Toxic Trace Elements in Selected Edible Rhizomes of Medicinal Plants Using INAA and ICP-MS Techniques

Abstract

Consumption the crop is one of the main sources of dietary exposure to toxic trace elements. In order to assess the level of toxic trace elements from selected herbs and to make an assumption on their consumption level of concern, fourteen elements in *Zingiber zerumbet* (lempoyang), *Boesenbergia rotunda* (temu kunci), *Zingiber officinale* var rubrum (halia bara) and *Zingiber officinale roscoe* (halia) have been determined via ICP-MS (Cd, Be, Ti, Pb) and INAA (As, Al, Ba, Cr, Co, Sb, Sr, Th, U and V) methods. It was revealed that the concentrations of these elements in most selected rhizomes did not exceed the standard dangerous toxic level. However, 47% cadmium accumulationa was detected in *Zingiber officinale* var rubrum grown in poly-bags.

Keywords: Toxic Elements; Herbs; Rhizomes; INAA; ICP-MS

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Abbreviation: Cd: Cadmium; Be: Beryllium; Ti: Thallium; Pb: Lead; As: Arsenic: Al: Aluminum; Ba: Barium; Cr: Chromium; Co: Cobalt; Sb: Antimony; Sr: Strontium; Th: Thorium; U: Uranium; V: Vanadium; MRL: Minimal risk level; INAA: Instrumental Neutron Activation Analysis; ICP-MS: Inductively Coupled Plasma/Mass Spectrometry

Introduction

The interaction between chemical extracted compounds from plants and the human body go through the same identical process to those well understood for the chemical compounds in conventional drugs. Similarly, the herbal medicine may cause the same potential to cause a side effect [1]. *Zingiber zerumbet* (lempoyang), *Boesenbergia rotunda* (temu kunci), *Zingiber officinale* var. rubrum (halia bara) and *Zingiber officinale roscoe* are belonging to Zingiberaceae or Ginger family consisting of aromatic perennial herbs with creeping horizontal or tuberous rhizomes planted locally. The same species can be found throughout tropical Africa, Asia, and the America. These rhizomes are a common edible ingredient in many countries, in South East Asia and serve as culinary herbs due to their aromatic flavor to promote appetite.

Their popularities as folk medicine have drawn further interest amongst researchers to investigate on various perspectives about their medicinal properties. Eng-Chong et al. [2], Sivasothy et al. [3] & Sontakke et al. [4] have been reported on medicinal crop areas. A list of possible treatments for illnesses using the described rhizomes is shown in Table 1.

Trace elements may take by medicinal plants from the surrounding mineral or contaminated soil environment via the root system and translocation within at various part of the plant such as in the rhizome, stems, and leaves. Some plants can absorb trace elements from the soil in high quantity and do not present toxicity symptoms or have their growth affected. These plants are suitable for remediation of polluted soils known as phytoremediation [5]. Accumulation of toxic trace element above minimal risk level (MRL) value or provisional tolerable index level, ingested by humans for a long term, will give an adverse effect to human health depending on the type of trace elements present.

Table 1: Medicinal plants and its uses.

Species	Parts used	Treatment of
Boesenbergia rotunda	Swollen roots and leaves	Rheumatism, pain killer, gout, gastrointestinal disorders, stomach ache, dyspepsia and peptic ulcer [2]
	Rhizome	Anti cancer [12]
		wound healing [12]
Zingiber zerumbet	Rhizome	Inflammation and pain-mediated diseases, worm infestation and diarrhea [3]
		Anti cancer [13]
Zingiber officinale var. rubrum	Rhizome	Anti cancer, nausea, antibacterial activity [14]
Zingiber officinale Roscoe	Rhizome	Nausea (seasickness), morning sickness and [4, 15]

The present study was undertaken to determine the level of toxic trace elements found in the rhizome samples and to make an assumption on their consumption level of concern if ingested by an adult based on the MRL tabulated by the Agency for Toxic Substances and Disease Registry [6].

Materials and Method

The rhizomes of four species, namely *Z.zerumbet* (A), *B.rotunda* (B, C)**,** *Z.officinale* var. rubrum (D, E) and *Z.officinale Roscoe* (F) were collected from the surrounding area in the University of Malaya. The *B.rotunda* was separated into a center piece or the top part and the tubular shape and *Z.officinale* var. rubrum were taken from the wild and planted in polybag condition.

The samples were pre-rinsed with deionized water and dried at 39 °C in the oven for five hours. The dried samples were powdered using mortar and pestle to make a dried rhizome powder. The powders were sieved using a standard set of sieves. About 15- 100 g of grounded sample was weighed and sealed in the polyethylene vials and tagged as A, B, C, D, E and F according to the species and their origin. A duplicate was used up for each analyte sample and blank for error correction.

Standard coal ash SRM – 1632a based on (NIST) [7] was employed as a multielement comparison standard. The Analyte and standard samples were stacked up in a rotary rack at different positions ready for irradiation.

