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Mineral Composition and Bioaccessibility in *Asteracantha longifolia (L.) Nees*

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Abstract

Asteracantha longifolia (L.) Nees. (AL) a small, spiny weed found in marshy places. It is reported for its "antitumor, hypoglycemic, aphrodisiac, antibacterial, free radical scavenging and lipid peroxidation, hepatoprotective activities"[3]. Accompanying its uses for a medicinal purpose the plant is also used as a leafy vegetable in some states of India. Hence study of mineral composition of *Asteracantha longifolia* (L.) Nees and its bioaccessibility is crucial along with studying phytoconstituents of this plant. Nutritional food value of the plant can be accessed by in-vitro gastrointestinal simulation in the form of Bioaccessible Fraction (BF). The bioaccessibility of minerals found in the plant is not investigated thoroughly, mainly in functional foods. Therefore, in this research work we have subjected shade-dried powdered material of AL to ICP-AES qualitative and quantitative analysis. The results revels presence of the essential elements like Calcium (Ca) at 814.5 ppm with %BF 96.5, Magnesium (Mg) at 169.2 ppm with %BF 102.8 and micronutrients like Iron (Fe) at 5.3 ppm with %BF of 25.0, Manganese (Mn) at 4.1 ppm with %BF 100.9 and Zinc (Zn) at 0.6 ppm with %BF 31.6. Successively based on the findings it is concluded that AL has good nutritional value.

Keywords: *Asteracantha longifolia (L.) Nees*, Bioaccessibility, ICP-AES, Mineral Composition

1. Introduction

"Traditional medicines are getting important contemplation in global health care considering their vital properties of being natural, low side effects, and significant in therapeutic actions"[1]. With these properties, traditional medicines can go hand in hand with modern synthetic medicines to cure diseases. "The medicinal plants are comprised of different therapeutic agents, various phytoconstituents like phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, and glycosides"[2]. In recent times extensive research work is going on to explore the therapeutic agents, phytoconstituents of medicinal plants, bioavailability and bioaccessibility of phytoconstituents and to study the mode of therapeutic action.

"*Asteracantha longifolia* (L.) Nees (AL) belong to a family Acanthaceae is known as Talamkhana in Unani and Kokilasha in Ayurveda system of medicine. It is a small, spiny weed found in moist places throughout India. The plant is widely distributed in India, Srilanka, Burma and Nepal"[6]. "It has been found that it is used as a leafy

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vegetable in some states of India like Odisha, Chhattisgarh and West Bengal. It has also been observed that boiled aerial parts of the succulent plant of pre-flowering and flowering stages are randomly used as haematinic by the rural people of these states"[4,5]. "The plant seeds, roots and leaves are used for medicinal purposes. Seeds are aphrodisiac, emmenagogue while roots and leaves are diuretic in nature"[6,7]. "The potential use of the aerial parts of AL as a vegetable source for human consumption is considered promising, given their high nutritional value in terms of natural minerals and trace elements, including Mg, Ca, Mn, Fe, Zn and Cr, dietary fiber and bioactive compounds"[7–10].

Due to the unavailability of precise information on mineral composition and its bioaccessibility fraction in aerial parts of AL, we have attempted to study its proximate composition, quantitative assessment of minerals and followed by study of bioaccessibility fraction for the minerals and trace elements.

2. Material and Method

2.1 Plant Collection

Sampling of the plant material of AL from Western Ghats of Maharashtra was conducted during its flowering season of September to November months.

2.2 Plant Material Preparation

The AL plant material after collection was washed thoroughly and dried under shade at room temperature for 2-3 weeks carefully. The dried material was powdered, sieved (through an ASTM 80 mesh) and homogenized powder was then stored in an air-tight container.

2.3 Chemicals and Reagents

Hydrochloric acid, Nitric acid (65%), and Karl Fischer reagents were purchased from Merck. Biorelavent media (FaSSIF/FeSSIF/FaSSGF powder) was purchased from Inveniolife Technology Pvt Ltd. Deionized water was obtained from a Milli-Q Plus system.

2.4 Proximate Analysis

Following parameters were studied for proximate analysis

- Ash values.
- Extractive values.
- Loss on drying.
- Moisture content by Karl Fischer Titration.

2.4.1 Ash Values

2.4.1.1 Total Ash

About 2 gm powdered sample was accurately weighed into a silica crucible. Incinerated for 3 hrs at 600°C, in a muffle furnace till it gets free from carbon. The sample was cooled weighed and the percentage of ash was calculated.

2.4.1.2 Acid-insoluble Ash

To the crucible from total ash, 25 mL of dilute HCl was added and boiled for 5 minutes and filtered through an ash-less filter paper. The collected insoluble matter was washed with hot water and dried carefully in the oven. The residue was then ignited to constant weight. The percentage of Acid insoluble ash was calculated against initial weight.

