Major and Trace Elemental Analysis of *Curcuma leucorrhiza* Roxb. (Zingiberacea Family) rhizome: A medicinal plant

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Abstract

Curcuma leucorrhiza Roxb. is a flowering plant and belongs to the zingiberaceae or ginger family having enormous medicinal properties such as antiseptic, anti-inflammatory, antibacterial, antioxidant, antacid, antifungal and carminative.Contents of major and trace elements in the medicinal plants also contribute to various therapeutic roles. Here, we have carried out the major and trace elemental analysis in the rhizome of this plant using the most sensitive techniques such as Flame atomic absorption spectroscopy (FAAS) to determine the elements Na (4.3 ppm), Mg (2.6 ppm), K (30.7 ppm), Fe (0.67 ppm), Ca (0.58 ppm), Inductively coupled plasma mass spectrometer (ICP-MS) to determine V (< 2 ppb), Cr (5 ppb), Mn (820 ppb), Co (< 2ppb), Ni (< 4ppb), Cu (48 ppb), As (< 5 ppb), Pb (14 ppb), Energy dispersive X-ray (EDX) to determine C (62.45 at.%), O (36.70 at.%) and Carbon - Hydrogen - Nitrogen – Sulphur (CHNS) to determine C (37.44 wt.%), H (7.04 wt.%), N (2.69 wt.%) and the presence of S was rule out. The elements C, O, N and H are the major elements, whereas Na, Mg, K, Fe, Ca, V, Cr, Mn, Co, Ni and Cu are the minor elements. Their presence in the medicinal plants plays a vital role to treat various diseases

***Index Terms-*:** *Curcuma leucorrhiza*, minor elements, Zingiberaceae, medicinal plant.

Abbreviations:

Atomic absorption spectroscopy (AAS), Flame atomic absorption spectroscopy (FAAS), Inductively coupled plasma mass spectrometer (ICP-MS), Relative standard deviation (RSD), Scanning electron microscopy (SEM), Energy dispersive X-ray (EDX), Carbon - Hydrogen Nitrogen – Sulphur (CHNS), X-ray diffraction (XRD).

1. Introduction

The plants belonging to Zingiberaceae or ginger family provide us the varied natural resources for the mankind such as food, perfume, spices, dyes and medicines (Riao & Gutiearrez, 2006; Chen, et al., 2007; Nelson, et al., 2017; Kress, Prince, & Williams, 2002). These comprise of about 53 genera and more than 1300 species (Kress, Prince & Williams, 2002). They are found in areas of the tropics and subtropics regions and are especially abundant in Southeast Asia (Yan-Qing, et al., 2018). The

parts of a plant such as flower, stem, leave, fluid and rhizome or root are very useful to us in many aspects. A few examples of these plants are *Zingiber officinale* (Ginger), *Curcuma caesia (*Black turmeric), *Curcumalonga* (Turmeric), *Alpinia zerumbet* (Shellflower), *Ravenala madagascariensis* (Traveler's tree), *Musa textilis* (Abaca), etc. India is one of the richest and diverse regions for Zingiberaceae where there are 20 genera and more than 200 species are available (Sabu,2006). Most of these plants had been used for medicinal purposes since ancient times. One of the interesting areas of this plant family is that they have the varied colours of rhizomes such as brown (*Kaempferia parviflora*), pale yellow (*Curcuma aromatica*), deep yellow (*Curcuma longa*), greenish blue (*Curcuma caesia*) and colourless (*Zingiber officinale*) in various species and accordingly, their medicinal properties are also different.

