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Short Communication

DETERMINATION OF MINERAL CONTENT IN *PRAECITRULLUS FISTULOSUS* BY USING ICP-MS AND SEM-EDX ANALYSIS

Archana Bollavarapu*, Gangaraju Rapaka, P. Felicity, Raghavarao Tamanam

Department of Biochemistry, Andhra University, Visakhapatnam, India

**Corresponding author: archanabollavarapu@gmail.com*

ABSTRACT

Vegetables and fruits are excellent sources of vitamins and minerals. Diets high in fruits and vegetables are linked to decrease risk of diseases (diabetes, cancer etc.) and their consumption ought to be inspired. Approximately thirty elements are recognized as essential to life, some are needed in macronutrient amounts, such as Ca, K, Mg, and Na others in trace or ultra-trace quantities. Copper, Iron, Nickel, Zinc and Manganese play a crucial role in biological systems. The objective of the study was to analyze the concentration of essential elements from *Praecitrullus fistulosus* for understanding its nutritive and medicinal values by using inductively coupled plasma mass spectrometry (ICP-MS) and scanning electron microscopy with an energy dispersive X-ray analytical system (SEM-EDX).

Keywords: *Praecitrullus fistulosus,* ICP-MS, SEM-EDX.

1. INTRODUCTION

In recent years, analysis on the role of trace elements and minerals in numerous metabolic processes and their impact on human health has become an area of particular concern and high priority in environmental research and protection. The functional role of trace elements is described in terms of their nutritionally essential role or their potential toxicity [1, 2]. Fruits and vegetables are valuable source of minerals. Diets high in fruits and vegetables are linked to decrease risk of diseases (diabetes, cancer etc.) and their consumption ought to be inspired [3]. Beside this, because of recent significant metal contamination of the surroundings the analysis of trace elements in fruit samples as well as in their products has gained considerable importance owing to health considerations. Minerals are of vital importance within the diet. An essential element is one required for maintenance of life when a deficient intake consistently results in an impairment of a function from optimal to suboptimal and when supplementation with physiological levels of this element but not of others prevent or cures this impairment [4]. Approximately thirty elements are recognized as essential to life, some are required in macronutrient amounts, such as Ca, K, Mg, and Na, others occur in trace or ultra-trace quantities. Copper, Iron, Nickel, Zinc, and Manganese play a crucial role in biological systems. Also Cr, Co and Se are essential for normal development and function of human cells.

Trace elements play a very important role as catalysts or a part of prosthetic groups for enzymes and consequently insufficient supply leads to element specific deficiency symptoms. However, when being present in enormous excess all of them can exert toxicity. In tissues and fluids, metals are mostly present as complexes with organic compounds like amino acids, proteins and peptides, organic acids or glutathione [5]. Fruits and vegetables contained considerable quantity of minerals that could be included in diets to support our daily allowance required by the body because of low cost and wide availability.

Cucurbitaceae is a plant family, also known as gourd family, which has crops like cucumbers, squashes, tinda, luffas and melons. The family consists of 118 genera and 825 species that have many medicinal and nutritional benefits. *Praecitrullus fistulosus* (Tinda) is a squash-like cucurbit grown for its immature fruit, a vegetable especially popular in South Asia, commonly known as Tinda in Hindi, Tindakaaya in Telugu and Indian round gourd in English. *Praecitrullus fistulosus* has been used as traditional medicine for curing heart diseases, strokes, controlling blood pressure, liver diseases and cancer etc.

To determine the trace element composition in plants, several techniques have been used such as graphite furnace, atomic absorption spectrometry, flame atomic absorptions spectrometry, inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), X-ray

fluorescence spectrometry, and scanning electron microscopy with an energy dispersive X-ray analytical system (SEM-EDX). Among the various techniques, two methods were chosen ICP-MS and SEM-EDX. Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful tool for the quantitative determination of a range of metals and non-metals (inorganic elements) in a wide variety of samples at trace and ultra-trace concentration levels [6]. SEM combined with EDX technique is highly qualified for identification and quantification of different elements in plants. The objective of the present study was to analyze the concentration of essential elements from *Praecitrullus fistulosus* for understanding its nutritive and medicinal values by using ICP-MS and SEM-EDX.

2. MATERIAL AND METHODS

2.1. Reagents

Concentrated Nitric acid (HNO_3), Concentrated Hydrochloric acid (HCl) (Merck, Mumbai), Hydrogen peroxide (30% H_2O_2) and double deionized water (Milli-Q) were used for all dilutions.

