Proximate and Elemental Analysis of Three Medicinal Plants: *Cuscuta reflexa*, *Cassia tora* and *Cassia fistula*

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Authors' Contributions

This work was carried out in collaboration between all authors. Author KS designed the research study and supervised the whole research work. Author NSA wrote the protocol, managed the literature, performed experimental work and wrote the manuscript. Author TT performed experimental work, managed the literature and wrote the manuscript. Author MNM performed the experimental work and managed the literature. Author MAH and M. A. Ahsan co-supervised the research work. Author MABS and M. A. Akbor managed the experimental work. All the Authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: The current study explores the proximate and elemental contents of three different medicinal plants, namely *Cuscuta reflexa* (whole plant)*, Cassia tora* (stem) and *Cassia fistula* (seed pot) that have been grown in Bangladesh.
Methodology: Macro (Na, K, Ca, Mg), micro (Fe, Cu, Mn, Zn, Ni, Cr) and heavy metal (Pb, Cd, As) elements, present in *C. reflexa* (Whole plant)*, C. tora* (stem)*,* and *C. fistula* (seed pod) were analyzed quantitatively by flame photometer and atomic absorption spectroscopy (AAS).
Results: The minerals of the plants were found in substantial amounts (Na: 13763.75-16419.42

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ppm, K: 6053.49-25864.92 ppm, Ca: 18912.16-2298.77 ppm, Mg: 1570.25-4602.23 ppm, Fe: 58.99-222.43 ppm, Cu: 3.16-11.61 ppm, Mn: 15.89-54.53 pm, Zn: 0.0367-50.2665 ppm, Ni: 0.9878-4.7186 ppm, Cr: 0.3279-0.8281 ppm, Pb: 0.3590-1.5030 ppm, Cd: 0.0016-0.0068 ppm and As: 0.0148-0.0675 ppm).

Conclusion: It is evident that the investigated medicinal plants are enriched in some macro and micro nutrient, such as Na, K, Ca, Mg, Fe etc which are important for biological metabolism and human health. On the other hand the heavy metals are present in trace amount which indicate the plants are safe for medicinal uses.

1. INTRODUCTION

Medicinal plants are rich in therapeutic properties and they are used for different medicinal purposes. Since the ancient time, medicinal plants are being used in this subcontinent for the treatment of various diseases. Plants provide minerals, vitamins and certain hormone precursors in addition to energy and protein to human body. Major and trace elements and minerals play pivotal role in the building up and restoration phenomenon of human health [1]. An important characteristic of metal is their tendency of bioaccumulation. Hence, bioaccumulation is very important in hazard evaluation techniques. According to the concept of modern pharmacology, direct correlation between elemental content of medicinal plants and their curative property is not understood yet [2]. Therefore, quantitative estimation of the concentration of different elements is significant for the determination of the effectiveness of medicinal plants in curing various diseases. In this experiment, proximate and elemental composition of three medicinal plants, namely *Cuscuta reflexa* (whole plant), *Cassia tora* (stem) and *Cassia fistula* (seed pod), were investigated. Although these medicinal plants are widely used in the traditional medicinal system as a source of drug to cure a variety of diseases, comprehensive elemental analysis on these plant materials have not been done yet in this region, especially in Bangladesh.

Cuscuta reflexa Roxb., known as dodder in English and Swarnalata in Bengali [3], is an important medical plant of *Cuscuta* genus and Convolvulaceae family. It is a parasitic plant and thus depends completely on the nutrient of the host plant for biological activity. Hence the presence and concentration of different minerals identified from this plant varies largely depending on the host plant. The whole Plant of *Cuscuta reflexa* is used for various therapeutic purposes. The plant is being used for the treatment of different diseases since ancient time. The plant is used in the treatment of jaundice, urination disorders, muscle pain and cough, and also used as blood purifier. It's warm paste is used to treat rheumatism and paste of whole plant is used for the treatment of headache [4]. Seeds of *Cuscuta reflexa* have carminative and antihelminthic properties and used to treat bilious disorder [5].

