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journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2015.10.003>Comparative studies of elemental composition in leaves and flowers of *Catharanthus roseus* growing in BangladeshShahin Aziz^{1*}, Koushik Saha², Nasim Sultana³, Husna Parvin Nur³, Md. Aminul Ahsan³, Shamim Ahmed³, Md. Kamal Hossain³¹Chemical Research Division, BCSIR, Dhaka, Bangladesh²Department of Chemistry, Jahangirnagar University, Savar, Dhaka, 1342, Bangladesh³Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka, Bangladesh

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ABSTRACT

Objective: To investigate the elemental composition of the leaves and flowers of *Catharanthus roseus* (*C. roseus*) due to the plant's wide application in the indigenous medicinal system and its chemical constituents' importance.

Methods: The atomic absorption spectrophotometer was used for quantitative analysis of various elements.

Results: Total 13 important elements were analyzed in leaves and flowers of *C. roseus*. Results indicated the presence of Na, K, Ca, Mg, Cr, Fe, Zn, Al, Cu, Ni, Pb, Cd and Mn in both leaves and flowers. The most important finding of the work was that, leaves of *C. roseus* showed high concentration of all elements except K and Zn while flowers of *C. roseus* showed higher concentration of K and Zn.

Conclusions: The elemental composition in both leaves and flowers of *C. roseus* were found to be different. Therefore, different parts of this medicinal plant are enriched in some micro and macro nutrients like Fe, Ca, Na, K, Zn, which are very important for biological metabolic system as well as human health.

1. Introduction

Medicinal plants have a long history of usage in traditional medicine. Ethnobotanical information on medicinal plants and their usage by indigenous cultures is useful in the conservation of traditional cultures, biodiversity, community health care and drug development [1]. The authentic knowledge of the usage of medicinal plants passed from one generation to another, after refining and addition [2]. The folk recipes are prepared either from the whole plant or from their different organs, like leaf, stem, bark, root, flower, seed, etc. and also from their secondary product such as gum, resins, and latex [3]. In the human body, medicinal plants interact directly or indirectly with the body chemistry by the chemical constituents. Once the

active constituents are absorbed into the blood, these constituents derive the required benefits by circulating and influencing the blood stream [4]. Plants supply minerals, vitamins, and certain hormone precursors in addition to protein and energy to human body [5,6]. Trace elements have significant roles in combating a variety of human ailments and disease was observed by the study of elements with respect to indigenous medicinal plants [7].

At the same time, major and trace elements play an important role in the building up and restoration phenomenon of health and disease of human body. Progress has occurred in this area of health sciences during the last few years [3]. Elemental research has definitely been part of this upsurge of scientific knowledge. Also a very important property of metals is, their tendency to bioaccumulation. This bioaccumulation is therefore essential in hazard evaluation strategies [8]. In terms of modern pharmacological concepts, direct correlation between elemental content of medicinal plants and their curative ability is not yet understood. So, the quantitative estimation of various elements' concentration is important for determining the effectiveness of the medicinal plant in treating various diseases

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and understanding their pharmacological action. The imbalance in human health has been linked with excess or deficiency of elements in soils, water, plants and animals [3]. The continuous intake of diets that are excessively high in a particular element can influence changes in the functioning, forms, activities of some organs or concentrations of such element in the body tissue and fluids can rise above the permissible limit [9].

As a medicinal plant *Catharanthus roseus* (family: Apocynaceae) (*C. roseus*) (common name: periwinkle, vinca; Bengali: Nayantara; synonyme: *Vinca rosea*) popularly known as Madagascar periwinkle is a potential source for anti-leukemic alkaloids. It is an evergreen subshrub or herbaceous plant growing up to 1 m tall [10]. This plant is a terpenoid indole alkaloids producing plant [11]. The plant is an important source of indole alkaloids, which are present in all plant parts. The leaves and stems of the plants are the source of dimeric alkaloids, vincristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine [12]. Vincristine and vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia [10]. All parts of the *C. roseus* are used for different medical purposes. It has been used as anti-diabetic (flowers and leaves), hypolipidemic and antioxidant. It has also been used for treatment of Alzheimer's disease [13–16]. Besides these medicinal usages, different plant parts were used as folk remedy for lowering the blood glucose level in Europe for centuries [17]. In India, juice from the leaves was used to treat wasp stings and also different part of the plant was used as antipyretic, antiulcer, antidiabetic and anticancer in Indian herbal medicine [18]. In Hawaii, the plant was boiled to make a poultice to stop bleeding. In China, it was used as an astringent, diuretic and cough remedy. In Central and South America, it was used as a homemade cold remedy to ease lung congestion and inflammation. Throughout the Caribbean, an extract from the flowers was used to make a solution to treat eye irritation and infections. It also had a reputation as magic plant. It was also observed that the leaves of the plant are used extensively in folk medicine for decreasing sugar level of blood and showed significant anti-hyperglycemic effect [10].

