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RESEARCH ARTICLE

Microwave assisted determination of minerals and toxic metals in traditionally used medicinal plant *Zingiber officinale* Roscoe by Inductively Coupled Plasma-Optical Emission Spectrometer

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Abstract

Seventeen minerals (Ba, Be, Bi, B, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Ag, Sr and Zn) and four toxic metals (As, Cd, Pb and Hg) were determined first time in the rhizome of *Zingiber officinale* Roscoe. The rhizomes were irradiated with microwave in a Microwave Reaction System with nitric acid and analysed by Inductively Coupled Plasma-Optical Emission Spectrometer. The mean concentration (mg/100g±SE) of minerals in the rhizome of *Z. officinale* were Ba 3.83±0.02, Be ND, Bi ND, B 0.47±0.24, Ca 39.70±0.57, Co 0.01±0.003, Cr 1.14±0.03, Cu 0.56±0.08, Fe 18.63±0.02, Mg 286.78±10.17, Mn 5.06±0.04, Mo 0.08±0.002, Ni 0.55±0.02, Se ND, Ag ND, Sr 2.11±0.21 and Zn 1.47±0.21 and toxic metals were As ND, Cd 0.01±0.003, Pb 0.20±0.16 and Hg ND. Results obtained revealed that *Z. officinale* rhizome has higher concentration of magnesium, calcium and iron with traces of other elements. Moreover, cadmium and lead were also present. On the basis of data obtained it can be concluded that *Z. officinale* rhizome has worthy nutritive elemental profile and can be a source of balanced elemental requisite of diet albeit it should be tested for toxic metals load before processing it further for medication.

Introduction

Zingiber officinale Roscoe (Ginger, Zingiberaceae) is a rhizomatous perennial plant used worldwide as a spice in foods and beverages and is commonly known for its medicinal properties. *Z. officinale* is cultivated commercially in India, China, South East Asia, West Indies and other parts of the world. It is an important ingredient in Ayurvedic, Tibbe-Unani and Chinese herbal medicines for the treatment of cataract, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Awang, 1992; Wang and Wang, 2005; Tapsell et al., 2006). About 25 species of Zingiberaceae are used to cure multiple disorders in human and animals (Tushar et al., 2010).

Role of micro and macro elements have been well recognized and their levels are of great concern to human health. In biological system, a number of minerals such as iron, magnesium, calcium, zinc, copper and manganese play a vital role. These elements are important for various biochemical and physiological mechanisms in living organisms, and they are recognized as essential elements for life. Moreover, arsenic, cadmium, lead and mercury are the non-essential metals which can be toxic even in trace amounts (Schroeder, 1973; Somer, 1974; Liang et al., 2004).

Several attempts have been made to determine the various elements in medicinal plants and herbal formulations. There have been reports of high levels of heavy metals in some therapeutically important Indian medicinal plants

(Rai et al., 2000; Haider et al., 2004; Naithani and Kakkar, 2005). The World Health Organization has also emphasized to ensure quality of plant products by using modern techniques and applications (WHO, 1998). Many analytical methods are available including atomic absorption spectrometry for the determination of trace elements in plant materials, but these methods require the decomposition of the sample (Tarley et al., 2004; Roychowdhury et al., 2003, Polkowska-Motrenko et al., 2000). Sample preparation is a critical step for the analysis of metals due to presence of different type of matrices. The wet and dry ashing procedures for sampling are common but they are quite slow and time consuming. Also, these procedures are difficult to follow consistently. With advancement of technology introduction of Microwave digestion is a rapid and efficient method for sample decomposition prior to the determination of trace metals (Tuzen, 2002; Kingston, 1986; Narin, 2004). Moreover, Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) is one of the most accurate and latest analytical technique for the determination of trace elements in numerous of sample types.

In this paper, we report for the first time, the determination of minerals Ba, Be, Bi, B, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, As, Sr and Zn along with toxic metals As, Cd, Pb and Hg in the rhizome of *Zingiber officinale* by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) with the help of Microwave Digestion System (MDS).

