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Determination of Major and Minor Elements in the Rhizome of *Kaempferiaparviflora* by Analytical Techniques

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Abstract: ***Kaempferiaparviflora (family: Zingiberaceae) is a perennial medicinal plant whose rhizomes have been used by the indigenous people of Manipur (one of the 29 states of India) for primary healthcare from the ancient time. Thus, in the present study, we have performed investigations on the rhizomes of Kaempferiaparviflora using differentanalytical techniques such as Flame atomic absorption spectroscopy (FAAS), Inductively-coupled plasma mass spectrometry (ICP-MS), Scanning electron microscopy with Energy dispersive x-rays analysis (SEM-EDX), X-Ray diffraction (XRD) and CHNS analyser to determine the presence of major and minor (trace) elements.The major elements were C (28.60 wt.%), H (5.02 wt.%), N (3.35 wt.%)on the basis of CHNS analysis or C (62.59 at.%) and O (36.69 at.%) on the basis of SEM-EDX and minor elements were Na (6.5 ppm), Mg (4.3 ppm), K (11.6 ppm), Fe (0.66 ppm), Ca (0.41 ppm), V (< 2 ppb), Cr (7 ppb), Mn (361 ppb), Co (< 2 ppb), Ni (32 ppb), Cu (55 ppb), As (<5 ppb), Pb (17 ppb) on basis of FAAS and ICP-MS. After the correlation of the various results, we have explored a comprehensive elemental fingerprint of the sample. In this study we have determined the presence of 17 elements (C, H, N, O, Na, K, Fe, Mg, Ca, Cr, Mn, Ni, Co, Cu, Pb, As and V) quantitatively in the rhizome. No study on elemental analysis of the rhizome of this plant was performed earlier. So, this study will definitely beneficial in further herbal based medicinal formulations.***

Keywords: *Kaempferiaparviflora*, major elements, minor elements, medicinal plant

1. Introduction

The United Nations has announced that 2020 is the International Year of Plant Health. The plant is one of the most important gifts of nature to the mankind. Medicinal plants are another important aspect of plants. According to the World Health Organization (WHO), about 80% of the world's population consumes traditional medicinal plants directly or indirectly to treat their diseases. The effectiveness of the medicinal plants depends on their chemical composition. Medicinal plants are not only the sources of phytochemicals such as polyphenols, flavonoids, proteins, vitamins, carbohydrates but also rich sources of several elements [1]-[3]. Many of these elements are essential for the good health and thereby deficiency or excessiveness of these elements may have significant health problems [4]. A study was discussed on the role of 29 elements in the human body [5]**.** According to the study, these 29 elements have been classified into 5 major groups: **Group I**: Major elements such as C, H, O and N essential for macromolecules such as carbohydrates, proteins and lipids. **Group II**: Nutritionally important minerals or elements such as Na, K, Ca, P, Mg, Cl and S. An adult person requires about 50-100 mg/day of it. **Group III**: Essential trace elements such as Fe, Zn, Cu, Co, Cr, Mo, Se and I. The deficiency of such trace elements shows symptomatic in the body. **Group IV**: Additional trace elements such as Cd, Ni, Si, Sn, V and Al. So far, their role in metabolism of body is unclear. **Group V**: Non-essential elements: Au, Hg, Pb.

Major elements (oxygen, carbon, hydrogen, nitrogen) account for 96% of the total body weight, and the minor (trace) elements (Zn, Cu, Se, Cr, Co, Mo, I, Mn, etc.) account for 3 to 4% of the total body weight [6]. Both the types of elements play significant roles in maintaining healthy body. Deficiency of certain element is symptomatic or problematic in the body. In this situation, herbal medicine takes a vital role to bring back normalcy without adverse effects. In medicinal plants generally, 16 elements are available which are essential in human body [7].The elements are major elements such as Carbon (C), Hydrogen (H), Nitrogen (N) and Oxygen(O) and minor elements are calcium (Ca), magnesium (Mg), phosphorus (P), sulphur (S), iron (Fe), potassium (K), zinc (Zn), boron (B), molybdenum (Mo), manganese (Mn), copper (Cu) and chlorine (Cl). The quantities of various elements in the medicinal plants vary depending upon various factors including soil and environmental conditions [8]. In recent years, with enhanced awareness of the importance of trace elements on health, an increasing number of reports on the role of trace elements in

traditional medicines has been published in many countries including India [9],[10], China [11],[12], Nigeria [13],[14], Greece [15]and Egypt [16].

