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Proximate and elemental analysis of two medicinal plants: *Cassia alata* and *Spermacoce latifolia*

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Abstract

Aim: The present study was conducted to evaluate the proximate and elemental analysis of two different medicinal plants namely *Cassis alata* and *Spermacoce latifolia* which are frequently grown in Bangladesh.

Methodology: Macro (Na, K, Ca, Mg), micro (Al, Fe, Cu, Mn, Zn, Ni, Cr) and heavy metal (Pb, Cd) elements were determined quantitatively in the leaves of *Cassia alata* and in the root and aerial parts of *Spermacoce latifolia* by using flame photometer and atomic absorption spectroscopy (AAS).

Results: The elemental contents of the plants were evaluated in considerable amounts (Na: 611.87-1223.88 ppm, K: 5532.37-8344.77 ppm, Al: 1349-9798.52 ppm, Ca: 13131.45-18591.65 ppm, Mg: 2165.71-5068.38 ppm, Fe: 924.93-1192.16 ppm, Cu: 8.36-12.89 ppm, Mn: 68.58-221.07 ppm, Zn: 116.96-323.60 ppm, Ni: 3.88-14.29 ppm, Cr: 1.59-4.56 ppm, Pb: 14.28-27.82 ppm and Cd: 0.0827-0.7313 ppm.

Conclusion: The mineral analysis showed that the macro (Na, K, Ca, Mg) elements are present in higher amount than the micro (Al, Fe, Cu, Mn, Zn, Ni, Cr) elements which indicate the effectiveness of these plants in treating various ailments. And the concentrations of the heavy metals (Pb, Cd) are in very trace amount which shows that these plants are safe for medicinal uses.

Keywords: C. alata, S. latifolia; moisture content; ash content; elemental analysis etc

1. Introduction

ince the old human civilization, people suffered a lot in lack of proper drug development and treatment. So, the exigent necessity of new therapeutic alternatives led to the rediscovery of plant based natural products. Medicinal plants have been used for thousands of years in order to treat various human ailments as they are enriched with many constituents that show strong therapeutic properties [1]. Conventional medicine systems are inferred to show anecdotic side effects which can be alleviated by increasing the uses of these medicinal plants [2]. Although the major and trace elements are required in tiny fraction, the remarkable influences of these elements on all the body functions in human body are of great importance. Most of them act as cofactor, catalysts, center of constructing stabilizing structures in various biochemical reactions [3]. So, the research on the concentrations of the elements present in different medicinal plants is very much important in order to find out whether the plant is effective in combating the human ailments or not.

Cassia alata Linn is one of the most important medicinal plants availably grown wild in Bangladesh which belongs to the family Fabaceae and is a significant member under the genus *Cassia*. It is commomly known as Dadmurdan in Bengali and Candle bush, Candle stick, Christmas candle, Candle Cassia in English [4]. It is a large shrubby, ornamental plant which normally grows in diverse habitats like waste lands, roadside, forest edges, coastal areas and other moist places like uncultivated crop fields. Literature reviews show that it possesses diuretic, antifungal, antimicrobial, analgesic activities. It is referred to as ringworm shrub as the leaves are being used for the treatment of ringworm and all skin diseases. Leaves also play pivotal role by combating heart failure, abdominal pains, convulsion, gonorrhea and are used as purgative and anti-parasitic [5]. Seeds are used as vermifuge [6]. Flowers are efficacious as a cure for herpes in venereal diseases as an expectorant in bronchitis, astringent, poisonous insect bites and a mouth wash in stomatitis [7].

Spermacoce latifolia Aubl is a broadleaf weed locally known as Ghuiojhil Shak distributed throughout Bangladesh. The plant is traditionally claimed as one of the most important medicinal plants under the genus *Spermacoce* found mostly in tropical and subtropical region [8]. Experimental reviews reported that it possesses hepatoprotective, antioxidant, antitumor, antimicrobial, anti-inflammatory and lavricidal activities. Malaria is cured by taking the root juice of this plant. The plant is broadly used in relieving headache, bladder stones, toothache, sores, trauma, constipation, arthritis etc [9]. Leaves show aptness in ophthalmic purposes, inflammation of eyes, blindness and are also used in the treatment of hemorrhoids, gallastones and conjunctivitis [10]. Seeds have anti-diarrhoeal properties and can be used to medicate dental problems, fevers and dysentery [9].

