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Elemental analysis of leaf and fruit powder of *Zanonia indica* L

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Abstract
Every organism requires nutrients for their overall growth and development. Animals, birds and human beings depends on plants in order to get their nutrients. Plants which are rich in these essential elements are identified using elemental analysis. Macro and micro elements present in plants also acts as medicine. The main objective of the present research to find out some major, minor and trace elements. The macro element Nitrogen is found to be rich in both fruit and leaf. The microelement iron was found rich when compared to other microelements.

Keywords: nitrogen, macronutrients, leaf, zinc, atomic absorption spectra

Introduction
Zanonia indica, belonging to the family Cucurbitaceae, a medicinal plant used in sidda and ayurveda (Khare CP 2008) [8]. Nutrients are essential for the human beings as well as animals for their normal functioning of the body, essential nutrients such as carbohydrates, minerals, vitamins, proteins, oils and fats are required for proper muscular activity and proper functioning of organs. Among them minerals such as Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Nitrogen (N), Phosphorous(P), Potassium(k) etc are required for human beings as well as animals for their proper growth and development. Zoroddu MA *et al* 2019) [14].
Elemental analysis of powdered plant samples exhibits the details of major and minor elements present in the samples. These elements are beneficent in preparation of medicines in order to overcome deficiencies. Essential elements and their role in human body metabolism can be analyzed utilizing the elemental analysis of the medicinal plant. Evaluation of micronutrients and essential trace elements levels of medicinal plants is a growing trend in nutritional studies throughout the world (Young EM 2012) [13].

Materials and Methods
Preparation of plant sample
Leaves and fruits of *Zanonia indica* L. were collected from the area of study, washed with tap water followed by distilled water, tap dried using tissue paper and then shade dried. Dried samples were ground to fine powder and used for analyzing mineral composition.

Elemental analysis
The element Nitrogen (N) was analyzed using kjeldahl digestion flask. Phosphorous (P) analyzed using spectrophotometer, Potassium (k) were analyzed by Flame photometer Jenway PEP7 FPM compressor unit-122.
The elements Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), were analyzed by Atomic Absorption Spectra GBC 932 AAS. The P, K, Ca, Mg, Fe, Mn, Cu, Zn were analyzed using diacid digestion method. The diacids (9ml nitric acid and 4ml perchloric acid) were mixed with 1gm of sample and kept on a hot plate until it reduced to 1ml.
This digested sample is cooled and mixed with distilled water. Filter the content to 50 ml volumetric flask then make up the volume to 50ml. This filtrate was used for determination of these elements.

Determination of Macronutrients
Determination of nitrogen (N)
Total Nitrogen in plant sample is commonly determined by Kjeldahl method using 4% boric acid indicator mixture [4g of boric acid is dissolved in 90ml of distilled water and 4 drops of mixed indicator (0.1g of methyl red and 0.5g of bromocresol green in 100ml of 95% ethanol) was added. Made up the volume to 100ml using distilled water.
About 0.2g of dried leaf/ fruit sample is taken in Kjeldahl's digestion flask. 10ml of sulphuric acid and sodium thiosulphate was added along with 2g of digestion mixture (16.8g of $CuSO_4$ and 82.66g of K_2SO_4 and 0.82g of selenium). The content was heated gently for 10 to 15min at 200ºC. The temperature was gradually increased to 420ºC for about 90 min until the liquid turned bluish green color. After completion of digestion, cool it up to the temperature reduced to room temperature.
Distilled water was added to digested sample, mixed well and made up the volume to 100ml (Sáez-Plaza 2013) [12].
In an another kjeldahl's flask, the aliquot of about 10ml was taken from the digested sample and 10ml of 40% NaOH was added into it.
The content flask was allowed to boil and evaporated content was collected in conical flask containing 10ml of 4% boric acid mixed indicator. This was titrated against standardized Sulphuric acid.
The percentage of nitrogen was calculated using the formula,

$$N\% = \frac{(B\text{-}T) \times N \text{ of } H_2SO_4 \times \text{Volume of digested sample}}{\text{Weight of sample} \times \text{aliquot taken}} \times 100$$

Where B is before titration volume in burette, T is after titration volume in burette

Determination of phosphorous (P)

The Phosphorous in the samples was determined by di-acid digestion method using Vanadomolybdate HNO_3 reagent [2.25g of ammonium molybdate was dissolved in 40ml of distilled water]. 0.125g of ammonium vanadate was dissolved in 30ml boiling distilled water. Ammonium molybdate solution and ammonium vanadate solution was mixed together and cooled to room temperature (Gliszczyńska-Świgło, A., & Rybicka, I. 2021) [13]. 25ml of concentrated HNO_3 was added and diluted to 100ml with distilled water.