A number of techniques such as atomic absorption spectrometry (AAS), voltammetry, inductively coupled plasma activation analysis are routinely used to determine trace elements in medicinal plants. AAS is a versatile, non destructive analytical tool widely used for the determination of minor and trace elements in complex biological samples.

Several studies have been carried out on the isolation of pharmacologically active compounds on leaves and flowers of *C. roseus*. However, little work has been reported about minor and trace elemental composition of the leaves and flowers of the plant. Some elemental screening works have been previously done on the leaves of this medicinal plant in some other countries like India, Pakistan, Thailand *etc.* But in Bangladesh, no systematic elemental screening was done on leaves and flowers of this plant so far. After all, Bangladesh is not only geographically but also zoologically different from other countries. It is well known that physiological changes do occur in plants due to changes in geographical sites, climatic and environmental conditions. This results in the production of nonidentical plant metabolites in the same plant grown in different geographical regions. Keeping in mind the wide application of different plant parts of *C. roseus* in traditional

medicine and Ayurvedic preparation, elemental screening on leaves and flowers of *C. roseus* was carried out by using atomic absorption spectrophotometer.

2. Materials and methods

2.1. Collection of plant material

Fully matured fresh leaves and flowers of *C. roseus* were collected from the gardens of Chemistry Department of Dhaka University, Bangladesh in June 2013 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka. Here a voucher specimen No. 39512 has been deposited. The leaves and flowers of *C. roseus* were separately dried at 100 °C for half an hour till a constant weight was achieved. These dried samples of leaves and flowers were powdered using 20 mesh screen in Willey mill and then used for subsequent analyses.

2.2. Reagents and standards

For digestion of our experimental sample, we used nitric acid (69%, Merck India) and perchloric acid (70%, Merck India) without further purification. For standard calibration of respective elements, we purchased Na, K, Ca, Mg, Cr, Fe, Zn, Al, Ni, Cd, Mn standard solution (100 mg/mL) from Hach (Germany). We prepared the respective desired standard from the stock solution using lab made double distilled water.

2.3. Ashing and digestion of plant parts

Accurately weight 2.0 g of leaves of *C. roseus* separately (previously cleaned) was taken in a porcelain crucible and was heated to about 650 °C and cooled and then it was weighed. The crucible with sample was placed in the Bunsen burner (at low flow rate gas) until the smoke ceased. Then the crucible was placed in a temperature controlled muffle furnace at 525 °C for about 8–10 h to obtain carbon free white ash. It was then cooled in desiccators and weighed. This procedure was repeated till a constant weight was obtained and the color of the ash was changed to almost white. The same was done for 2.0 g of flowers of *C. roseus*. About 1.0 g ash sample for both leaves and flowers of *C. roseus* were taken separately in 50 mL volumetric flask and then 15 mL of 1 mol/L HNO₃ acid was added. Then the flask was placed on magnetic stirrer heater in fume hood for 4 h at 250 °C. When the color of the solution was changed to milky solutions, it was cooled for 10 min and then 7.5 mL concentrated perchloric acid was added. Then it was heated until colorless solution was obtained. For the determination of dissolved elements, the sample was filtered through 0.45 micron filter paper. In all the 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. Absorption for a sample solution used the calibration curves to determine the concentration of particular element in that sample [19].

2.4. Analytical procedure

Among all elements, only Na and K were estimated by using flame photometer (Model AnA-135, OSK, Japan). Most of the elements like Ca, Mg, Cr, Fe, Zn, Al, Cu, Ni, Pb, Cd and Mn in

leaves and flowers of our plant samples were analyzed by using atomic absorption spectrophotometer (Varian, AA 240FS, Australia) which was equipped with flame and graphite furnace. For our experiment, we choose air acetylene flame mode. The condition fixed acetylene 1.8 L/min and air 15 L/min, argon gas flow for inert atmosphere. The instrumental default temperature parameters were automatically fixed for each element analysis. For quantitative measurement of each element with its linear working range, its respective wavelength and statistical calibration graph of correlation coefficient were listed in Table 1. Data recorded respective elements in triplicate measurements for its authentication was used for standard deviation calculation.