2. Materials and Methods

2.1 Experimental Section

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

2.2 Collection of Raw Materials

Reclined on traditional medicine system preferred by the locals of our region, two of the medicinal plants namely *Cassia alata* Linn and *Spermacoce latifolia* Aubl were picked to examine their mineral concentrations. The fresh sample of the leaves of *Cassia alata* Linn and roots & aerial part of *Spermacoce latifolia* Aubl were collected separately from different parts of Jahangirnagar University, Savar, Dhaka. The plants were then identified and authenticated by taxonomist of Bangladesh National Herbarium (BNH). Voucher specimen of these two plants was deposited at BNH under the accession number DACB-38723 and 37755 for *Cassia alata* Linn & *Spermacoce latifolia* Aubl respectively.

2.3 Preparation of Samples

Firstly the fresh plant samples (*C. alata* & *S. latifolia*) were cut severally into small pieces. Before drying the samples, it was ensured that the samples were cleaned properly. At the temperature 110°±5°C, the fresh samples were dried in the oven. It was continued until a constant weight was achieved. Then the samples were moved from the oven, cooled and the final mass was taken accurately [11].

2.4 Ashing and Digestion of Plant Samples

The plant samples were accurately measured 14.50 g and taken separately into three authenticated porcelain crucibles. It should be noted that the porcelain crucibles were previously cleaned and heated to about 650°C. The crucibles were then cooled and weighed carefully. The crucibles with samples were placed in Bunsen burner at flow rate gas until the smoke ceased. For obtaining the expected carbon free white ash, the crucibles were moved to a muffle furnace and heated to 525°C for about 8-10 hours. The crucibles with ash were cooled in the desiccators and weighed carefully. The whole process was continued until a fixed weight was acquired and the color of the ash was changed to almost white. About 1.50 g, 0.99 g & 1.52 g white ash for the leaves of *C. alata*, roots & aerial part of *S. latifolia*, respectively were then treated individually with 5-7 ml conc. HNO_3 and evaporated for the removal of the organic matter remained. Then 15-20 ml 1M HNO_3 acid was added to the ash and after cooling the solution, about 7.5 ml conc. $HClO_4$ was added into it. After that, the solution was evaporated under a BIOBASE fume hood on a magnetic stirrer heater for 4 hrs at 250°C to make it half of its volume. The evaporated mass was then cooled and diluted with distilled water to 50 ml in volumetric flasks. For the determination of dissolved elements, the sample was filtered through 0.4 micron filter paper. In all cases, the pH of the sample was maintained and verified to be less than 2.0 prior to analysis. The standard working solution of interest was prepared to make the standard calibration curve [11].

2.5 Analytical Procedure

In this analysis, atomic absorption spectroscopy (AAS) was used to evaluate the concentrations of about 13 elements. Among all the elements, Al, Ca, Mg, Cr, Fe, Zn, Cd, Pb, Cu, Ni & Mn were analyzed by atomic absorption spectrometer (Varian AA 240FS) equipped with flame and graphite furnace and Na & K were estimated by using flame photometer (Model Ana-135, OSK, Japan) in each of the plant sample (*C. alata* & *S. latifolia)*. Air acetylene mode was chosen for this experiment where the flame was operated with the following conditions of 1.8 L/min, air 15 L/min and for creating the inert atmosphere, Argon gas flow was used. A standard solution was prepared previously for each of the element to plot a standard calibration curve. With respect to this curve, each experimental sample curve was estimated. The instrumental default temperature parameters were automatically fixed for each of the element. The stock solution was passed through the AAS to observe the corresponding curve. Then the solutions which were prepared for each of the element were passed through the AAS individually and compared to the standard curve. If the concentration of any of the minerals was seemed to be high, then the solution of that mineral was diluted again because its corresponding curve exceeded the standard curve. In order to obtain the percentage of every specific element for each of the plant sample, data obtained from the AAS was converted. For the determination of the elements, following equation was used: ppm (mg/kg) of the elements = [elemental content collected (ppm) / sample taken (g)] × 1000 [11].