The 5ml of each leaf and fruits aliquot (filtrate) prepared by diacid digestion was taken separately in 50ml volumetric flask, 10ml of Vanadomolybdate HNO3 reagent was added to each flask and the volume was adjusted to 50ml using distilled water (Ganesh, S *et al* 2012) [12]. The mixture was allowed to stand for 30min at room temperature. Yellow color was developed after incubation and it was measured using spectrophotometer at 420nm. The sample concentration was calculated by using standard graph and the percentage of phosphorous was calculated using the formula,

$$\% \text{ of } P = \frac{\text{Graph ppm} \times \text{Volume of digestion made} \times \text{Volume of digested sample}}{10^6 \times \text{Aliquot taken} \times \text{Weight of the leaf/ fruit sample}} \times 100$$

Determination of potassium (K)

For about 2ml of aliquot digested fruit and leaf material is taken in 50ml of volumetric flask separately and adjusted to final volume by adding distilled water (Khandpur, R.S., 2005) [7]. The solution is aspirated at the wave length 589nm of flame photometer. The concentration of potassium was determined by using standards of potassium chloride and the percentage was calculated using the formula,

$$\% \text{ of } K = \frac{\text{Graph ppm} \times \text{Volume of digestion made} \times \text{Volume of digested sample}}{10^6 \times \text{Aliquot taken} \times \text{Weight of the leaf/ fruit sample}} \times 100$$

Determination of calcium (Ca) and magnesium (Mg)

In 50ml volumetric flask, 1ml of leaves and fruits di-acid digested aliquot was taken and made up the volume to 50ml by addition of distilled water. Calcium was determined at the wavelength 422nm and magnesium was determined at wavelength of 229nm of atomic absorption spectrum (Prkić, A *et al* 2017) [13]. The percentage of magnesium and calcium were calculated using the formula,

$$\% \text{ Of Mg/Ca} = \frac{\text{Graph ppm} \times \text{Volume of digestion made} \times \text{Volume of digested sample}}{10^6 \times \text{Aliquot taken} \times \text{Weight of the leaf/ fruit sample}} \times 100$$

Determination of micronutrients

Micronutrients was determined by taking 1ml of di-acid digested aliquot of leaves and fruits sample in 50ml volumetric flask and final volume was adjusted with distilled water. The concentration of Manganese (Mn), Zinc (Zn), Copper (Cu) and Iron (Fe) was determined using atomic absorption spectrum at the wavelength of 279.5nm, 213.9nm, 324.7nm and 248.3nm respectively (Prkić, A *et al* 2017) [13]. The values of micronutrients are expressed in ppm using the formula,

$$\text{Ppm of Mn/Zn/Cu/Fe} = \frac{\text{Graph ppm} \times \text{Volume of digested sample}}{\text{Weight of the leaf/ fruit sample}}$$

Result and Discussion

The elemental analysis shows the presence of nitrogen, phosphorous, potassium, calcium, magnesium, iron, manganese, zinc, and copper.

Macronutrients were calculated and represented in percentage. Nitrogen was 5.25% in fruit powder and 2.87% in leaf powder.

Nitrogen content was rich when compared to other macro nutrients such as Phosphorous, potassium, calcium, and magnesium. The percentage of macronutrients of fruit and leaf powder of *Zanonia indica* L. were depicted in table1.

Table 1: Macronutrients of Fruit and Leaf powder of *Zanonia indica* L.

Macronutrients	Nitrogen	Phosphorous	Potassium	Calcium	Magnesium
Fruits powder	5.25%	0.284%,	1.715%	0.50%,	0.16%
Leaves powder	2.87%	0.271%	1.38%	2.042%	0.215%

Micronutrients were calculated and represented in ppm (parts per million). Iron content in fruit powder was 78.7ppm and in leaf powder 185.25ppm.

Iron was the abundant mineral compared to other micronutrients such as manganese, zinc, and copper.

The amount of micronutrients of fruit and leaf powder of *Zanonia indica* L. were depicted in Table 2.

Table 2: Micronutrients of Fruit and Leaf powder of *Zanonia indica* L.

Micronutrients	Iron	Manganese	Zinc	Copper
Fruits powder	78.70 ppm	24.05 ppm	17.90 ppm	9.75 ppm
Leaves powder	185.25 ppm	61.30 ppm	17.85 ppm	13.40 ppm

Essential elements perform several complementary vital functions in the body to keep the organism healthy. Copper helps in the formation of protective covering around nerves. Copper is necessary for the absorption of iron in the formation of haemoglobin. Copper is regarded as anti-inflammatory agent [Nrc 1989] [10].

Potassium helps in regulation of the water balance in the body (Andrerson *et al* 2008). Magnesium and Calcium is an essential element which maintaining healthy bones and teeth.

They extensively used in chemotherapy (Khan, 1996; Ogugbuaja *et al*., 1997) [6]. Zinc helps in proper working of genetic materials, proteins. It has wound healing property; it also helps in development of the fetus and sperm production. Manganese acts as antioxidant and helps in Nourishment of the nerves and the brain, it also helps in managing fats and cholesterol [Hamilton *et al* 1988] [4].

Phosphorous forms the sugar-phosphate backbone of DNA and RNA, generates adenosine triphosphate (ATP). High consumption is toxic to human beings and animals.

Nitrogen is essential for all living beings, it is a main part of amino acids, without aminoacids plants cannot make proteins. Iron (Fe) is an essential element for human beings and animals because it is an important part of haemoglobin. It facilitates the oxidation of protein, carbohydrates and fat and control body weight. Low Fe content causes anaemia, nose bleeding, and gastrointestinal infection [Hunt JR1994] [5].

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