



Proximate composition and element contents of selected species of *Ganoderma* with reference to dietary intakes

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Received: 22 August 2019 / Accepted: 26 March 2020 / Published online: 8 April 2020
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Abstract *Ganoderma* is a wood-degrading mushroom that is treasured as a functional food since primitive times. Monitoring of macronutrient and element levels in mushrooms collected from the natural environment provides basic information in terms of safety, regulation, and nutrition. A comparative study was developed on the proximate and element contents of *Ganoderma applanatum*, *G. brownii*, *G. lucidum*, and *G. philippii* collected from different zones of the natural forests in Uttarakhand, India. These mushrooms revealed high amounts of proteins (9.29–12.4%) and carbohydrates (75.5–80.3%) and low contents of fats (1.62–2.87%), but ash (6.14–8.32%) and fibre (4.92–8.07%) were available in significant amounts. Element concentrations were determined by wavelength dispersive X-ray fluorescence (WDXRF) spectrometry. Calcium (5400–19,250 mg/kg) and potassium (2602–5601 mg/kg) were the predominant elements in mushrooms. The mushroom samples provided

significant percentage contribution to reference recommended dietary intakes (RDIs) of essential elements such as calcium (27.0–96.3%), copper (58.2–95.8%), and manganese (37.3–62.3%), for adult males and females; and iron (35.3–97.1% for males and 28.6–78.6% for females), magnesium (7.06–11.5% for males and 7.74–12.6% for females), and zinc (6.35–19.8% for males and 7.65–23.7% for females). The studied mushrooms have no health risks as toxic metals such as aluminium and lead were detected below the legislated respective provisional tolerable intake values. Nutritional quality index (NQI) values revealed that mushrooms are densely rich in calcium, copper, iron, magnesium, manganese, and zinc.

Keywords Mushrooms · NQI · Provisional tolerable intake · RDIs · Uttarakhand · WDXRF

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10661-020-08249-7>) contains supplementary material, which is available to authorized users.

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Introduction

In recent years with increased interest in a healthy lifestyle and disease prevention, there has been a vast and rapidly growing consumer demand for functional foods with high nutritional and medicinal value. Mushrooms are highly admired for their nutritional value and beneficial health effects (Barros et al. 2008; Gogavekar et al. 2014). Mushrooms have excellent nutritional value as they are good sources of carbohydrates, proteins, fibre, minerals, vitamins, and low in fat content (Kalač 2009; Wang et al. 2014). Apart from their use as food, mushrooms also have numerous pharmacological effects owing to the presence of relatively high levels of

nutraceuticals such as unsaturated fatty acids, phenolic compounds, alkaloids, steroids, terpenoids, ascorbic acid, and carotenoids (Kozarski et al. 2011; Ma et al. 2018).

Ganoderma is a wood-inhabiting mushroom with poroid hymenophore having rich diversity in tropical and sub-tropical forests, whereas some species are confined to temperate regions (Bakshi 1976). *Ganoderma* has been perceived as one of the most important medicinal mushrooms, and the herbal history regarding its amazing beneficial effects has been archived in ancient scripts (Bishop et al. 2015). The bioactive constituents extracted from *Ganoderma* include polysaccharides, terpenoids, proteins, alkaloids, phenolic compounds, and fatty acids, with wide-ranging biological activities such as anti-allergic, anti-cancer, anti-diabetic, anti-inflammatory, anti-microbial (anti-bacterial, anti-fungal, and anti-viral), anti-oxidant, hepatoprotective, and immunomodulatory (Paterson 2006; Baby et al. 2015). Many *Ganoderma* products such as coffee, tea, dietary supplements, toothpastes, soaps, tonics, pills, spore products, and lotions are available in the market which has been popularised for promoting health benefits (Lai et al. 2004; Bishop et al. 2015). More than 90 brands of *G. lucidum* products have been registered and being marketed globally with significant economic impact (Lin 2000; Bishop et al. 2015). In India, the dried fruiting bodies of *Ganoderma* are sold in the market at a rate of Rs 600–700/kg, with an annual business of about Rs 120 cores (Kapoor and Sharma 2014).

The mushrooms collected from natural habitats can be very effective in the accumulation of essential and undesirable elements from the ecosystem due to which there might be the probability of some toxic elements intrusion into the food chain (Sarikurkcu et al. 2011). Thus, mushrooms represent reliable bioindicators to evaluate the level of environmental pollution. Mushrooms contain a large amount of essential minerals and trace elements that are required for proper growth, normal functioning, and good health. The essential macroelements such as calcium, magnesium, phosphorus, potassium, and sodium aid in specific human body functions for the osmotic regulation of fluid, maintenance of acid-base balance, and transport of oxygen (McDowell 2003). Copper, iron, manganese, zinc, and molybdenum are essential micronutrient minerals since they act as cofactors of key enzyme complexes in many metabolic pathways relevant to the different biological systems (Keen et al. 2004). Aluminium, arsenic, cadmium, lead, and mercury are non-essential elements that can create serious toxicological

effects on humans, even at very low levels (Garcia et al. 2009; Aloupi et al. 2012). Excessive intake of the essential elements can also have toxicological effects on health (Tuzen et al. 2007).