Materials and Methods

Reagents

The standards for ICP-OES were prepared from stock solutions of studied elements at 1000 mg/L concentration obtained from Perkin Elmer (USA). All other reagents and solvents used in this study were of analytical grade obtained from Fischer Scientific (USA). Milli-Q water was used for washing laboratory glassware and in preparation of sample and standard solutions.

Plant material

Rhizomes of *Z. officinale* were procured from Mubarakpur vegetable market, Azamgarh (India). Collected rhizomes were identified by Dr. M. M. A. A. Khan, Associate Professor, Department of Botany, Shia P. G. College, Lucknow, India. Rhizomes were air dried and grinded to fine powder for the study.

Microwave digestion

Digestion of *Z. officinale* air dried rhizome powder was carried out with a Multiwave Reaction System (Multiwave 3000, Anton Paar, Perkin Elmer) with the Rotor 16HF100 (100 ml PFA vessels, 40 bar) and Pressure, Temperature (p/T) sensor.

0.2g of powdered rhizome was digested with 2.0mL of HNO_3, 1.0 ml of H_2O_2 and 3.0 ml of H_2O in microwave digestion system, according to the digestion program presented in Table 1. The resulting clear solution was cooled and diluted to 20.0 mL with Milli-Q water.

Table 1 Operating conditions for the Microwave Digestion System

S. No.	Power [W]	Ramp [min]	Hold [min]	Fan
1.	400	5	10	1
2.	800	5	10	1
3.	0	0	10	3

ICP-OES analysis

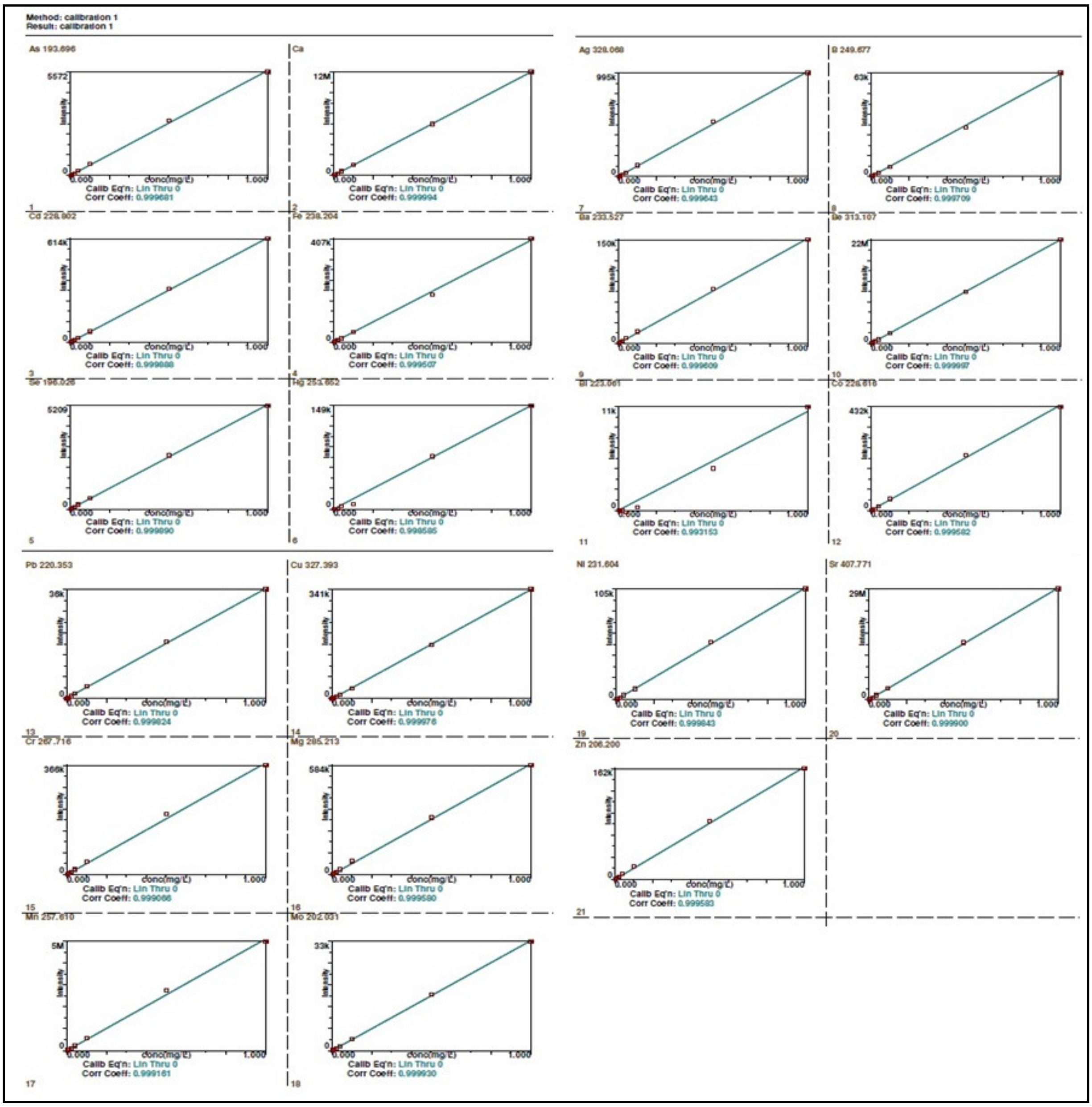
The calibration standards for ICP-OES were prepared by diluting the stock standard solution (1000 mg/L) in 0.2% (v/v) nitric acid. Working solutions were prepared from the stock as necessary. The calibration curves for all the studied elements were prepared by different concentrations of standards in the range 0.005 mg/L to 1.0 mg/L from working solution (Figure 1). The above clear solution obtained after Microwave Digestion was analysed by ICP-OES (Optima 8000, Perkin Elmer) for elemental analysis with following operational conditions (Table 2):

