한국환경과학회지 제16권(제1호), 21~26, 2007
*Journal of the Environmental Sciences*

# Determination of Mineral and Trace Elements in *Ganoderma Lucidum* Consumed in China, Vietnam and Korea

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*(Manuscript received 5 January, 2007; accepted 20 January, 2007)*

The concentrations of fourteen mineral and trace elements (Al, Ca, Fe, K, Mg, Se, Ba, Co, Cu, V, Pb, Hg, Cd and As) were determined in Ganoderma Lucidum and their infusions consumed for medical purposes collected from Vietnam, China and some places in Korea. Concentrated acid digestion procedure was applied under optimized conditions for dissolution of these medicinal fungi. Element concentrations in these fungi and their infusions were then determined by ICP-AES. The mineral and trace element content of these samples and their infusions showed a wide variability. However, distribution of some elements in the infusions is not high.

Key Words : Ganoderma lucidum, ICP-AES, Mineral and trace elements, Medicinal herbs

## 1. Introduction

Interest in traditional medicine has increased over the last decades and seems likely to continue to do so in the future. People now are more prepared to look for alternative approaches to maintain and promote their health. With the increase of public demand, governments have begun to pay more attention to traditional medicine and many areas in the world they have shown a willingness to promote its proper use and to try to integrate it into formal health-care systems. The integration of traditional medicine into the mainstream health-care delivery system is a challenge for countries and areas where allopathic health-care predominates. Some traditional medicine systems are highly developed and well documented. They are based on systematized knowledge, a comprehensive methodology and rich clinical experience. Traditional Chinese medicine falls into this category. It originated in China and was later introduced into neighbouring countries such as Japan, Vietnam, the republic of Korea and others, which then developed their own variations[1]. In the Chinese culture, many medicinal materials and health products were used. For example, reishi (Ganoderma lucidum) is a type of fungi that is widely used as medicine in anticancer and immunomodulatory therapy. Since on of the proteins in reishi (LZ-8) has been shown to have immunnomodulatory effects, it is also used as a raw material for many health products[2,3].

Reishi, already mentioned as a sacred mushroom in ancient China, has come to occupy a leading place in present-day medicinal mushroom development. The market values of reishi-based natural healthcare products in 1995 were estimated as 215 millions USD in Taiwan,350 millions USD in China, 600 millions USD in Korea, and 350 millions USD in Japan reference[4]. The physiologically active substances of reishi are water-soluble polysaccharides and alcohol- soluble triterpenoids. Today, 119 different triterpenoids are identified in reishi, about 80 of which are biologically active. Reishi dietary supplements are valued for their immunomodulating, anticancer, antiviral properties. They are used during remission of cancer and by hepatitis B patients. They also have anti-hyperlipidemic, hypotensive, and hypoglycemic actions.

The 1995 production of reishi in Japan was estimated to about 500 tons dry weight. Reishi cultivation has also prospered in China, Taiwan, Korea, Thailand and Vietnam. In addition, attempts are being made to obtain useful cellular materials or to

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produce effective substances from cultures mycelia[5].

Because of the increasing interest in traditional Chinese medicine products such as reishi, it is important to determine whether they are safe for consumption. Levels of toxic elements such as As, Hg, and Pb in the plant samples must be determined. Furthermore, some common elements such as K, Ca, Fe are essential for health and the quantification of these elements in the plant is important for nutrition purposes.

The objective of this study is to establish the levels of some mineral and trace elements (Al, Ca, Fe, K, Mg, Se, Ba, Co, Cu, V, Pb, Hg, Cd and As) in some samples of Ganoderma lucidum and their infusions collected in China, Vietnam and Korea.

## 2. Materials and Methods

### 2.1. Standard reference materials, samples and reagents

Unless stated otherwise, all chemicals of the highest purity were used and they were purchased from Daejung Chemicals & Metals Co. Ltd, Korea. For preparation of all solutions and samples, high purity water from a Mili-Q system (Millipore, Milford, USA) was used. $HNO_3$, $H_2SO_4$, $H_2O_2$ are analytical grade. During the experiments, all glassware and equipment were carefully cleaned starting with 2-4% $HNO_3$ and ending with repeated rinsing distilled deionized water to prevent contamination.

