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Mineral composition of some wild mushrooms from Eastern Anatolia, Turkey

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Abstract: Within this study 40 samples including sequential extracts, water extracts and drug samples obtained from five mushroom samples wild grown from Turkey were evaluated for their mineral composition analysis. All samples were found as rich sources of minerals notably Ca, K, Si, Mg, Se and Si which might contribute health enhancing properties. The levels of heavy metals were detected in low amounts in the extracts of mushroom species (except *T. populinum*). Acetone and ethyl acetate were detected as the most efficient solvents in the isolation of minerals from mushroom samples. Our findings showed that extracting of mineral compounds were varied due to the type of solvents applied and mycochemical diversity and the reported mineral compounds profiles suggest that these wild-edible mushrooms might be potential sources of therapeutic nutraceuticals.

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1. INTRODUCTION

Mushrooms have been utilized for centuries by humankind for various purposes particularly for food and medicine. They are utilized as fresh material, drugs, infusions, decoctions or crude extracts [1]. *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga) are among the most used mushroom samples as therapeutic based nutraceuticals [2].

Extracts obtained from several mushroom species have been reported for their pronounced health enhancing activities which are associated to the presence of active chemical compounds such as phenolics, glycosides, tocopherols, polysaccharides, carotenoids, ergothioneine, vitamins, ascorbic acid and minerals [3-5].

Among these mycochemical compounds, minerals have specific properties due to their essential roles in biological reactions as catalyst. They are essential compounds in all cognitive and physiological processes in several tissues. For instance, iron, copper, selenium and zinc enrolling to the antioxidant enzymes as cofactor. Na and K are constituents of acid-base balance and nerve stimulation. Fe joins to the structure of haemoglobin. Zn present in some enzymes structure and plays significant roles in protein synthesis. Moreover, mineral compounds are

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necessary in the absorption of vitamins; Ca for Vitamin C, Zn for Vitamin A, Mg is for Vitamin B and Se for Vitamin E [6].

Addition to the health benefits of mineral compounds, there are also some hazardous effects of some mineral compounds called heavy metals such as Cd, Pb and Ni. Pb is well known for its harmful effects on cardiovascular system. Cd has side effects on kidney, urinary and skeleton system [7]. The accumulation of heavy metals particularly mercury, cadmium, copper and arsenic in mushroom species collected from wild such as *Boletus badius*, *Suillus variegatus* and *Rozites caperata* was reported previously [8].

Tricholoma scalpturatum (Fr.) Quél., *Tricholoma populinum* J.E. Lange, *Neolentinus cyathiformis* (Schaeff.) Della Maggiora & Trassinelli, *Chlorophyllum agaricoides* (Czern.) Vellinga and *Lycoperdon utriforme* Bull. are among mushroom species that most utilized for nutritional and medicinal purposes in Turkey. However, studies regards to chemical analysis particularly mineral components are limited in scientific literature. Therefore, this study focused on evaluating mineral composition of these mushroom species comprehensively by using various organic solvents. For this purpose, three different extraction methods including sequential, water and drug extracts were prepared for analysis.

Sequential extracts were used in order to understand the effect of polarity on mineral extraction capability of various organic solvents including n-hexane, chloroform, ethyl acetate, acetone, ethanol and pure water. Water extract was applied in order to reveal the traditional utilization of these species. Moreover, drug samples were directly analysed for mineral compound analysis.