Irradiation and counting

The irradiation facility was carried out by TRIGA MK II (Malaysian Institute of Nuclear Technology, MINT) for 6 hours at a thermal flux (2×10^{12} n cm^{-2} Sec^{-1}). After irradiation, the vial is returned from the reactor by atmospheric pressure and allowed to decay to the level of activity is within acceptable limits for handling. Cooling time was arranged according to the nuclear properties of radioisotopes. Medium-lived species were counted after four days of cooling while a decay time of nearly a month was set aside for a long-lived isotope. Each sample was placed in the appropriate position on the detector for counting.

For the gamma spectra measurement, a solid state horizontal hyperpure germanium (HPGe) detector was used. A nuclear Data 66 multichannel analyzer was used for pulse height analysis. The gamma spectra analyses were carried out by using the software Genie 2000 and gamma vision supplied by ORTEC and Canberra, respectively.

Heavy metals and non-measurable by Instrumental Neutron Activation Analysis (INAA), such as Pb, Be, Tl, and Cd were analyzed using a Perkin-Elmer SCIEX Elan 6000 Inductively Coupled Plasma/Mass Spectrometry (ICP-MS).

The dry duplicate sample was weighed (0.5g) and transferred into a beaker (10mL) followed by concentrated HNO_3 (5 mL). The sample was pre-digested for one hour, and the beaker was heated up on a hot plate at 60-70 °C.

A 2mL of HNO_3 and 3 mL of $HClO_4$ were dropped slowly until the solution becomes clear and dissolved. The solution was filtered out using filter paper then was added up with deionized water (30mL). The blank was prepared using the same reagents undergoing the similar digestion procedure. If necessary, the sample and blank were diluted accordingly prior analysis. Moreover, enough samples based on IAEA-SOIL-7 were digested with some digestion groups to assure the accuracy of the analytical procedures.

The elemental compositions for the four selected rhizome species, determined by ICP-MS and INAA are shown in Table 2. The samples are labeled as A, B, C, D, E and F with all concentrations are expressed in parts per million (ppm). A total of 14 elements was detected. The Cd, Be, Tl and Pb by ICP-MS, and As, Al, Ba, Cr, Co, Sb, Sr, Th, U and V were revealed by INAA technique.

Result and Discussion

The high concentration of aluminum (>200ppm) was observed in *Z.zerumbet* and *B.rotunda* in center piece and tubular shape. However, the trace amount was recorded in *Z.officinale* var rubrum (polybag and wild environment) and Al ions in *Z.officinale Roscoe* translucent very slowly to the upper parts of plants [8]. The distribution of toxic trace elements in the studied rhizomes showed that the Al and Ba are found to be present at the major level, Cr, Pb, and Tl at the minor level and Cd, Co, Sb, Sr, Th, U and V at the trace level. Most plants contain no more than 0.2mg Al g^{-1} dry mass, and this caused that the rhizomes of sample A, B and C is not an Al accumulator.

B. rotunda sp. (samples B and C) was accumulated with a higher mean of Ba concentration compared to *Z.officinale* var rubrum sp. (samples D and E). A little amount was detected from *Z.zerumbet* (sample A). Sample D was recorded rich of Cd, Tl, As, Ba and Cr compare to other specimens. Even compared to the sibling sample E, the ratio of these toxic minerals can go from 1.5-8 folds higher. In a contrary, sample E absorbed more Pb and Sr. Since sample D and E grew in different cultures thus the sources of toxic elements up taken by the plants may come from the soil compost, the fertilizer or the polybag. Heavy metals are often used in pigments or as stabilizers in polymer materials. Thus, polymers inevitably contain hazardous metals or toxic elements such as As, Cd, Cr and Zn to a certain degree [9].

Similarly, sample B showed higher concentrations of Pb and Ba over sample C that in contrary marks higher concentration in Cr. It led to Pb and Ba translocated at the centerpiece of the rhizome while Cr resides more in the tuberous shape. Other elements observed a balanced distribution throughout the rhizome of *B.rotunda* sp.

The Sr was only detected in sample E and F. Concentration of Be, Sb and V were below the detection level (undetected) for all samples. Comparatively, sample A, B and C contain a high amount of toxic trace mineral observed, whereas sample D, E and F are due to the negligible contribution from Al.

The estimation of tolerable dietary intake of the selected rhizomes was calculated based on the published data from [6] & [10]. According to the guideline for the medicinal plant [10], the concentrations of As, Cd and Pb do not exceed the limits of 1, 0.3 and 10ppm respectively. Based on Table 2&3, the concentration of As, Cd and Pb were measured below the recommended limit except in sample D where it registered 47% higher for Cd.

Table 2: Result for samples analyzed using ICP-MS and INAA techniques.