Particularly, the North-East states of India have many genera (19) and many species (90) of Zingiberaceae (Kumar, Asish, Sabul & Balachandran, 2013; Dutta, 2015; Linthoingambi, Asem, Singh & Laitonjam, 2013). Manipur is one of the North-East states of India which lies in the Indo-Burma Biodiversity Hot – Spot Region which ranks 6th among the 25 biodiversity hotspots of the world (Singh, Singh & Singh, 2009). The area of Manipur covers longitude of 93°03 – 94°78 East and a latitude of 23°83– 25°68 North. The ratio of plain to hill areas is about 1:9. The maximum rainfall ranges from 933 mm to 2600 mm and temperature varies from 0 ^{o}C in winter (January) to 32 ^{o}C in summer (May-August). Altitude of plain area varies from 746 to 850 meters lying above sea level and that of hilly regions varies from 900 to 3000 meters lying above sea level. Owing to different climatic conditions varying from tropical, subtropical, and temperate zones, Manipur is rich in biodiversity which happens the major existence of medicinal plants (Devi, Devi & Singh, 2015) such as *Curcuma caesia*, *Kaempferia perviflora*, *Curcuma aromatica* and so on.

In Manipur, a wild medicinal plant, *Curcuma leucorrhiza* Roxb. (zingiberaceae) has been extensively used in traditional medicines for its remarkable therapeutic properties such as antiseptic, anti-inflammatory, antibacterial, antioxidant, antacid, antifungal, enlarged liver and spleen, ulcer in stomach and carminative (Linthoingambi, Asem, Singh & Laitonjam, 2013; Devi, Devi & Singh, 2015; Sinha, 1996; Pal & Srivastava, 1976; Theanphong, Mingvanish & Jenjittikul, 2016). In fact, medicinal plants contain both organic and inorganic constituents. Organic constituents such as alkaloids, amines and glycosides are highly responsible for medicinal property (Jyothsna, Manjula, Suthari & Rao 2020). There are reports on the studies of organic components (secondary metabolites) in the *Curcuma leucorrhiza* rhizome (Asem, 2014) but the studies of the inorganic components in the treatment of various diseases has received less attention. However, macronutrients and trace (micro-) elements present in medicinal plants also play a significant role to prevent various diseases. Trace elements are very important components in the plants for various physiological activities and cofactors in the production of enzymes (Sattar, et al., 2012). The deficiency and excess amount of trace elements are also responsible for toxicity in plants (Chrzan, 2016). A study reported on the presence of the elements Fe (0.971 ppb), Zn (0.414 ppb), Cu (1.70 ppb), Mo (0.013 ppb), Cr (5.091 ppb), Mn (0.015 ppb) in the *Curcuma leucorrhiza* Roxb rhizome using atomic absorption spectroscopy (AAS) (Singh, Singh & Phucho, 2014). But, their results are not reliable because AAS technique cannot determine concentration of elements below 1 ppm.

In this study, we have carried out analyses of the trace and macro elemental concentrations in the rhizome of the *Curcuma leucorrhiza* plant using different experimental techniques. This will help in understanding of the effect of these elements on human health and also environmental impacts on the selected plant.

2. MATERIALS AND METHODS

2.1 Plant sample

Curcuma leucorrhiza Roxb. is a perennial and aromatic herb of Zingiberaceae family. The plant started growing in the month of April-May and its full growth completed within 2-3 months (**Fig. 1a**). The plant bears white flower with partly pink at the top. The rhizomes are colorless with distinctive characteristic which is shown by transverse sectional view of fresh rhizomes

(**Fig. 1b**). The plant had been authenticated by Department of Life Sciences, Manipur University, Imphal, whose voucher specimen was (000815) (Asem, 2014).

2.2 Collection of Rhizomes

About 45 kg of freshly harvest tubers (*Curcuma leucorhiza* Roxb. Rhizomes) was collected from Waphong Inthan Village of Senapati District of Manipur, North-East, India, in the month of December, 2017. The collected tubers (rhizomes) were washed thoroughly with running tap water and then with distilled water to remove impurities present in the rhizomes. Finally, it was washed with deionized water. The cleaned rhizomes were sliced into small pieces (**Fig. 1c**). It was shed air dried at room temperature (i.e., maximum effort was done to avoid incorporation of unwanted impurity that might change chemical composition of the sample). After some days i.e. 2-3 weeks, the colour of the sample was observed to slight change from white (**Fig. 1c**) to yellowish (**Fig. 1d**). The sample was found completely dried after nearly 30 days of drying; and the sample was easily breakable and dark yellow in colour (**Fig. 1e)**. Finally, the dried rhizomes were stored in an air tight container. The dried rhizomes were made fine powder with an electric grinder. The powdered rhizomes were stored in the glass bottles for various analyses.

