound healing potential, anti-fertility effect, modulation of humoral immunity, hepato-protective effect, purgative action and anti-tubercular activity were present in different parts of this plant.[6] The stem bark of this plant has a variety of medicinal properties, including laxative, anthelmintic, emetic, anti-tubercular, febrifuge, diuretic and depurative. It can also be used to treat pustules, leprosy, ring worm, colic, dyspepsia, constipation, fever, diabetic, cardiac problems, and dysentery.[7,8] Managing and preventing coronary artery disease as well as lowering blood sugar levels are further benefits of stem bark.[9] Previous phytochemical investigation also resulted in the isolation of two anthraquinone derivatives such as 1,4-dihydroxy-2-methylanthraquinone and 6,7,11-trihydroxynaphtalene-5,12-dione from the roots of *Cassia fistula*.[10] Besides 5,7,3,4-tetrahydroxy-6,8-dimethoxyflavone-3-O-$\alpha$-arabinopyranoside, 5,7,4-tridydroxy-6,8,3-trimethoxyflavone-3-O-$\alpha$-L-Rahamonosyl-O-$\beta$-D-glucopyranoside, 1,8-difydroxy-3,7-dimethoxyanthone-

4-O- $\alpha$-L-rhamnosyl (1→2) O- $\beta$-D-glucopyranoside, lupeol, $\beta$-sitosterol, 1-hexacosanol, 1-octacosanol, palmitic acid, linoleic acid, heptacosyl eicosanate, glyceryl-1-tetra eicosanate, epifriedelinol and its glucoside were reported to be found in the stem barks of this plant.[11-13] Due to its acclaimed pharmacological importance, this paper is aimed at analyzing phytochemical screening, proximate contents and analyzing the essential elements of the stem barks of *Cassia fistula* Linn.

## MATERIALS AND METHODS

### Sample Collection and Preparation

The stem barks of matured plant *Cassia fistula* Linn were collected from Jahangirnagar University campus. A taxonomist from the Bangladesh National Herbarium in Dhaka recognized the plant and a voucher specimen (Specimen no. 41562) was put there as proof. Cut into little pieces, the stem bark was thoroughly dried underneath the shed. After that, a grinder was used to grind the dry bark fragments into powder. The dried powder (1.55 kg) was then successively extracted at room temperature with n-hexane, chloroform, ethyl acetate and methanol. Using a rotary evaporator to evaporate the solvents, the dried crude extracts were produced and denoted as CFSH, CFSC, CFSE & CFSM respectively for stem barks.

### Phytochemical Screenining of Stem Barks of *Cassia fistula* Linn

The following qualitative assays were used to phytochemically screen the various extracts from stem barks of Cassia fistula.[14-16]

### Test for Steroids and Terpenoids *(Libermann-Burchard Test)*

10 mg of extract solution was made by using chloroform. A few drops of $Ac_2O$ were added, and then 1 ml of pure sulfuric acid was added. The blue chloroform layer that turned green indicates the existence of steroids, while the formation of the pink $CHCl_3$ layer reveals the existence of terpenoids.

### Test for Flavonoids *(Shindo's Test)*

First, methanol was used to dissolve 10 mg of the extract. Next, magnesium turnings were added, and then a significant amount of HCl was added. The color pink indicates the presence of flavonoids.

### Test for Phenolic compounds

A mixture of 10 mg of extract dissolved in methanol was added with a few drops of a 2.5% $FeCl_3$ solution. The reddish-brown color suggested that the phenolic component was present.

### Test for Coumarins

After 10 mg of the extract had dissolved in methanol, alcoholic KOH was added. Coumarin was identified when a yellow color developed that turned gray when strong HCl was added.

### Test for Quinones

The extract, 10 mg, was dissolved in methanol and treated with sulfuric acid. Quinone was present due to the color development.

### Test for Alkaloids (*Mayer's Test)*

Alkaloids were initially detected using Mayer's reagent. KI (5.0 g) and mercuric chloride (1.36 g) were mixed together in water to prepare the reagent (100 ml). Ten mg of the extracts were dispersed in hydrochloric acid separately. In the middle of the watch glass, a few solution droplets were put. Mayer's reagent and the sides of the watch glass were added using a glass rod. Positive results are indicated when a gelatinous white precipitate forms.

### Tests for Saponins

The extract was dissolved in distilled water and the mixture was then agitated ferociously. Foam formation is a sign that saponins are present.

### Test for Carbohydrates (*Molisch's Test)*

The extracts were filtered after being shaken ferociously with water. The Molisch's reagent (95% ethanol + 5% naphthol) was then added after the aqueous filtrate had been vigorously shaken. Concentrated $H_2SO_4$ (1mL) was carefully added to form a layer beneath the aqueous solution. A brown ring at the interface indicated the positive test.