2.2. Elemental analysis

Samples were subjected to elemental analysis using inductively coupled plasma mass spectrometry (ICP-MS) optimized instrumentation conditions given in table 1 and scanning electron microscopy with an energy dispersive X-ray analytical system (SEM-EDX).

Table 1: Optimal instrumentation condition for ICP-MS analysis

Required parameters
Plasma conditions:
Rf frequency: 27 MHz; Rf power 1kW
Gas flow rate:
Carrier gas: Ar – 0.8 L/min
Auxillary gas: Ar 1.1 L/min
Coolant gas: Ar 15 L/min
Sampling conditions:
Sampling depth: 7mm from work coil
Sampling cone: Nickel 1.0mm orifice diameter
Skimmer cone: Nickel 0.4 mm orifice diameter
Nebulizer: cross flow type sampling uptake rate :0.4 ml/min
Data acquisition:
Data point: Multi element hope by peak hopping dwell time :3 points /peak
Integration: 50ms/point
Reputation: 1000 times

2.3. Collection of Plant material

The fresh fruits of *Praecitrullus fistulosus* were collected from the local market of Hyderabad. The fruit is a type of berry called a pepo. The fruits are approximately spherical, and 5-8 cm in diameter. The fruits were washed under running water to remove adhering dirt. The fruits were then sliced and shade dried for 20 days. The dried fruit were grinded. The powdered samples were then analyzed for various parameters.

2.4. ICP-MS

0.05 g (dry mass) of powdered plant sample was weighed into the digestion vessels. Then 4.0 ml of concentrated HNO_3 and 1.0 ml of 30% H_2O_2 were added to each sample. Samples were pre-digested overnight at 70°C. Repeated the step again and added 4ml of concentrated HNO_3 & 1.0 ml of 30% H_2O_2 and incubated at 70°C for 1 hour. After cooling, the entire digest was transferred into plastic bottles and diluted to 25 ml with double deionized water. Reagent blanks were prepared similarly without plant sample. All sample solution was clear.

2.5. SEM-EDX analysis

The sample was mixed properly and carefully placed on a glass cover slip followed by air-drying. The cover slip itself was used throughout scanning electron microscopy (SEM) analysis. The samples were then gold coated using a coater. The images of powdered sample were obtained in a scanning electron microscope (JSM-6610LV, Jeol Asia PTE Ltd, Japan).The details relating to applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

The presence of elements in the plant powder was confirmed through EDX. Energy dispersive analysis X-ray spectrometer takes advantage of the photon nature of the light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable pulse X ray; the output of an ultra-low noise pre-amplifier connected to the low noise is a statistical measure of the corresponding quantum energy. A semiconductor material is employed to sight the X-rays alongside process physical science to analyses the spectrum. The EDX observations were carried out in INCA energy 250, Oxford, Japan (JOEL ModelJED-2300).

3. RESULTS AND DISCUSSION

Elemental analysis of *Praecitrullus fistulosus* was done using ICP-MS and SEM-EDX. Powdered sample was subjected to acid digestion prior to the analysis. The ICP-MS analysis confirmed the presence of different elements like Mn, Fe, Co, Ni, Cu, Zn, As, Rb, Sr, Tl, and U. The results of ICP-MS analysis were given in Table 2.

Table: 2 Elemental analysis of *Praecitrullus fistulosus* using ICP-MS.

Element	Mass	Atomic number	*P. fistulosus*
Manganese (Mn)	55	25	25.678
Iron (Fe)	56	26	318.887
Cobalt (Co)	59	27	0.431
Nickel(Ni)	60	28	0.131
Copper(Cu)	63	29	22.0695
Zinc (Zn)	66	30	88.662
Arsenic (As)	75	33	0.098
Rubidium (Rb)	85	37	2.533
Strontium (Sr)	88	38	57.423
Thallium(Tl)	205	50	0.002
Uranium (U)	238	358	0.0385

Units: concentration expressed in ppm (Parts per million)

Manganese (Mn) derivatives have antipyretic, antibiotic and hypocholesterolemic properties [7]. Iron (Fe) is essential element in formation of blood haemoglobin in human body [8]. Manganese (25.678 ppm), Iron (318.887 ppm) was found in sufficient amount in *Praecitrullus fistulosus*. Zinc is an essential growth supplement and for insulin production [9]. Copper plays active role in metabolic processes, which was found in adequate concentration in the extract of *Praecitrullus fistulosus*, Zn was 88.66 ppm and Cu was 22.06 ppm respectively. Cobalt is an essential trace element and a central component of the vitamin cobalamin, vitamin B_{12} and is required for the synthesis of hemoglobin. Nickel is a trace element in multiple vitamins. Nickel is employed for increasing iron absorption, preventing iron-poor blood (anaemia), and treating weak bones (osteoporosis). Cobalt and Nickel were found to be 0.431 ppm and 0.131 ppm respectively. Strontium is a trace mineral found naturally in body. Although strontium is often overlooked, it's incredibly beneficial to bones and is an important factor in overall bone health (Sr 57.423 ppm). Other elements like As, Rb, Tl, U were also found in various proportions in the extract of *Praecitrullus fistulosus*.

