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Determination of Trace Elements in *Rumex nepalensis*, *Inula discoidea*, *Tripleurospermum callosum*, and *Thymus migricus* Plants Using ICP-MS Application

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ABSTRACT: The form and distribution of trace elements in tissues and fluids of the body have become key details in life sciences and medicine, and they may be used to discover disease biomarkers. In the current study, concentrations of twelve elements in *Rumex nepalensis, Inula Discoidea, Tripleurospermum callosum,* and *Thymus migricus* plants samples were detected by inductively coupled plasma mass spectrometry (ICP-MS). All elements the range of correlation coefficient (R) obtained from 0.993 to 1. For *R. nepalensis* plant extract Zn (45.457 µg ml^{-1}), Mn (41.357 µg ml^{-1}), and Cu (13.628 µg ml^{-1}); for *I. Discoidea* plant extract Zn (62.175 µg ml^{-1}), Mn (53.288 µg ml^{-1}), and Cr (16.904 µg ml^{-1}); for *T. callosum* plant extract Mn (50. 594 µg ml^{-1}), Cu (12.712 µg ml^{-1}), and Cr (2.662 µg ml^{-1}); for *T. migricus* plant extract Mn (53.893 µg ml^{-1}), Zn (23.768 µg ml^{-1}), and Cu (9.568 µg ml^{-1}) were identified as the most intensive elements. These plants extracts could be a novel source of mineral constituents in various industries and pharmaceutical industries.

Keywords: Trace element, ICP-MS, R. nepalensis, I. discoidea, T. callosum, T. Migricus

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INTRODUCTION

The trace elements such as manganese, copper, zinc, and selenium act as cofactors of antioxidant enzymes to defend the body from free oxygen radicals that are made during oxidative stress (F. Y. Leung, 1998). Essential elements zinc (Zn), copper (Cu), manganese (Mn), and selenium (Se) also called micronutrients to have significant effects in the processing of some critical enzyme system for the living organism (Nuapia et al., 2018). These antioxidant response elements, regulate the network of genes, offer cytoprotection under various stress conditions (Hayes,Dinkova-Kostova, 2014), and play a critical role in redox homeostasis (Raghunath et al., 2018). Plant micronutrients and secondary metabolites such as flavonoids, phenolic- compounds are AChE inhibitors (AChEIs) and thought to have a therapeutic effect in the treatment of Alzheimer's disease (AD) (Türkan et al., 2020).

Approximately 60% of the plants have medicinal value (Hao,Xiao, 2018). Medicinal plants are defined to possess chemical components that could be used for therapeutic purposes (Köksal et al., 2017). The aromatic and pharmacological properties of the medicinal plants allow them to be used for several medical purposes (S Takshak,SB Agrawal, 2019). These natural properties such as anthocyanins, vitamins, carotenoids, flavonoids, tannins, and volatile compounds are broadly used in cosmetics. They have antioxidant, wound-healing, anti-inflammatory, and anti-aging activities (Donglikar,Deore, 2016; Saewan,Jimtaisong, 2013; S Takshak,S Agrawal, 2019; Traverso *et al.*, 2013). According to the World Health Organization (WHO) 2011 data (Salhi et al., 2019), approximately of 70% of the world population in developing countries still consuming the traditional medicines for their primary health care and about 25% part of current modern medicines we use is from natural sources (De Luca et al., 2012; Lee et al., 2019).

MATERIALS AND METHODS

Plant sample and extract preparation

Rumex nepalensis spreng. was collected between Bingöl - Sancak 10. km, opening *quercus* forests, scrub, on 04.05.2018, at an altitude of 1650-1850 m., by Omer Kilic, collected number 5896 (Davis, 1967). *Thymus migricus* was collected between Adaklı (Bingöl) and Bağlarpınarı village, steppe areas and slopes on 01.07.2017, at an altidude of 1500-1600 m., collected number 5923. *Tripleurospermum callosum* plant was collected from Bingöl; Karlıova, vicinity of Göynük village, steppe, slopes and damaged *Quercus* forest, 1850-2000 m, 19.06.2017, with collected number 5197. *I. discoidea* was collected from the mountain slopes in Karlıova Bingöl district, on July 10th 2017, at an altitude of 1850-1950 m. The identification of plant sample was made by Dr. Omer Kilic according to volume 5 of the Flora of Turkey (Davis, 1970).