	Elements	Concentration (ppm)					
		A	B	C	D	E	F
ICP-MS method	Cd	0.02 ± 0.01	0.04 ±0.02	0.04 ± 0.01	0.44 ± 0.01	0.10 ± 0.01	0.22 ± 0.01
	Be	<0.01	<0.01	<0.01	<0.01	0.02 ± 0.01	<0.01
	Tl	<0.01	0.02 ± 0.01	<0.01	0.07 ± 0.01	0.02 ± 0.01	<0.01
	Pb	1.04 ± 0.10	1.45 ± 0.07	0.66 ± 0.07	0.62 ± 0.20	2.29 ± 0.11	1.07 ± 0.12
INAA method	As	0.09 ± 0.01	0.07 ± 0.01	0.13 ± 0.01	0.40 ± 0.02	<0.05	0.05 ± 0.01
	Al	254 ± 40	242 ± 35	213 ± 54	<1.0	<1.0	<1.0
	Ba	<1.0	23.7 ± 0.7	8.74 ± 2.01	14.7 ± 1.6	10.1 ± 1.9	4.05 ± 0.81
	Cr	1.52 ± 0.94	1.55 ± 0.21	3.44 ± 0.28	3.84 ± 0.43	0.54 ± 0.09	0.38 ± 0.08
	Co	0.61 ± 0.05	0.23 ± 0.01	0.20 ± 0.01	0.27 ± 0.01	0.36 ± 0.01	<0.1
	Sb	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	Sr	<1.0	<1.0	<1.0	<1.0	10.1 ± 1.0	9.3 ± 1.0
	Th	0.26 ± 0.01	0.99 ± 0.19	1.21 ± 0.07	0.55 ± 0.02	0.62 ± 0.08	1.03 ± 0.03
	U	<0.05	0.09 ± 0.02	0.11 ± 0.01	0.05 ± 0.02	<0.05	<0.05
	V	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

A: Z.zerumbet (lempoyang); B: B.rotunda (temu kunci)- centre piece; C: Boesenbergia rotunda (temu kunci)- tubular shape; D: Z.officinale var rubrum (halia bara) - polybag environment; E: Z.officinale var rubrum (halia bara)- wild environment; F: Z.officinale Roscoe (halia)

Table 3: Estimation of tolerable dietary intake of rhizomes.

Elements	ATSDR (MRL)[a]	@ 60 Kg BW	WHO (1999)[b]	Consumption Level of Concern
	ppm BW/day	mg/day	ppm	g/day (sample)
Cd	0.0005 (Int.)	0.03	0.3	68 (D)
Be	0.002 (Chr.)	0.12		6000 (E)
Tl	0.002	0.12		1714 (D)
Pb	0.007[c]	0.42	10	183 (E)
As	0.005 (Acute)	0.3	1	750 (C)
Al	1 (Int.)	60		236 (A)
Ba	0.2 (Int.)	12		506 (B)
Cr	0.005 (Int.)	0.3		78 (D)
Co	0.01 Int.)	0.6		983 (A)
Sb	0.0004 (	0.024		480 ()
Sr	2 (Int.)	120		1200 (E)
Th	15 pCi/L[d]	0.13		109 (C)
U	0.002 (Acute)	0.12		1090 (C)
V	0.01 (Int.)	0.6		6000 ()

For Duration, Acute = 1 to 14 days, Intermediate = 15 to 364 days, and Chronic = 1 year or longer.
[a][6], [b][10], [c][16], [d][17]

The minimal risk level (MRL) tabulated by ATSDR was used to estimate the number of rhizomes may be consumed per day. For example, the MRL of Cd taken orally continuously for 15 days to a year without giving adverse health effect is 0.0005 ppm BW/day. Assuming an adult of 60 kg BW may require 0.03 mg of the Cd/day for biosystem sustainability function. The consumption level of concern for Cd was calculated from the ratio of adult MRL to the maximum value in Cd column detected from the samples. As shown in Table 2, sample D was recorded the highest concentration of Cd, and the tolerable dietary intake of rhizome D was only 68g. Similarly, the MRL for Cr was attained when someone ingested a dry weight of 78 g of the same rhizome.

An herbal infusion of these rhizomes may dilute the concentration of toxic trace element to some extent due to low extraction efficiency and in some cases the inorganic toxic trace element such as were not detected in water infusion [11].

Conclusion

The obtained amount of toxic trace elements in medicinal rhizomes is being less than the permissible levels allowed by the MRL standard of ATSDR. Cadmium, Tellurium, Arsenic, and Chromium showed high concentration in *Z. officinale* var rubrum (growth in polybag environment). However, the level of Cadmium detected higher than the recommended level. In *Z.officinale* var rubrum (growth in the wild environment) the same pattern did not copy. It could be a coincidence the sample that grown in Agra-polybag may contain specific toxic trace element used for strengthening and stabilizing poly bags.

Ingestion of these rhizomes as a medicinal herb is considered safe as along as the period of ingestion is kept short and with the quantified amount. Water infusion further reduced the risk of metal toxic poisoning possibly due to low extraction efficiency.

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