The calibration curves were made from a multi-element standard solution (Plasma CAL, SCP Science, USA). Nine reishi samples were purchased from different local pharmaceutical stores in Korea, China and Vietnam. The nine samples were either black or brown in color.

The nine reishi samples were numbered from Sp-1 to Sp-9, respectively. The five samples from 1 to 5 in which samples number 4 and 5 are natural Korean reishi, samples 1 to 3 are cultured Korean reishi and all of them were purchased from some oriental herb stores in Gyeongsang-Buk province, Korea. Sample number 6 is Chinese reishi was collected in Vietnam. Sample number 7 is Chinese reishi, and sample number 9 is Korean reishi, both were purchased from Daegu Haany University Oriental Hospital, Daegu, Korea. Sample 8 is Vietnamese reishi and was supplied by Vietnamese Agricultural Genetic Research Institute, Hanoi, Vietnam.

### 2.2. Apparatus

A Thermo-inductively coupled plasma with the next generation CID high frequency plasma luminous spectroscopic analysis device IRIS was used for simultaneous multi-element detection of Al, Ca, Fe, K, Mg, Se, Ba, Co, Cu, V, Pb, Hg, Cd and As. Instrument configuration and general experimental conditions for ICP-AES are given in Table 1 below.

### 2.3. Procedures

#### 2.3.1. Sample preparation

Weight 0.50g sample to the beaker, add 5ml nitric acid and 3ml sulphuric acid (analytical grade), cover with a watch-glass and leave to cold soak for approximately 30 min, then place the beaker into an aluminium block. Raise the temperature steadily up to 120°C and heat with reflux for 2 hours. Remove the beaker from the block and cool to room temperature. Add 10ml $H_2O_2$ to the beaker and heat with reflux for more 2 hours then cool down to room temperature. Dilute the digest to 50ml volume with deionized water. Shake the solution by a mini shaker for 2 minutes and transfer to the sample tube. The resulting solution is then ready for ICP-AES analysis. Each sample was prepared three times and measured three times. The mean concentration was reported.

#### 2.3.2. Infusion preparation

To prepare the infusion of the samples, 0.50g dried sample was placed in a beaker, then 25ml of deionized water at 95°C was poured into the same

Table 1. ICP-AES operating conditions for determination of some elements in reishi

| Items | Condition |
|---|---|
| Rf power(W) | 115 |
| Gas flow rate (ml/min) | |
| Coolant gas | 15 |
| Auxiliary gas | 0.5 |
| Nebuliser gas | 0.5 |
| Nebuliser pump | |
| Flush pump rate (ml/min) | 2.03 |
| Analysis pump rate (ml/min) | 2.03 |
| Pump relaxation time (sec) | 5 |
| Pump tubing type | Tygon-Orange |
| Nebuliser flow (PSI) | 20.1 |

beaker and it was kept in water bath for 30 min. After cooling, the extract was filtered through the Whatman No 541 filter paper and the volume of infusion was filled up to 50ml with deionized water. The resulting solution is then ready for ICP-AES analysis. Each sample was prepared three times and measured three times. The mean concentration was reported.

## 3. Results and Discussion

In the present work, fourteen elements were determined in the nine samples numbered from Sp-1 to Sp-9 respectively. In the samples, as well as the infusions and blanks, the quantitative determinations were carried out by a calibration curve obtained by using multi-element standard solutions in which the concentrations of the elements were in the optimal measurement range (0.1, 1, 10 ppm).

The concentrations of fourteen elements determined in nine samples and their infusions are collectively listed in Table 2. It is also the percentage of each element reporting to the infusion. It was observed that all samples contain significant value of elements and their infusions presented a wide variability.

In spite of high content Al(Aluminium) in all samples percent Al reported to infusions was very low or not detected, except for infusion 3. The Al contents are especially high in sample 4 and sample 5 which are natural reishi. Most investigated foodstuff (vegetables, meat and dairy products) contained less than 5mg/kg of Al and high concentrations were determined in cocoa products (33mg/kg), spices (145mg/kg) and black tea leaves (899mg/kg). In general, the Al content of frequently consumed food increased in the following order: beverages>food of animal origin>food of plant origin but studies including Al are rare[6,7]. The Al content ranged from 6.90 mg/kg to 41.70 mg/kg.