2. MATERIAL AND METHODS

2.1. Mushroom Material

Fruiting bodies of mushroom samples (*Tricholoma scalpturatum* (Fr.) Quél. (GPS coordinates 38º34ʾ09.81ʾʾN, 43º16ʾ53.23"E), *Tricholoma populinum* J.E. Lange (GPS coordinates 37º17ʾ24.35ʾʾN, 44º35ʾ47.56"E), *Neolentinus cyathiformis* (Schaeff.) Della Maggiora & Trassinelli (GPS coordinates 38º17ʾ31.69ʾʾN, 43º 05ʾ25.12"E), *Chlorophyllum agaricoides* (Czern.) Vellinga (GPS coordinates 37º23ʾ59.2ʾʾN, 44º29ʾ49.02"E) and *Lycoperdon utriforme* Bull. (GPS coordinates 37º23ʾ59.92ʾʾN, 44º29ʾ49.02"E)) were harvested from Eastern Anatolia Region of Turkey, on 5-19 May 2016. Mushroom materials were isolated in clean polythene bags and transferred to the laboratory within a maximum of 3 h after harvest. The identities of mushroom materials were confirmed by Yusuf Uzun, PhD at Mcygology Research Fungarium, Science Faculty, Van Yuzuncu Yil University, Turkey and a voucher specimen was stored at the university's fungarium (Fungarium codes: 7484, Acar 481, 7485, Acar 636 and 7486 respectively). The mushroom materials were properly cleaned from dust and contaminants by minimizing the loss of chemical components and left at room temperature in the dark until dry. The dried mushroom materials were subsequently ground for a fine powder and stored at -20 °C until analysed.

2.2. Reagents

Unless otherwise stated, all chemicals were purchased from Sigma–Aldrich (Istanbul, Turkey) and were of analytical or HPLC grade.

2.3. Preparation of sequential lyophilized extract

The ground mushroom materials were extracted sequentially using a range of organic solvents with increasing degrees of polarity; n-hexane, chloroform, ethyl acetate, acetone, ethanol and pure water respectively, as recommended by Dai and co-authors [9]. Firstly, the ground air-dried mushroom samples were mixed with a 10-fold volume of n-hexane (gr/ml) shaken for 2 h at room temperature (22°C) and centrifuged for 20 min at 15320g (10000 rpm)

at 4°C (Sorvall RC-5B; DuPont, Wilmington, DE, USA; rotor Beckman JA14 (137 mm) serial no. 02U8152, USA) with the supernatant collected.

The same extraction procedures were applied to the pellet using aqueous 10-fold those of chloroform, ethyl acetate, acetone (80%), ethanol (80%) and pure water (gr/ml) respectively, with the supernatants collected. The supernatants from n-hexane, chloroform, ethyl acetate, acetone, ethanol and pure water fractions were evaporated individually under reduced pressure at 37°C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The derived concentrated fractions were dissolved in a minimum amount of purified water and freeze-dried under a vacuum at -51°C to obtain fine lyophilized powders.

2.4. Preparation of water extract

The water extracts were prepared as described previously [10] using pure water as solvent. Briefly, the ground mushroom material was mixed with a 10-fold volume of pure water, shaken for 2 h at room temperature (22°C) and centrifuged for 20 min at 15320g (10000 rpm) at 4°C with the supernatant collected. The extraction was repeated one more time. The supernatants from the consecutive extractions were combined and the solvent evaporated under reduced pressure at 37°C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The derived fraction was dissolved in purified water and freeze-dried under a vacuum at -51°C to obtain a fine lyophilized powder.

2.5. Mineral Composition Analysis

The mineral composition of the extracts were prepared as described previously [11]. The analysis were conducted using AAS (Thermo Scientific, ICE-3000 series, USA), ICP-MS (Thermo Scientific, ICAP RQ series, USA) and ICP-OS (Thermo Scientific, ICAP-7200 series, USA). The analysis solutions were prepared by dissolving the lyophilized extracts and drug material in HNO_3 individually. Subsequently, the solution was subjected to microwave assisted extraction procedure and identities of mineral compounds were confirmed by comparison of authentic standards.