Table 2 Operating conditions for the ICP-OES

Operating Conditions	Value
Plasma Gas Flow (L/min)	8

Auxiliary Gas Flow (L/min)	0.2
Carrier Gas Flow (L/min)	0.55
RF Power [W]	1300
Plasma view	Axial
Sample flow rate (ml/min)	1.0

Figure 1 ICP-OES calibration curves of different elements



Results

Elemental analysis showed that the concentration of Barium (Ba) was 0.47±0.02 mg/100g, Beryllium (Be) ND, Bismuth (Bi) ND, Boron (B) 3.83±0.24 mg/100g, Calcium (Ca) 39.70±0.57 mg/100g, Cobalt (Co) 0.01±0.003 mg/100g, Chromium (Cr) 1.14±0.03 mg/100g, Copper (Cu) 0.56±0.08 mg/100g, Iron (Fe) 18.63±0.02 mg/100g, Magnesium (Mg) 286.78±10.17 mg/100g, Manganese (Mn) 5.06±0.04 mg/100g, Molybdenum (Mo) 0.08±0.002 mg/100g, Nickel (Ni) 0.55±0.02 mg/100g, Selenium (Se) ND, Silver (Ag) ND, Strontium (Sr) 2.11±0.21 mg/100g and Zinc (Zn) 1.47±0.21 mg/100g (Table 3). Moreover, the level of toxic metals were Arsenic (As) (ND), Cadmium (Cd) 0.01±0.003 mg/100g, Lead (Pb) 0.20±0.16 mg/100g and Mercury (Hg) (ND) (Table 4).

Table 3 Concentration (mg/100g) of different minerals in the rhizome of *Z. officinale*

S. No.	Element	Symbol	Wavelength (nm)	Concentration (mg/100g±SE)
1	Barium	Ba	233.527	0.47±0.02
2	Beryllium	Be	313.107	ND
3	Bismuth	Bi	223.061	ND
4	Boron	B	249.677	3.83±0.24
5	Calcium	Ca	317.933	39.70±0.57
6	Cobalt	Co	228.616	0.01±0.003
7	Chromium	Cr	267.616	1.14±0.03
8	Copper	Cu	327.393	0.56±0.08
9	Iron	Fe	238.204	18.63±0.02
10	Magnesium	Mg	285.213	286.78±10.17
11	Manganese	Mn	257.610	5.06±0.04
12	Molybdenum	Mo	202.031	0.08±0.002
13	Nickel	Ni	231.604	0.55±0.02
14	Selenium	Se	196.026	ND
15	Silver	Ag	193.69	ND
16	Strontium	Sr	407.771	2.11±0.21
17	Zinc	Zn	206.200	1.47±0.21