Manipur is one of 29 states of India and 70% of area is hill [17]. It lies at a longitude of 93°03 – 94°78 East and a latitude of 23°83 – 25°68 North. It has rainfall between 933 mm to 2600 mm and temperature ranges from 0^{o}C in winter (January) to 32^{o}C in summer (May-August). Plain area is about 746 to 850 meters lying above sea level, whereas hilly regions vary from 900 to 3000 meters lying above sea level. It has rich varieties of flora, therefore many medicinal plants are available and many of them are still unexplored. *Kaempferiaparviflora* Wall.ex Baker (Common name: black ginger, Family: Zingiberacea) is an aromatic perennial medicinal plant found in Manipur, North-East India. The rhizomes of the plant have been used by the indigenous people of Manipur, for treatment of various ailments such as stomach pain, headache, rheumatism, sore throat and renal disease etc. without proper scientific knowledge since ancient time. The plants are found in the adjoining places of Tamenglong and Senapati Districts (hilly regions) of Manipur. The tribal people of the area called the plant as Ai-kabomnu (Kabumnu means black in their dialect). It is known Singmu in Manipuri language. The plant was also found in tropical areas such as Malaysia, Sumatra, Borneo Island, and Thailand [18],[19]. It was reported that its brown rhizome possesses various medicinal properties [20].

Since minor (trace) elements contain in small amount in different parts of the medicinal plants, a sensitive and reliable analytical techniques are important for determining precise and accurate elemental data of the sample. And also, determination of various elements in medicinal plant is important to judge their nutritional as well as therapeutic values and also to prevent any probable adverse effects after consumption. Considering the above facts, qualitative and quantitative elemental analysis of *Kaemferiaperviflora* rhizome was performed by Flame atomic absorption spectroscopy (FAAS), Inductively-coupled plasma mass spectrometry (ICP-MS), Scanning electron microscopy with Energy dispersive x-rays analysis (SEM-EDX), X-Ray diffraction (XRD) and CHNS analyser. FAAS is so sensitive that it can measure down to ppm (parts per million) in a sample. But the technique fails to determine the elements like arsenic (As) and zirconium (Zr) because the maximum atomization temperature is not sufficient to induce complete atomisation. ICP-MS is an excellent quantitative multi-element measuring system that offers wide detection range of elements in ppb label. XRD technique will provide the presence of crystalline phase or amorphous nature of sample. SEM-EDX is also one of the analytical techniques used for elemental analysis which is highly qualified for the identification and the quantification of different elements in the sample [21]. Besides, the method is non-destructive and is more advantageous in multi-elementary analysis compared to other existing methods such as ICP-MS, FAAS in one time experiment. However, it has limitation in detecting the light atomic numbers or less than 1 at.%. Correlation of the results given by the above techniques, we have explored the presence of 17 elements (C, H, N, O, Na, K, Fe, Mg, Ca, Cr, Mn, Ni, Co, Cu, Pb, As and V). The experimental data of the present work will be of immense importance in the synthesis of new Ayurvedic formulations.

2. Materials and Methods

2.1 Biological Sample

*Kaempferiaparviflora*Wall.ex Baker is an aromatic and perennial herb which belongs to Zingiberaceae family. The plant started growing during the month of April-May (2017) that is time of rainy season (Figure 1a). After2-3 months, plant completed its full growth (Figure 1b) bearing purplish flowers (Figure 1c). A plant sample consisting of flower, rhizome, stem, leaves was collected and prepared a herbarium and then sent to BSI Shillong, India for authentication of the plant (Figure 1d). Consequently, the plant had been authenticated by Botanical Survey of India, and Shillong on 10th January, 2020 with No.BSI/ERC/Tech/19-20/726.