ICP-MS analysis

The determination of elements was done by an inductively coupled plasma tandem mass spectrometer (Agilent 7800 ICP-MS). The multi-element calibration standard was prepared using a matrix of 5% HNO_3. The element standard was calibrated 1.25 to 500 ng ml^{-1}. For standards, different concentrations were prepared with distilled water. For all elements the range of correlation coefficient (R) obtained from 0.993 to 1. *R. nepalensis, T. migricus, T. callosum, and I. discoidea* plant samples were homogenized and blended before they eliminate with sieves (4 mm – 20 µm of mesh sizes). 3 mL HCl and 1 mL HNO_3 were added to 0.2 g of plant samples were digested with Ethos UP Microwave Digestion System using the program that given pressure (10 bar), hold time (15 min), temperature (200°C), and energy (1500w) (Gray et al., 2015). The mixture was then filtered with 4-7 µm filter papers. By adding 50 ml of pure water volume of the sample completed (Millipore Simplicity instrument (Millipore)).

RESULTS AND DISCUSSION

ICP-MS analysis

Trace elements such as Mn, Cu, Zn, and Se were determined to have effects in human cell defense mechanisms against reactive oxygen species. These elements function as cofactors of some antioxidant enzymes. For instance, by interaction with selenium-glutathione peroxidase eliminates H_2O_2 (Negi *et al.*, 2012; Spears,Weiss, 2008). Also, Zn has protective property against the lipid peroxidation and oxidative damage in the cellular environment (Cikim et al., 2003; Ngamdee et al., 2016). Zinc accumulation in edible plant parts grown in Zn-rich soils have crucial exceptional importance due to the human body has no private Zn storage system (Rink, 2000; Sofo et al., 2018). The essential elements in proper amount have beneficial effects on the antioxidant defense system. Micronutrients such as manganese (Mn), selenium (Se), copper (Cu), and zinc (Zn) have substantial importance in the functioning of some crucial enzyme system (Nuapia et al., 2018) but, at the excess concentrations, they have phytotoxic effects (Tokalıoğlu et al., 2019). Heavy metal in high concentrations in food can cause harm to many biochemical processes such as nervous, liver, kidney, bones, brain and cardiovascular problems (Pena-Bautista et al., 2019). Mn, Cu, Mo, Fe, and Zn are transition element that presents in plants (Samreen et al., 2017).

Elemental analysis of the plant samples using ICP-MS method has become a very interesting technique because by using ICP-MS, the analysis of the elements in the environmental samples can be performed at lower limits and faster. In this way, the concentrations of twelve elements in *R. nepalensis, I. discoidea, T. callosum,* and *T. migricus* samples were analyzed by ICP-MS (Inductively Coupled Plasma – Mass Spectrometer). Digestion of the plant sample was performed in a microwave system.

Antioxidant potential and enzyme inhibitory properties of *R. nepalensis* were reported previously. In that study, it is demonstrated that plant methanol and water extract had remarkable inhibition and antioxidant results (Bursal, et al., 2021). These biological activities are may due to trace elements (Fred Y Leung, 1998). The decreasing sequence of the average element concentrations (µg ml-1) was as follows: for *R. nepalensis* Zn (45.457 µg ml^{-1}) > Mn (41.357 µg ml^{-1}) > Cu (13.628 µg ml^{-1}) > Cr (3.796 µg ml^{-1}) > Ni (1.062 µg ml^{-1}) > As (0.549 µg ml^{-1}) > Co (0.229 µg ml^{-1}) > Cd (0.074 µg ml^{-1}) > Se (0.045 µg ml^{-1}) > Be (0.044 µg ml^{-1}) as shown in figure 1. The elements in *R. nepalensis* plant which is investigated were quite good sources of Zn, Cu, and Mn, while other elements were low of levels of concentration. But, the most significant problem concerning elements is the amounts of the toxic elements such as As, Cd, and Pb, whose dietary excess may be injurious to health. Some elements' permissible levels of medicinal plants are as follows (Stanojkovic-Sebic et al., 2012). In terms of elemental content, *R. nepalensis* is in the permissible range except for Cr. Mean cadmium exposure for adults ranged from 2.2 to 12 µg/kg body weight per month, for Pb mean exposures ranged from 0.02 to 3 µ kg^{-1} body weight per and for the arsenic lower limit was determined from epidemiological studies to be 2–7 µg kg^{-1} body weight per day (Joint et al., 1997).