Calcium(Ca) content in all samples and infusions was very high. Ca in the nine samples and their infusions was in the range of 523-2398.4 mg/kg and 386.7-637.3 mg/kg, respectively. The highest Ca content was determined in sample 4 and 5 but their infusions were determined the lowest. Chan and Lo[8] determined the Ca content in reishi in the range 748-1637 mg/kg. Iron(Fe) content of the samples and their infusions was in the wide range of 8.47-240.88 mg/kg and 0.79-36.3 mg/kg, respectively. Fe concentration found in all samples was lower than those reported by Chan and Lo[8], who determined Fe content in the range 63.9-123.9 mg/kg.

Magnesium(Mg) in the nine samples and their infusions was in the range of 299.5-785.15 and 99.03-422.8 mg/kg, respectively. Mg concentration in natural samples was determined higher than those in cultured samples but percent Mg reported to infusion was in the contrary. And Mg concentration found for all samples was similar to those reported by Chan and Lo[8], who determined Mg content in the range of 418-775 mg/kg.

Potassium(K) concentration in all samples and their infusion was particularly high. K content of the samples and their infusions was in the range of 653.8-3354.6 mg/kg and 570.6-2761.5 mg/kg, in turn. Copper(Cu) content in all samples and their infusions was determined in the range of 2.48-18.78 mg/kg and 0.24-1.13 mg/kg, respectively. The quantity of Cu in infusions was very low to those in the samples. For all of the samples, Barium(Ba) concentration in the samples varied between 1.82 mg/kg to 14.6 mg/kg, whereas infusions contained this metal in the range of 0.53-5.37 mg/kg.

Selenium (Se) concentration in nine samples and their infusions was in the range of 0.02-2.22 mg/kg and 0.01-0.17 mg/kg, respectively. Se content found for all samples was higher than those reported by Yang-ying Chan et al, who determined Se content in the range of 0.043-0.076 mg/kg. Hg and As were not detected in these nine samples. Chan and Lo[8], determined Hg and As in some Reishi samples by using ICP-MS. These concentrations were in the range of 0.003-0.037 mg/kg and 0.021-0.085 mg/kg, respectively.

Vanadium (V) content in the samples was in the wide range of 0.0-10.66 mg/kg. V content found in some samples was higher than those reported by Yang-ying Chan et al, who determined V content in the range of 0.012-0.098 mg/kg.

Cobalt, Lead and Cadmium (Co, Pb and Cd) were detected in the range of 0.0-0.26 mg/kg, 0.38-2.24 mg/kg and 0.0-0.775 mg/kg, respectively.

Previous studies have shown that some of the 14 elements such as Ca, Mg and K are necessary to human

Table 2. Concentration of elements in samples of *Ganoderma Lucidum* and their infusions (mean ± S.D mg/kg, $n$=3), and the percent of element in the samples reported to infusion ($n$: number of assay for each of sample; ND: Not detected)

