Determination on the Concentration of Elements in Kassod Tree (Me` Zale) Using EDXRF and AAS Techniques

Aye Aye Mon

Abstract

Flowers and Leaves of Me` Zale (*Cassia siamea* Lam. Syn) are often used for preparation of the indigenous medicines. X–ray fluorescence spectrometer Shimadzu (EDX-700) and Atomic Absorption Spectrophotometer (AAS) are used to determine the elements contained in it. According to the results, toxic elements such as mercury (Hg), lead (Pb), arsenic (As) and so on, were not found in it. The elements, potassium (K), calcium (Ca), sodium (Na) and iron (Fe) were also present in both samples.

Keywords: Me` Zale (*Cassia siamea* Lam), indigenous medicines, EDXRF, AAS, toxic elements

INTRODUCTION

Since ancient times, plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestocks. Considerable indigenous knowledge, from the earliest times, is linked to the use of traditional medicine in different countries. *Cassia siamea* is a shrub belonging to the Fabaceae family, native of Southeast Asia and better known in folklore medicine, feeding, agriculture and manufacture all over the world. *C. siamea* has recently been shown to have antimicrobial, antimala rial, antidiabetic, anticancer, hypotensive, diuretic, antioxidant, laxative, anti-inflammatory, analgesic, antipyretic, anxiolytic, antidepressant, and sedative activities. Chromone (anhydrobarakol), Chromone alkaloids (barakol, cassiarin A-B), anthraquinones (chrysophanol, emodin), bianthraquinones (cassiamin A-B), flavonoids and phenolics compounds are the main constituents of this plant which are reported. Barakol was identified as the major constituent of *C. siamea* of leaves and flowers of the world. Due to the easy collection of the plant, it's widespread and also remarkable biological activities, this plant has become a worldwide medicine.

Heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury, cadmium, arsenic, chromium, tin and lead. Heavy metals are dangerous because they tend to bioaccumulate in a biological organism over time, compared to the chemical's concentration in the environment. Compounds which accumulate in living organisms can be taken up any time and stored faster than they are broken down (metabolized) or excreted.

Exposure to various metal containing components of plants varied widely and may have varying health implications. Furthermore, the consumption of heavy metal contaminated food and medicinal plants can seriously deplete some essential nutrients in the body causing a decrease in immunological defenses, intrauterine growth etardation, impaired psycho-social behavior, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer. In this study, elemental contents present in flowers and leaves of Me` Zale (*Cassia siamea* Lam. Syn) were analyzed by the Energy Dispersive X-ray Fluorescence (EDXRF) method and Atomic Absorption Spectroscopy (AAS) method.

Associate Professor, Dr., Department of Physics, Hinthada University

MATERIALS AND METHODS

Sample Preparation

Both Flower and leave samples of Me` Zale (*Cassia siamea* Lam. Syn) were collected from Yangon Region. Samples were cleaned with water and then dried under the room temperature. These dried samples were crushed and ground in order to get fine powder by using grinding machine. They were ground until fine enough to meet the conditions for homogenous dense materials, and to ensure reproducibility in measurements. For EDXRF measurement, these powders were poured into a die made of steel and pressed into pallet in 3-ton weight of hydraulic press. The diameter of each pallet is 2.5 cm. For AAS measurement, the samples were weighed and then pre-ashing was carried out on a hot plate until all the combustible materials were burnt. Pre-ash samples were placed inside the electric muffle furnace and heated gradually raising the temperature until 450ºC. The process of heating, cooling and weighing were repeated, until constant weight of ash samples were obtained. A 0.5 g of the dried sample was placed into a porcelain crucible. 5 mL of HNO_3 : HCl (1:4) concentrated acid mixture was also added to it. The solution was evaporated to dryness overnight on a hot plate. To leach the residue of the sample, it was mixed with 10 mL of HNO_3 weak acid mixture at a temperature of about 70ºC for 30 minutes. The solution was stirred by using vortex mixer. The solution was filtered and aspirated on an atomic absorption spectrophotometer (AAnalyst-7000, Perkin Elmer Co. Ltd.,) at Universities' Research Centre.