3. Results

3.1 Determination of Proximate Composition

3.1.1 Moisture Content

The moisture content determined of the required samples of the leaves of *C. alata*, roots and aerial parts of *S. latifolia* were 63.83%, 78.50% & 80.95% consecutively. In this case, the moisture content was ascertained on the fresh weight basis. This data confirms the less chances of developing microorganisms such as fungus, bacteria etc in these samples [12].

3.1.2 Dry matter content

The dry matter content involves all of the constituents of the plant samples excluding water. This content was also resolved on the fresh weight basis. The dry matter content for the sample of the leaves of *C. alata*, roots and aerial part of *S. latifolia* were 14.83%, 14.70% & 11.51%, respectively.

3.1.3 Ash content

The term ash content indicates the measurement of the total amount of minerals present within the plant samples [12]. In this experiment, the ash content for the all the samples were evaluated on the dry weight basis. The ash content estimated for the leaves of *C. alata* and roots & aerial part of *S. latifolia* were 1.50%, 0.99% & 1.52%.

3.1.4 Elemental Analysis

In order to find out the curative effects of the medicinal plants, it is necessary to evaluate the concentrations of the minerals and trace elements present in the plants. The elemental contents for the plant sample of *C. alata* and *S. latifolia* are shown in Table-2.

Table 1. Proximate composition (percentage) of *C. alata* & *S. latifolia*

Test parameters	Cassia alata (leaves)	Spermacoce latifolia	
		Roots	Aerial part
Moisture content	63.83 ± 0.17	78.50 ± 0.50	80.95 ± 0.30
Dry matter	14.83 ± 0.16	14.70 ± 0.14	11.51 ± 0.15
Ash on drying	1.50 ± 0.04	0.99 ± 0.06	1.52 ± 0.03

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

Table 2. Elemental composition (mg/Kg or ppm) of C. alata & S. latifolia

Metal	Cassia alata (leaves)	Spermacoce latifolia	
		Roots	Aerial part
Sodium (Na)	611.87 ± 2.78	1223.88 ± 4.99	1055.12 ± 4.57
Potassium (K)	5532.37 ± 1.07	5787.72 ± 5.04	8344.77 ± 5.08
Aluminum (Al)	1349 ± 3.27	2168.68 ± 4.86	9798.52 ± 5.98
Calcium (Ca)	18591.65 ± 1.58	13131.45 ± 5.05	18566.24 ± 8.06
Magnesium(Mg)	4550.05 ± 0.55	2165.71 ± 3.03	5068.38 ± 3.09
Iron (Fe)	1192.16 ± 0.270	924.93 ± 0.26	1019.12 ± 0.29
Copper (Cu)	8.36 ± 0.055	12.89 ± 0.05	9.54 ± 0.02
Manganese (Mn)	68.58 ± 0.009	202.45 ± 1.05	221.07 ± 1.06
Zinc (Zn)	116.96 ± < 0.002	274.94 ± 1.07	323.60 ± 1.09
Nickel (Ni)	3.88 ± 0.087	7.83 ± 0.044	14.30 ± 0.06
Chromium (Cr)	2.76 ± 0.022	1.589 ± 0.045	4.56 ± 0.048
Led (Pb)	27.82 ± 0.078	14.28 ± 0.085	24.87 ± 0.087
Cadmium (Cd)	0.0827 ± < 0.001	0.4079 ± < 0.001	0.7313 ± < 0.001