2.3 Sample preparation for analysis

1 gm of the powder sample was mixed with 10 ml of ultra-pure nitric acid in a volumetric flask and warmed to dissolve it. It was then added deionized water to make the volume up to 100 ml (**Fig. 1f**). This solution was kept for FAAS and ICP-MS studies. For analysis of elements present in part of plant, the solvents such as HNO_3, HNO_3–HCl and HNO_3–$HClO_4$ were used for dissolution (Uddin, et al., 2016; Daran, et al., 2017; Deschamps, & Matschullat, 2011).

1 cm diameter pellets of dried powders of rhizomes were prepared under hydraulic pressure and these pellets were given for SEM-EDAX study. Also, some (about 200 mg) of dried powders of rhizomes were kept for CHNS and XRD studies.

2.4 Methods

For determination of elements such as Na, K, Fe, Mg, Ca up to ppm (μg/ml), the Flame atomic absorption spectroscopy (FAAS) with model GBC 906AA AAS unit with deuterium-arc background correction was employed. The air-acetylene flame was used. Nanopure water (18.3 Mega ohm) was used as diluent in this estimation.

For determination of elements such as Cr, Mn, Co, Cu, Pb, As, V up to ppb (ng/ml), the Inductively coupled plasma mass spectrometer (ICP-MS) with model VG PQ Ex Cell, VG Elemental, UK was used.

Relative standard deviation (RSD) = (SD × 100)/x, where x is provided in ppm or ppb as mean. In this study, RSD values for FAAS and ICP-MS techniques are calculated to be 2-5 % and 5-10%, respectively. All experiments were performed after standardization for every element.

The scanning electron microscopy (SEM) images were recorded using model TESCAN VEGA3; and energy dispersive x-ray (EDX) analysis of elements present in the sample was analysed using same instrument.

For determination (quantitatively) of elements such as C, H, N, and S, Thermo-Fischer Flash EA 1112 Series CHNS Analyzer was used.

X-ray diffraction (XRD) data of the dried power sample was recorded using PAN analytical powder X-ray diffractometer with Ni-Filtered Cu-Kα (1.5405 Å) at 40 kV and 30 mA.

3. Results and Discussion

FAAS technique provides the presence of elements such as Na, K, Fe, Mg, Ca in ppm level, whereas ICP-MS technique provide the presence of elements such as Cr, Mn, Cu, Pb in ppb level and As, V, Ni, Co in below 5 ppb level in the *Curcuma leucorrhiza* rhizome (**Table 1)**.

Figure 2(a, b) shows the SEM image of the dried powder of sample (*Curcuma leucorrhiza* rhizome) along with its EDX spectrum and **Table 1** gives elemental composition of sample. This provides the content of C and O in the sample and the amount of C is about 1.7 times than that of O. SEM-EDX detected the following elements also in the sample but they are treated as negligible in amount as their concentrations are less than 1 at.%; viz. Cl (0.01 at.%), Br (0.04 at.%), I (0.01 at.%), P (0.12 at.%), Zr (0.02 at.%), Mo (0.02 at.%), Hg (0.02 at.%), Se (0.05 at.%). Notably, this technique cannot provide the exact contents of light elements such as Li, B, C, N, etc. ($Z < 10$) because the intensity of X-ray characteristics lines is low.

From CHNS data (**Table 1**) indicates the presence of C, H and N but the presence of S is ruled out in the sample. This weight percentage of the elements is expressed with respect to the mass of sample taken. Only 47.17 wt. % is found contributed from C, H and N. The XRD pattern of the dried rhizome is shown in **Fig. 2c**. It shows a broad peak with maximum at $2\theta = 23^{o}$ indicating the amorphous nature of the test sample. Even if crystalline compounds are present, their amount per mass of powder sample is very less, which cannot be determined by XRD technique. Usually, such crystalline compounds are obtained after solvent extraction through multi-step processes.

