

## Comparison between XRF, PIXE and ICP-OES Techniques Applied For Analysis of Some Medicinal Plants

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**Abstract:** In this study trace element concentrations (Ca, Cu, Cr, K, Fe, Mn, Ni, Sr, and Zn) in two Sudanese wild plants namely, *Ziziphusspina Christi* and *Ziziphus Abyssinica* were determined using X-ray fluorescence (XRF), PIXE and ICP-OES techniques. The results showed that, XRF, PIXE and ICP-OES are equally competitive methods for measuring Ca, K, Fe, Sr and Zn elements. Unlikely to ICP-OES, PIXE and XRF techniques tend to be appropriate methods for Cu determination in plant samples however, for Mn element PIXE and ICP-OES are advisable techniques for measuring this element rather than XRF method. On the other hand, ICP-OES seems to be the superior technique over PIXE and XRF methods for Cr and Ni determination in plant samples. It was found that, there was no difference between the two plants for Ca, K, Cu, Fe, and Sr element content. Moreover, significant variations were observed for Mn and Zn concentrations implying the possible use of these two elements as additional discriminate criteria for plant taxonomy purposes.

**Key words:** Trace element, Competitive methods, *Ziziphusspina*, XRF, PIXE, ICP.

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### I. Introduction

Plants have highly important roles in human lives. Many ethnic groups rely on wild-collected plants for food and many other purposes from birth to death. Traditional knowledge of plant uses has accumulated and has been passed on from ancestors to new generations again and again by spoken word and by life style (Inta et al., 2013). According to the World Health Organization (WHO), the use of traditional herbal medicine has spread not only in the developing countries but also in the industrialized ones, as a complementary way to treat and to prevent diseases. The pharmacological properties of the medicinal plants have been attributed to the presence of active constituents which are responsible for important physiological function in living organisms (Özcan, 2004; Yamashita et al., 2005). (WHO) estimates that 80% of the developing world's people rely on herbs for their primary health care needs (Mosihuzzaman and Choudhary, 2008). Practitioners of traditional African medicine claim to be able to cure a wide range of diseases including cancer, Acquired Immunodeficiency Syndrome (AIDS), psychiatric disorders, high blood pressure, cholera, infertility, and most venereal diseases (Khalid et al., 2012). Medicinal plants contain both the organic and inorganic constituents. Abundant research work has been carried out on the organic constituents of the medicinal plants while little attention has been paid on the role of inorganic elements in the medicinal use of these plants. Most of these plants are found to be rich in one or more individual elements, thereby providing a possible link to the therapeutic action of the medicine (Nafiu et al., 2011). Trace elements play an important role in the formation of the active chemical constituents present in medicinal plants and are therefore responsible for their medicinal as well as toxic properties (Kumar et al., 2005; Singh and Garg, 1997).

Sudanese medicinal plants in dried form have been exported to different African, Asian, European, North and South American countries since 1952. Variation in marketed species and quantity of plant material exported is subjected to international demands. (Khalid et al., 2012). *Ziziphus* species have wide distribution and uses worldwide traditionally as medicine (Niamat et al., 2012). The genus *Ziziphus* belongs to the buckthorn family Rhamnaceae that consists of small trees. It is a genus of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical of the world regions including India (Lawton, 1985; Nyaberi et al., 2010).

Three commonly used methods for characterizing trace elements in medicinal plants are based on X-ray fluorescence (XRF), Particle Induced X-ray Emission (PIXE) and Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES). PIXE allows analysis of minute samples less than one milligram without complex chemical preparation. This technique allows for rapid, simultaneous detection of elements between Na and U with a large dynamic range (mg/kg to  $\mu\text{g/kg}$ ). Consequently, it is used in a variety of fields, including environmental research, medicine, geology and archaeology (Johansson and Campbell, 1988; Lucarelli et al., 2011). On the other hand, (ICP-OES) is routinely used by many research and analysis institutions. In the field of environmental research, ICP-MS and ICP-AES have become synonymous with multi-element analysis even at very low concentrations (Saitoh et al., 2002). ICP-OES has lower detection and quantification limits (range LoQ 50–1200  $\mu\text{g/kg}$ , after digestion of e.g.