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

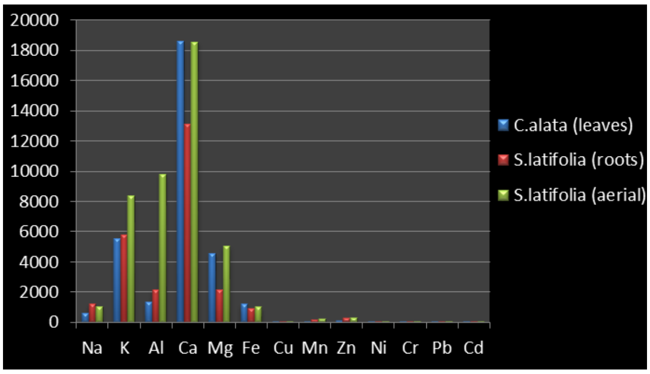

Fig 1: Elemental composition (mg/Kg or ppm) of *C. alata* & *S. latifolia*

4. Discussion

Minerals have a great necessity in human body for maintaining chemical reaction and intestinal absorption (magnesium), normal growth, muscular activities, cellular activities, development of the skeleton, oxygen transport (copper & iron), fluid balance & nerve transmission (sodium & potassium) [13]. Apart from these minerals, there may be the presence of trace elements which have important role in growth but their increasing concentrations may make the corresponding plant toxic for human [14].

The plants *C. alata* (leaves) and *S. latifolia* (roots & aerial part) were selected for the proximate and elemental analysis. These two plants are serving the human as a source for curing different diseases but significant elemental analysis on these two medicinal plants has not done yet specially in Bangladesh. A total 13 elements i.e, Sodium (Na), Potassium (K), Aluminum (Al), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn), Nickel (Ni), Chromium (Cr), Led (Pb) & Cadmium (Cd) were analyzed by using Atomic Absorption Spectrometer. It should be mentioned that each of the results is an average of three individual measurements. The result of this analysis is shown in Table-2.

Sodium (Na) governs the muscles function, acts as the principal cation of extracellular & intracellular fluids and is one of the major electrolytes in the blood [15]. Too much intake of sodium causes the breakdown of the cells and hypertension [16]. Deficiency of plasma sodium results in tissue oedema, raised intracranial pressure and neurological symptoms [17]. Lawal *et. al* reported the concentration of Na in the leaves of *C. alata* as 302.5 ppm [18]. The present investigation showed that the concentration of Na was 611.87 ppm in the leaves of *C. alata*. On the other hand concentration varied from 1223.88 ppm to 1055.12 ppm in case of the roots and aerial part of *S. latifolia*.

Potassium (K) facilitates the release of essential chemicals which conducts nerve impulses, reins the rhythm of heart. Insufficiency of this important electrolyte causes low blood sugar, insomnia, coma and nervous irritability mental disorientation [19]. The concentration was found to be lowest as 5532.37 mg/kg in the leaves of *C. alata*. Previously performed investigation by Lawal showed the concentration as 376.0 ppm in *C. alata* [18]. On the contrary, the highest concentration was carried by the aerial part of *S. latifolia* as 8344.77 ppm and the root of this plant contained the moderate concentration in the present investigation as 5787.72 ppm.

In the regular dietary list, the amount of aluminum (Al) is comparatively lower than the other minerals as it may cause diverse effects causing various diseases. The systematic absorption of this element from foods, air, drugs is controlled by the healthy human body which has effective barriers [20]. The present experiment indicated the highest concentration of Al in the aerial part of *S. latifolia* as 9798.52 ppm and the lowest in the leaves of *C. alata* as 1349 ppm. The moderate amount was found in the root of *S. latifolia* as 2168.68 ppm.

Calcium (Ca) is said to be the essential mineral which is required for many health purposes including the development of bones & teeth and a normal heart rhythm. This element is also requisite for muscles contractions & relaxation, blood pressure regulation, nerve & hormone function. Deficiency of Ca leads to osteoporosis [21]. In this investigation maximum concentration was found in the leaves of *C. alata* as 18591.65 ppm and the lowest was found in the root of *S. latifolia* as 13131.45 ppm. The concentration found in the aerial part of *S. latifolia* was 18566.24 ppm.

The moderate concentration of Magnesium (Mg) was found as 4550.05 ppm in the leaves of *C. alata*. Maximum concentration was found in the aerial part of *S. latifolia* as 5068.38 ppm and minimum concentration was found to be 2165.71 ppm in the root of that plant. It should be noted that Mg plays a vital role acting as a cofactor in more than 300 enzymes controlling a number of fundamental functions such as glycemic control, neuromuscular conduction, myocardial contraction, muscle contraction and blood pressure [22, 23].