Cassia tora Linn. is a small annual herb or under shrub growing as common weed in Asian countries. It is a very much common weed that grows wild in Bangladesh and is locally known as Chakunda in different regions over the country. It belongs to the family Fabaceae and genus *Cassia* [6]. Different parts of the plants are being used for the treatment of different diseases. The roasted seeds are a good substitute for coffee. *C. tora* tea is a herbal, pure, natural and non-polluted green health beverage. In the Republic of Korea it is believed to rejuvenate human vision and improve the liver condition [7]. The tea has created a new term "coffee-tea", because of its mysterious but very rich texture and coffee-like aroma. It does not contain any caffeine and could be healthy substitute for tea and coffee. Decoction of the leaves is used as laxative [8]. The seeds of *C. tora* has been used in Chinese medicine as aperients, antiasthenic and diuretic agent. It is also given to improve visual activity and to treat liver disorders [9].

Cassia fistula Linn. is known as Golden shower in English and locally known as sonalu, banderlathhi, amultas etc [10]. The plant has therapeutic importance in health care since ancient times. In Ayurvedic medicine, Golden Shower Tree is known as "disease killer". Its fruit pulp is used as mild laxative as well as for cardiac diseases and stomach problems such as acid reflux [11]. The bark and leaves are used for skin diseases. The seeds are

recognized as antibilious, aperitif, carminative, and laxative while the root is used for curing adenopathy, burning sensations, leprosy, skin diseases, syphilis, and tubercular glands [12]. The leaves of the tree are used for erysipelas, malaria, rheumatism, and ulcers, the buds are used for biliousness, constipation, fever, leprosy, and skin disease and the fruit for abdominal pain, constipation, fever, heart disease, and leprosy. Thus every part of this plant is recognized for its medicinal properties [13,14].

2. MATERIALS AND METHODS

2.1 Experimental Section

All the reagents and chemicals used were of analytical grade and were purchased from E. Merk.

2.2 Collection of Raw Materials

The plants *C. reflexa* (whole plant)*, C. tora* (stem) and *C. fistula* (seed pod) were investigated for the current experiment. The whole plant of *C. reflexa* was collected from the host plant *Ziziphus mauritiana* (Kul) from Dinajpur (north eastern district of Bangladesh), while the plant materials of *C. tora* and *C. fistula* were collected from some areas of Jahangirnagar University, Savar, Dhaka. All the plants were identified by Bangladesh National Herbarium at Dhaka and voucher specimen number 43869, 39529 and 41562 for *C. reflexa, C. tora* and *C. fistula,* respectively, were deposited at the herbarium.

2.3 Preparation of Samples

The plant materials (*C. reflexa, C. tora, C. fistula*) were separately cut into small pieces, properly cleaned under running tap water and dried in an electric oven at 105°C until a fixed weight was attained. The dried plants were then converted into powdered samples using 20 mesh screen in Willey mill and were preserved in sealed containers. The samples were kept in a cool place for further analysis.

2.4 Ashing and Digestion of Plant Samples

Accurately weighed 20.0 g of each plant materials were taken separately in three individual cleaned porcelain crucibles which were heated previously to about 650°C and cooled and then weighed. The crucibles along with samples were placed in Bunsen burner (at low flow rate gas) until the smoke finished. Then the crucibles were placed in a muffle furnace whose temperature was controlled at 525°C for around 8-10 hours to get carbon free ash. The samples were then cooled properly in desiccators and weighed. This method was repeated until a constant weight was attained. About 1.0 g ash of each sample was taken in three different 50 ml volumetric flasks and then 15 ml of 1M HNO3 acid was added to the flasks. Then the flasks were placed in magnetic stirrer heater under fume hood for about four hours at 250°C. When the color of the solutions was altered to milky solutions, the samples were cooled for 10 minutes and 7.5 ml of concentrated HClO4 acid was then added. The solutions were heated until the formation of colorless solutions. The samples were then filtered through 0.4 micron filter paper to determine the dissolved elements. In every case, the pH of the samples were monitored and confirmed to be less than 2.0 before analysis. The standard solutions were prepared to make the standard calibration curve.