| Samples / mg/kg | | Sp-1 | Sp-2 | Sp-3 | Sp-4 | Sp-5 | Sp-6 | Sp-7 | Sp-8 | Sp-9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Al | Contents | 21.70±1.68 | 41.70±0.18 | 11.44±1.43 | 272.69±23.41 | 269.59±12.21 | 10.24±2.63 | 17.16±0.00 | 6.90±4.54 | 14.62±0.32 |
| | Infusion | ND | ND | 7.63±0.63 | ND | 10.04±5.27 | ND | ND | ND | ND |
| | % in infusion | - | - | 66.69 | - | 3.72 | - | - | - | - |
| Ca | Contents | 865.55±2.14 | 1170.95±15.94 | 861.50±1.89 | 2398.45±0.57 | 2028.45±15.44 | 523±9.49 | 420.7±64.49 | 1110.45±1.44 | 882.40±3.89 |
| | Infusion | 602.24±21.36 | 637.34±2.96 | 509.69±47.11 | 185.09±47.01 | 179.29±78.51 | 386.69±0.61 | 364.09±23.31 | 421.54±33.86 | 450.89±38.01 |
| | % in infusion | 69.57 | 54.43 | 59.16 | 7.72 | 8.84 | 73.94 | 86.54 | 37.96 | 51.1 |
| Fe | Contents | 49.75±5.42 | 61.81±2.51 | 44.87±1.04 | 230.88±19.80 | 240.88±20.10 | 31.45±2.08 | 19.58±2.57 | 43.57±5.94 | 8.47±5.45 |
| | Infusion | 0.79±0.19 | 36.30±6.30 | 4.40±1.40 | 8.27±1.05 | 9.57±5.94 | 8.53±2.53 | ND | 2.86±1.86 | 4.17±1.03 |
| | % in infusion | 1.6 | 58.73 | 9.8 | 3.58 | 3.97 | 27.12 | - | 6.56 | 49.23 |
| K | Contents | 653.88±7.46 | 2361.58±15.95 | 1867.08±22.45 | 1441.58±8.95 | 2459.08±15.45 | 1744.08±49.45 | 2252.08±149.5 | 3354.58±15.95 | 2633.6±91.95 |
| | Infusion | 570.59±2.73 | 2026.54±0.53 | 1592.04±95.98 | 1007.04±8.98 | 1746.54±67.48 | 1385.04±77.98 | 1936.54±269.5 | 2761.54±59.48 | 1439.04±112 |
| | % in infusion | 87.26 | 85.81 | 85.27 | 69.86 | 71.02 | 79.41 | 85.99 | 82.82 | 54.64 |
| Mg | Contents | 396.20±2.96 | 446.65±12.81 | 345.95±5.41 | 785.15±4.31 | 700.75±9.41 | 391.15±8.41 | 435.55±15.51 | 299.55±0.5 | 348.35±3.31 |
| | Infusion | 302.13±0.95 | 330.33±1.45 | 262.38±17.2 | 99.03±23.36 | 124.93±49.86 | 295.28±5.7 | 422.78±0.0 | 219.33±13.75 | 205.17±16.89 |
| | % in infusion | 72.26 | 73.96 | 75.84 | 12.61 | 17.83 | 75.5 | 97.07 | 73.22 | 58.9 |
| Se | Contents | 0.15±0.01 | 2.22±0.24 | 0.18±0.02 | 2.65±1.24 | 0.02±0.01 | 0.71±0.61 | 1.13±0.04 | 2.02±0.54 | 3.22±0.24 |
| | Infusion | 0.02±0.005 | 0.17±0.05 | 0.10±0 | 0.17±0 | 0.01±0 | 0.07±0 | 0.15±0.03 | 0.13±0.01 | 0.13±0.10 |
| | % in infusion | 13.33 | 7.66 | 55.56 | 6.64 | 50 | 9.86 | 13.27 | 6.44 | 4.04 |
| Ba | Contents | 7.31±0.88 | 10.98±3.38 | 9.64±1.96 | 14.60±0.23 | 7.28±1.54 | 1.88±0.21 | 2.03±1.79 | 1.82±0.09 | 4.47±1.46 |
| | Infusion | 3.37±0.88 | 5.37±0.02 | 4.87±1.09 | 0.80±0.13 | 0.53±0.27 | 0.32±0.03 | 1.99±0 | 1.56±0.25 | 4.17±0.75 |
| | % in infusion | 46.1 | 48.9 | 50.52 | 5.48 | 7.28 | 17.02 | 98.03 | 85.71 | 93.29 |
| Co | Contents | 0.03±0 | 0.06±0.01 | ND | 0.26±0.02 | 0.21±0.01 | 0.05±0 | 0.04±0 | 0.04±0 | 0.03±0.01 |
| | Infusion | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | % in infusion | - | - | - | - | - | - | - | - | - |
| Cu | Contents | 2.48±0.11 | 4.48±0 | 7.21±0 | 2.36±0.16 | 18.78±0.13 | 5.17±2.0 | 3.70±0.06 | 12.48±0.43 | 4.30±0.12 |
| | Infusion | 1.13±0.88 | 0.25±0.06 | 0.56±0.25 | 0.19±0 | 1.00±0.08 | 1.75±0 | 0.25±0 | 0.39±0.12 | 0.24±0.02 |
| | % in infusion | 45.56 | 5.58 | 7.77 | 8.05 | 5.32 | 33.85 | 6.76 | 3.13 | 5.58 |
| V | Contents | ND | 3.92±0.79 | 1.32±79 | 7.03±1.31 | 4.43±0 | ND | 10.66±1.81 | 6.77±5.71 | 9.37±5.71 |
| | Infusion | ND | 0.79±0.01 | 0.28±0.01 | ND | ND | ND | 3.39±0.01 | 1.66±0.32 | 6.52±3.89 |
| | % in infusion | - | 20.15 | 21.21 | - | - | - | 31.8 | 24.52 | 69.58 |
| Pb | Contents | 0.685±0.005 | 0.7±0.55 | 0.605±0.605 | 2.245±0.165 | 1.4±0.22 | 1.13±0.05 | 0.375±0.075 | 0.495±0.055 | 1.61±0.04 |
| Hg | Contents | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| As | Contents | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Cd | Contents | ND | 0.65±0.25 | 0.35±0.05 | 0.775±0.015 | 0.375±0.02 | 0.075±0.005 | 0.095±0.025 | 0.105±0.005 | 0.035±0.005 |

