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Investigation of Heavy Metal Contents in Thyme *(Thymus vulgaris)* and Ginger (*Zingiber officinale)* Sold in Bingöl Herbalists

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Abstract

Objective: In our country, which is rich in medicinal plant diversity, there is an increase in heavy metal accumulation with the increase of industrial development and environmental pollution. The aim is to evaluate the health safety of heavy metal content of thyme and ginger plants, which are frequently used for therapeutic purposes, sold in herbalists.

Methods: In this study, heavy metal (Cr, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) levels in thyme (Thymus vulgaris) and ginger (Zingiber officinale) plant samples obtained from three different herbalists in Bingöl were determined by ICP-MS.

Results: Fe, Zn, Cd and Pb levels in both thyme and ginger samples, Cr levels in thyme samples and Cu levels in ginger samples were above the safe limit values for health. In the thyme and ginger samples, Co, Ni, Cr levels in ginger samples and As levels in thyme samples were found to be in the safe range for health

Conclusion: As a result; it is noteworthy that some heavy metals in medicinal plants used for therapeutic purposes in this study are above the recommended critical levels. Considering the possibility of exposure to heavy metals while consuming medicinal plants, regular monitoring of heavy metal concentrations in plants is important in order to minimize the risks that may adversely affect human health.

Keywords: Thyme, Ginger, Heavy metal, ICP-MS

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INTRODUCTION

Plants have been used throughout history to heal people and protect them from diseases. In Far Eastern countries, some plants are widely used for medicinal purposes in the treatment of diseases (1). In recent years, it is seen that the use of natural medicinal plant species has increased in western countries. In the mid-1970s, the use of herbal materials was below 5%, while at the beginning of the 20th century, more than 40% of medicines were of plant origin. On the other hand, with the increasing consumer awareness about health in the 1980s and 1990s, the demand for organic and naturally grown medicinal and aromatic plants has also increased (2).

Aromatic plants constitute 1/3 of Turkey's flora, which includes more than 10,000 plant species, and 3,000 of them are endemic. Today, there are around 300 plant varieties sold in herbalists and 70 to 100 of them are exported (3). Especially thyme, bay leaf and cumin, as well as sage, anise, acacia, fennel, sumac and rosemary are among the most important export products (4). Investigation of heavy metal accumulation in foodstuffs and medicinal plants is important for human and environmental health due to the increase in environmental pollution with technological developments (5). Consumption of medicinal and aromatic plants is low compared to other food products; however, they can be dangerous due to their possible high heavy metal content and misuse. For this reason, it is important to establish a database for the compositions of widely used medicinal and aromatic plants in terms of standardization (4). *Thymus vulgaris* (thyme), belonging to the Lamiaceae family, relieves pain types such as gastralgia, abdominalgia and cephalgia, and has carminative, digestive, diuretic, sedative properties as well as gastroenteritis, neurological diseases, trigeminal neuralgias, typhoid, diarrhea, rheumatism, epilepsy and upper respiratory tract (bronchitis, sinusitis, pharyngitis, etc.) is used in diseases (6,7). It is reported that *Zingiber officinale* (ginger) from the Zingiberaceae family has sedative, antispasmodic, antiemetic, expectorant, anticolitis, anticoagulant and regulating circulation, lowering cholesterol, and relieving abdominal pain. In addition, it provides the removal of toxins due to its diaphoretic effect against poisoning (7).

The aim of this study is to determine whether the heavy metal contents of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) sold in herbalists are among the limits recommended by International Organizations such as the World Health Organization (WHO) / Food and Agriculture Organization (FAO).

METHODS

Plant material

In this study, two different plant species, which are mostly used for treatment in upper respiratory tract infections, were obtained from three different herbalists in Bingöl between October-November of 2019. These plants, taken from herbalists, were taxonomically defined by Bingöl University Biology Department Lecturer Alpaslan Koçak (8). The scientific names, family names, English names, Turkish names and used parts of the plants that make up the material of the study are presented in Table 1.

Table 1. Scientific, common names and used parts of medicinal plants

Scientific name	Family name	Common name	Plant Part Used
Thymus vulgaris	*Lamiaceae*	Thyme	Branches with leaves and flowers
Zingiber officinale	*Zingiberaceae*	Ginger	roots and rhizomes

Sample Preparation

In the research, different parts of thyme and ginger plants were used as material. Approximately 0.5 g of the grinded samples were weighed and placed in the CEM-Mars 6 240/50 (Corp. Mathews NC, USA) Teflon containers of the microwave unit and 10 mL of HNO_3 was added to it. It was kept in the microwave for 25 minutes at 200 ºC for 15 minutes and then the temperature was lowered to make the samples soluble. At the end of the combustion process, the volumes of 0.5 mL samples cooled to room temperature were completed by diluting up to 15 mL with 1% (HNO_3-ultrapure water).