100mg sample) for most metals in comparison to PIXE but it requires 100–300mg sample for the digestion of the sample with strong acids prior to analysis. This digestion step increases the limits of quantification as typically the exactly weighed sample mass (100–300mg) gets digested in a final digestion volume of 10–30 mL. Further, there is a risk for losses of volatile elements or insolubility of some metal oxides or silicates. On the other hand, the sensitivity of PIXE is affected by matrix effects which enhance or decrease the limit of detection of an element; consequently it can affect its measured concentration. The advantages of PIXE are that it is a non-destructive technique and that little to no chemical preparation of samples is needed, which permits for rapid analysis (Saitoh et al., 2002). Additionally the third technique X-ray Fluorescence has some advantages like simple spectra, spectral positions are almost independent of the chemical state of the analyte, minimal sample preparation; it is non-destructive, applicable over a wide range of concentrations, good precision and accuracy, can be used to measure solid, powdered and liquid samples. And has some disadvantages such as X-ray penetration of the sample is limited to the top 0.01 - 0.1 mm layer, light elements (below Na) have very limited sensitivity although C is possible on new instruments, inter element (MATRIX) effects may be substantial and require computer correction, limits of detection are only modest, and instrumentation is fairly expensive (Salamó Clapera, 2006). This work was conducted to determine trace elements concentration in some Sudanese wild edible medicinal plants namely *Ziziphus spina Christi* and *Ziziphus Abyssinica*. These mentioned plants were determined in parallel by XRF, PIXE and ICP-OES in order to find complementarities between the three techniques for the determination of a range of trace elements.

## II. Experimental

### 2.1 Sample Collection

Four samples of some Wild edible medicinal plants were collected from Khartoum and Kordofan states. Botanical identification and authentication of the collected species with deposition of herbarium specimens has been done by the Medicinal and Aromatic Plants Research Institute (MAPRI) - National Centre for Research-Sudan. Common, scientific names and collection areas of studied plants are given in (Table 3.1).

Table 2.1.: A brief review about the studied plants

Botanical Name	Local Name	Family	Collection Area	Part used
			Khartoum, North	Fruits
<i>Ziziphus spinachristi</i>	(Ar) Sedir, Nabag	Rhamnaceae	Kordofan and Nuba Mountains	
<i>Ziziphus Abyssinica</i>	(Ar) Sedir Nabag Alfil	Rhamnaceae	Nuba Mountains	Fruits

### 2.2 Chemicals

All chemicals used in this research were of analytical grade type.

### 2.3 Method

#### 2.3.1 Sample drying and homogenizing

Samples were dried at room temperature before transportation to the laboratory, then in the oven at 110 centigrade to vaporize the water in them for one hour, Aliquots of the plant material were grind and homogenized using a mortar and sieving. The resulting fine powder was used for the further analysis.

#### 2.3.2. Sample preparation for XRF analysis

1.0 g of the powdered sample was pressed by (15 ton/cm<sup>2</sup>). For quality control, four Standard Reference Materials are used, and had been treated in the same manner. One of them is IAEA-V-10 (Hay powder) and other three are ISE 2012-1.

#### 2.3.3. Sample preparation for ICP-OES measurement

For ICP-OES technique, the dried and homogenized samples had been properly weighed into quartz vessels. Subsequently, 1 mL HNO<sub>3</sub>, suprapure, sub-boiling distilled was added. The vessels were closed and introduced into a micro-wave digestion system. The resulting clear solutions were ready for element determination.