Table 4 Concentration (mg/100g) of toxic metals in the rhizome of *Z. officinale*

S. No.	Element	Symbol	Wavelength (nm)	Concentration (mg/100g±SE)
1	Cadmium	Cd	228.802	0.01±0.003
2	Lead	Pb	220.353	0.20±0.16
3	Mercury	Hg	253.652	ND
4	Silver	AS	193.69	ND

ND-Not Detected

Discussion

Our results showed that Mg is the most abundant element in the rhizome of *Z. officinale*, while Ca had the second highest concentration followed by Fe. In biological process Mg, Ca and Fe play a vital role in controlling various functions and disturbance in their levels may have detrimental effects. Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control and blood pressure regulation. Magnesium deficiency results in hypocalcaemia or hypokalemia due to mineral homeostasis disruption leading to tingling, muscle contractions, cramps and abnormal heart rhythms (IOM, 1997; Rude RK, 2010a, 2012b). Ca plays an important role in building stronger and dense bones. Approximately 99 percent of the body's calcium is stored in the bones and teeth and its deficiency is closely associated with osteoporosis (NIH, 2011). Iron is important for oxygen transport, apart from being the cofactor of many enzymes and proteins, and also being involved in the formation of red blood cells (Aggett PJ, 2012; Murray-Kolbe, 2010). The functional deficits of iron are associated with anaemia and it also leads to gastrointestinal disturbances. Moreover, iron is also important for growth development, cognitive function, immune function, work performance and body temperature regulation (Clark SF, 2008).

As per the results of the present study, the levels of Mn, B, Sr, Zn and Cr were lower in comparison with Ca and Fe in the rhizome of *Z. officinale*. These elements have a significant role in maintaining and regulating various cellular processes. Mn is a component of several enzymes and contributes to metabolism of carbohydrates, amino acids and cholesterol. Signs of manganese deficiency include impaired growth, skeletal abnormalities, disturbed or depressed reproductive function. However, when in excess it can cause a Parkinson-type syndrome (Food and Nutrition Board, 2001; Aschner, 2000). Boron has significant interaction with mineral metabolism, especially, calcium and magnesium, and deficiency is to be reported related to arthritis (Nielsen, 1990). Recently strontium has come into vogue as a “natural” dietary supplement for bone health. Some studies indicate that strontium ranelate may improve bone density in osteoporosis or osteopenia. The effect of strontium on bone is mainly related to its similarity to calcium (Ortolani and Vai, 2006; Reginster and Meunier, 2003). Zn is an essential metal which is required for the normal functioning of many enzyme systems, it improves immune system. Zn deficiency, particularly in children, can lead to loss of appetite, growth retardation, weakness, and even stagnation of sexual growth (Keen and Gershwin, 1990; Luara et al., 2010). Chromium is an essential trace element required for normal carbohydrate metabolism. The biological function of chromium is closely associated with that of insulin and most chromium-stimulated reactions are also insulin dependent. Moreover, deficiency or excess Cr causes atherosclerosis, diabetes, asthma etc. (Anderson, 1981).