Calibration Procedure and Analysis Method

Calibration solutions used for ICP-MS were prepared at specific concentrations by diluting commercially purchased multi-element standards with 1% nitric acid-ultrapure water. Element standards with atomic masses ^{53}Cr, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{111}Cd, ^{202}Hg, ^{208}Pb were used. The determination of Cr, Fe, Co, Ni, Cu, Zn, As, Cd, Hg and Pb heavy metals in medicinal plant samples was performed with ICP-MS in triplicate. Perkin Elmer NexION 2000 model ICP-MS operating conditions for heavy metal analysis are given in Table 2.

Table 2. Working conditions for ICP-MS detection

Parameters	Description / Value
Nebulizer	Glass Type C
Nebulizer Flow	< 2% optimized for oxide
Nebulizer gas flow rate	0,93 L/min
Spray room and temperature	Glass, 2 ºC
Injector	2.0 mm i.d.
Deflector voltage	−12 V
Analogue step voltage	−1750 V
RF power	1600 W
Rinse Time	45 second
Standby time	50 ms
Aerosol dilution	Set to 2.5x
Sample delivery speed	350 µL/min
Threshold of discrimination	26
Alternating current bar offset	−4
Sampling Cone	Ni
The number of repetitions	3

Data Analysis

Calibration functions were determined for each element and heavy metal concentrations in plant samples were calculated using calibration functions. Heavy metal levels in plant samples are given as the heavy metal mean, three reconcentrations (µg/g) and standard deviation (mean ± standard deviation). Syngistix for ICP-MS software version 2.2 instrument software was used to control the instrument, including tuning, data acquisition, and data analysis in analyzes.

RESULTS

In this study, heavy metal amounts were determined in µg/gr in plant samples by ICP-MS method. Limit of determination (LOD), limit of measurement (LOQ) and recovery (R, %) values of the calibration obtained in the study are in Table 3, mean and standard deviation values of heavy metal concentrations of thyme and ginger plants are given in Table 4 and Table 5.

Cd in thyme samples analyzed by the device, and Hg in both thyme and ginger samples could not be read. It is seen that the thyme samples taken from the herbalist no. 3 have the highest Fe, Cr, Co, Ni, As, Pb contents, while the thyme samples taken from the herbalist no. 1 have the lowest Cr, Co, Ni, Cu contents. Likewise, it is seen that the ginger samples taken from the herbalist no. 2 have the highest content and the ginger samples taken from the no. 3 plant have the lowest Fe, Cu, Cr, Co, Ni, Cd, Pb contents (Table 4, Table 5).

Table 3. Limit of detection (LOD), limit of measurement (LOQ) and recovery (R, %) values selected for heavy metals investigated in the study

Element	LOD (mg/L)	LOQ (mg/L)	Recovery (R,%)
Cr	0,017	0,056	97,380
Fe	0,223	0,746	98,743
Co	0,00032	0,00103	96,521
Ni	0,008	0,025	98,968
Cu	0,022	0,073	96,784
Zn	0,043	0,142	97,417
As	0,001	0,004	95,826
Cd	0,000165	0,00055	97,608
Hg	0,0016	0,0054	95,950
Pb	0,010	0,033	94,882

Table 4. Mean contents of Fe, Zn, Co, Ni, Cu elements in *Thymus vulgaris* and *Zingiber officinale*

Herbalists	Plant	Fe (µg/g)	Zn (µg/g)	Co (µg/g)	Fe (µg/g)	Zn (µg/g)
Herbalist 1	Thyme 1	1785,99±1,04	44,42±0,02	0,34±0,00	0,76±0,00	2,29±0,00
	Ginger 1	535,99±0,46	13,59±0,11	0,44±0,01	2,16±0,00	4,47±0,00
Herbalist 2	Thyme 2	1740,05±2,42	37,26±0,05	0,38±0,01	0,92±0,00	3,14±0,01
	Ginger 2	700,99±1,03	62,02±0,07	0,56±0,01	2,58±0,01	5,63±0,01
Herbalist 3	Thyme 3	5109,90±6,75	42,65±0,06	0,45±0,01	1,11±0,00	2,71±0,01
	Ginger 3	415,65±0,13	75,12±0,01	0,30±0,00	0,38±0,00	3,15±0,00