3. RESULTS

Tricholoma scalpturatum had a rich mixture of mineral compounds as shown in Table 1. Na, Ca, K, Fe, Si, S, Zn and Cu were the dominant mineral compounds of the extracts and drug. Acetone was more efficient than other apolar and polar solvents used in sequential fractions in terms of mineral compounds extraction, followed by ethyl acetate and ethanol. Interestingly hexane fraction was found as efficient in the extraction of Ag, B and Mo. No significant difference was detected between water extract and drug as shown in Table 1. The levels of heavy metals such as Cd, Ni and were detected in low levels. As and Cr were not detected in the extracts. Among heavy metals Pb was detected in high levels particularly in drug and acetone fraction (Table 1).

Table 2 presents mineral composition of *Neolentinus cyathiformis* extracts and drug. The results showed that water was the most efficient solvent in terms of mineral extraction. The levels of heavy metals such as Cd, Ni and Pb were detected in low levels. As and Cr were not detected in the extracts. The highest levels of Ag, B, Mo, Na, Se and Si were found in hexane fraction.

As shown in Table 3, sequential fractions were more efficient than those of water extract and drug in terms of mineral extraction. n-Hexane extracted B and Mo effectively than those of organic solvents. Relatively high amounts of minerals were extracted by chloroform, followed by ethyl acetate, water and acetone. Drug was the less efficient solution compare to water and sequential extracts except of Pb. Heavy metals including, Ni, As and Cr were not found, while the amount of Cd was detected at low level (Table 3).

Table 1. Mineral Composition of *Tricholoma scalpturatum* (µg/g extract)*

	Sequential Fractions						Water Extract	Drug
	n-Hexane	Chloroform	Ethyl Acetate	Acetone	Ethanol	Residue		
Ag	**48.2**	ND	ND	ND	ND	ND	ND	ND
B	**2151**	371.3	ND	ND	195.3	ND	ND	ND
Ba	27.3	1.3	8.3	**54.1**	ND	ND	ND	ND
Be	**26.3**	ND	6.4	ND	ND	ND	ND	ND
Ca	1966.8	795.7	8488.9	**36800**	289.5	1983.4	2190.5	2612.8
Cd	8.9	1.5	18.6	**120.2**	T	T	T	T
Co	6.6	1.3	6.2	**58.2**	T	T	T	T
Cu	43.9	5.5	41.5	**464.6**	7	T	1.4	1.9
Fe	459.7	56.5	441.5	**5264**	11.5	1.8	244.9	785.3
K	25807	5736.9	ND	**34960**	**35197**	18.95	6294.2	3256.1
Mg	1368	357.9	429	**3336**	153.8	ND	184.1	180.6
Mn	40.1	3.9	12.5	**78.9**	T	T	T	T
Mo	**404**.7	T	8.1	18.4	ND	ND	ND	ND
Na	2607.2	1337.5	5896.3	**45616**	412.3	2.8	494.4	249.1
Ni	ND	T	3.4	**43.2**	T	T	T	ND
Pb	5.9	1.4	99.4	245	1	T	287.9	**406.7**
Sb	21.6	3	6.9	**223.5**	T	T	ND	T
Se	9.1	ND	46.8	**108.2**	ND	T	ND	ND
Si	2296.9	187.8	1376	**8718.4**	134.4	ND	213.6	401.6
Ti	**53.2**	ND	ND	ND	T	ND	T	2.1
V	51.6	26.7	419	**2088**	6	5.3	39.6	39.9
Zn	157.4	65.8	192.1	**1985.6**	23.8	ND	62.3	67

ND: Not detected. T: Traces. * Bold written values indicate the highest content of mineral compounds.