2.2 Collection of Rhizomes

About 15 kg of fresh wild *Kaempferiaparviflora*Wall.ex Baker were collected from WaphongInthan Village of Senapati District of Manipur, India, during the month of November–December, 2017 (Figure 1e). The collected mature rhizomes were washed thoroughly with running tap water followed by distilled water to remove foreign matters attached to the surface of the rhizomes and finally washed with deionized water (Figure 1f).The cleaned rhizomes were sliced into small pieces (Figure 2a) and shed air dried at room temperature so that not to affect chemical constituents in the sample. During the drying process, the colour of the rhizomes changed into different colours such as yellowish-green (Figure2b), brownish-green (Figure 2c), brownish colour (Figure 2d). Finally, the dried rhizomes were ground into fine powder by an electric grinder. The rhizome powder was stored in the glass bottles till further analyses.

2.3 Sample preparation for analysis

1 g of the dried powder of rhizomes was mixed with a 10 ml of ultra-pure nitric acid [22]-[24]in a volumetric flask and warmed to dissolve it. The solution was added deionized water to make the final volume of 100 ml (Figure 2e) in a volumetric flask. Then it was performed for FAAS and ICP-MS studies. The various solvents such as HNO_3, HNO_3–$HClO_4$ and HNO_3–HCl were used for dissolution of part of plant for analysis of elements present in it [22]-[24]. The typical picture of wither plant having flower, leaves and stem along with scale bar was shown in Figure 2f.

The pellets of dried powders of rhizomes were prepared and this was used for SEM-EDAX study. And some rhizome powers were used to perform CHNS analysis and XRD studies.

2.4 Methods

Flame atomic absorption spectroscopy (FAAS) with model GBC 906AA AAS unit with deuterium-arc background correction was used for determination of elements Na, K, Fe, Mg, Ca up to ppm (μg/ml). The air-acetylene flame and

nanopure water (18.3 ohm) as diluent were used in this estimation. FAAS has limitations in ppb level.

Inductively coupled plasma mass spectrometer (ICP-MS) with model VG PQ Ex Cell, VG Elemental, UK was used for determination of elements Cr, Mn, Co, Cu, Pb, As, V up to ppb (ng/ml). ICP-MS has limitations from isotropy effects, very light gases such as H_2, He and very heavy elements such as La, Nd.

Relative standard deviation (RSD = (SD × 100)/x, where x is provided in ppm or ppb as mean). Here, RSD values for FAAS and ICP-MS techniques are 2-5 % and 5-10%, respectively.

The scanning electron microscope (SEM) with model TESCAN VEGA3 was used to record SEM image and for the elemental analysis through energy dispersive x-ray (EDX).

Investigation of the presence of elements C, H, N, and S in the sample was carried out using a Thermo-Fischer Flash EA 1112 Series CHNS Analyzer.

X-ray diffraction (XRD) study of the dried power sample was carried out using PAN analytical powder X-ray diffractometer with Ni-Filtered Cu-Kα (1.5405 Å).

3. Results and Discussion

FAAS and ICP-MS data of *Kaempferiaparviflora* rhizomeare presented in Table 1.The techniques established the presence of Na, K, Fe, Mg, Ca, Mnelements in ppm level; Cr, Ni, Cu, Pbin ppb level and As, V, Co elements less than 5 ppb in the rhizome.

SEM image of dried powder of rhizome is shown in Figure 3 along with EDX spectrum. SEM-EDX data (Table 1) show the presence of C and O in quantities in the rhizome in which amount of C is about 1.7 times that of O. The elements observed less than 1 at.% by the technique were Na, Si, Mg, K, Cl, Br, P, Mo, Se in which Na, Mg and Mg are detected by FAAS in ppm label. It is to be noted that elements whose concentrations are less than 1 at.% and elements of light atomic numbers cannot be determined for their exact concentrations by SEM-EDX. This is the limitation of SEM-EDX [25].

CHNS data (**Table 1**) provide the presence of C, H and N and the presence of S is ruled out. The study indicates that the contribution of C, H and N together is 37 wt. % of the total sample weight. The data will be very helpful to establish the structure of the isolated bioactive compounds obtained from the rhizome extracts in the further research activities.