Table 5. Mean contents of Cr, Cd, Hg, As, Pb elements in *Thymus vulgaris* and *Zingiber officinale*

Herbalists	Plant	Cr (µg/g)	Cd (µg/g)	Hg (µg/g)	As (µg/g)	Pb (µg/g)
Herbalist 1	Thyme 1	2,25±0,00	NR	NR	0,22±0,00	4,52±0,01
	Ginger 1	0,92±0,00	0,34±0,00	NR	1,37±0,00	11,52±0,01
Herbalist 2	Thyme 2	2,38±0,01	NR	NR	0,17±0,01	4,09±0,01
	Ginger 2	1,49±0,00	1,37±0,01	NR	1,25±0,00	16,40±0,02
Herbalist 3	Thyme 3	2,56±0,01	NR	NR	0,27±0,00	6,28±0,01
	Ginger 3	0,83±0,00	0,08±0,00	NR	0,93±0,00	6,12±0,00

DISCUSSION

The use of natural medicinal plants is increasing day by day in our country as well as in the world. Medicinal plants are mostly consumed in autumn and winter by brewing (infusion) and boiling (decoction) methods (1). In addition to many health benefits of medicinal plants, mistakes made in their use, excessive consumption, and the risk of contamination due to uncontrolled collection, diagnosis and stocking pose a threat to health. The presence of factors such as industrial pollution, pesticides, traffic pollution increases the exposure of plants to heavy metals. The different concentrations seen in plants vary depending on the type of plant, the parts used, soil content, water pollution, air pollution, industrial activities, and the use of fertilizers as well as other chemicals (9).

Elements such as iron, chromium, copper, zinc, cobalt, manganese and nickel, which play an important role in biological processes in metabolism, are vitally essential. Heavy metals such as mercury, lead and cadmium can be toxic even at very low concentrations. Heavy metals such as lead, cadmium, chromium and arsenic are recognized as potential pollutants commonly found in our environment (10). Heavy metal contamination with medicinal plants is attributed to environmental pollution and should be limited as they have the potential to pose a health hazard (11-16). Plants grown in areas contaminated with heavy metals may have high metal contents (17). In addition, the use of cadmium-containing fertilizers and the use of organic mercury or lead-containing pesticides in agricultural lands increase the heavy metal rates in grown plants (18). Heavy metals taken in high doses through the food chain also affect human health negatively (19,20).

Elements that fall into the category of micronutrients that the human body needs less than 100 mg/day are considered to be vanadium, chromium, manganese, iron, cobalt, copper, zinc, molybdenum, selenium, fluorine and iodine. Micronutrients such as Cu, Cr, Mo, Ni, Se, and Zn can be toxic at high concentrations (21-24). High zinc intake from contaminated food or drink and acute zinc poisoning have been associated with nonspecific gastrointestinal symptoms such as abdominal pain, diarrhea, nausea and vomiting. In addition, exposure to high zinc interferes with the metabolism of other trace elements such as copper absorption (25). The mechanism of cellular and tissue damage of excess iron is thought to be modulated by Fenton reaction, as it is a potent free radical generator. In vivo free radical generation has been associated with cancer, cardiovascular disease and arthritis (26).

In the study, thyme and ginger samples, whose heavy metal content was determined, were dissolved in the microwave, then content analysis was performed with ICP-MS and the results are given in Table 4 and Table 5. Permissible limit values for Fe and Zn determined by FAO/WHO in edible plants are 20 ppm and 27.4 ppm (1ppm=µg/g), respectively (27). Fe and Zn values detected in thyme and ginger samples were above the limit values allowed by FAO/WHO. While Fe values of thyme and ginger samples reported in the literature were mostly above the limit values allowed by FAO/WHO (4,28-31), Okut et al. found that Fe and Zn values were within the limit values in the thyme sample they studied (30).

High copper levels can cause hair and skin discoloration, dermatitis, upper respiratory tract irritation, metallic taste in the mouth and nausea. Copper deficiency results in anemia and congenital failure (11). Chronic exposure to chromium can cause liver, kidney, stomach and lung damage (31).

In edible plants, 3.0 ppm for Cu by FAO/WHO (27) and 2.0 ppm for Cr by WHO (32) are allowable limit values. Except for ginger Cr levels and thyme 1st and 3rd Cu values, chromium and copper values of all thyme and ginger were found above the determined limits. In addition to the studies in which the Cu values in thyme samples were found below the limit values allowed by FAO/WHO (30), there are also studies that were found above the limit values (4,29). In the literature, while the Cr values in some ginger samples are below the limit values allowed by WHO (4,29), there are also studies that are above the limit values (33).