Table 2. Mineral Composition of *Neolentinus cyathiformis* (µg/g extract)*

	Sequential Fractions						Water Extract	Drug
	n-Hexane	Chloroform	Ethyl Acetate	Acetone	Ethanol	Residue		
Ag	**20**	3.4	ND	ND	ND	ND	2.3	ND
B	**2588**	ND	ND	ND	ND	ND	ND	ND
Ba	**7.2**	T	3.5	4.1	T	1.9	ND	ND
Be	1190.3	547.6	804.4	883.6	232.4	**1109.4**	**1517.9**	731.3
Ca	**3.7**	1.2	**3.7**	3,4	T	T	T	T
Cd	2.4	T	1.2	**3.2**	T	1.3	T	0.5
Co	11.9	1.7	**14.3**	5.9	1	7.5	T	T
Cu	60.6	40.8	**230.3**	168.4	9.2	90.4	71.8	**367.1**
Fe	10947.9	1625.6	ND	691.4	11787.8	**52487.5**	2046.9	1868
K	835.9	559.3	198	123.2	109.2	**3170.3**	166.7	156.5
Mg	4.3	5.1	3.5	4.5	T	**15.7**	ND	T
Mn	**138**	T	7.5	3.8	T	1.5	ND	ND
Mo	**4128**	574.8	1143.4	1559	431.1	1142.4	528.5	295.4
Na	ND	T	**5.7**	ND	T	T	ND	ND
Ni	5.1	T	4.8	2.1	T	1	233.1	**491**
Pb	3.4	1.9	**12.3**	T	1	T	ND	0.1
Sb	**8.8**	ND	ND	2	T	T	ND	ND
Se	**865.9**	120.2	380.8	446.2	65.9	237.9	142.4	465.9
Si	**7**	1	ND	ND	ND	2	ND	ND
Ti	26	26	**49**	44	6	13	40.2	40
V	32.9	23.7	**86.4**	82	5.3	49.1	43.2	46.4
Zn	**20**	3.4	ND	ND	ND	ND	2.3	ND

ND: Not detected. T: Traces. * Bold written values indicate the highest content of mineral compounds.

Table 3. Mineral Composition of *Chlorophyllum agaricoides* (µg/g extract)*

	Sequential Fractions						Water Extract	Drug
	n-Hexane	Chloroform	Ethyl Acetate	Acetone	Ethanol	Residue		
Ag	ND	ND	ND	ND	ND	ND	T	T
B	**1169**	ND	ND	ND	ND	14.2	ND	ND
Ba	3.4	3.5	8.6	**236**	T	1.3	ND	ND
Be	614.9	**3281.4**	1644.7	1363.6	385.4	1915.1	2102.9	1079.7
Ca	2.7	**10.9**	4.1	4.8	T	T	T	T
Cd	T	6.5	ND	1.9	T	T	**11.1**	T
Co	4.8	3.1	18.3	**27.9**	13.7	27.2	T	6.4
Cu	13.1	**282**	187.4	153.3	16.6	30.9	56.4	193.4
Fe	5155.2	11600	6131.6	7812.1	23180	**76582**	5023	3988.1
K	302.4	675.5	934.2	446	46.3	**2306.6**	176.5	185.7
Mg	3.4	7.6	**11.9**	3.1	T	8.1	11.5	T
Mn	**48**	36	9.1	2.6	T	T	ND	1.1
Mo	1696.1	3258.3	**3494.7**	2925.5	387.6	1133.9	486.5	215.3
Na	ND	ND	ND	ND	T	T	ND	ND
Ni	6.6	4.9	13.4	3.5	T	T	179	**469**
Pb	7.2	**27.1**	5.5	ND	T	T	ND	ND
Sb	8.1	**21**	8.4	ND	6.5	3.8	2.2	3.5
Se	770	**1006**	667	870	66	84	82.2	438
Si	ND	ND	ND	**1.8**	ND	ND	ND	T
Ti	17	**136**	61	88	6	14	40.1	39
V	22	119	**161**	79	10	76	57.6	100
Zn	ND	ND	ND	ND	ND	ND	T	T

ND: Not detected. T: Traces. * Bold written values indicate the highest content of mineral compounds.