#### 2.3.4. Sample preparation for PIXE

The dried and homogenized plant samples were properly weighed. 1.5 g per sample was pressed (5 ton/cm<sup>2</sup>) on a substrate of 2 g of boric acid, which acts as an external binder agent to form a pellet of 25 mm diameter. A very thin ultra pure carbon layer was evaporated on the surface of the sample in order to ensure good surface conductivity as required for vacuum ion beam analysis (IBA).

## 2.4. Sample analysis

### 2.4.1. XRF measurement

In this study an energy dispersive XRF spectroscopy equipped with Si (Li) detector and radioisotope <sup>109</sup>Cd with energy 22.1 KeV as a primary source is used for measurement of elements (Ca, Cr, Cu, Fe, K, Mn, Ni, Sr, and Zn) in some Sudanese wild edible plants and standard reference materials. X-Ray Fluorescence has long been recognized as a powerful technique for the qualitative and quantitative elemental analysis. It has the advantage of being non-destructive, multi-elemental, fast and cost-effective. Furthermore, it offers a fairly uniform detection limit across a large portion of the Periodic Table and is applicable to a wide range of concentrations. As illustrated in the method involves the excitation of the atoms by a primary X-ray irradiation – originating either from a radio isotopic source, the main of XRF spectrometer (Abubaker Sulyman, 2015).

### 3.3.2. ICP-OES measurement

An ICP-OES ‘SpectroCiros Vision’ system (SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, Germany) was used for Cr, Zn, Cu, Mg and Mn determination in digested samples. Sample introduction was carried out using a peristaltic pump equipped with an “anti-pulse-head” (SPETEC, Erding, Germany), connected to a Meinhard nebulizer with a cyclone spray chamber. The measured spectral element lines were Cr 267.716 nm, Zn 213.956 nm, Cu 324.754 nm, Mg 279.079 nm and Mn 257.611 nm. The RF power was set to 1400 W; the plasma gas was 13 L Ar /min, whereas the nebulizer gas was 0.6 L Ar/min.

### 3.3.3. PIXE measurement

Proton beam was delivered by the 1.7 MV tandem accelerators, model 5SDH from NEC, of the Lebanese Atomic Energy Commission. The beam fluence was 1 μC. The spot diameter of the beam on the target was about 3 mm, defined by a Ta anti-scatter collimator. The X-ray emission from target was detected using Si (Li) detector with 30 mm<sup>2</sup> active area, 12.7 μm thick Be windows and 170 eV measured energy resolution at 5.9 keV, situated at 135° referring to the beam direction. A Kapton filter, used as X-ray absorber, with thickness value 131 μm, was inserted between the sample and the detector.

For RBS measurements, a silicon PIPS detector from Canberra with 14 keV of energy resolution and 25mm<sup>2</sup> of active area, detected the backscattered particles of 3 MeV proton beam, at a scattering angle θ of 165°. A detailed description of the experimental set-up has been reported elsewhere (Roumié et al., 2004).

PIXE spectra were processed with the Gupix package (Guelph PIXE software package) (Strivay et al., 2008). This program is based on the fundamental parameter approach including X-ray production cross-sections, X-ray attenuation coefficients, proton stopping powers, detector efficiency, and collected charge and geometry effects to produce an output of elemental concentrations in ppm. RBS spectra were processed with the SIMNRA simulation code (Mayer and Guide, 1997) to drive out the matrix composition.

## III. Results

Table (3.1) elements concentration in some Standard Reference Materials (SRM) measured by XRF