It has been reported that acute exposure of the human body to nickel can cause liver, kidney, spleen, brain and tissue damage, vesicular eczema, lung and nose cancer (25,31). The systemic effects of cobalt are mainly characterized by a complex clinical syndrome involving neurological (hearing and visual impairment), cardiovascular and endocrine (34).

While the limit value allowed for Ni by FAO/WHO (31) is 1.63 ppm, there is no regulatory limit for Co. However, in a study conducted on seven plants by Başgel and Erdemoğlu in Turkey, it was reported that the Co concentration ranged between 0.14 ppm and 0.48 ppm. Ni values determined in thyme samples were found below the limit value (35). There are studies in which Ni values detected in thyme and ginger samples reported in the literature are above the value allowed by FAO/WHO (4, 28). The daily Co intake determined by the Agence Française de Sécurité Sanitaire des Aliments (AFSSA) is 1.6–8 μg/kg body weight/day (36). Except for the ginger 2 plant sample obtained from Herbalist 2, the cobalt contents of all samples were within the determined safe range. In the literature, Co values in thyme samples were found to be between 0.00285-0.66 mg/kg (4,28,29). In the literature, ginger Co content has been determined between 0.16-0.41 mg/kg (4,28).

On the other hand, it is known that toxic elements such as As, Cd, Hg and Pb have harmful effects on human health (24). Lead accumulates mainly in the skeletal system and soft tissue. Also, lead tends to accumulate in gray matter and certain centers in the central nervous system. Lead inhibits porphobilinogen synthase and ferrochelatase, preventing the formation of porphobilinogen, incorporation of iron into protoporphyrin IX, causing ineffective heme synthesis and microcytic anemia. In addition, lead poisoning negatively affects the kidney, liver, vascular and immune systems (33). Low levels of Cd exposure can cause damage to the kidney, liver, skeletal and cardiovascular systems, as well as impaired vision and hearing. Cadmium, which is known to have teratogenic and mutagenic effects, also causes delay in puberty and menarche, steroidogenesis, menstrual cycle, reproductive hormone disorders, miscarriage, preterm birth and low birth weight (37). Elemental and methyl mercury are toxic to the central and peripheral nervous systems, its inorganic salts are corrosive to the skin, eyes, and gastrointestinal tract, and can cause kidney toxicity when taken orally (25). Exposure to inorganic arsenic is associated with an increased risk of skin lesions and developing skin, lung, liver, and kidney cancer (38).

The limit values allowed for Cd and Pb by FAO/WHO (27) are 0.21ppm and 0.43 ppm, respectively, while the limit allowed for As by FAO/WHO (39) is 0.9- 1.1 ppm. It was determined that cadmium content was high in one of the ginger plant samples, while the cadmium content was found in the reference range in thyme samples. The amount of Pb was found above these allowable limit values in both thyme and ginger samples. The amount of As in thyme samples was determined below the limit value. It was determined that arsenic content was high in one of the ginger plant samples. Cd values detected in ginger samples reported in the literature ranged between 0.05-2.39 mg/kg (4, 28). While thyme Pb values are in the range of 0.001-1.02 ppm in the literature (4, 30), it has been determined as 0.05-1.6 mg/kg for ginger samples (4, 40). In the literature, As values determined in thyme samples were determined as 0.001-0.38 ppm (28, 29, 30), and in ginger samples as 0.03-0.84μg/g (28)

Heavy metals pollute soil and water through the use of coal as fuel, industrial fertilizers, pesticides and industrial flue gases, exhaust fumes, industrial oxidation and water pipes. Plants have the capacity to collect heavy metals from soil and water throughout their development (20). Based on the findings of our study, high heavy metal contents in plants exposed to environmental pollutants can reach toxic levels for humans and animals.

CONCLUSION

As a result; it is noteworthy that some heavy metals in medicinal plants used for therapeutic purposes in this study are above the recommended critical levels. Adverse environmental conditions in which medicinal plants are grown and stocked can lead to heavy metal contamination that is harmful to human health. Considering geographical conditions and different factors in industrial activities, metal levels in plants should be carefully monitored. Herbal materials, including medicinal plants and imported products sold in herbalists, should be checked regularly and heavy metal contamination risks should be evaluated continuously.

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