The analysis of nutrients in natural products is highly relevant for consumers' demand not only from a nutritional perspective but also to evaluate quality and toxicity from their consumption (Bhat et al. 2010). Adequate dietary intake of essential elements is essential for human health and wellness. Further, there is a need to standardise the permissible levels of elements in natural products to evaluate the potential health risks associated with their consumption. With all these attributes, the purpose of the study was to determine the levels of elements in *Ganoderma applanatum*, *G. brownii*, *G. lucidum*, and *G. philippii* collected from Uttarakhand, India, by wavelength dispersive X-ray fluorescence (WDXRF) spectrometry. The use of WDXRF technique for various solid biological samples has increased over the last few years since the analytical performance of the method was proved to be effective and robust (Salvador et al. 2002; Kaymak et al. 2010). WDXRF is a simple, non-destructive, fast, multi-element analysis technique with adequate sensitivity and relatively short analysis time as compared with conventional spectroscopic techniques. WDXRF can be directly applied in dried samples without any chemical pre-treatment, thus decreasing the consumption of reagents used for analysis (Margui et al. 2005).

This study gives a comprehensive evaluation of elements and their contribution to dietary intakes of elements with a view to provide information on the nutritional qualities and safety aspects of the mushrooms.

Materials and methods

Mushroom samples

The fruiting bodies of the mushroom samples were collected during the monsoon months (July–August) from different forest sites of Uttarakhand, India, and authenticated on the basis of morphological and microscopic characters (Singh et al. 2014). Characteristics of the examined mushrooms regarding sampling sites and hosts are shown in Table 1. Voucher specimens of the mushrooms were deposited at the herbarium of the Department of Botany, Punjab University, Patiala (PUN).

Table 1 Herbarium number, sampling sites, coordinates, host, and substratum of examined species of *Ganoderma*

Species	Herbarium number	Site of collection	Coordinates	Host	Substratum
<i>G. applanatum</i>	PUN 6563	Forest Research Institute, Dehradun	30.3165° N, 78.0322° E	<i>Mangifera indica</i>	Stem
<i>G. brownii</i>	PUN 6564	Tilwara, Rudraprayag	30.3445° N, 78.9762° E	<i>Grevillea robusta</i>	Stem
<i>G. lucidum</i>	PUN 5077	Chaukori, Pithoragarh	29.8385° N, 80.0332° E	<i>Quercus incana</i>	Root
<i>G. philippii</i>	PUN 5078	Mussoorie, Dehradun	30.4599° N, 78.0664° E	<i>Quercus incana</i>	Root

Proximate composition

The content of crude protein was analysed by the macro-Kjeldahl method (AOAC 1990). The fat content was investigated by successive Soxhlet extraction of the powdered sample with petroleum ether (AOAC 1995). The crude fibre estimation was based on the treatment of the dried and fat-free powdered sample with dilute acid and alkali (Maynard 1970). The ash content was determined by incineration of mushroom samples at 450 °C until free of carbon (Indian Pharmacopoeia Commission 2007). Total carbohydrates were estimated by using the following formula:

$$\text{Total carbohydrates (\% dry weight)} = 100 - [\text{crude protein (\%)} - \text{crude fat (\%)} - \text{ash content (\%)}].$$

The energy values were calculated by the relations as suggested by Crisan and Sands (1978).

$$\text{Energy(Kcal/100 g)} = \text{Protein} \times 4 + \text{Fat} \times 9 + \text{Carbohydrates} \times 4$$

Sample preparation for element analysis

The fruiting bodies of mushrooms were grounded into powder using a SPEX 6870 Freezer Mill (SPEX Sample Prep, Metuchen, USA). Powdered samples were dried in an oven at a temperature not exceeding 60 °C until the uniform weight was attained. Powdered sample (8 g) was compacted and pressed using a hydraulic press machine by applying 20 tons of pressure for 2 min. The sample pellets were 1.5 mm thick and 20 mm in diameter. Three pellets of each sample were prepared. The sample pellets were dried at 60 °C for 40 min. Each pellet was analysed three times by WDXRF by altering their position and orientation after each measurement (Kim et al. 2016).