Table 4. Mineral Composition of *Tricholoma populinum* (µg/g extract)*

	Sequential Fractions						Water Extract	Drug
	n-Hexane	Chloroform	Ethyl Acetate	Acetone	Ethanol	Residue		
Ag	ND	9.7	**11.3**	ND	0.9	ND	ND	2.1
B	**451**	ND	ND	ND	ND	ND	ND	ND
Ba	4	1.4	2.1	**171.8**	11.7	1.1	ND	ND
Be	42.2	21.1	59.2	**96.3**	6.5	95.5	ND	ND
Ca	ND	T	ND	**18.1**	ND	T	**1528.5**	**1285.6**
Cd	1009.2	803.9	1890.4	**32400**	618.1	1362.8	1.8	3
Co	3.4	1.7	8	**67.6**	T	2	T	T
Cu	T	T	3.5	**25**	T	T	T	1.5
Fe	6.7	6.4	**18.7**	ND	T	14.8	92.3	325.9
K	132.9	211.5	200.6	3530	3.9	183	**5389.2**	5614
Mg	4739.8	1084.4	3686.6	14060	29820.7	**74975.3**	109.3	120.7
Mn	460.6	160.8	227.3	654.1	88.9	**1422.8**	T	T
Mo	10.4	5.2	4	**45.8**	T	14	ND	ND
Na	34.1	2.3	ND	ND	ND	T	**216.6**	94.6
Ni	1392.8	620.3	2919.5	**23170**	193.6	388.3	ND	ND
Pb	T	18.4	ND	16.3	ND	ND	**153**	**444.9**
Sb	6.3	2.5	7.8	ND	T	1.7	T	T
Se	7.5	1.7	16.6	**98.9**	T	T	T	1.1
Si	5.1	1.2	32.9	**168.5**	1.6	1.4	53.5	415.7
Ti	279.6	269	440.7	**3871**	98.1	386.5	ND	ND
V	ND	1.6	ND	ND	ND	4.45	**39.6**	38.5
Zn	21.7	31.6	141.5	**1295**	6.3	7.9	50.6	51.4

ND: Not detected. T: Traces. * Bold written values indicate the highest content of mineral compounds.

Mineral composition of *Tricholoma populinum* was presented in Table 4. Relatively higher levels of minerals were detected in water-based fraction and extracts and drug compare to organic solvents-based fractions. Similar to other mushroom samples, drug contained the highest level of Pb. Interestingly, Cd and Ni levels of acetone-based fraction, water extract and drug were detected in pronounced levels. Mg, K, Ca and Zn were detected as the major mineral compounds. Additionally, Ag, B, As, Cr were not detected in all extracts (Table 4).

Table 5. Mineral Composition of *Lycoperdon utriforme* (µg/g extract)*

	Sequential Fractions						Water Extract	Drug
	n-Hexane	Chloroform	Ethyl Acetate	Acetone	Ethanol	Residue		
Ag	**12.2**	ND	ND	7.4	T	T	ND	T
B	**271**	ND	ND	ND	ND	ND	ND	ND
Ba	24.9	T	22.6	**322**	T	1.44	ND	ND
Be	5540	4566.9	**6720**	2609.1	214.3	1350.4	2127.6	1353.3
Ca	24.3	3.8	**28.2**	6.8	0.4	0.5	T	0.7
Cd	**10.3**	1.8	7.7	1.3	T	T	T	T
Co	**54**	13	5	4	9	35	4.2	9
Cu	692	253.2	**764**	262.7	25.3	143.3	244.2	224.6
Fe	5496	3403	ND	3623	9722	**72560**	3368.7	1640
K	1136	364	611	796	30	**2144**	124.6	126
Mg	11	4.2	ND	7.4	T	**15**	ND	T
Mn	**266**	3.17	41.7	6.13	T	2.3	ND	ND
Mo	**11060**	1865	9608	5649	284	740	651.5	117
Na	ND	ND	**16**	ND	ND	ND	ND	ND
Ni	75.4	2.8	ND	ND	T	1.2	**532.5**	418
Pb	4.2	9.4	**53**	6.2	T	1.07	ND	ND
Sb	**132**	9	47	10	1.1	T	ND	T
Se	**3870**	380	1936	936	218	176	104	337
Si	ND	6	**11**	ND	ND	1.6	ND	T
Ti	317	65	**478**	130	6.6	9.3	148.5	38
V	161	159	**1412**	133	8	44	54.3	96
Zn	**12.2**	ND	ND	7.4	T	T	ND	T

ND: Not detected. T: Traces. * Bold written values indicate the highest content of mineral compounds.