The XRD pattern of the rhizome is shown in Figure 3. It shows a broad peak indicating amorphous nature of sample. Even, if crystalline compounds are present in sample, its amount will be very less per mass of sample for detection.

Our elemental analysis of the rhizome of the plant is compared with elemental analyses of two rhizomes of the same family, reported elsewhere in Table 1[26]. Our study covers a wider range of elemental analysis as compared to others reported ones. After the correlation of the results given by the different instrumental techniques, we have explored a comprehensive elemental fingerprint of the rhizome under study. The study determines quantitatively the presence of 17 elements (C, H, O, N, Na, K, Fe, Mg, Ca, Cr, Mn, Ni, Co, Cu, Pb, As and V). Among the various elements, C, H, O and N are found to be present at the major level and Na, K, Fe, Mg, Ca, Mn, Cr, Ni, Cu, Pb ,As, V and Co are at minor level.

The chemical characteristic of each element obtained in this study is described here.

Sodium (Na): Na is essential to all living organisms. It is one of the major electrolytes in the blood. Without Na in the body, it cannot be hydrated. Na is of great importance for many regulation systems in the body. The minimum daily intake of Na is 2.4 g [27].

Potassium (K): The high concentration of K plants is required for enzyme activation, photosynthesis, water uptake, starch formation and protein synthesis. K participates actively in the maintenance of the cardiac rhythm [28],[29].

Iron (Fe): Fe is an essential element for human beings and also an essential component of haemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is a very important factor in diabetes. Low Fe content causes gastrointestinal infection, nose bleeding, and myocardial infarction. Iron deficiency causes anaemia in human [30], [31].

Magnesium (Mg): Mg works with calcium to help transmitting nerve impulse in the brain. Magnesium has an important role in the phosphorylation reactions of glucose and its metabolism. Its deficiency has been implicated in insulin resistance, carbohydrate intolerance, dyslipidemia and complications of diabetes [32]**.**

Manganese (Mn): Mn is a trace mineral which is present in the body in tiny amounts. It helps the body to form connective tissue, bones, blood-clotting factors, and sex hormones [33], [34]. It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation [35],[36]. **A**lso, it is an important component of enzyme systems, including oxygen handling enzymes. It is a component of the antioxidant SOD, which reduces free radicals [37],[38].

Nickel (Ni): Ni deficiency particularly affects carbohydrate metabolism [39]. Significant amounts of Niare found in DNA and RNA. Nickel may act as a stabilizer of these nucleic acids [40].

Calcium (Ca): Ca helps in preventing and curing all bone related issues [41]. It also helps to repair worn out cells, strong teeth in humans, building of RBCs and body mechanism. Therefore it has been extensively used for treatment of various diseases.

Chromium (Cr): Cr has significant effect in controlling sugar level in the blood of human beings. The high Cr contents in the tissues of Africans may be due to the use of raw sugar, which contains an appreciable amount of Cr [42].

Cobalt (Co): Cois a part of vitamin B-12. Its deficiency in human beings seriously affects some biological processes [43].

Copper (Cu): Cu is an element associated with many enzymes such as ceruloplasmin, cytochrome oxidase, lysyl oxidase, superoxide dismustase. Alzheimier's disease, Wilson's disease and Prion disease are due to the excess of Cu present in body. For normal synthesis of haemoglobin, traces of copper are required. Cu is needed for neurotransmitter synthesis and formation of myelin [44].

Vanadium (V): V affects carbohydrate metabolism including glucose transport, glycolysis, glucose oxidation, and glycogen synthesis [45]**.**

The other elements such as Si, Cl, Br, P, Mo and Se were also determined in the present study but the concentration of these elements are found comparatively less and negligible (less than 1a.t%). Toxic heavy metals Pb and As are detected in the investigated rhizome but their concentrations are negligible, so there will be no effect of these metals in the therapeutic properties of the rhizome [46].The variation in elemental concentration of the rhizome is mainly attributed to botanical structure, mineral composition of the soil, absorbability of the plant and climatological conditions [7]**,** [47].