Element	SRM(1) Measured	SRM(1) Certified	Ratio	SRM(2) Measured	SRM(2) Certified	Ratio	SRM(3) Measured	SRM(3) Certified	Ratio	Overall Ratio
Ca	23300	23150	1.01	7760	8615	0.90	18350	16530	1.11	1.01
K	26850	26200	1.02	39100	41700	0.94	5095	4495	1.13	1.02
Cr	6.45	1.038	6.21	234.1	39.65	5.90	15.75	2.539	6.20	6.20
Cu	4.3	4.257	1.01	8.79	10.7	0.82	7.98	8.66	0.92	0.92
Fe	225	231.6	0.97	180.5	191.5	0.94	479	466.9	1.03	0.97
Mn	45.75	40.2	1.14	1270	1510	0.84	628.5	629.1	1.00	1.00
Ni	2.595	1.088	2.39	13.225	6.49	2.04	3.91	1.79	2.18	2.18
Sr	56.3	57.39	0.98	21	26	0.81	39.7	44.82	0.89	0.89
Zn	19	19.66	0.97	22.2	26.75	0.83	91.2	88.56	1.03	0.97

Table 3.2: Analysis of Hay powder (IAEA-V-10) using XRF

Elements	SRM (HAY)Measured	SRM (HAY)Certified
Ca	21600	21600
K	22000	21000
Cr	44.4	6.5
Cu	8.69	9.4
Fe	177	186
Mn	41.5	47
Ni	8.81	4.2

Sr	37.6	40
Zn	20.1	24

Figure(1).Ca and K

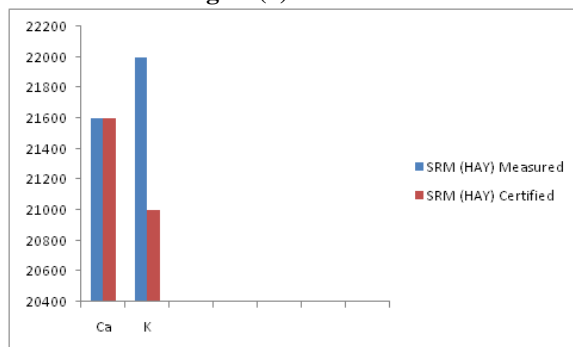


Figure (2).Other elements

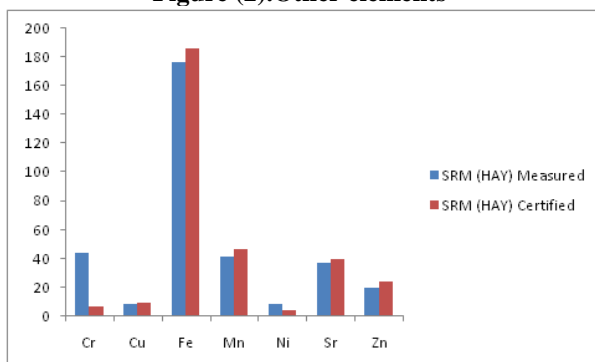


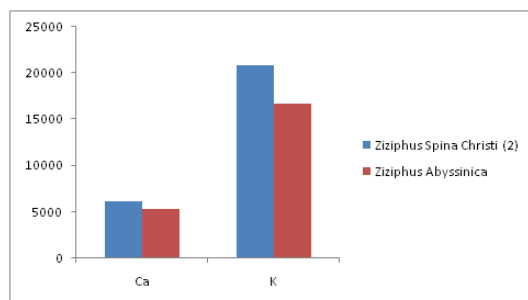
Table (3.3) Elements concentrations mg/kg in ZiziphusSpina Christi (1) measured by XRF, ICP-OES and PIXE

Element	XRF	ICP-OES	PIXE	XRF/ICP-OES	XRF/PIXE	PIXE/ICP-OES
Ca	4392.5	3740.0	4372.0	<b>1.2</b>	<b>1.0</b>	<b>1.2</b>
K	21325.0	18500.0	17818.0	<b>1.2</b>	<b>1.2</b>	<b>1.0</b>
Cr	32.3	0.5	ND	<b>64.6</b>	-----	-----
Cu	9.9	3.0	9.5	<b>3.3</b>	<b>1.0</b>	<b>3.2</b>
Fe	28.2	20.0	22.0	<b>1.4</b>	<b>1.3</b>	<b>1.1</b>
Mn	24.7	13.0	10.0	<b>1.9</b>	<b>2.5</b>	<b>0.8</b>
Ni	4.6	0.5	ND	<b>9.1</b>	-----	-----
Sr	23.4	22.3	25.5	<b>1.1</b>	<b>0.9</b>	<b>1.1</b>
Zn	5.6	6.0	4.9	<b>0.9</b>	<b>1.1</b>	<b>0.8</b>