### Test for Tannins (*Lead Acetate Test)*

A small amount of the 1% solution of lead acetate was added to the aqueous extract (5 mL) (previously boiled in a water bath). Yellow or reddish precipitation was a sign of a successful test.

## PROXIMATE CONTENT ANALYSIS

The proximate analysis of stem barks of *Cassia fistula* was conducted to determine moisture content, dry matter and ash content in the sample. Moisture content of the sample was determined on the fresh weight basis. At first the fresh barks were cut into small pieces and dried under the shed. Using a grinder, it was then reduced to powder. The powder was dried in the oven at 110± $5^0$C. When constant weight was achieved (6-10 hrs), it was removed from the oven, cooled in the desiccators and weighed to record the final mass. The experiment was repeated three times. The following equation was used to determine the sample's moisture content.[17]

*Moisture content (%) = (Weight of Moisture ÷ Weight of the sample taken)* ✕ *100*

*Dry Matter (%) = 100 - Moisture content (%)*

The sample's ash content was calculated on a dry weight basis. The oven dried sample was placed in a muffle furnace overnight where the temperature was gradually increased up to 500 $^0$C for ashing. The weight of the ash was determined after cooling in the desiccators. The procedure was repeated three times. The sample's ash content was calculated using the equation below.[18]

*Ash Content (%) = (Weight of the Ash Content ÷ Weight of the dry Sample) × 100*

## ELEMENTAL ANALYSIS

### Ashing and Digestion of Plant Samples

6.017 g of plant material precisely weighed were added to a porcelain crucible that had been cleaned and heated to a temperature of roughly 650°C before cooling and being weighed. The crucible and sample were both placed in a Bunsen burner with low gas flow until the smoke stopped. To obtain carbon-free ash, the crucible was then placed in a muffle furnace with a temperature control of 500°C for about 8 to 10 hours. In desiccators, the sample was adequately cooled before being weighed. Until a steady weight was reached, this procedure was repeated. 50 ml volumetric flasks containing 1.0 g of sample ash were then filled with 15 ml of 1M $HNO_3$ acid. The flask was then placed in a magnetic stirrer heater beneath a fume hood and heated to 250°C for roughly four hours. The sample was cooled for 10 minutes after the solution's color changed to milky, and 7.5 ml of concentrated $HClO_4$ acid was then added. The solution was boiled until a colorless solution formed. The material was then filtered through 0.4 micron filter paper to identify the dissolved components. Every time, the samples' pH was checked and verified to be less than 2.0 before analysis. The standard calibration curve was created using the standard solutions.[19]

## ANALYTICAL PROCEDURE

Atomic absorption spectroscopy was utilized to examine a total of 14 elements Na and K among these elements were examined using a flame photometer (Model AnA-135, OSK, Japan). Using an atomic absorption spectrometer (Varian AA 240FS, Australia) built with a flame and graphite furnace, Ca, Mg, Cu, Fe, Mn, Zn, Cr, Ni, Cu, Co, Pb, and Cd in the sample (*Cassia fistula*) were quantified. Atomic absorption spectrometer was used to quantify the amount of As (Varian 220 AAS). A standard solution was prepared for each element in order to build a standard calibration curve, from which each experimental sample curve was approximated. An adequate volume of the stock solution was run through the AAS to view a comparable curve of that solution. The stock solution was diluted with solvent and re-examined in order to determine whether the matching curve would surpass the standard curve if the concentration of any of the minerals in the solution was too high. The percent elements concentrations in dried samples were determined using data acquired from the AAS technique. The elements were then determined by the use of the following equation;

ppm (mg/Kg) of elements = [elemental content collected (ppm)/ sample taken (g)] X 1000

## RESULTS AND DISCUSSION

### Phytochemical Screening

The qualitative result of the phytochemical screening of stem barks of *Cassia fistula* has been shown Table-1.

**Table 1: Qualitative phytochemical content present in different extracts of the stem barks of *Cassia fistula*.**

| Tests for | n-Hexane extract (CFSH) | Chloroform extract (CFSC) | Ethyl acetate extract (CFSE) | Methanol extract (CFSM) |
|---|---|---|---|---|
| 1. Steroids | +Ve | +Ve | +Ve | +Ve |
| 2. Terpenoids | +Ve | +Ve | +Ve | +Ve |
| 3. Flavonoids | +Ve | +Ve | +Ve | +Ve |
| 4. Phenolic Compound | -Ve | +Ve | +Ve | +Ve |
| 5. Coumarins | -Ve | +Ve | +Ve | +Ve |
| 6. Quinones | -Ve | -Ve | +Ve | +Ve |
| 7. Alkaloids | -Ve | -Ve | -Ve | -Ve |
| 8. Saponines | -Ve | -Ve | -Ve | +Ve |
| 9. Carbohydrate | -Ve | -Ve | -Ve | +Ve |
| 10. Tannis | -Ve | -Ve | +Ve | +Ve |

The results showed that steroids, terpenoids and flavonoids were found in every extracts. The phenolic compounds and coumarines were also found in all extracts except n-hexane extract but alkaloids were absent in the extracts.