According to WHO, our values reported here are within the permissible limits for trace elements [48].The literature survey revealed that optimal intake of elements such as Sodium, Potassium, Iron, Magnesium, Calcium, Manganese, Copper, Chromium, Nickel, Cobalt and Vanadium could reduce individual risk factors, including those related to cardiovascular disease for human beings [49]-[51].

4. Conclusions

The present study on major and minor elemental analysis of the rhizome of *Kaempferiaparviflora*Wall.ex Baker, collected from WaphongInthan Village of Senapati District of Manipur, India, have determined 17 elements qualitatively and quantitatively. Many therapeutic activities of rhizome of this plant may be attributed and correlated to the presence of these potential minor/trace elements. The data will be very useful in deciding the dosage of the drugs prepared from the rhizome and thus will be helpful in synthesis of new modern drugs with various combinations to treat various diseases ethno-medicinally. However, in order to produce a stronger basis for effective the curative effects of the rhizome, there is a need to study the effect of soil and climatic conditions on the elemental composition of this rhizome.

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6. Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Table 1: Comparison of elemental content in the rhizomes of some zingiberaceae plants

Elements	*Kaempferiaparviflora* (our study)				*Zingiberzerumbet* **[22]**		*Zingiberofficinale* (Ginger) **[23]**
	FAAS	ICP-MS	SEM-EDX	CHNS	ICP-MS	INAA	PIXE
	µg/mL Or ppm	ng/mL or ppb	at.%	wt.% per mass of sample	ppm	Ppm	% or ppm
C	-	-	62.59	28.60	-	-	-
H	-	-	-	5.02	-	-	-
N	-	-	-	3.35	-	-	-
S	-	-	-	0	-	-	-
O	-	-	36.69	-	-	-	-
Na	6.5	-	0.02	-	-	-	-
Si	-	-	0.21	-	-	-	-
Mg	4.3	-	0.20	-	-	-	-
K	11.6	-	0.06	-	-	-	0.77%
V	-	<2	0.00	-	-	<0.1	-
Cr	-	7	0.00	-	-	1.52 ±0.94	-
Mn	-	361	0.00	-	-	-	313.4
Fe	0.66	-	0.00	-	-	-	216.6
Co	-	<2	0.00	-	-	0.61 ±0.05	-
Ni	-	32	0.00	-	-	-	-
Cu	-	55	0.00	-	-	-	4.5
Zn	-	-	0.00	-	-	-	72.5
Cl	-	-	0.01	-	-	-	-
Br	-	-	0.02	-	-	-	-
I	-	-	0.00	-	-	-	-
P	-	-	0.15	-	-	-	-
Zr	-	-	0.00	-	-	-	-
Mo	-	-	0.02	-	-	-	-
As	-	<5	0.00	-	-	0.09 ±0.01	-
Hg	-	-	0.00	-	-	-	-
Se	-	-	0.02	-	-	-	-
Pb	-	17	0.00	-	1.04 ± 0.10	-	-
Ca	0.41	-	0.00	-	-	-	0.165%

Cd	-	-	-	-	0.02 ± 0.01	-	-
Be	-	-	-	-	<0.01	-	-
Tl	-	-	-	-	<0.01	-	-
Al	-	-	-	-	-	254 ± 40	-
Ba	-	-	-	-	-	<1.0	-
Rb	-	-	-	-	-	-	12.9
Sb	-	-	-	-	-	<0.05	-
Sr	-	-	-	-	-	<1.0	-
Th	-	-	-	-	-	0.26 ±0.01	-
U	-	-	-	-	-	<0.05	-