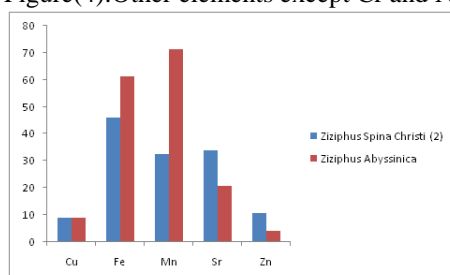
Table (3.4): Elements concentration in some studied Sudanese medicinal plants under study in mg/kg.

Element	ZiziphusSpina Christi (2)	ZiziphusAbyssinica
	Ca	6190 ± 325
Cu	8.9 ± 0.7	8.9 ± 0.3
Fe	46 ± 3	61.2 ± 1.6
K	20900 ± 1556	16750 ± 3465
Mn	32.7 ± 3	71.5 ± 2.8
Sr	34 ± 0.2	20.8 ± 3.1
Zn	10.6 ± 4.8	4.3 ± 0.6

Figure (3).Ca and K



Figure(4).Other elements except Cr and Ni



The quality of obtained results by XRF were realized using different standard reference materials of plant (plant 2012-1), provided by Wageningen Evaluating Programs for Analytical Laboratories(WEPAL) as well as Hay powder (IAEA-V-10), The results were presented in Table (3.1) and figures (1), (2) in term of mean concentrations in mg/kg. Ratios between the certified and the measured values were calculated for each element and the results included in the same table.

From table (3.1) and figures (1), (2), results for Ca, K, Cu, Fe, Mn, Sr and Zn showed a trend of consistency to their corresponding certified values. This obviously can be seen in the values of ratios reported for each studied element; most of the values were approximately around one, indicating strong agreement between XRF measurements and the actual concentrations. It is worth to mention that, most of these elements were found in a considerable amount; however, in spite of low concentrations for Cu, Sr and Zn elements, excellent ratios were reported.

Concerning elements that found in low concentrations (e.g. Cr and Ni), a discrepancy for their measured values in relative to their certified ones were recognized. Cr concentration was found to be six orders higher than their corresponding certified values. This enhancement might be attributed to the spectral interferences that expected to take place between K- $\beta$  of Cr (5946 eV) and K- $\alpha$  of Mn (5898.8 eV). Chromium overestimation should be taken into our consideration when a sample with expected high amount of Mn, such as plants, is analyzed. Specifically, for medical practitioners who interesting in the anti- diabetic activity of plants, they should look keenly into the actual concentration of this element, since Cr is known to be involved in the secretion of insulin (Lokhande et al., 2014).

The calculated ratios presented in table (3.1) could be valuable for correcting of the concentrations of some elements that expected to be significantly deviated from their actual values. Hay powders standard reference material was analyzed by the same XRF technique to examine if the estimated ratios are useful for such corrections or not. See results in table (3.2). As an example, the measured concentration of Cr in hay powder was found to be 44.4mg/kg. This concentration was divided by the calculated ratio for Cr which is equal to 6 then compared to the certificated value. The obtained results were 7.4 mg/kg instead of 44.4 which is very close to 6.5mg/kg. Another example for Ni, the calculated concentration shifted to 4.4 mg/kg instead of 8.81 mg/kg, which are in agreement with its certified values 4.2 mg/kg. From these results one can conclude that, ratios estimated in this work could be serve as additional parameters to correct the obtained results for Cr and Ni in plant samples using XRF techniques; however, further studies using a series of plant standard reference materials is needed to establish correction factors for each element.