Table 3: Elemental analysis of *Praecitrullus fistulosus* using SEM-EDX

Element	Weight %	Atomic %
O	84.45	92.99
K	15.55	7.01

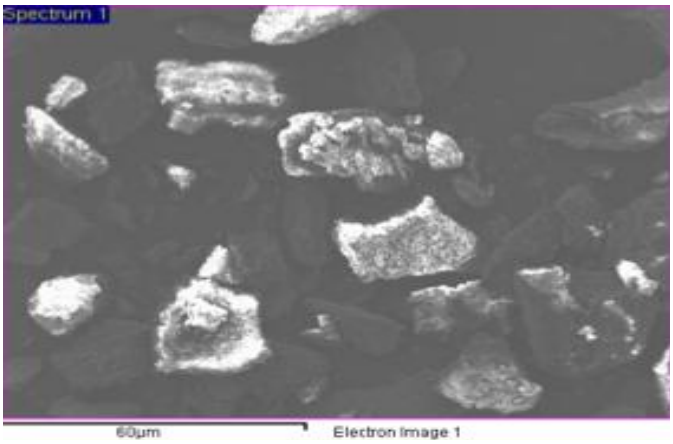

Fig.1: SEM micrographs of *Praecitrullus fistulosus*

SEM-EDX analysis of *Praecitrullus fistulosus* detected two main elements (O, K) (Fig 1, 2). Atomic concentration of these elements (in percentage) Oxygen (O) (92.99), Potassium (K) (7.01) is given in Table 3. The Energy Dispersive X-ray (EDX) microanalysis maybe a technique of elemental analysis associated to electron microscopy supported the generation of characteristic X rays that reveals the presence of elements present in the specimens. Our analysis confirmed the presence of oxygen and potassium. These elements have various functions in physiological processes. Potassium is important body mineral, important to both cellular and electrical function. The diet high in fruits, vegetables, and whole grains is rich in potassium and low in sodium, serves to maintain normal blood pressure and typically lowering elevated blood pressure.

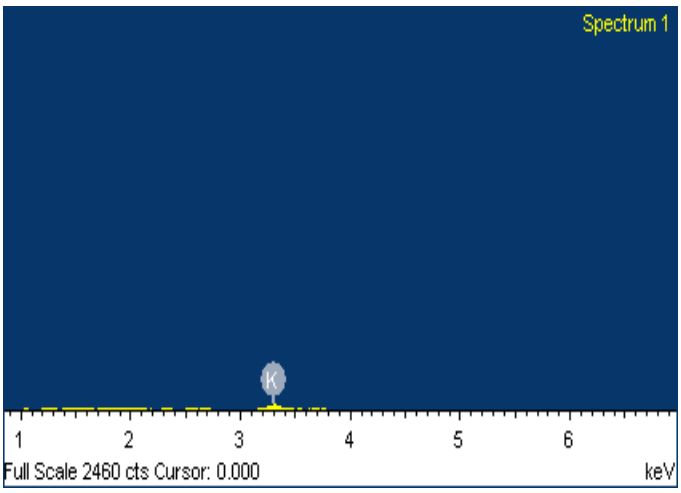

Fig. 2: EDX Analysis of *Praecitrullus fistulosus*

4. CONCLUSION

Results of elemental analysis highlight the importance of *Praecitrullus fistulosus* as rich source of biologically

essential elements that may play most important role in therapeutic activity and curative ability of plant. Essential trace elements play an important role as a cofactor for certain enzymes, involved in metabolism, cell growth, normal cellular functioning, and synthesis of some hormones and connective tissue. The plant contained active trace elements like Copper, Iron, Nickel, Zinc, Manganese etc., whose activity may be responsible for curing certain diseases. ICP-MS and SEM-EDX analysis identified different elements in extract of *Praecitrullus fistulosus* but ICP-MS is more sensitive than SEM-EDX analysis as EDX analysis doesn't detect mineral, which have lower concentration. O and K were only detected, as their concentration was more. Atomic Absorption Spectroscopy Analysis maybe needed for further exploration of minerals in *Praecitrullus fistulosus*.

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