EDXRF measurement

In order to determine the heavy toxic metals and macronutrient elements in plant samples, elemental contents in Me` Zale (*Cassia siamea* Lam. Syn) were determined by ED-XRF method at the University Research Centre, Yangon.

Pellets of the samples (2.5 cm diameter) were first made by using a pellet making machine. The X-ray spectrometer permits simultaneous analysis of light element to heavy element. The energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX-700) (Figure 1) can analyze the elements from Na to U under vacuum condition. The X-ray fluorescence uses X-rays to excite an unknown sample. The individual elements comprising in the sample reemit their own characteristic X-rays. They are detected by using semiconductor detector [Si(Li)] that permits simultaneous analysis of multi elements within the sample. In this way, EDX-700 spectrometer determines the elements that are present in the sample. It can perform two kinds of quantitative analysis: the Fundamental Parameter (FP) Method and the Calibration Curve Method. In the FP method, theoretical results can be calculated even when standard samples are not available. It can be applied to most samples, but the accuracy must be checked in advance. In the calibration curve method, experimental results can be obtained by using standard samples. Although limited samples can be applied, the accuracy is high. In the present study, the FP method was applied for the elemental analysis.

Figure (1). Photograph of EDX -700 spectrometer

AAS measurement

Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. The atoms absorb ultraviolet or visible light and make transitions to higher energy levels. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample. A detector measures the wavelengths of light transmitted by the sample, and compares them to the wavelengths which originally passed through the sample. A signal processor then integrates the changes in wavelength absorbed, which appear in the readout as peaks of energy absorption at discrete wavelengths. The energy required for an electron to leave an atom is known as ionization energy and is specific to each chemical element. When an electron moves from one energy level to another within the atom, a photon is emitted with energy E. Atoms of an element emit a characteristic spectral line. Every atom has its own distinct pattern of wavelengths at which it will absorb energy, due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample. The concentration is calculated based on the Beer-Lambert law. Absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions. The concentration is usually determined from a calibration curve, obtained using standards of known concentration. However, applying the Beer-Lambert law directly in AAS is difficult due to: variations in atomization efficiency from the sample matrix, non-uniformity of concentration and path length of analyte atoms (in graphite furnace AA). AAS is a technique used mostly for determining the concentration of a particular metal element within a sample. AAS can be used to analyze the concentration of over 62 different metals in a solution. Figure 2 shows the photograph of AA – 7000 spectrometer.

Figure (2). Photograph of AA – 7000 spectrometer

RESULTS AND DISCUSSION

Heavy metals are known to affect biological communities. When the levels of heavy metals exceed certain level, in plants and animals, it can induce a variety of acute and chronic effects in wide range of organisms in various ecosystems. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The effects of toxicity vary between metals; for instance, while lead poisoning typically may cause cognitive impairment, abdominal pain, vomiting and anaemia; mercury poisoning may cause peripheral neuropathy, desquamation and psychological disturbances. Other factors, such as the chronicity of exposure may also affect symptoms and clinical presentation. There is, however, considerable overlap between the clinical syndromes associated to heavy metal poisoning, often involving gastrointestinal disturbances and specific identification of metals is required for diagnosis. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, medicinal plants, or residential settings.

Certain heavy metals are nutritionally essential for a healthy life; they are referred to as the trace elements (e.g., Fe, Cu, Mn and Zn). These elements are commonly found naturally in foodstuffs, in fruits and vegetables, and in commercially available multivitamin products.