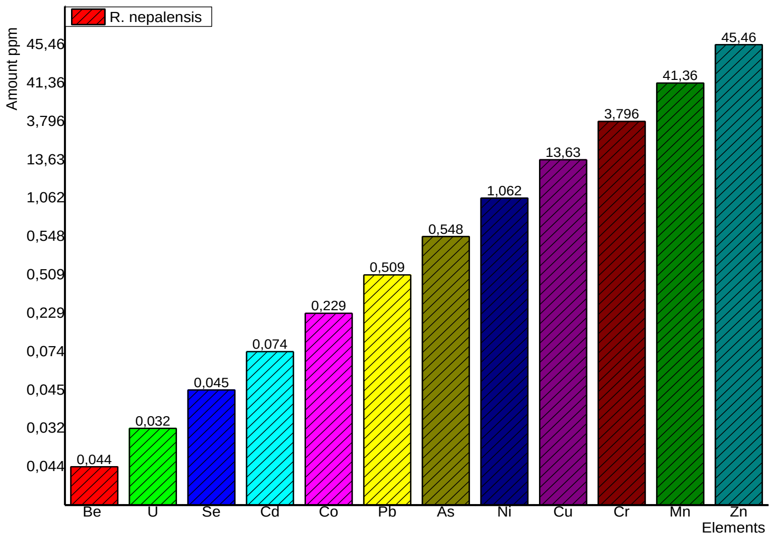

Figure 1. Trace elements and their amounts in *R. nepalensis*

We previously studied the *I. discoidea* plant aqueous and methanol extracts enzyme inhibitory and antioxidant activities. Results demonstrated sufficient enzyme inhibition of the methanol extract against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and glutathione S-transferase (GST), and α-glycosidase (α-Gly) enzymes and effective antioxidant activities (Bursal et al., 2021). For *I. discoidea* was Zn (62.175 µg ml^{-1}) > Mn (53.288 µg ml^{-1}) > Cr (16.904 µg ml^{-1}) > Cu (10.695 µg ml^{-1}) > Ni (3.0134 µg ml^{-1}) > Co (0.422 µg ml^{-1}) > Se (0.317 µg ml^{-1}) > As (0.297 µg ml^{-1}) > Cd (0.074 µg ml^{-1}) > Be (0.034 µg ml^{-1}) as shown in figure 2.

