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Heavy Metal Screening Using Atomic Absorption Spectroscopy (AAS) in *Breynia androgyna* (L.) Chakrab. and N.P. Balakr. Leaves



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ABSTRACT

Breynia androgyna (L.) Chakrab. & N.P. Balakr is a shrubby plant belonging to the phyllanthaceae family. In India, it is also known as multivitamin plant as it contains an excellent source of vitamins A, B, C, D, E and rare vitamin K, carotenoid and contains phytochemicals which can act as antioxidant. The present study was aimed to analyze the heavy metals in leaves of Breynia androgyna (L.) Chakrab. & N.P Balakr. Elemental composition of leaves has been determined using atomic absorption spectroscopy (AAS). A total of 9 elements Mg⁺², Ca ⁺², Fe⁺², Cu ⁺², Mn ⁺², Zn⁺², Pb⁺², Cd+2, P -3 have been analysed. The results of analysis showed that Magnesium has the highest concentration, which illustrates the ability of the plant to maintain cholesterol levels, heart rhythm in the human body, the ability to convert blood sugar into energy and also to prevent headaches and attention deficit hypersensitivity disorder. The concentration of calcium and iron meets the dietary requirement of man which reflect their nutritional and beneficial values. The plant can be utilized for pharmaceutical purposes.

INTRODUCTION

Breynia androgyna (L.) Chakrab & N.P Balakr., a multivitamin plant is distributed in South Asia and Southeast Asian countries including China, India, Srilanka, Indo-China, Indonesia, Malaysia, Philippines etc. It is highly nutritious leafy vegetables. Fresh leaves are excellent source of antioxidants (Hemalatha et al., 1997; Mahuya De Ghosh et al., 2011), carotenoids (Tee et al., 1996), pro vitamin A, Vitamin B, C, D, E (Ling Soon Ching et al., 2001). Leaves are also rich in carbohydrates and minerals like calcium, potassium, phosphorus, copper, iron, sodium and are rich sources of fibre (Padmavathi et al., 1990 and Singh et al., 2011). A green dye obtained from leaves is used as food colouring for pastries, rice and preserves. (Padma, 2013) The leaf extract of Breynia androgyna (L.) Chakrab. & N.P. Balakr was used for bodyweight reduction. In order to enhance lactation in feeding mothers, the leaves are given to women after delivery. (Tamanna Arif and Raviraja Shetty G,2020). The enhancement in breast milk production probably derived from the hormonal effects of chemical compounds that are estrogenic sterols (Sa'roni et al., 2004). The leaf juice is used to treat cholecystitis, diarrhoea and other forms of fever, rhinosis. Decoction of the leaves and roots is remedy for epistaxis and oriental sores. Leaf juice is used as an eye lotion for eye complaints. (Kanchanapoom et al., 2003). The leaf extract was used for bodyweight reduction known as a Slimming agent in Asian countries specifically in Taiwan, Malaysia, etc., (Tamanna Arif and Raviraja Shetty G, 2020).

Breynia androgyna (L.) Chakrab & N.P Balakr. is an erect, glabrous, perennial, monoecious shrub which can reach a height of about 3m. The leaves are simple, alternately arranged, lanceolate or oblong in shape 2-7 cm long and 1-3cm wide, entire. The upper surface is dark green in colour as compared to lower surface. Flowers are unisexual with the female appearing first before the male, in axillary clusters on the underside of the branch. Pedicle of male flower is slender with 5-7 mm in length. Calyx is yellow coloured, shallowly disk shaped and is 5-12 mm in diameter. Sepals are obovate, disk segments are 6 in number, opposite to sepals and incurved distally covering anthers. Stamens are 3 in number filaments are connate and anthers are extrose. Pedicles of female flower have 6-8 mm length. Calyx is red, 6-lobed. Sepals are obovate or obovate and are triangular, base attenuate into a short claw. Disk is absent. Trilocular ovary, with white to pinkish fruit, dehiscing with 3 thinly crustaceous valves.

Atomic absorption spectroscopic technique makes use of the atomic absorption spectrum in a sample in order to assess the concentration of specific analytes within it. Atoms in metallic elements tend to absorb UV light when they are exposed to ultraviolet rays. Each and every metal element has a unique absorption peak at a particular wavelength under all circumstances. This is due to the unique electronic configuration of the valence shell of its atoms. If a particular metal is present in any sample, there will be a maximum absorption at a particular wavelength which is characteristic of the metallic atom. Atomic absorption spectroscopy (AAS) is a spectro analytical procedure of the quantitative determination of chemical elements using the absorption of optical radiation (light) by the free atoms in the gaseous state.

Systematic analysis of the macro and micro mineral elements thus facilitates evaluation of nutritional composition and mineral accumulated by the edible plants. The main objective of the present study is to quantify the non-essential heavy metals like Copper, Lead, cadmium and essential minerals like Iron, Magnesium, manganese, Calcium and Phosphorus in the plant *Breynia androgyna* (L.) Chakrab. & N.P Balakr using Atomic Absorption Spectroscopy. The present study reveals the nutritional potential in the plant *Breynia androgyna* (L.) Chakrab. & N.P Balakr.