Determination of Elements in Me` Zale (*Cassia siamea* Lam. Syn) Flowers and Leaves by EDXRF Method

The elemental analysis of the flowers and leaves sample Me` Zale were determined by using Energy Dispersive X-ray Fluorescence (ED XRF) spectrometry. X-ray spectrometry has since long been recognized as a powerful method of multi-elemental analysis. The major advantage of X-ray spectrometry is that it offers a satisfactory compromise between, on the other hand, economy, speed and ease of operation. The general advantages of ED XRF are: simultaneous analysis, faster multi-element screening, detection of unexpected elements, high X-ray tubes of low beam currents or radioisotopes excitation, little sample damage by

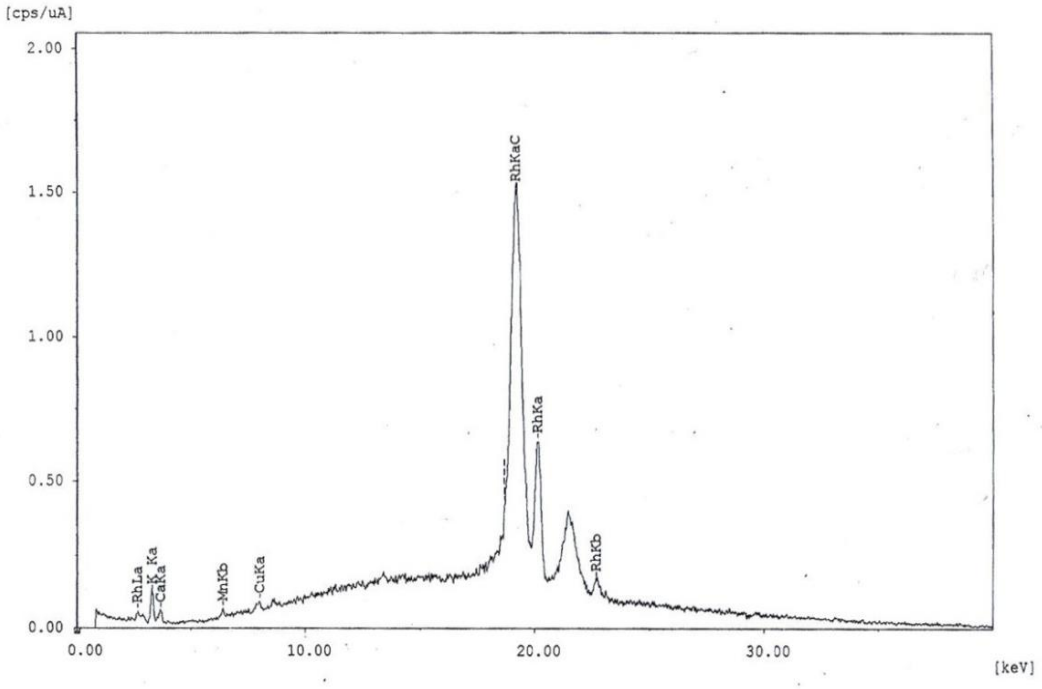
radiation. The ED X-700 spectrometer quantitatively determines which elements are present in the samples. This makes the fastest possible analysis of a sample even in the ppm range. Metals, powders, ceramics, rubber, plastics and liquids can be measured directly. The EDXRF spectra of Me` Zale sample was shown in Figures 3 and 4.

According to the result, it was found that the major elements contained in both samples were potassium (K) and calcium (Ca). The element potassium is an extremely important element in the human body. Potassium is important in controlling the heart, muscles, and the nervous system. Without potassium, the nerve cells couldn't send those messages to brain. Calcium is important to bone growth and formation, blood clothing, nerve and muscle functioning. It also helps regulate the passage of nutrients in and out of the cell walls, lower blood pressure. Calcium is important to normal kidney function and reduces blood cholesterol level. Iron is used by the body to make tendons and ligaments. It can be found that these predominant elements can be very effective to human health.

Measurement Condition

Instrument: EDX-700	Atmosphere: Air	Collimator: 10(mm)	Spin: Off

Analyte	TG kV	uA	FI	Acq.(keV)	Anal.(keV)	Time(sec)	D.T.(%)
Si-U	Rh 50	15-Auto	--	0 - 40	0.0 - 40.0	Real - 99	24