## PROXIMATE ANALYSIS

The proximate composition of the barks of *C. fistula* is presented in following table. The experiment was repeated three times and all the data are expressed as mean.

**Table 3: The result of proximate composition of the barks of *C. fistula*.**

| Test Parameters | Percent (%) Composition |
|---|---|
| Moisture Content | 11.37 ± 0.12 |
| Dry matter | 84.99 ± 0.24 |
| Ash on drying | 3.64 ± 0.32 |

Measured values are mean ± SD of three replicate analyses. The ash content was determined using the dry weight method, while the moisture content was calculated using the fresh weight method

The proximate study of the barks of *Cassia fistula* shows that the barks of the plant have 11.37% moisture content, 84.99% dry matter and 3.64% ash content. The plant sample's showed significant mineral content indicated by the ash content.

## ELEMENTAL ANALYSIS

Atomic Absorption Spectrometer evaluated a total of 14 different elements. It should be emphasized that each outcome is the average of three different measurements.

The result of the analysis is presented in **Table 2** and **Figure-1**.

**Table 2: Elemental composition (mg/Kg) of *C. fistula*.**

| Serial No | Metal | Result (mg/Kg) |
|---|---|---|
| 1. | Sodium (Na) | 182.9237±2.750 |
| 2. | Magnesium (Mg) | 2187.4560±3.040 |
| 3. | Potassium (K) | 7206.3998±4.040 |
| 4. | Calcium (Ca) | 25953.1005±4.040 |
| 5. | Chromium (Cr) | Non Detectable |
| 6. | Manganese (Mn) | 168.6575± 2.040 |
| 7. | Iron (Fe) | 3.9097±0.025 |
| 8. | Cobalt (Co) | 0.1330 ±0.035 |
| 9. | Nickel (Ni) | 0.657±0.050 |
| 10. | Copper (Cu) | 1.3900±0.050 |
| 11. | Zinc (Zn) | 41.4926±1.350 |
| 12. | Arsenic (As) | Non Detectable |
| 13. | Cadmium (Cd) | 0.2911±0.020 |
| 14. | Lead (Pb) | 1.3309±0.045 |

Measured values are mean ± SD of three replicate analyses.

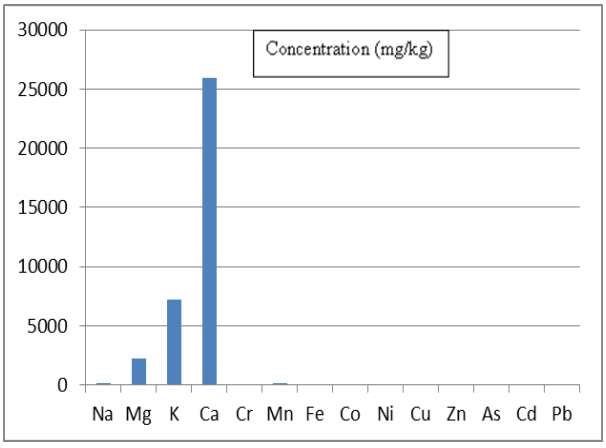


**Fig. 1: Elemental Compositions (mg/Kg) of *C. fistula.***

In the study of the elements, it was observed that the pant showed higher concentration of calcium (Ca), potassium (K) and magnesium (Mg). Potassium (K), a macro element found which helps to control body weight and improve water and electrolyte balance in the blood and tissues.[20] The role of Ca is to maintain strong bones and teeth. It lowers the risk of osteoporosis in elderly people.[21,22] Magnesium (Mg) also plays necessary roles in most reaction involving phosphate transfer. It helps in improved insulin sensitivity and work against complications related to diabetes.[23] The analysis also shows that lower concentration of cobalt (Co), cadmium (Cd) and nickel (Ni). The most important finding of the works was that the concentration of some of the essential elements in the plant is very high. On the other hand, the concentration of toxic elements was very low, i.e., within the tolerance limit, which indicates that the plant is safe for the medicinal use.

## CONCLUSION

The analysis of phytochemical screening, proximate and elemental composition revealed that the stem bark of *Cassia fistula* containing phytochemicals may be responsible for some of its reported pharmacological activities. It also shows that the barks of the plants have high nutritional value and safe for medicinal use.

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