2.4.1.3 Water-soluble Ash

To the crucible from total ash 25 mL water was added and boiled for 5 minutes and filtered through an ash-less filter paper. The collected insoluble matter was washed with hot water and dried carefully in the oven. The residue was then ignited to constant weight. The weight of this residue was subtracted from the weight of Total ash and the percentage of water-soluble ash was calculated against the initial weight.

2.4.2 Extractive Value

2.4.2.1 Water-soluble Extractive Value

5% aqueous solution was prepared by weighing accurately about 5 gm powdered sample in a conical flask. The solution was allowed to stand for a day by stirring the solution from time to time. The solution was then filtered through the grade-41, Whatman filter paper. The filtrate was evaporated to dryness in a previously weighed empty evaporating dish on a water bath and the residue was discarded. The evaporating dish was weighed to calculate the water-soluble extractive value.

2.4.3 Alcohol-soluble Extractive Value

5% ethanolic solution was prepared by weighing accurately about 5 gm powdered sample in a conical flask. The solution was allowed to stand for a day by stirring the

solution from time to time. The solution was then filtered through the grade-41, Whatman filter paper. The filtrate was evaporated to dryness in a previously weighed empty evaporating dish on a water bath and the residue was discarded. The evaporating dish was weighed to calculate the alcohol-soluble extractive value.

2.4.4 Loss on Drying

Loss of weight was calculated in percentage on about 2 g powdered sample at 100-105°C for 3 hours.

2.4.5 Moisture Content by Karl Fischer Titration

Percent water content was calculated for the 100 mg powdered sample with Karl Fischer titration in specially dried Methanol and pre-neutralized Karl Fischer reagent. Calibration/ titration factor for the Karl Fischer reagent was determined with Disodium Tartrate.

3. Mineral Composition by Inductively Coupled Plasma (ICP)-AES and Bioaccessibility

3.1 Sample Preparation

About 100 mg of powdered sample was digested for about 12 hours with 5 mL of nitric acid into a 25 mL volumetric flask. This digested solution was then diluted up to the mark with deionized water. This solution was filtered through grade-41, Whatman filter paper. The filtrate was subjected to ICP-AES to determine the mineral composition qualitatively and quantitatively.

3.2 In-vitro Gastrointestinal Digestion and Bioaccessibility of Minerals

Bioaccessibility of minerals was evaluated from two chronological stages of in-vitro gastrointestinal digestion system i.e. gastric digestion and intestinal digestion

3.3 Preparation of Gastric and Intestinal Solutions[11]

3.3.1 Buffer Preparation for in vitro Gastric Digestion Solution

In about 0.9 L of purified water dissolve 2 g of Sodium chloride (NaCl). Adjust the pH to 1.6 with Hydrochloric acid 1N makeup to volume (1 L) with purified water at room temperature.

Add 0.06 g of FaSSIF/FeSSIF/FaSSGF powder to about 0.5 L of buffer. Stir until powder is completely dissolved. Makeup to volume (1 L) with buffer at room temperature

3.3.2 In-vitro Gastric Digestion

About 200 mg of the powdered sample was added with 50 mL of the gastric digestion buffer (pH 1.6). The mixture was sonicated for 1–2 min for degassing and then incubated for 2 hours in a thermostatic water bath at 37°C.

3.3.3 Buffer Preparation for in vitro Intestinal Digestion Solution

In about 0.9 L of purified water dissolve 0.42 g of Sodium hydroxide pellets (NaOH), 3.95 g of Monobasic sodium phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), 6.19 g of Sodium chloride (NaCl). Adjust the pH to 6.5 with Sodium hydroxide 1N or Hydrochloric acid 1N. Makeup to volume (1 L) with purified water at room temperature

Add 2.24 g of FaSSIF/FeSSIF/FaSSGF powder to about 0.5 L of buffer. Stir until powder is completely dissolved. Makeup to volume (1 L) with buffer at room temperature.

3.3.4 In-vitro Intestinal Digestion

About 200 mg of the powdered sample was added with 50 mL of the Intestinal digestion buffer (pH 6.5). The mixture was sonicated for 1–2 min for degassing and then incubated for 2 hours in a thermostatic water bath at 37°C.

Centrifuge both the mixtures at 3000 rpm for 30 min at the end of the incubation period. The supernatants were subjected to ICP-AES analysis to determine the elemental composition and bioaccessibility.

3.4 Calculation of Bioaccessibility Fraction (%)

"The bioaccessible fraction is the maximum amount of a nutrient or contaminant that can be absorbed through the intestinal epithelium, reaching the bloodstream (Souza *et al.,* 2018)"[12].