HUMAN

MATERIALS AND METHODS

Plant Materials

Breynia androgyna (L.) Chakrab. & N.P Balakr. leaves were collected from Thiruvananthapuram district for the present investigation. The micronutrient content present in the plant was analysed through Atomic Absorption Spectroscopy. The flower, leaf and aerial parts of the *Breynia androgyna* (L.) Chakrab & N.P Balakr. were identified and noted their morphological characters. After the collection, the whole plant was washed and dried in the shade. Test Method used was Association of Analytical Chemical International 20th Edition 2016 (3.2.05). Samples are stored in tight sealed pack and analysed within a day if any delay occurred samples are sorted without contamination and leakage. The whole sample is mixed well and a portion is blended to fine powder.

PREPARATION OF SAMPLE AND EXTRACTION

1 to 3 g of well ground mixed sample is weighed into a pre weighed crucible and heated for overnight at 500 degree centigrade and let cool. Wet ash with 10 drops water and 1-2ml nitric

acid is carefully added. Evaporated excess nitric acid on a hot plate set at approximately 100 degree centigrade. Returned crucible to furnace and heated for 1 hr at 500 degree centigrade. Ash is dissolved in 10ml HCL, filter to volumetric flask and made up to 10ml with distilled water. Read the concentration by aspirating sample and reference standard solution in Atomic Absorption Spectroscopy. Calibration is done by aspirating standard metal solutions and noted the absorbance. Heat must be applied to the sample to break the bonds combining atoms in molecules as most of the atoms for elemental analysis cannot exist in the free ground state at room temperature. A liquid sample is aspirated and mixed as an aerosol with combustible gasses (acetylene and air or acetylene and nitrous oxide.) in flame atomic absorption spectroscopy. The mixture is ignited in a flame of temperature ranging from 2300 °C to 2700 °C (depending on the fuel gas used.) During combustion, atoms of the element of interest in the sample are reduced to the atomic state. The Perkin Elmer Lambda 45 Atomic Absorption Spectrophotometer was used to record the spectra. The liquid sample is fed into the flame with a nebulizer where the sample is converted into atom at approximately 2300 °C. The wavelengths are element specific and accurate to 0.01-0.1 nm.

RESULTS AND DISCUSSION

The leaves of Breynia androgyna (L.) Chakrab. & N.P Balakr are a source of almost all vitamins including A, B, C, D, E and rare vitamin K found in only few taxa. The leaves are also rich source of essential amino acids such as lysine, methionine, tryptophan, phenylalanine, threonine, valine, leucine and isoleucine. Apart from being nutritious vegetable it was promoted as being effective for weight reduction, in controlling hypertension, gynaecologic problems, gallstones, diabetes, constipation, cholecystosis, diorrhea, rhinosis, epistaxis, oriental sores, nasal ulcers, yaws and leaf extract is said to be active against some breast cancer cell lines. Leaves extract are also used as an eye lotion for eye complaints and to increase lactation in feeding mothers. Breynia is usually propagated vegetatively since the plant grows readily from cuttings. People have become much calorie conscious and are fascinated towards being slim and Breynia androgyna is proved to be an effective slimming agent. Nutrients are needed in sufficient quantities to survive a healthy life and to obtain best productivity. For the present study, the parameter used for nutritional analysis is heavy metals. Heavy metals are defined as elements with metallic properties and an atomic number greater than twenty. Metals are natural components in soil. Some metals are micronutrients necessary for plant growth like Zn, Cu, Mn, Ni, and Co, while some others

have unknown biological function, like Cd, Pb, and Hg. These micronutrients play a central part in metabolism and in the maintenance of tissue function.

Heavy metals show a major role in plant nutrition. The daily mineral requirement of an adult man (70 kg person) are 15 mg iron, 2.8mg manganese, 15mg zinc, 2.5 mg copper, 0.0025 mg nickel, 0.057mg cadmium. A number of these elements have been reported with high biochemical essence and are involved in up regulating the formation of secondary metabolites which are responsible for pharmacological actions of vegetal species (Tanghu et al., 2011). Trace elements are also essential to all cells and deficiencies of essential elements may cause several disease in humans. The present study indicates the presence of magnesium greater than that of other metals. It is 310 mg/ 100 g. Magnesium is the major part of chlorophyll. Magnesium play an important role in human body that maintain cholesterol levels, maintains heart rhythm. Magnesium converts blood sugar into energy and also prevents headaches and attention deficit hypersensitivity disorder. The concentration of calcium in Breynia androgyna (L.) Chakrab. & N.P Balakr was 40mg/100g. Calcium is a mineral necessary for life. It helps in building bones and keeping them healthy, it helps to clot our blood, our muscles to contract, and our heart to beat. About 99% of the calcium in our body is deposited in our bones and teeth. Tingling Fingers, Muscle cramps, Lethargy, Poor appetite, Weak or brittle fingernails, Difficulty swallowing, fainting are the conditions that cause due to deficiency of calcium. The recommended daily allowance of Calcium for children is between 500mg and 1000 mg and for adults 800 mg.