Iron (Fe) is an indispensible element for human body as it takes part in various metabolic functions including oxygen transport, electron transport, synthesis of Deoxyribonucleic acid (DNA) and its requirement is very high in case of the pregnant women & in adolescents at the time of period of growth spurt [24]. Abdulwaliyu researched on the element present in the leaves of *C. alata* and found the concentration of Fe as 420.35 ppm [25]. In our present study, the concentration of the leaves of *C. alata* was found as the highest one showing the value 1192.16 ppm. On the contrary, the lowest concentration was found as 924.93 ppm in the root and the middle concentration was 1019.12 ppm found in the aerial part of *S. latifolia*.

The necessity of Copper (Cu) lies in sustaining the strength of blood vessels, skin, epithelia and connective tissues throughout the body and can act as both antioxidant & pro-oxidant [26]. The concentration of this element was found as highest in the root of *S. latifolia* as 12.89 ppm whereas as lowest in the leaves of *C. alata* as 8.36 ppm. The concentration of the aerial part of *S. latifolia* was found to be the moderate concentration as 9.54 ppm.

Manganese (Mn) is an essential mineral engaged in intracellular activities and regulates the antioxidant defense, reproduction, energy production, development, digestion and immune response [27]. The concentration of Mn was found to be highest to lowest as 221.07 ppm, 202.45 ppm, 68.58 ppm in case of the aerial part, root of *S. latifolia* & the leaves of *C. alata*. The concentration was previously ensured by Lawal as 5.4 ppm in *C. alata* [18].

The importance of Zinc (Zn) is mainly in normal growth and development during pregnancy, childhood, adolescence and takes part in cell division, wound healing, immune function, protein synthesis [26]. Lawal found out the concentration of this element as 350.47 ppm in the leaves of *C. alata* whereas the present investigation showed it as 116.96 ppm [18]. And the highest concentration was 323.60 ppm in the aerial part and the root showed the concentration as 274.94 ppm in *S. latifiolia*.

Trace elements are normally the elements which are required for human body in small amount usually between 1 to 100 mg/day. But its high intake may give rise to various diseases and toxicity [28]. So the concentration of these trace elements should be within tolerance limit in the sample. In our present investigation, Ni, Cr, Pb & Cd were found in the plant samples. The concentrations showed by the leaves of *C. alata*, roots & aerial part of *S. latifolia* of Ni, Cr, Pb, Cd were evaluated as for Ni (3.88, 7.83, 14.30 ppm), Cr (2.76, 1.59, 4.56 ppm), Pb (27.82, 14.28, 24.87 ppm), Cd (0.0827, 0.4079, 0.7313 ppm). For dietary intake the concentration of Cd should be 10 mg/day according to WHO [29]. In the examined samples, the highest concentration of Cadmium was found in the aerial part of *S. latifolia* as 0.7313 ppm which is within the limit. So, this is safe for medicinal uses.

If the present investigation data is compared with the literature review, it can be seen the concentration of the elements vary very often from each other. This may be due to the pollution of different areas i.e, air, water, soil pollution. Because of the pollutants present in these sectors cause the difference in concentration of the minerals present in that plant. Higher concentrations of these trace elements make the plant toxic for human intake.

5. Conclusion

Medicinal plants are indispensible in combating the human illness and are sources of the precursors for the synthesis of useful drugs that can be used as therapeutic purposes. These plants are also enriched with the essential elements that contribute immensely in the formation of secondary metabolites which are claimed to be responsible for pharmacological actions of these elements in the plants. So the research in this field has been turned into a great interest. The interest has been forwarded to the elemental analysis, isolation, characterization and synthesis of new drug leads.

But the destination of the present investigation was targeted on the proximate and elemental analysis of different parts of the two medicinal plants namely *C. alata* (leaves) and *S. latifolia* (roots and aerial part). This experiment was conducted using Atomic Absorption Spectrometer. From the evaluated data, it was noticed that the plants are enriched with high nutritional values including the higher concentration of Na, K, Ca, Mg, Fe which greatly contribute to the proper growth and development of the human body. It should be also noted in case of this experiment that the plant samples contain considerable amount of trace elements i.e, the concentrations are within the tolerance limit. So, the plants are safe for medicinal uses and are not cautious for human health.

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