Quantitative Result

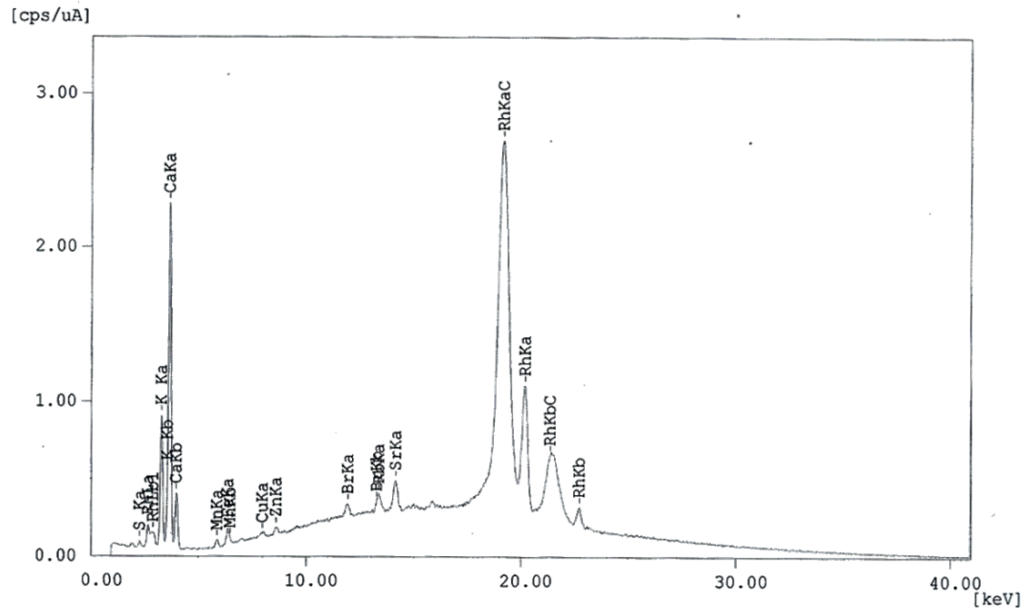
Analyte	Result	Std.Dev.	Proc.-Calc.	Line	Int.(cps/uA)
====[No. 1 Layer]====< Layer1		>==========			
Layer1	6.000 um	--------	Fix	--------	--------
C10H8O4	100.000 %	--------	Fix	--------	--------
====[No. 2 Layer]====< Base		>==========			
K	51.589 %	1.745	Quan-FP	K Ka	0.773
Ca	48.411 %	2.138	Quan-FP	CaKa	0.454

Figure (3). The EDXRF spectrum of Me` Zale flower sample.

Sample : Dr Aye Aye Mon Mazali
Operator: NTD
Comment : Pallet
Group : Solid Air
Date : 2017-09-07 15:10:45

Measurement Condition

Instrument: EDX-720	Atmosphere: Air	Collimator: 10(mm)				
Analyte	TG kV	uA	FI	Acq.(keV)	Anal.(keV)	Time(sec) DT(%)
Si-U	Rh 50	44-Auto	----	0 - 40	0.02-40.00	Live- 100 40



Quantitative Result

Analyte	Result	[3-sigma]	Proc.-Calc.	Line	Int.(cps/uA)
Ca	0.580 %	[0.006]	Quan-FP	CaKa	15.4571
K	0.399 %	[0.007]	Quan-FP	K Ka	5.8531
S	0.048 %	[0.011]	Quan-FP	S Ka	0.1965
Fe	0.003 %	[0.000]	Quan-FP	FeKa	0.8159
Mn	0.002 %	[0.000]	Quan-FP	MnKa	0.4146
Sr	0.001 %	[0.000]	Quan-FP	SrKa	2.3100
Rb	0.001 %	[0.000]	Quan-FP	RbKa	1.3593
Br	0.001 %	[0.000]	Quan-FP	BrKa	0.9836
Zn	0.001 %	[0.000]	Quan-FP	ZnKa	0.4457
Cu	0.001 %	[0.000]	Quan-FP	CuKa	0.3069
C H	98.963 %	[------]	Balance	--------	--------

Figure (4). The EDXRF spectrum of Me` Zale leaves sample.