health, whereas others have been shown to be toxic, such as Pb, Cd and Al. The other elements are not toxic to human unless they are present in high concentrations. There are no standards for medical raw plant materials, which establish a permissible level of metals in such materials. The World Health Organization mentions maximum permissible levels in raw plant materials only for arsenic, cadmium and lead which amount to 1.0, 0.3 and 10 mg/kg, respectively[9-11].

It must be noted that all of the samples considered in this study are not cooked or consumed directly but are prepared as hot beverages such as tea. In spite of the fact that concentration of the elements such as Ca, K, Mg and Al in the samples seems high, they do not completely report to infusion. Therefore, rather than the concentrations in the samples those in infusions are more significant for taking into consideration the daily uptake.

The level of element concentration in infusions may be affected from a number of parameters such as organic matrix of corresponding sample, original mineral content and natural pH of water used to prepare the infusion. In addition, since no any further acidic or basic reagent treatment was applied to the infusions, solubility characteristics of mineral and organic matrix of the fungi in water maybe the most important one.

In order to discuss the contributions of the different herbal beverages on the average daily dietary intake (ADDIs) for the studied elements Table 3 was given.

When the intake values listed in Table 3 were compared with those given in Table 2 depending on the metal levels of infusions, reishi tea infusions may be a good source of essential elements. However, consumption rate of this herbal tea should be under strict control. Toxic elements such as Pb, Cd, Co, Hg and As either were in low concentration or not found in the infusions. Nevertheless, it must be realized that, in Table 2, the element concentrations in the fungi and their infusions were given for 1 kg of the corresponding fungi. Normally, it is not expected that one directly consumes 1 kg of any fungi in a day. These medicinal fungi are generally used in average 2-5g. This means that only 10g of these fungi is to be consumed in several times a day and therefore ADDI is never reached and there is no danger mainly for toxic elements originating from these medicinal fungi.

Table 3. Daily need of the world's daily average uptake of elements by a person weighing 70 kg[12]

| Elements | ADDIs mg/day (Range) |
|---|---|
| Ca | 1000 (800-1200) |
| Mg | 350 (300-400) |
| Fe | 15 (10-28) |
| Al | 5 (2-10) |
| Cu | 2.5 (2-3) |
| Ba | 1.1 (0.65-1.7) |
| Co | 0.04 |
| Pb | 0.415 |
| Cd | 0.057 |
| K | 3800 (1900-5600) |

## 4. Conclusions

A method for detection of fourteen elements (Al, Ca, Fe, K, Mg, Se, Ba, Co, Cu, V, Pb, Hg, Cd and As) in the medicinal fungi reishi collected in China, Vietnam and Korea was established.

This study demonstrated that all the samples of reishi widely used in China, Vietnam and Korea did not contain toxic elements and their infusions might be a good source of essential elements.

## Acknowledgements

The authors also would like to thank Vietnamese Agricultural Genetic Research Institute for their supplying the reishi samples.

This work was supported by the Regional Innovation Center Program of the Ministry of Commerce, Industry and Energy through the Research Center for Biomedical Resources of Oriental Medicine at Daegu Haany University, Korea.

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