Table 1 gives the results of elemental analysis of sample using the various instrumental techniques along with reported ones. As and Pb are also detected in the sample and they are within the permissible limit. So there will be no effect of these metals in the therapeutic properties of the rhizome (Karayil, Bhavani & Vivek, 2014). Trace elements are essential micronutrients that exist in very low concentrations in the body, forming less than 0.01% of the total body weight (Sherbeny, Behairy, Mohammad & Elsayed,2016). But, they play very important roles in various physiological processes and are crucial for proper functioning of the immune system (Gray, et al.,2010).

After correlating the various results of the different instrumental techniques, we have explored a comprehensive elemental fingerprint data of the rhizome. The study have determined the presence of 17 elements: Na (4.3 ppm), Mg (2.6 ppm), K (30.7 ppm), Fe (0.67 ppm), Ca (0.58 ppm), V (< 2 ppb), Cr (5 ppb), Mn (820 ppb), Co (< 2 ppb), Ni (< 4 ppb), Cu (48 ppb), As (< 5 ppb), Pb (14 ppb), O (36.70 at.%), C (37.44 wt.%), H (7.04 wt.%) and N (2.69 wt.%). These results are compared with those of 2 rhizomes reported elsewhere in **Table 1** (Daran, et al.,2017; Devi, Sarma & Kumar,2007). Our study covers a wider range of elemental analysis as compared to the reported ones. The literature shows that many plants have the presence of different elements, which are broadly classified as major and minor or trace. In this view, C, O, H and N are major elements and other elements in ppm or ppb range are classified as trace/minor elements (**Fig. 3**). The present study showed three categories of elements

1) **Major elements**: C, O, H and N.

2) **Minor elements**: Cr, Na, Mg, K, Co, Fe, Ca, V, Mn, Ni, and Cu.

3) **Toxic elements**: As and Pb.

Here, we are discussing on the importance of each element obtained in this study:

Chromium (Cr): The daily requirement of chromium is about 0.005 mg/day. Cr is an essential micro nutrient which potentiates insulin action and hence influences carbohydrate, lipid and protein metabolism (Raju, Sarita, Rao, Rao & Reddy,2013). Its deficiency causes impairment of glucose tolerance and its toxicity causes in renal failure, dermatitis, and pulmonary cancer (Cefalu & Hu, 2004). **Sodium (Na):** It is one of the important electrolytes of blood which is very important for many regulation systems in the body. The daily minimum requirement of Na in the body is 2.4 g (Parab & Vaidya, 2016). **Magnesium (Mg):** Magnesium acts as a cofactor of many enzymes which are involved in energy metabolism, protein synthesis, DNA and RNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes (Classen,1984). Deficiency of it may be lead to the etiologies of cardiovascular problems, diabetes, hypertension and atherosclerosis in humans (Jing, et al.,1995). **Potassium (K):** Potassium is extremely important to cells and without it we could not survive. K participates actively in the maintenance of cardiarhythm (Babu et al., 2015). and deficiency of it may cause weakness as cellular processes are affected. Excess of K can cause Hyperkalemia (Bihl & Meyers, 2001). **Cobalt (Co):** The daily requirement of cobalt is 0.0001