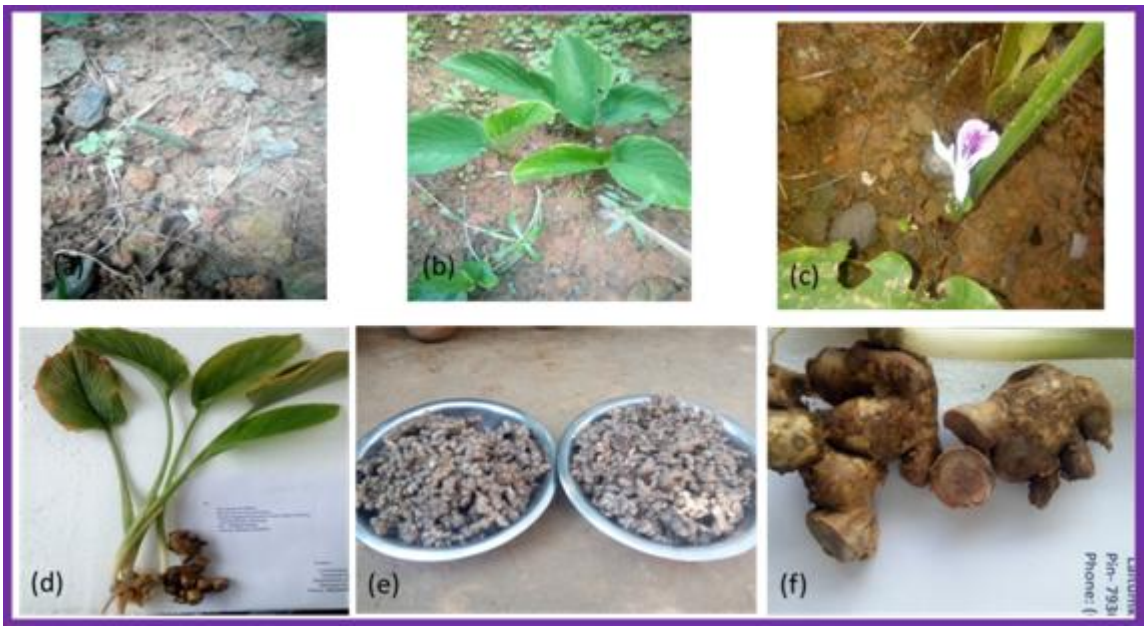

Figure 1: *Kaempferiaparviflora*:**(a)** April-May 2017 (plant started shooting), **(b)** June-July 2017 (full growth of the plant), **(c)**flower of the plant, **(d)** authentication process of the plant, **(e)**collection of 15 kgs of fresh rhizomes, **(f)** transvers sectional view of fresh rhizome