Table 1

Operating parameter for working element.

Element	Wavelength (nm)	Lamp intensity (mA)	Slit width (nm)
Na	589.0	12	0.2
K	766.5	10	0.2
Ca	422.7	10	0.5
Mg	285.2	4	0.5
Cr	357.9	7	0.2
Fe	248.3	5	0.2
Zn	213.9	5	1.0
Al	396.2	10	0.5
Cu	324.8	4	0.5
Pb	217.3	10	1.0
Cd	228.8	4	0.5
Mn	279.5	5	0.2
Ni	232.0	4	0.2

3. Results

3.1. Minerals and heavy metal contents

The elemental compositions of leaves and flowers of *C. roseus* were determined by using atomic absorption spectrophotometer. A total of 13 elements *i.e.* Na, K, Ca, Mg, Cr, Fe, Zn, Al, Cu, Pb, Cd, Mn and Ni were analyzed from both leaves and flowers of the plant which were accountable for curing various diseases. The result of the analyses was presented in Table 2. It may be noted that each result was an average of at least three independent measurements. These elements played a vital role in the formation of secondary metabolites which were

Table 2

Elemental compositions (dry weight basis, mg/g) of leaves and flowers of *C. roseus*.

Elements	Leaves	Flowers
Na	4.7200 ± 0.530	2.3100 ± 0.2600
K	23.0700 ± 4.980	23.4200 ± 5.0500
Ca	36.1900 ± 3.040	6.0500 ± 5.0500
Mg	5.1300 ± 0.260	1.7500 ± 0.0900
Cr	0.0090 ± 0.001	0.0020 ± 0.0004
Fe	1.0400 ± 0.083	0.5590 ± 0.0440
Zn	0.0230 ± 0.002	0.0480 ± < 0.0050
Al	0.0350 ± 0.004	0.0220 ± 0.0050
Cu	0.0060 ± < 0.001	0.0020 ± < 0.0010
Pb	0.0008 ± < 0.001	0.0003 ± < 0.0010
Cd	0.0002 ± < 0.001	0.0001 ± < 0.0010
Mn	0.1300 ± 0.009	0.0200 ± 0.0010
Ni	0.0060 ± 0.001	0.0024 ± < 0.0010

Measured values are mean ± SD of three replicate analyses.

responsible for pharmacological actions of these elements in both leaves and flowers of the plant.

4. Discussion

In the present study, it was observed that leaves showed higher concentration of Na, Ca, Mg, Cr, Fe, Al, Cu, Pb, Cd, Mn and Ni compared to flowers of *C. roseus*. But flowers showed higher concentration of K and Zn.

Na maintains the osmotic equilibrium between the extra cellular fluid and the tissue cells and maintains the pH of blood within normal limit. It is also concerned with the conduction of nervous impulses, muscle contractility and control of heart muscle conduction [20]. The average concentration of Na was 4.72 mg/g for leaves and 2.31 mg/g for flowers. Some researches have been reported elemental analysis on this plant part in India and Pakistan by AAS. In Pakistan, Sahito *et al.* found that the average concentration of Na was 0.66 mg/g for leaves and 0.38 mg/g for flowers [21].

K is helpful in reducing hypertension and maintaining cardiac rhythm. In the human body, K plays vital role in many physiological reactions and its deficiency or excess can affect human health [22]. The average concentration of K was 23.07 mg/g for leaves and 23.42 mg/g for flowers in the present study. Whereas in India Singh *et al.* found average concentration of K to be 6.50 mg/g for leaves [23]. And Sahito *et al.* found the average concentration of K to be 1.90 mg/g for leaves and 1.76 mg/g for flowers [21].

Ca overcomes the problems of high blood pressure, heart attack, premenstrual syndrome, colon cancer and keeping the bones strong and reduces the risks of osteoporosis in old age [3,24]. The average concentration of Ca was 36.19 mg/g for leaves and 6.05 mg/g for flowers in the present study. Whereas in India Singh *et al.* found average concentration of Ca to be 29.09 mg/g for leaves [23]. And Sahito *et al.* found the average concentration of Ca to be 10.08 mg/g for leaves and 1.40 mg/g for flowers [21].

Mg improves insulin sensitivity, protects against diabetes and its complications and reduces blood pressure [5]. Also Mg involves in many enzymatic reactions of oxidative metabolism of nutrients and cell constituents synthesis, transmission of nerve impulses, body temperature regulation, detoxification, energy production and the formation of health bones and teeth [25]. The average concentration of Mg was 5.13 mg/g for leaves and 1.75 mg/g for flowers. Sahito *et al.* found the average concentration of Mg to be 1.41 mg/g for leaves and 0.85 mg/g for flowers [21].