mg/day. It is a component of Vitamin B12. The deficiency of it in human beings affects seriously in some biological processes (Sullivan, 2002). **Iron (Fe)** Iron is an essential trace element found in living organisms. The total content of iron in the body is about 3-5 g. Out of which 75% is in blood while the rest is in liver, bone marrow and muscles (Vasudevan & Sreekumari,2007). Average daily requirement of Fe is 1-2 mg. Iron is absorbed from food when it is being needed. Enzymes and proteins which contain Iron, often consist of heme prosthetic groups perform various biological oxidations and transportation. Excess of Fe causes rapid increase pulse rate, coagulation of blood vessels, drowsiness and hypertension (Naziri, et al., 2015). Deficiency of Fe causes severe disorders; most important among them is anaemia (Lieu, Heiskala, Peterson & Yang, 2001). **Calcium (Ca)**: Calcium is an essential nutrient which helps in preventing and curing all bone related issues (Chattopadhyay & Eddouks, 2012). It also helps to repair worn out cells, building of RBCs, strong teeth in humans and body mechanism. Therefore, it is very useful for treatment of various diseases. **Vanadium (V)**: Vanadium is an inhibitor of enzymes, minimise plasma cholesterol levels, influence glucose metabolism. It is important for toxic interactions of chemicals (WHO.2000). Excessive vanadium intake causes gastrointestinal disturbances (WHO,1996). **Manganese (Mn):** It is a trace element used for reproduction and normal ducting of the central nervous system (Kaur, et al., 2012). It is present mainly in mitochondria. Deficiency of manganese causes human myocardial infarction and other cardiovascular diseases. And excess of manganese is toxic in brain and causes a Parkinson type syndrome (Aschner, 2000). **Nickel (Ni): D**eficiency of nickel particularly affects carbohydrate metabolism (Anke, Groppel, Kronemann & Grün, 1984). Nickels are found in DNA and RNA in significant amounts. It may act as a stabilizer of these nucleic acids (Phipps et al., 2002). **Copper (Cu):** After Iron and Zinc, the third largest trace element found in human body is Cu. Copper plays a vital role in our metabolism because it allows many critical enzymes to function properly (Harris, 2001). It is the main constituent of bone, connective tissues, brain, heart and other body organs (Morabad, Patil & Tapash, 2013). Copper deficiency causes the liver damage malnutrition, malabsorption disorders, depigmentation of hair and skin.

4. Conclusions

In the present study on elemental analysis of *Curcuma leucorrhiza* rhizome, various elements of interest are identified and quantified. The main factors of the varied elemental concentrations are attributed to the differences in chemical structure, environment effect, age, water in which the plants are grown. Major elements: C, H, N and O are the backbone of organic compounds such as organic oils, alcohols, protein and carbohydrates present in plant. The minor or trace elements: Na, Mg, K, Fe, Ca, V, Cr, Mn, Co, Ni and Cu are therapeutically important. Further, the present study has evaluated that no excess quantities of toxic elements were detected. The study will be very useful to pursue further study in the area of herbal and alternative medicines.

Conflict of Interest Statement:

The authors declare no competing financial interest.

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Fig. 1 *Curcuma leucorrhiza* Roxb.: (a) Growth of plant during April-July. (b) Transverse sectional view of fresh rhizomes. It appears colorless. (c) The cleaned rhizomes were sliced into small pieces. It appears white/ colourless. (d) After some days i.e. 2-3 weeks, the colour of the sample appears yellowish. (e) It completely dried after nearly 30 days of shed dry at room temperature. It appeared dark yellow in colour. (f) Solution of 1 g of dried powder per 100 ml deionized water after dissolution in ultrapure HNO_3.

