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### **Elemental Analysis of Two Varieties of Wild Date Palm Seeds.**

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#### **ABSTRACT**

Wild plant species provide important energy and micronutrient needs during drought and social and political unrest. In spite of the role of edible wild plants in the bridging period of food shortages and providing dietary variety, very little attention has been given to the inventory and conservation of such species. Dietary minerals cannot be produced by the human body and need to be taken on a daily basis from outside in sufficient amounts. Seeds and nuts are recognized as one of the main dietary mineral source for humans. They have a broad dietary mineral spectrum, consisting of both macro-minerals (i.e. Ca, Mg, Na and K) and micro-minerals or trace elements (i.e. Cu, Fe, Zn, Mn and Se) with requirements of no more than few milligrams per day. The present work reports the simultaneous determination of macro-minerals and micro-minerals in the two selected seeds of wild date palm species (*Phoenix sylvestris* and *Phoenix loureiroi*) using inductively coupled plasma mass spectrometry (ICP-MS) and scanning electron microscopy with an energy dispersive X-ray analytical system (SEM-EDX).

**KEYWORDS:** *Phoenix sylvestris*, *Phoenix loureiroi*, ICP-MS, SEM-EDX.

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## **INTRODUCTION:**

The importance of wild plants in subsistence agriculture in the developing world as a food supplement and as a means of survival during times of drought, famine has been overlooked. Local people know about the importance and contribution of wild plants in their daily diet. Wild plant species continue to provide important energy and micronutrient needs during drought and social and political unrest. In spite of the role of edible wild plants in the bridging period of food shortages and providing dietary variety, very little attention has been given to the inventory and conservation of such species. Compositional knowledge of these plant materials could help in developing technological processes to make the plant material edible and more digestible<sup>1</sup>. Recently, there is very much demand on fruits and seeds which have well-known storehouse of polyphenols and nutrients which possess antioxidant activities, antimicrobial activities and anticancer activities<sup>2</sup>. Dietary minerals cannot be produced by the human body and need to be taken on a daily basis from outside in sufficient amounts. Seeds and nuts are recognized as one of the main dietary mineral source for humans. They have a broad dietary mineral spectrum, consisting of both macro-minerals (i.e. Ca, Mg, Na and K) and micro-minerals or trace elements (i.e. Cu, Fe, Zn, Mn and Se) with requirements of no more than few milligrams per day. Each individual mineral has a specific role in metabolism. The type and amount of essential elements are very important factors for human health. For example, iron deficiency, low selenium amount and disruption of sodium/potassium balance in the body may cause many physiological problems. When the amounts of the essential elements are excessive as compared to the recommended daily intake (RDI) values, they may become harmful or may have a toxic effect. Excessive ingestion of these elements may cause adverse physiological effects<sup>3</sup>. Therefore, the analyses and nutritional values of minerals in seeds and nuts are important for human beings to determine whether the seeds and nuts have the recommended or toxic mineral contents. Recent studies have shown that some of the seeds could find application as animal feeds, based on their nutritional values, and raw materials for paint formulation based on the amounts and nature of the oils contained in them<sup>4</sup>. Based on the importance of seeds and nuts as a mineral source, it is necessary to determine their mineral composition and nutritional values. However, the mineral nutritional values of seeds have not been examined in some studies.

To determine the trace element composition in plants, many 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. ICP-MS is effective and widely used due to its

excellent precision, accuracy and wide dynamic range in determining multiple elements at trace levels. SEM combined with EDX technique is highly qualified for identification and quantification of different elements in plants.

The present work reports the simultaneous determination of macro-minerals and micro-minerals in the two selected seeds of wild date palm species (*Phoenix sylvestris* and *Phoenix loureiroi*) using inductively coupled plasma mass spectrometry (ICP-MS) and scanning electron microscopy with an energy dispersive X-ray analytical system (SEM-EDX).

## MATERIALS AND METHODS:

### **Reagents:**

Concentrated Nitric acid ( $\text{HNO}_3$  Merck, Mumbai), Hydrogen peroxide (30%  $\text{H}_2\text{O}_2$ ), Concentrated Hydrochloric acid (HCl) (Merck, Mumbai) and double deionized water (Milli-Q) were used for all dilutions.

### **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).

<b>Table: 1</b>
<b>Optimal instrumentation conditions for ICP-MS analysis</b>
<b>Required Parameters with values</b>
<u>Plasma conditions</u>
Rf frequency: 27 MHz
Rf power: 1kW
<u>Gas flow rates</u>
Carrier gas: Ar 0.8 L/min
Auxiliary gas: Ar 1.1 L/min
Coolant gas: Ar 15 L/min
<u>Sampling conditions</u>
Sampling depth: 7mm from work coil
Sampling cone: Nickel 1.0 mm orifice diameter
Skimmer cone: Nickel, 0.4mm orifice diameter
Nebulizer: Cross flow type sampling uptake rate: 0.4 ml/min
<u>Data acquisition</u>
Data point: Multi-element hope by peak hopping dwell time: 3points/peak
Integration: 50ms/point
Reputation: 1000 times

### Sample preparation:

#### Collection of Plant Material:

Mature fruits of *P.sylvestris* and *P.loureiroi* were collected from Ramannapalem, West Godavari District, Andhra Pradesh. Seeds were separated, air dried, powdered and stored for further use.