Zn is a component of a wide variety of enzymes, including the ribonucleic polymerases, alcohol dehydrogenase, carbonic anhydrase and alkine phosphate. Studies in animals have shown that Zn deficiency during pregnancy may lead to development disorders in the offspring. Zn is an important constituent of viable sperm especially human sperm. It is necessary for the growth and multiplication of cells (enzymes responsible for DNA and RNA synthesis, for skin integrity, bone metabolism and functioning of taste and eyesight) [26]. Zn deficiency may contribute to arrested sexual maturation, growth retardation and hair loss, delayed wound healing and emotional disturbance [3]. The average concentration of Zn was 0.023 mg/g for leaves and 0.048 mg/g for flowers. Singh *et al.* found average concentration of Zn to be 0.054 mg/g for leaves, which is higher than our present study [23]. And

Sahito *et al.* found the average concentration of Zn to be 0.026 mg/g for leaves and 0.068 mg/g for flowers, which supports present study for leaves [21].

For the formation of the oxygen carrying protein haemoglobin and myoglobin, the human body needs Fe. It is an essential mineral to prevent anemia and cough associated with angiotensin-converting enzyme inhibitors [27]. Also in the synthesis of neurotransmitters such as dopamine or epinephrine and serotonin, Fe is an essential cofactor [4]. The average concentration of Fe was 1.04 mg/g for leaves and 0.559 mg/g for flowers. Singh *et al.* found average concentration of Fe to be 0.424 mg/g for leaves [23]. And Sahito *et al.* found the average concentration of Fe to be 0.049 mg/g for leaves and 0.105 mg/g for flowers [21].

Mn can help to assist the body in metabolizing protein, helps the diabetic also metabolizes carbohydrates and in treating diabetes [28]. The average concentration of Mn was 0.130 mg/g for leaves and 0.020 mg/g for flowers in the present study. Singh *et al.* found average concentration of Mn to be 0.183 mg/g for leaves [23]. And Sahito *et al.* found the average concentration of Mn to be 0.03 mg/g for leaves and 0.027 mg/g for flowers [21].

Pb is toxic metal and nonessential element for human body as it causes a rise in blood pressure, kidney damage, miscarriage and subtle abortion, brain damage, declined fertility of men through sperm damage, diminished learning abilities of children and disruption of nervous systems [3,9]. The average concentration of Pb was 0.0008 mg/g for leaves and 0.0003 mg/g for flowers in our present study. Sahito *et al.* found the average concentration of Pb to be 0.004 mg/g for leaves and 0.001 mg/g for flowers [21].

The value of toxic element Cd for the case of leaves is 0.0002 mg/g and for the case of flower is 0.001 mg/g. The maximum limit for Cd is 0.3 mg/kg in herbal medicines and products while the dietary intake limit is 10.3 mg/kg which is prescribed by World Health Organization [29]. Sahito *et al.* found the average concentration of Cd to be 0.002 mg/g for leaves and 1.40 mg/g for flowers [21].

Cr showed the value for leaves 0.009 mg/g whereas for the case of flowers the value is 0.002 mg/g. In the present experiment, the toxic elements found to be below the prescribed limits. Excessive intake can cause poisoning in human body. Pb and Cd cause acute and chronic poisoning, adverse effects on the kidney, liver, heart vascular and immune system, brain damage, subtle abortion, declined fertility of men through sperm damage, diminished learning abilities of children and disruption of nervous systems [30,31].

The elemental composition determined in present study differs in some cases with some literature values. It may be due to the environmental factors including atmosphere and pollution, season of collection samples, age of plant and soil condition in which plant grows. These factors may affect the concentration of elements as it varies from plant to plant and region to region [5].

Elemental uptake by a plant is influenced by various factors, including types of plant, nature of soil, climate and agriculture practices [32,33]. The concentration of elements is not uniformly distributed throughout the plant. In the current study, different elemental concentrations vary due to those factors. The present investigation will be helpful in the synthesis of new modern drugs with various combinations of plant parts which can be used in the cure of many diseases ethnomedicinally. Also the different concentration of elements in different parts of *C. roseus* leads to the conclusion that the plant will have

different specific roles in the treatment of different diseases. However, more detailed analysis of chemical composition of different part of this important medicinal plant is required.

Conflict of interest statement

We declare that we have no conflict of interest.

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