2.5 Analytical Procedure

A total of 13 elements were analyzed by atomic absorption spectroscopy. Among these elements, Na K and were analyzed by using flame photometer (Model AnA-135, OSK, Japan). Ca, Mg, Fe, Cu, Mn, Zn, Ni, Cr, Pb and Cd in each plant samples (*C. reflexa, C. tora, C. fistula*) were estimated by using atomic absorption spectrometer (Varian AA 240FS, Australia) designed with flame and graphite furnace. As (Arsenic) was estimated with Atomic Absorption Spectrometer (Varian 220 AAS).

For each element, a standard solution was prepared to plot a standard calibration curve, with respect to which each experimental sample curve was estimated.

The stock solution of an appropriate volume was passed through the AAS to observe a corresponding curve of that solution. If the concentration of any of the minerals in the solution is too high then the corresponding curve will exceed standard curve, at that point the stock solution was diluted with solvent and reexamined.

Data obtained from the AAS procedure were converted to collect the percent elemental contents in dried samples. 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

3. RESULTS

3.1 Determination of Proximate Composition

3.1.1 Moisture content

The moisture contents of all the samples were determined on the fresh weight basis, which are for *C. reflexa, C. tora* and *C. fistula* are 87.20%, 65.32% and 51.97%, respectively, which indicates the growth of microorganism and life period of stored materials would be low [15].

3.1.2 Dry matter content

The dry matter content of the plant samples, *C. reflexa, C. tora* and *C. fistula* were determined on the fresh weight basis and the amounts were 12.80%, 34.68% and 48.03%, respectively.

3.1.3 Ash content

The ash contents of all the samples were calculated on the dry weight basis. The ash contents of *C. reflexa, C. tora* and *C. fistula* are 7.76%, 5.43% and 3.25% respectively which indicates the presence of high minerals and metal contents in the plant samples [15].

3.1.4 Elemental analysis

The elemental contents of plants *C. reflexa, C. tora* and *C. fistula* are summarized in Table 2.

4. DISCUSSION

Literature studies on these medicinal plants revealed that the plants demonstrated a decent percentage of ash contents. Based on the literature survey, it can be reported that the percentage of ash content of the experimental plant samples ranges between 4.3% and 8% [16].

The elemental composition of *C. reflexa, C. tora* and *C. fistula* were analyzed by the use of Atomic Absorption Spectrometer. A total of 13 elements, i.e., Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn), Nickel (Ni), Chromium (Cr), Led (Pb), Cadmium (Cd) and Arsenic (As) were investigated, which are responsible for curing various diseases. The result of the investigation is presented in Table 2. It can be noted that, each of the results is an average of at least three independent measurements. These elements play an important role in the development of secondary metabolites which are accountable for pharmacological actions of these elements in the plant.

Table 1. Proximate composition (percentage) of *C. reflexa*, *C. tora* and *C. fistula*

Test parameters	*C. reflexa* (whole plant)	*C. tora* (stem)	*C. fistula* (seed pod)
Moisture content	87.20 ± 0.28	65.32 ± 0.23	51.97 ± 0.32
Dry matter	12.80 ± 0.15	34.68± 0.21	48.03 ± 0.12
Ash on drying	7.76 ± 0.08	5.43 ± 0.10	3.25 ± 0.05

Measured values are expressed as Mean ± SD of three replicate analyses

Table 2. Elemental composition (mg/Kg or ppm) of *C. reflexa*, *C. tora* and *C. fistula*

Metal	*C. reflexa* (whole plant)	*C. tora* (stem)	*C. fistula* (seed pod)
Sodium (Na)	16419.42 ± 5.08	13763.75 ± 5.50	14979.12 ± 4.80
Potassium (K)	25864.92 ± 5.05	6633.70 ± 4.98	6053.49 ± 4.99
Calcium (Ca)	2298.77 ± 5.50	18912.16 ± 5.60	5043.19 ± 5.59
Magnesium (Mg)	1570.25 ± 3.04	4602.23 ± 3.01	2208.21 ± 3.30
Iron (Fe)	171.37 ± 0.26	222.43 ± 0.25	58.99 ± 0.25
Copper (Cu)	11.61 ± 1.05	6.63 ± 1.01	3.16 ± 0.95
Manganese (Mn)	17.83 ± 0.50	54.53 ± 0.72	15.89 ± 0.49
Zinc (Zn)	0.0367 ± < 0.002	50.2665 ± 0.4050	0.0894 ± < 0.002
Nickel (Ni)	4.7186 ± 0.0900	0.9878 ± 0.0890	3.5904 ± 0.0906
Chromium (Cr)	0.6209 ± 0.0488	0.8281 ± 0.0481	0.3279 ± 0.0490
Led (Pb)	1.5030 ± 0.0833	0.8087 ± 0.0188	0.3590 ± 0.0793
Cadmium (Cd)	0.0068 ± < 0.001	0.0016 ± < 0.001	0.0034 ± < 0.001
Arsenic (As)	0.0675 ± < 0.001	0.0301 ± < 0.001	0.0148 ± < 0.001