#### ICP-MS:

0.05 g (dry mass) of seed samples was weighed into the digestion vessels. Then 4.0 ml of concentrated HNO<sub>3</sub> and 1.0 ml of 30% H<sub>2</sub>O<sub>2</sub> were added to each sample. Samples were pre-digested overnight at 70°C. Repeat the step again add 4ml of concentrated HNO<sub>3</sub> and 1.0 ml of 30% H<sub>2</sub>O<sub>2</sub> and incubate at 70°C for 1 hour. After cooling, the entire digest were transferred into plastic bottles and diluted to 25 ml with double deionized water. Reagent blanks were prepared similarly without seed samples. All sample solution was clear.

#### SEM-EDX analysis

The powdered seed sample was mixed properly and carefully placed on a glass cover slip followed by air-drying. The cover slip itself was used during 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 regarding applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

The presence of elements in the seed 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 used to detect the X-rays together with processing electronics to analyses the spectrum. The EDX observations were carried out in INCA energy 250, Oxford, Japan (JOEL Model JED-2300).

### RESULTS AND DISCUSSION:

Elemental analysis of wild date palm seeds was done using ICP-MS and SEM-EDX. Seed powder of wild date palm was subjected to acid digestion prior to the analysis.

The ICP-MS analysis identified different amount of elements in wild date palm seeds. Two varieties of date palm seeds showed the presence of Li, Be, Mn, Fe, Cu, Ga, As, Rb, Sr, Cs, Ba, U (Table 2). It was observed that *Phoenix sylvestris* have higher amount of manganese (49.815 ppb) while in *Phoenix loureiroi* (11.09 ppb). The amount of Iron present in *Phoenix loureiroi* (37.491

ppb) however iron was not detected in *Phoenix sylvestris* seeds. Copper was observed in both varieties of wild date palm seeds 6.696 and 5.652 ppb respectively. The levels of rubidium detected in both species were 23.843 and 5.991 ppb. Strontium was found higher in *Phoenix sylvestris* (5.488 ppb) than *Phoenix loureiroi* (0.699 ppb). Arsenic was detected in both varieties 0.021 and 0.277 ppb. Other elements like Ga, Ba, and U were also found in various proportions in *Phoenix sylvestris*, these elements were not detected in *Phoenix loureiroi*. Li, Be, Cs were detected in *Phoenix loureiroi* and absent in *Phoenix sylvestris*.

**Table : 2 Elemental analysis of two varieties of wild date palm seeds using ICP-MS.**

Name of the element	Mass	Atomic number	<i>Phoenix sylvestris</i>	<i>Phoenix loureiroi</i>
Lithium (Li)	7	3	ND	0.006
Beryllium (Be)	9	4	ND	0.022
Manganese (Mn)	55	25	49.815	11.09
Iron (Fe)	56	26	ND	37.491
Copper (Cu)	63	29	6.696	5.652
Gallium (Ga)	69	31	1.498	ND
Arsenic (As)	75	33	0.021	0.277
Rubidium (Rb)	85	37	23.842	5.991
Strontium (Sr)	88	38	5.488	0.699
Cesium (Cs)	133	55	ND	0.001
Barium (Ba)	137	56	2.991	ND
Uranium (U)	238	358	0.068	ND

ND: Not Detected, Units: concentration expressed in ppb (Parts per billion)

Our study suggests that two varieties of wild date palm seeds have good number of micronutrients. Manganese is present in high amount in *Phoenix sylvestris*. Manganese is a mineral found in plant and animal tissue. It plays an important role in physiological processes. It helps to form connective tissue, bones, blood clotting factors and activator of enzymes. Iron level in *Phoenix loureiroi* was found to be higher than other elements. Iron is an essential element for blood production and Heme synthesis. Iron is necessary for proliferation and maturation of immune cells.

SEM-EDX analysis of two wild date palm seeds detected three main elements (O, K, Cl) (Fig 1, 2 and 3). Atomic concentration of these elements (in percentage) Oxygen (O) (97.92), Potassium (K) (2.08) in *Phoenix Sylvestris* while *Phoenix loureiroi* Oxygen (O) (94.62), Potassium (K) (8.64) atomic percentage respectively (Table 3). Chlorine (Cl) (3.28) was detected only in *Phoenix loureiroi*. The Energy Dispersive X-ray (EDX) microanalysis is a technique of elemental analysis associated to electron microscopy based on the generation of characteristic X rays that reveals the

presence of elements present in the specimens<sup>5</sup>. Our analysis conformed the presence of oxygen and potassium in both seeds. These elements have various functions in physiological processes.