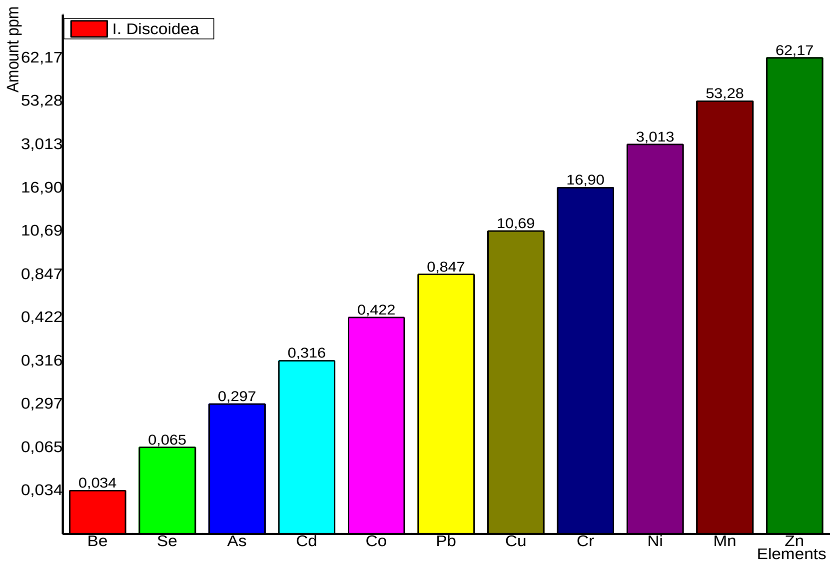

Figure 2. Trace elements and their amounts in *I. discoidea*

For *T. callosum* Mn(50.594 µg ml^{-1}) > Zn (22.962 µg ml^{-1}) > Cu (12.712 µg ml^{-1}) > Cr (2.662 µg ml^{-1}) > Ni (0.957 µg ml^{-1}) > As (0.295 µg ml^{-1}) > Co (0.106 µg ml^{-1}) > Cd (0.086 µg ml^{-1}) > Se (0.036 µg ml^{-1}) > Be (0.0221 µg ml^{-1}) as shown in figure 3. Besides, uranium, selenium, and beryllium elements have been identified in trace amounts. Mn is a necessary element during the initial development periods (Lima et al., 2008), acts as an activator or enzyme cofactor for many reactions of metabolism, but excessive Mn effect toxic accumulation in brain tissue and resulting extrapyramidal symptoms as seen in Parkinson's patients (Martin et al., 2008). Zn is an essential trace element that acts as a cofactor in many enzyme systems (Jiang et al., 2016) and functions for the linkage of these enzymes to their corresponding substrates (Samreen et al., 2017).

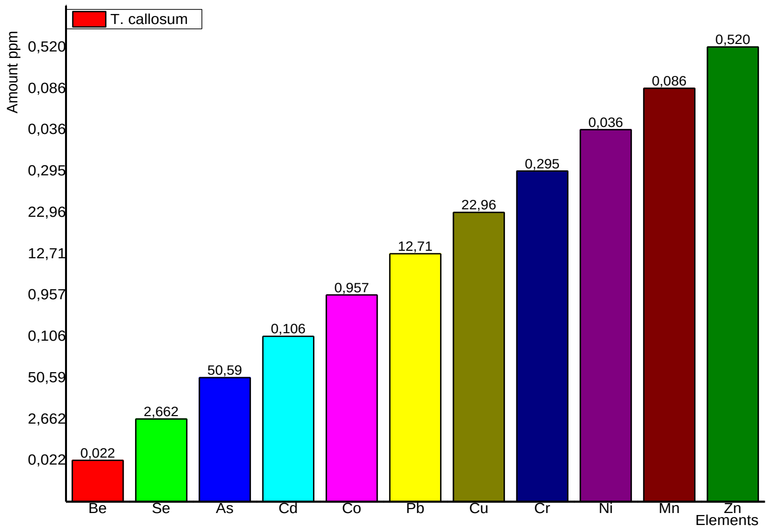

Figure 3. Trace elements and their amounts in *T. callosum*

In our previous work, we tested the antioxidant activity and enzyme inhibition of the *T. migricus* plant. Results values showed that methanol and water extract had inhibition effects against the glutathione S-transferase (GST), α-glycosidase (α-Gly), acetylcholinesterase, and butyrylcholinesterase enzymes that are linked with certain metabolic diseases. Also, had potent antioxidant effects (Aras et al., 2021). For *T. migricus* plant extract Mn (50.59 µg ml^{-1}) > Zn (22.962 µg ml^{-1}) > Cu (12.712 µg ml^{-1}) Cr (2.662 µg ml^{-1}) > Ni (0.957 µg ml^{-1}) > As (0.295 µg ml^{-1}) > Co (0.106 µg ml^{-1}) > Se (.036 µg ml^{-1}) > Cd (0.086 µg ml^{-1}) > Be (0.020 µg ml^{-1}) as shown in figure 4. The content of metals can be toxic to human's health if higher than certain limits. Some elements' permissible limits of medicinal plants are as follows (Stanojkovic-Sebic et al., 2012; Stanojkovic-Sebic et al., 2015). They are 15-100 mg kg^{-1}, 15-150 mg kg-1, 3-15 mg kg^{-1}, 0.1-1 mg kg^{-1}, 0.1-5 mg kg^{-1}, 10-60 mg kg^{-1}, 1-5 mg kg^{-1}, and 0.05-0.5 mg kg-1 for: Mn, Zn, Cu, Cr, Ni, As, Pb, and Co respectively, versus 53.893 mg kg-1, 23.768 mg kg^{-1}, 9.568 mg kg^{-1}, 4.321 mg kg^{-1}, 3.602 mg kg^{-1}, 1.065 mg kg^{-1}, 0.971 mg kg^{-1}, 0.565 mg kg^{-1} in the studied *T. migricus* samples. Thus, in terms of elemental content, *T. migricus* is in the permissible range except for Co.