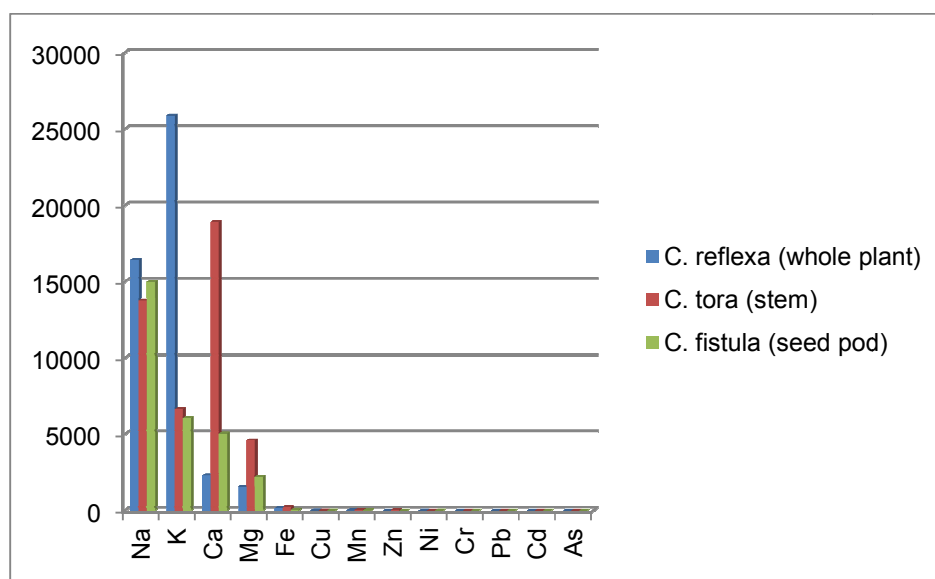*Measured values are expressed as Mean ± SD of three replicate analyses*

Fig. 1. Elemental compositions (ppm) of *C. reflexa*, *C. tora* and *C. fistula*

Sodium (Na) plays a key role in controlling blood pressure and regulates the function of muscles. Excess Na intake can lead to hypertension [17]. Ismet et al. found the amount of Na in *C. reflexa* to be 7.260 ppm [16] whereas the present study has shown the amount as 16419.42 ppm which is highest among the three plant samples. Furthermore, Shaikh et al. carried out similar kind of experiment in case of *C. tora* Seeds [15] and found the presence of Na, while the value in this study was found out to be13763.75ppm in *C. tora* which is the minimum in this experiment. Moreover, the moderate amount was found in *C. fistula* as 14979.12 ppm.

Potassium (K) was another macro element found which helps to control body weight and improve water and electrolyte balance in the blood and tissues [18]. The concentration of K was found as lowest as 6053.49 ppm in *C. fistula* and as highest as 25864.92 ppm in *C. reflexa* which is also the most abundant element amongst all. The moderate amount was found in *C. tora* as 6633.70 ppm in the current experiment. Previous study has shown the presence of K in *C. reflexa* (27.160 ppm) and *C. tora* [15,16].

The role of Ca is to maintain strong bones and teeth. It lowers the risk of osteoporosis in elderly people [19,20]. The concentration of Ca was found to be the highest in *C. tora* as 18912.16 ppm and lowest as 2298.77 ppm in *C. reflexa* whereas the moderate amount was found in *C.*

fistula as 5043.19 ppm. Similar studies carried out by Shaikh et al. which showed the presence of Ca in *C. tora* seeds [15].