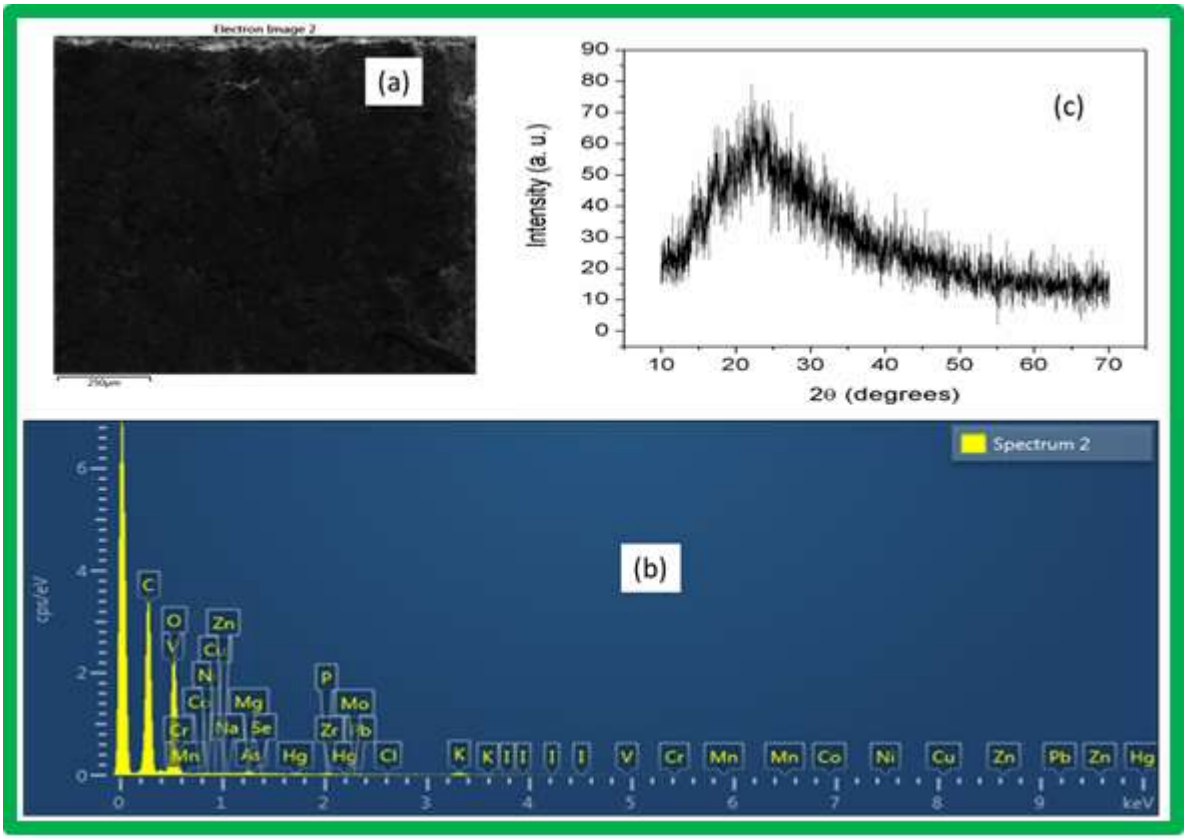

Fig. 2 (a) SEM image of dried power of rhizome, (b) EDX spectrum and (c) XRD pattern.

Fig. 3 Major and minor elements present in the rhizome of *Curcuma leucorrhiza* Roxb.

Table1: Comparison of the sample with standard material and two other elsewhere studies

Elements	*Curcuma leucorrhiza* Roxb (our study)				*Zingiber zerumbet* [26]		*Zingiber officinale* (Ginger) [27]
	FAAS	ICP-MS	SEM-EDX	CHNS	ICP-MS	INAA	PIXE
	µg/ml	ng/ml	at.%	wt.% per mass of sample	ppm	ppm	% or ppm
C	-	-	62.45	37.44	-	-	-
H	-	-	-	7.04	-	-	-
N	-	-	-	2.69	-	-	-
S	-	-	-	0	-	-	-
O	-	-	36.70	-	-	-	-
Na	4.3	-	0.00	-	-	-	-
Si	-	-	0.00	-	-	-	-
Mg	2.6	-	0.19	-	-	-	-
K	30.7	-	0.36	-	-	-	0.77%
V	-	<2	0.00	-	-	<0.1	-
Cr	-	5	0.00	-	-	1.52 ±0.94	-
Mn	-	820	0.00	-	-	-	313.4
Fe	0.67	-	0.00	-	-	-	216.6
Co	-	<2	0.00	-	-	0.61 ±0.05	-
Ni	-	< 4	0.00	-	-	-	-
Cu	-	48	0.00	-	-	-	4.5
Zn	-	-	0.00	-	-	-	72.5
Cl	-	-	0.01	-	-	-	-
Br	-	-	0.04	-	-	-	-
I	-	-	0.01	-	-	-	-
P	-	-	0.12	-	-	-	-
Zr	-	-	0.02	-	-	-	-
Mo	-	-	0.02	-	-	-	-
As	-	<5	0.00	-	-	0.09 ±0.01	-
Hg	-	-	0.02	-	-	-	-
Se	-	-	0.05	-	-	-	-
Pb	-	14	0.02	-	1.04 ± 0.10	-	-
Ca	0.58	-	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	-