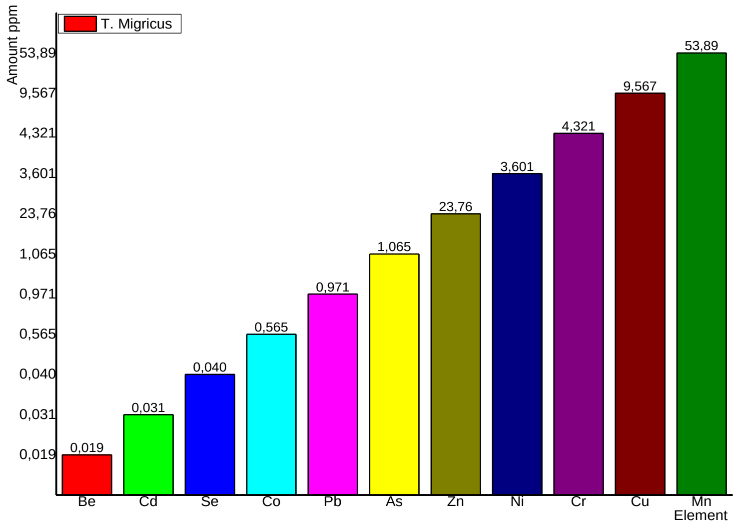

Figure 4. Trace elements and their amounts in *T. migricus*

Table 1. Trace element analysis of I. discoidea, R. nepalensis, T. migricus, and T. callosum plants by ICP-MS

	Be (µg ml^{-1})	**Cr** (µg ml^{-1})	**Mn** (µg ml^{-1})	**Co** (µg ml^{-1})	**Ni** (µg ml^{-1})	**Cu** (µg ml^{-1})	**Zn** (µg ml^{-1})	**As** (µg ml^{-1})	**Se** (µg ml^{-1})	**Cd** (µg ml^{-1})	**Pb** (µg ml^{-1})	**U** (µg ml^{-1})
I. discoidea	0.0337	16.904	53.288	0.4221	3.0134	10.695	**62.175**	0.2974	0.06538	0.31692	0.8475	0.0382
R. nepalensis	0.0442	3.7963	41.357	0.2291	1.0623	13.628	**45.457**	0.5485	0.04526	0.07416	0.5091	0.0323
T. migricus	0.0197	4.3218	**53.893**	0.5655	3.6015	9.5678	23.768	1.0651	0.04071	0.03156	0.9714	0.03046
T. callosum	0.02214	2.6621	**50.594**	0.1063	0.9573	12.712	22.962	0.2954	0.03644	0.08612	0.5201	0.02135

Zinc (Zn) is an essential trace element that acts as a cofactor in many enzyme systems (Jiang et al., 2016). Zn, Cu, and Co have biologic activities such as antitumor (Alexander et al., 2019). A number of these elements play a primary role in the body's antioxidant process, protecting the tissues from harmful oxygen-free radicals (OFRs) (Fred Y Leung, 1998). Copper and zinc are a trace element found in the organism and in the structure of some enzymes (Mehmetoğlu et al., 2019). Also, Zn acts as a divalent cation in, metalloenzymes and function linkage of these enzymes to their corresponding substrates. Organic compounds Zn links to S and also forms tetrahedral complexes with O and N (Samreen et al., 2017). Mn is necessary as an enzyme cofactor or activator for many reactions, but excessive Mn exposure can effect in toxic accumulation in brain tissue and resulting extrapyramidal symptoms as seen in Parkinson's patients (Martin et al., 2008). Manganese dioxide (MnO_2) has catalytic activity on the oxidation of the different substrates, in particular, one including O_2 as the electron acceptor (Wu et al., 2019). Cu acts as a cofactor and is necessary for catalytic and structural properties of some important enzymes, including dopamine beta-hydroxylase, tyrosinase, cytochrome c oxidase, p-hydroxyphenyl pyruvate hydrolase and Cu-zinc superoxidase dismutase (Cu, Zn-SOD) (Gaetke,Chow, 2003).

CONCLUSION

In this paper, we investigated the elemental content of four mentioned plant using ICP-MS. To the best of our knowledge, the plants used in this study were not analyzed in regards to the trace element content so far. It was observed that the four plants used were quite rich in terms of element contents. Manganese, zinc, copper, and chromium were found to be the most abundant elements in all plants. These plants extracts could be a novel source of mineral constituents in various industries and pharmaceutical industries.

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