Magnesium (Mg) also plays necessary roles in most reaction involving phosphate transfer. It helps in improved insulin sensitivity and works against complications related to diabetes [21]. The concentration of Mg was found as lowest as 1570.25 ppm in *C. reflexa* and as highest as 4602.23 ppm in *C. tora* whereas the moderate amount was found in *C. fistula* as 2208.21 ppm. Similar studies have done previously on *C. reflexa* [16] (5.58 ppm) and *C. tora* which ensured the presence of Mg in these plants [15].

Iron (Fe) is an important element in the diet of pregnant woman, nursing mothers, infants and the elderly to prevent anemia diseases [22]. The moderate concentration of Fe was found to be 171.37 ppm in *C. reflexa* whereas the highest concentration was found in *C. tora* (222.43 ppm) and the lowest concentration was found in *C. fistula* (58.99 ppm). Previous studies performed by Ismet et al. showed the amount of Fe in *C. reflexa* as 0.4394 ppm [16]. Another study carried out on *C. tora* seeds indicated the presence of Fe [15].

Copper (Cu) is necessary for the proper growth, development and maintenance of bone, connective tissue, brain, heart, and many other body organs. The concentration of Cu was

found as lowest as 3.16 ppm in *C. fistula* and as highest as 11.61 ppm in *C. reflexa* while the moderate amount was found in *C. tora* as 6.63 ppm. The presence of Cu (0.2117 ppm) in *C. reflexa* was confirmed by a previous study carried out by Ismet et al. [16].

Manganese (Mn) is an essential nutrient, because the body requires it to function properly. It is useful in chronic obstructive pulmonary disease and as a trace element in total parenteral nutrition preparations [23]. The average concentration of Mn was found from highest to lowest as 54.53 ppm, 17.83 ppm, 15.89 ppm in *C. tora, C. reflexa* and *C. fistula,* respectively; even though similar experiment showed negative result for *C. reflexa* [16].

Zinc (Zn) is needed for the proper growth and maintenance of the human body. It is needed for immune function, wound healing, blood clotting, thyroid function and some other biological functions [24]. The highest concentration of Zn was found to be 50.2665 ppm in *C. tora* which is quite deviant from the other two plant samples, *C. reflexa* (0.0367 ppm) and *C. fistula* (0.0894 ppm).

Ni, Cr, Pb, Cd and As are the toxic elements found in the plant samples and cause chronic poisoning and adverse effects on the liver, kidney, heart, vascular and immune system. The average concentration of these toxic metals were found in this study are below the prescribed limit of World Health Organization (WHO) [25]. According to WHO, the maximum limit for Cd should be 0.3 mg/kg in herbal medicines and goods whereas the dietary intake limit is 10.3 mg/kg [26]. The highest average concentration was found as 1.5030 ppm of Pb in *Cuscuta reflexa* which is below the tolerance limit. So, they can be considered as safe. However, consumption of them in an excess amount can be proven to be poisonous for human body.

The elemental composition found in the present study might vary from those determined in other places. This is due to the differences in the environment, climate, nature of soil and atmosphere, age of the plant, host plant (in case of parasitic plants) etc. Also, concentrations of the elements showed different results in this very study as well. One of the reasons for that is difference in the nature of the uptake of elements from soil. Not all plants take up same elements at the same rate. These

elements are not uniformly distributed in all the parts of a plant as well. This could be another reason for the variation of concentration of elements.

5. CONCLUSION

The four elements, carbon, hydrogen, oxygen and nitrogen are considered as main components of human body. These components made up of the 96% of human body weight. The remaining percentage of human body is comprised of minerals, for example, sodium, potassium, calcium, magnesium, iron, manganese chromium etc. Human body requires these elements in trace amounts to form tissue or to maintain metabolic processes. Furthermore, plants require minerals in varied amount depending on different conditions, such as, weather, soil, light etc.

The present study indicates that the investigated plants contain both macro (Na, K, Ca, Mg) and micro (Cu, Zn, Fe, Mn, Cr, Ni) elements in considerable amount. Thus the plants can be considered as potential sources of food and drug. These plants can be used in the ayurvedic sector in order to synthesize new drugs. However, further studies are required to isolate, characterize, quantify and to estimate microbial activity of the pharmaceutically active compounds for the development of new drugs from these plant sources.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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