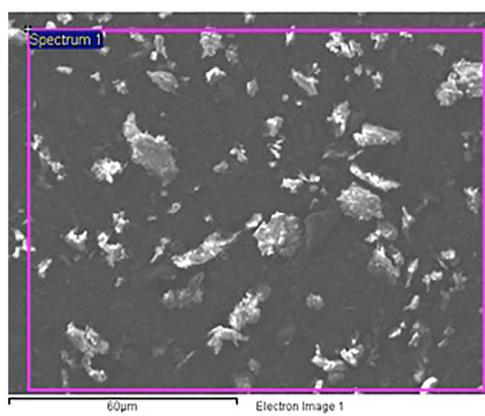
**Table 3: Elemental analysis of two varieties of wild date palm seeds using SEM-EDX.**

	<i>Phoenix Sylvestris</i>		<i>Phoenix loureiroi</i>	
Element	Weight %	Atomic %	Weight %	Atomic %
O	95.07	97.92	88.09	94.62
K	4.93	2.08	8.64	3.80
Cl	ND	ND	3.28	1.59

ND: Not Detected.

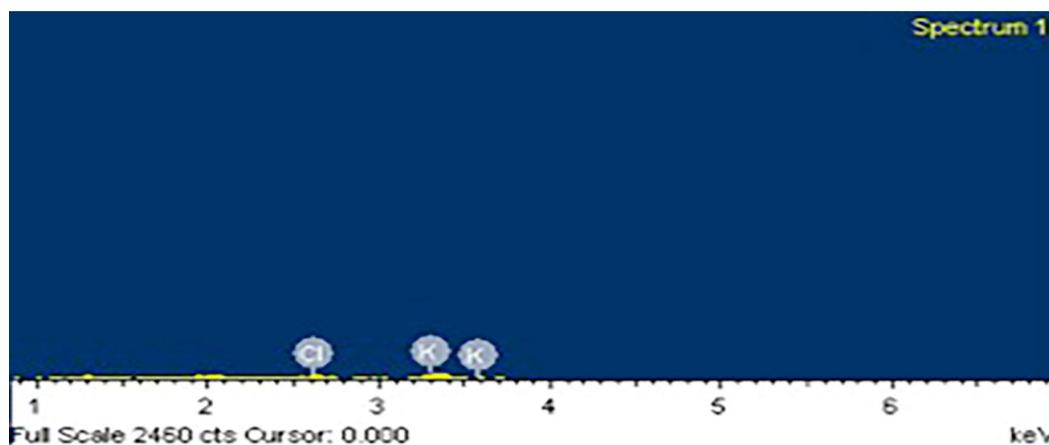


*Phoenix sylvestris*



*Phoenix loureiroi*

**Figure: 1 SEM micrographs of two wild date palm seed powder.**



**Figure: 2. EDX Analysis of *Phoenix loureiroi*.**

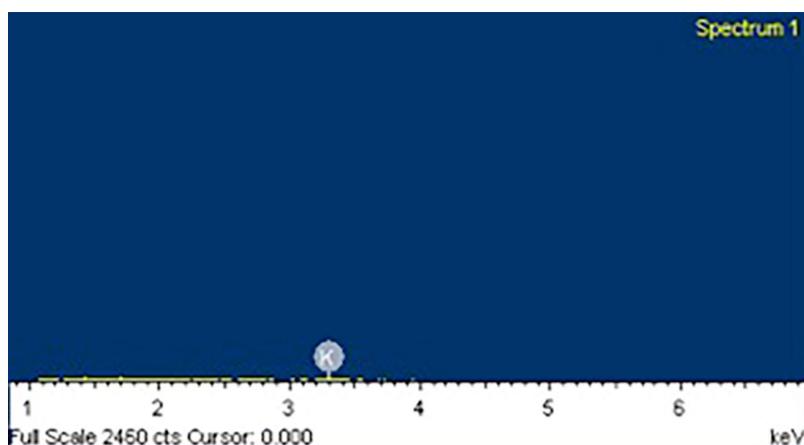


Figure: 3 EDX Analysis of *Phoenix sylvestris*.

## CONCLUSION:

The results of present study highlight the importance of wild date palm seeds as rich source of essential elements. Two varieties of wild date palm seeds have adequate amount of minerals. These seeds have nutritional benefits to the mankind. ICP-MS and SEM-EDX analysis identified different elements in wild date palm seeds but ICP-MS is more accurate than SEM-EDX analysis as EDX analysis doesn't detect mineral, which have lower concentration. In palm seeds O, K and Cl were only detected, as their concentration was more. Atomic Absorption Spectroscopy Analysis maybe needed for further exploration of minerals in these seeds.

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