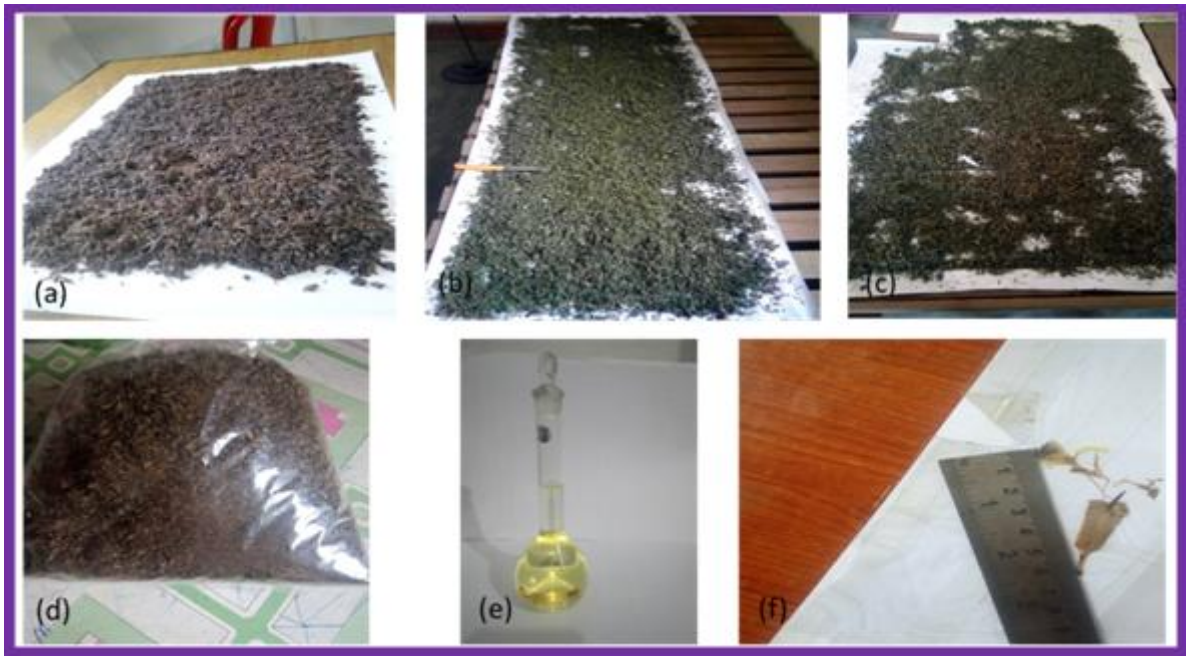

Figure 2: Drying process: **(a)** shed drying process of the rhizomes at room temperature after chopping (it appears as black-brownish), **(b)** change of colour after 15 days of shed dried (it appears as yellowish-green, **(c)** after 20 days of shed dried (it appears as brownish-green), **(d)** complete dryness after 30 days (it appears as brownish) **(e)** solution of brownish dried and chopped rhizomes after dissolution in ultrapure HNO_3 followed by addition of deionized water, **(f)** wither flower with scale

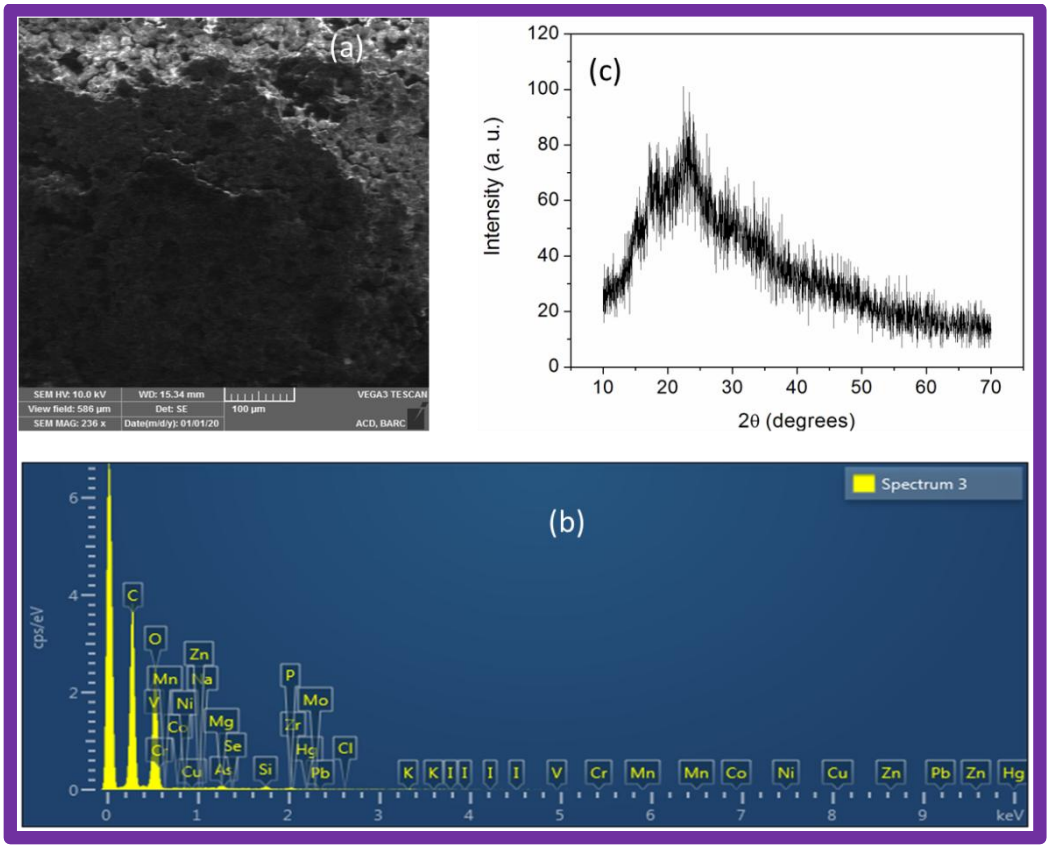

Figure 3: (a) SEM image of dried power of rhizome, **(b)** EDX spectrum and **(c)** XRD pattern.