Very low concentration of Ba, Co, Cu, Mo and Ni were found in the rhizome of *Z. officinale*. However, human body requires low amount of Cu and Mo, while Ba, Co and Ni have no definite RDA (Table 5), however, they do have considerable biological value. Barium role in human it as yet not much clear although there are some reports demonstrating that Barium can cause stomach and intestinal problems and muscular weakness in people exposed to its high concentration (ATSDR, 2007). Cobalt has both beneficial and harmful effects; it is a part of vitamin B12, which is essential for various biological functions. Serious adverse effects have been reported on the lungs, including asthma, pneumonia and wheezing, have been found in people exposed to high concentration of cobalt (ATSDR, 2004). Copper is a critical functional component of several essential enzymes known as cuproenzymes, and its deficiency can produce anaemia-like symptoms, bone abnormalities, hypo pigmentation, impaired growth, and increased incidence of infections, osteoporosis, hyperthyroidism, and abnormalities in glucose and cholesterol metabolism. Conversely, Wilson’s disease causes accumulation of copper in body tissues (Prohaska JR, 2011). Molybdenum is an essential component of various enzymes and disruption in its metabolism leads to molybdoenzymes improper function (Wuebbens et al., 2000). Contact with Nickel compounds can cause a variety of adverse effects on human health. The most important and frequent are nickel allergy in the form of contact dermatitis, lung fibrosis, cardiovascular, kidney diseases, lung and nasal cancers. In 2008, nickel received the name of the “Allergen of the Year” (Aleksandra and Urszula, 2008). More recent evidence indicates that Ni is required in small amounts for normal plant growth and development. Ni is an essential component of urease in plants and microorganisms (Dalton et al., 1988).

Table 5 Recommended Dietary Allowances (RDAs) and Upper Level of intake (UL) (mg/day) of different elements for humans

Element	Male	Female	UL (mg/day)
Barium	NA	NA	NA
Boron	NA	NA	20

Beryllium	NA	NA	NA
Bismuth	NA	NA	NA
Calcium	1000	1000	2500
Cobalt	NA	NA	NA
Chromium	0.035	0.025	NA
Copper	0.90	0.90	10
Iron	8.0	18.0	45
Magnesium	420.0	320.0	350
Manganese	2.30	1.80	11
Molybdenum	0.045	0.045	2
Nickel	NA	NA	1
Selenium	0.055	0.055	0.4
Silver	NA	NA	NA
Strontium	NA	NA	NA
Zinc	11.0	8.0	40

Source: Annette Dickinson. Council for Responsible Nutrition, 2002.
NA-Not Available

Our results illustrated that the rhizome of *Z. officinale* lacks Be, Bi and Se. Selenium has an important role in thyroid hormone metabolism, as an essential component of the three deiodinase. Also the selenium deficiency has been reported to be correlated with thyroid dysfunctions (Miniero et al., 1998). It is obvious from above discussion that minerals both micro and macro play a vital role in variety of functions in human body and a proper balance has to be maintained for the health. Insufficient intake or excess intake of any of them can have calamitous effects on health. Other elements such as As, Cd, Hg and Pb, which are not used directly by the plants and are detrimental to human health, also accumulate in these plants (Baker et al., 1989; Lasisi et al., 2005, Annan et al., 2013). More importantly they exert diverse toxicological effects in animal and human systems. Our results demonstrated that *Z. officinale* rhizome showed no concentration of As and Hg, however, Cd (0.01 mg/100g) and Pb (0.2 mg/100g) were detected but it was found below the permissible limit of 0.03 mg/100g and 1.0 mg/100g respectively, as prescribed by WHO (WHO, 1998). Cadmium is primarily toxic to the kidney, especially to the proximal tubular cells, the main site of accumulation. Cd can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction (Bernard A., 2008). Pb is a highly toxic metal and it affects the central and peripheral nervous systems, renal function, and the vascular system. The toxic effects of lead vary greatly, manifesting as subtle changes in neurocognitive function in low-level exposure or as the potentially fatal encephalopathy of acute lead poisoning (Needleman, 2004). The present study screening of *Z. officinale* indicated that its rhizome contained cadmium and lead but within the permissible limit, however, with increasing use of cadmium and lead chances of their contamination in earth crust are more and therefore continuous screening of the plants is required.

Conclusion

On the basis of above study it may be concluded that *Z. officinale* rhizome has higher concentrations of magnesium, calcium and iron with traces of other elements. Moreover, cadmium and lead were also present but within the limit specified by WHO. *Z. officinale* rhizome has valuable nutritive elemental profile and can be a source of balanced elemental requirement of diet albeit it should be tested for toxic metals load before processing it further for medication.

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