Another important aspect of this work is to compare the results obtained from some elemental analysis techniques that employed intensively in this field (XRF, ICP-OES and PIXE). This comparison may provide information either on the total content of metal examined by XRF and PIXE or its content after extraction (i.e. which technique is preferable at different matrices and concentrations).

ZiziphusSpina Christi is a wild edible plant used in Sudanese traditional medicine for treatment of liver and chest Diseases(Orwa et al.). This plant was selected for analysis using the above mentioned techniques. Nine elements (namely Ca, K, Cr, Cu, Fe, Mn, Ni, Sr and Zn) were screened in this plant and the results were showed in table (3.3) in term of mean mg/kg. Calcium, potassium, iron, strontium and zinc tend to have same

values from all employed techniques, which may indicate the possibility of using each of these techniques to determine such elements in plant samples. For these elements, XRF has an advantage over ICP-OES and PIXE, since it is straightforward technique, while ICP-OES requires labour sample preparations and PIXE needs installation of an accelerator.

Copper concentration in *Ziziphus Spina Christi* determined by both XRF and PIXE techniques found to be in the same level; however, its concentration reported by these two techniques was lower by three times than its corresponding values measured by the ICP-OES method this might imply that, XRF and PIXE techniques are suitable for determining Cu concentration compared with ICP-OES.

Mn concentration in the same plant obtained by XRF technique was found to be two times higher than those concentrations obtained with ICP-OES and PIXE techniques. This difference might indicate that, it is preferable to determine Mn in plant samples with ICP-OES and PIXE rather than XRF.

There is overestimation in the concentration of Cr and Ni obtained by XRF technique, which might be resulted from spectral interference as mentioned before. On the other hand, these two elements were not detected with PIXE technique which could be due to the low concentration (below the detection limits). According to the result for Cr and Ni, ICP-OES seems to be a superior technique for measuring these elements in plant samples. Based on the above results, XRF seems to be reliable for measuring elements such as Ca, Cu, K, Fe, Sr and Zn in plant samples. This may support involving this technique in further examinations regarding plant research.

In general, variation in the elemental concentrations of the analyzed plants 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 the elemental content are preferential absorbability of the plant, use of the fertilizers, irrigation water and climatologic conditions (Narendhirakannan et al., 2005). Also, it has been found that in many cases the origin of the plant samples from different plant species or from different geographical locations has impact on their chemical composition (Arceusz et al., 2010). In order to study the influence of geographical location and genotype on the alteration of trace element contents in plant, it was decided to study plants with different genus groups but grown in the same locations (*Ziziphus Spina Christi* (2), *Ziziphus Abyssinica*).

These two plants were collected from the same place at Nubian mountains. Same elements (mentioned before) were quantified and their concentrations were listed in table 3.4. and figures (3,4), the results showed that, there is no difference between the two plants for Ca, K, Cu, Fe, and Sr. Significant variations were observed for Mn and Zn elements in the two different plant implying the effect of genus specie on the absorption or/and accumulations of these two elements in plants. These elements could be used as additional criteria for plant taxonomy purposes since there are different views in plant classification for certain classes that have more than one distinct taxonomy feature. However further clarification is recommend with more number of plants.

#### **IV. Conclusion**

- XRF, ICP-OES and PIXE are competitive equal for measuring Ca, K, Fe, Sr and Zn elements.
- The elevation of the measured concentration of Cr in plant samples could be due to spectral interference with Mn element.
- Unlikely to ICP-OES, XRF and PIXE techniques are suitable for determining Cu concentration in plant samples.
- ICP-OES seems to be the preferable technique over XRF and PIXE for measuring low concentrated elements such as Cr and Ni.
- It is recommended to determine Mn element in plant with ICP-OES and PIXE rather than XRF.
- The geographical location has no significant impact on the alteration of trace elements content in these plants.
- Mn and Zn elements could be saved as additional discriminate factor for plant taxonomy.

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