Determination of Elements in Me` Zale (*Cassia siamea* Lam. Syn) Leaves by AAS Method (Atomic Absorption Spectrophotometry)

Metal ions are required for many critical functions in humans. The shortage of metal ions can lead to serious metabolic disorders. Without metal ions the cell can not retain its integrity or carry out metabolism. Therefore, metal ions play an important role in biological systems. Among them, iron, potassium, sodium, calcium and magnesium play a central role in the normal regulation of blood pressure. Atomic absorption spectrophotometry is the most widely used technique for trace elemental analysis. It is a comparative method and is also

capable of a complete analysis. This method has high sensitivity and several elements are easily analyzed and measured in the range of ppm. In the present work, contents of Fe, Na, K, Ca and Mg in the sample were determined by using atomic absorption spectrophotometer. The results obtained are shown in Table (1) and Figure (5).

Table (1). Elemental Analysis of Me` Zale (*Cassia siamea* Lam. Syn) Leaves by Atomic Absorption Spectrophotometry (AAS).

No.	Elements	Elemental Content (ppm)
1	Fe	3.18
2	Na	10.29
3	K	11.48
4	Ca	5.24
5	Mg	7.18

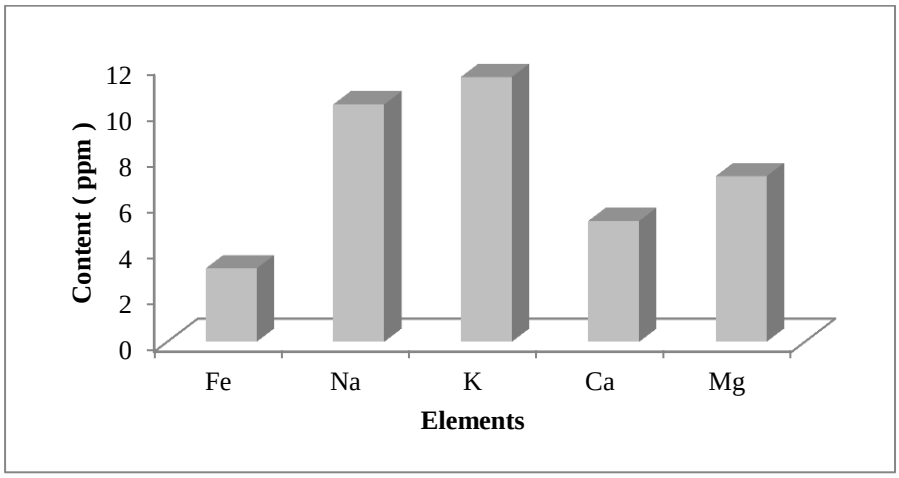


Figure (5). The elemental contents present in Me` Zale (*Cassia siamea* Lam. Syn) leaves by AAS Method.

CONCLUSION

The chemical analysis of inorganic materials used as raw materials nowadays can be made in different ways. In this study, different methods are compared with the different elements. The first method is the analysis that is relatively low cost and energy-dispersive X-ray fluorescence spectrometer (EDXRF) can analyze without standards. In this method the analysis is completed in a short time and gives the semi-quantitative results. The wet chemical and atomic absorption spectroscopy method is quantitative but EDXRF method is semi-quantitative. According to the result, it was found that the major elements contained in both samples (flowers and leaves of Me` Zale) were potassium (K) and calcium (Ca). The second method is atomic absorption spectroscopy and it is used principally for the quantitative determination of metal elements in aqueous and solid samples from a wide range of fields including medicine, food and geology since 1950s. So in this study, the wet chemical and atomic absorption spectroscopy analytical results are accepted as reliable. From the quantitative determination of the elements by Atomic Absorption Spectrophotometry (AAS), the samples were found to have high concentration of Fe (3.18 ppm), Na (10.29 ppm), K (11.48 ppm), Ca (5.24 ppm) and Mg (7.18 ppm). It can be seen that sodium and

potassium are the most predominant mineral elements in flowers and leaves of Me` Zale. In reality, the scope of Myanmar traditional medicine is very wide and is firmly rooted as a distinct entity. By making indigestion, hypertension, flatulence, antipyretics and tonic have been systematically produced and efforts have been made to export these traditional medicines. On the other hand, while conducting a scientific research on the production of traditional medicines, I should take every effort should be taken to preserve my Myanmar traditional medicine.

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