The levels of mineral compounds present in *Lycoperdon utriforme* were presented in Table 5. K, Na, Mg, Si and Zn were the dominant compounds of mineral composition. With regards to heavy metal contents; As and Cr were not detected, while Ni and Cd were at low levels except Pb (Table 5). n-Hexane based fraction was the most efficient in the context of extracting Ag, B, Cu, Mo, Na, Se and Si. Water-based extracts were found as more efficient in extraction of K, Mg and Pb than those of organic solvents (Table 5).

4. DISCUSSION AND CONCLUSION

Mushrooms have been used as important dietary supplements because of their pleasant tastes, nutritional and pharmaceutical constituents such as rich protein, low-fat content, secondary metabolites, vitamins and minerals. Minerals are vital chemical compounds for humans which have crucial functions such as maintaining acid-base balance, the osmotic regulation of fluid and oxygen transport in the body and also playing significant roles in the catalytic processes [12].

Wild grown edible mushroom species are able to cumulate significant amounts of mineral constituent's specifically K, P, Ca, Mg, Na and Fe which are essential to fungi and its consumers. Mushrooms can also be enriched with toxic elements such as As, Hg and Cd [13], which can cause several health risks and have no any significant biological roles. The toxic effects of these hazardous elements are harmful for the human body and its proper functions.

For instance, Cd is the seventh most toxic heavy metal which has adverse effects on the enzymatic systems of cells and oxidative stress [14].

Previous analysis carried out on mineral composition of mushroom species were generally focused only on drug materials which have directly treated with HNO_3 [11, 15, 16]. The utilization of a single solvent (HNO_3) restricted the extraction of mineral compounds from mushroom tissues and therefore various extractions including different solvent systems with different polarities should be applied in order to analyse the mineral composition comprehensively.

Our findings showed that extracting of mineral compounds were varied due to the type of solvents applied and mycochemical diversity. For instance, hexane is a proper solvent in the extraction of Ag, B, Be, Mo, Ti, while ethyl acetate is more appropriate in the extraction of Zn and Fe. Deductively it can be suggested that, acetone and/or ethyl acetate were relatively more efficient solvents than those of other organic solvents.

Mushrooms rich in K and Mg and poor in Na are recommended for the treatment of hypertension [17]. Our findings were in accordant with this aspect and it can be suggested that all mushrooms analysed in this study (except *T. populinum*) might be helpful in the management of hypertension. Moreover, the extracts contained high levels of Ca and Mg, which might help for the maintenance of nerve transmission, glandular secretion, muscle contraction and hinder the biochemical abnormalities and clinical manifestations [18].

All samples were found as rich sources of minerals notably Ca, K, Si, Na and Mg. The levels of heavy metals were generally in low amounts except Pb. However, *Tricholoma populinum* extracts were accumulated significant amounts of Cd. With regards to Pb accumulation, organic solvent fractions were found as more selective than those of the drug and water extract which indicate that polarity of solvents are a significant factor of Pb isolation from mushroom tissues. Although *T. populinum* were detected as a rich source of mineral compounds, it also contained an excessive amount of Cd and therefore care should be taken in the consuming of this mushroom species.

This study confirms the presence of some important bioactive mineral compounds, including K, Mg, Si, Se and Fe. The reported mineral compounds profiles propose the use of wild mushrooms as potential sources of curative nutraceuticals.

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Conflict of Interests

Authors declare that there is no conflict of interests.

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