The amount of manganese present in *Breynia androgyna* (L.) Chakrab. & N.P Balakr was 35.6 mg/100g. Manganese plays an important role in bodily functions, including the metabolism of amino acids, cholesterol, glucose, and carbohydrates. It also plays a vital role in bone formation, blood clotting, and also in reducing inflammation. It is found that the concentration of iron in *Breynia androgyna* (L.) Chakrab. & N.P Balakr is 16.5mg/100g. Iron is necessary to preserve many vital functions in the body, including general energy, gastrointestinal processes, the immune system, and body temperature regulation. Anemia can cause fatigue, heart palpitations, pale skin, breathlessness etc. The rich iron and calcium content help to build a healthy body and helps to avoid anemia like diseases.

The amount of zinc present in *Breynia androgyna* (L.) Chakrab. & N.P Balakr leaves was 3.8mg/100gm. Zinc is essential for the activity of over 300 enzymes that aid in metabolism. It helps in digestion, nerve function and many other processes. In addition, It also helps in

development and function of immune cells and aids in maintaining skin health, DNA synthesis and protein production. Zinc is necessary for our senses of taste and smell. Zinc deficiency can reduce ability to taste or smell. The results show that the amount of copper content in *Breynia androgyna* (L.) Chakrab. & N.P Balakr. was 0.8mg/100g. Copper is also an essential nutrient for the body. Along with iron, it enables the body to form red blood cells. It helps to maintain healthy bones, blood vessels, nerves, and immune function, and it contributes to iron absorption. (Koche D,2011) The concentration of phosphorous in *Breynia androgyna* (L.) Chakrab. & N.P Balakr. leaves was found to be 0.23g/kg. Phosphorous play an important role in the formation of bones and teeth. It is essential for the body to make protein for the growth, maintenance and repair of cells and tissues.

The amount of lead in *Breynia androgyna* (L.) Chakrab. & N.P Balakr. leaves was found to be 0.5mg/100g. Long-time exposure to lead has been reported to cause anaemia, along with an increase in blood pressure, and that is mainly in old and middle-aged people. Severe damage to the brain and kidneys, both in adults and children, were found to be linked to exposure to heavy lead levels which results in death. The toxic heavy metal cadmium in *Breynia androgyna* (L.) Chakrab. & N.P Balakr. leaves was found to be 0.1mg/100g. Test Method used for the present study was Association of Analytical Chemical International 20th Edition 2016 (3.2.05).

Table No. 1: Chemical analysis of *Breynia androgyna* (l.) hakrab. & N.P Balakr. leaves by atomic absorption spectroscopy by A.O.A.C. International 20th edition 2016(3.3.05) method

SL NO.	PARAMETERS	RESULTS	UNIT
1	Phosphorous	0.23	g/100g
2	Cadmium	0.1	mg/100g
3	Copper	0.8	mg/100g
4	Manganese	35.6	mg/100g
5	Iron	16.5	mg/100g
6	Calcium	40.1	mg/100g
7	Lead	0.5	mg/100g
8	Magnesium	310	mg/100g
9	Zinc	3.8	mg/100g

CONCLUSION

Breynia androgyna (L.) Chakrab & N.P Balakr., a member of Phyllanthaceae is a shrub grown in some tropical regions as a leafy vegetable. The leaves are used as food as well as medicine. It is a multi-vitamin plant, leaves having an excellent profile of various important minerals like calcium, potassium, phosphorus, copper, iron, sodium, antioxidants and is a good source of proteins, carbohydrates, carotenoids etc. The leaves are traditionally used as antibacterial, analgesic and anti-inflammatory antihypertensive and wound healer. The leaves are rare source of almost all vitamins including A, B, C, D, E and rare vitamin K found in only few taxa. The leaves are also rich source of essential amino acids such as lysine, methionine, tryptophan, phenylalanine, threonine, valine, leucine and isoleucine. Further, the leaves are rich in rare source of combination of lutein and zeaxanthin in along with ligan and metastigmane glycosides. The present study indicates the presence of minor quantities of lead and cadmium also. According to the present study, Breynia androgyna (L.)Chakrab.& N.P Balakr. leaves was found to be the better variety for safer and healthy consumption although it had considerably less amount of heavy metals cadmium and lead. The variation in elemental concentration is mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated. Other factors responsible for the variation in elemental content are preferential absorbability of the plant, use of fertilizers and irrigation water (Rajurkar, and Pardeshi, 1997). The consumption of this green leafy vegetable should be promoted, especially among the vulnerable sections of the population, since the plant is easily available. The merits of this plant can be used as a better strategy to combat micronutrient deficiency and malnutrition in our country. Since it is really essential to study and research to analyse and recognise the nutrient and medicinal properties of this less explored plant at various levels.

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