"The percentage (%) of bioaccessibility was defined as the fraction (concentration) of the element released in the in vitro digestion compared to the total amount (concentration) of the element, and the value was

calculated according to the formula (Leufroy *et al.*, 2012)"[10] as given below:

$$\% \text{ Bioaccesibility Fraction} = \frac{\text{Fraction of total element}}{\text{Total element concentration}} \times 100$$

4. Results and Discussion

The proximate analysis of powdered sample includes Ash values, Extractive values, Loss on drying and Moisture content by Karl Fischer titration.

Ash value is the indication of total amount of minerals present in the sample under study. Usually it consists of inorganic components like Sodium, Potassium, Calcium and Chlorides. Determination of the ash is significant for a number of reasons like Nutritional value, Quality, Microbiological stability and processing of the material.

The mineral composition of plant material is indicated by the Ash value which includes Total ash (12.44%), the Water-soluble (11.02%) and the Acid-insoluble (9.03%). The Water-soluble extractive value here was found to be higher than the Alcohol-soluble extractive value. It was found to be 16.5%. It exhibits that the powdered sample has higher water-extractable constituents than the alcohol. Moisture content is also important which plays a vital role in controlling the decay of the powder sample during storage and in its formulations. Lower moisture content is advisable for higher stability. The moisture content for the plant material was 13.07%. Results of proximate analysis are illustrated in Table 1.

Table 1. Proximate analysis results

Sr. No.	Parameter	Result
01.	Total ash	12.44% ± 0.08
02.	Water soluble ash	11.02% ± 0.07
03.	Acid-insoluble ash	9.03% ± 0.06
04.	Water-soluble extractive	16.5% ± 0.21
05.	Alcohol-soluble extractive	3.2% ± 0.32
06.	Loss on drying	10.75% ± 0.12
07.	Moisture content	13.07% ± 0.05

4.1 Mineral Composition and Bioaccesibility

A qualitative and quantitative analysis was performed with ICP-AES for elements of *Asteracantha longifolia* (L.) Nees. Results of the analysis are illustrated in Table 2. The elements Aluminum (Al), Calcium (Ca), Cobalt (Co), Iron (Fe), Magnesium (Mg), Manganese (Mn) and Zinc (Zn) were detected and quantitatively measured for their bioaccessibility as observed in the table given below.

Calibration curve was plotted for response of the element against the predetermined concentrations. The correlation coefficient of 0.99 is indicative of good agreement between response and concentration for each element.

Table 2. Qualitative and Quantitative analysis results

Sr. No.	Element	Conc. found ($\mu g\ g^{-1}$)	% Bioaccesibility
01	Aluminum (Al)	9.4	9.3
02	Calcium (Ca)	814.5	96.5
03	Cobalt (Co)	0.1	83.3
04	Iron (Fe)	5.3	25.0
05	Magnesium (Mg)	169.2	102.8
06	Manganese (Mn),	4.1	100.9
07	Zinc (Zn)	0.6	31.6

Calcium (Ca) is found to have a %BF of 96.5 and Magnesium (Mg) has a %BF of 102.9 "Calcium and Magnesium plays essential role in plants as well as human body. Calcium in plant is important in formation of and stability of cell walls and in maintenance of membrane structure and permeability, activates some enzymes, regulates many responses of cells to stimuli, in human calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin and also takes part in milk clotting"[13].

"Magnesium is a vital component of chlorophylls, activates many enzyme and deficiency of Magnesium lead to poor growth, decreased muscle tone, ataxia, progressive in coordination and convulsions"[13].

The micronutrients or trace elements are present in very small quantities. Iron (Fe) has %BF of 25.0, Manganese (Mn) and Zinc (Zn) are having %BF of 100.9 and 31.6 respectively. "Iron is a component of cytochromes, electron transport, activates some enzymes, plays a role in chlorophyll synthesis in plants and Iron functions as hemoglobin in the transport of oxygen, In cellular respiration, it functions as an essential component of enzymes involved in biological oxidation"[13] in the human body.

"The % bioaccessibility is the absorbed nutrient which plays a vital role in routine physiological functions. Factors affecting bioaccessibility are the elemental oxidation state and its adherence with the food matrix i.e. its interactions with food components"[10].

5. Conclusion

The results of mineral composition, mineral concentration and % bioaccessibility derived from in-vitro gastrointestinal digestion of *Asteracantha longifolia* (L.) Nees reveals that the plant is a rich source of essential elements like Calcium (Ca), Iron (Fe), Magnesium (Mg) and Manganese (Mn) with a considerable amount of bioaccessibility. This indicates that the plant has good nutritional value.

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