Instrumentation

WDXRF system consists of the X-ray source, sample holder, collimators, crystals, detectors, amplifier, and recorder. In this study, a wavelength dispersive X-ray fluorescence spectrometer

(WDXRF, S8 Tiger Bruker Germany with rhodium anode as X-ray source) was used. All measurements of the WDXRF were implemented under the conditions as tube maximum power (W) 4000; tube voltage (kV) 20–60; tube current (mA) 5–170; analytical line K α ; crystal LiF(200); collimators 0.23 dg, 0.46 dg, 1.0 dg, and 2.0 dg; detectors for light elements-gas proportional counter and for heavy elements-scintillation counter; peak angle (degree) 3; and counting time (s) 40.

Irradiating the samples by an X-ray beam is the basis of the WDXRF analysis. Element concentrations can be determined by measuring the energy and intensity of the fluorescent X-ray radiations emitted by the element present in the sample. The emitted X-rays passed through collimators and were reflected by crystals. The detector converted the emitted X-rays to measurable pulses. The numbers of pulses (counts per second) were plotted against the energy of emitted photons. The height of a peak profile determined the intensity of an element (Costa et al. 2019). The concentrations of different elements were determined by measuring the intensities of the emitted energies using the software ‘Quant Express’.

Estimated daily intakes (EDIs) of elements

The estimated daily intake (EDI) values of different elements by the consumption of the mushrooms analysed were determined by the equation

$$EDI = c \times m$$

where EDI is the estimated dietary intake of the element (mg/day), *c* is the mean concentration of element (mg/kg), and *m* is the average daily consumption (kg/day).

Based on the recommendations of the European Scientific Committee for Food Adult Weight parameter, an adult person having an average body weight of 60 kg reaches a tolerable daily intake by average daily consumption of 300-g portion of fresh mushrooms, which contains 30-g dry matter (Kalač and Svaboda 2000; Çayir et al. 2010).

Dietary intakes of essential elements

To evaluate the essential element contribution by mushroom consumption, the recommended dietary intakes (RDIs) for adults were drawn from a report published by the Indian Council of Medical Research (ICMR 2009). The percentage contribution to RDI was calculated as

$$\%RDI = \frac{EDI}{RDI} \times 100$$

where EDI is the estimated dietary intake (mg/day) and RDI is the recommended dietary intake (mg/day) of element.

Dietary intakes of toxic elements

Dietary intakes of toxic elements were determined in terms of EDI (estimated daily intake) and EWI (estimated weekly intake) and compared with the PTDI (provisional tolerable daily intake) and PTWI (provisional tolerable weekly intake) reference values established by the Food and Agriculture Organization/World Health Organization (FAO/WHO 1993, 1999) and Joint Expert Committee on Food Additives (JECFA 2007).

Estimated weekly intake of the specific element through consumption of mushrooms was calculated as

$$EWI = EDI \times 7$$

where EDI is the estimated dietary intake (mg/day) and EWI is the estimated weekly intake (mg/week) of element.

Nutritional quality index (NQI) of essential elements

The nutritional quality index of a specific essential element was estimated by the formula proposed by van Heerden and Smith (2013).

$$NQI = \frac{C \times ERDI}{E \times RDI}$$

where NQI is the nutritional quality index, C is the mean concentration of element (mg/100 g), ERDI is the energy in terms of recommended dietary intake (2730 kcal/day for men and 2230 kcal/day for women), E is the calculated energy (kcal/100 g), and RDI is the recommended dietary intake of the element (mg/day).

Statistical analysis

The results of the proximate and elemental contents were determined in triplicates and expressed as mean \pm SEM. Data were standardised by setting mushroom samples in columns and proximate components or elements in rows. The first analysis comprised of Student's t distribution test of significance to predict the significant differences among mean contents of macronutrients and elements across all the mushroom species at $p < 0.05$.

The next step consisted of Pearson's linear correlation analysis to establish the strength of the relationship between the analysed element contents of the mushrooms. Significant correlation coefficients ($r \geq \pm 0.514$ at 0.05 probability level) were established between concentrations of elements.

Additionally, element diversity was also studied by plotting the content of elements on the logarithmic scale (Mleczek et al. 2018).

Finally, the Friedman rank-sum test was performed to indicate differences between the studied species of mushrooms concerning levels of essential macroelements, trace elements (essential as well as toxic), and all elements jointly followed by post hoc Nemenyi test. All statistical analyses were implemented with SPSS 16 software.

Results and discussion

Proximate composition

The results of the proximate analysis of mushroom samples are depicted in Table 2. On the basis of dry biomass, *G. lucidum* revealed the highest values of ash (8.32%) and crude protein (13.3%) among the studied mushroom samples. The ash content values in the current study are comparable with the literature values ranging from 1.00 to 8.90% (Cohen et al. 2014) and 3.66 to 9.70% (Takshak et al. 2014).

Proteins are the most important group of biomolecules contributing to the nutritional value of mushrooms (Chang and Hayes 1978). According to earlier reports, crude protein content has been determined in the range of 9.93–16.8% (Ogbe and Obeka 2013; Stojković et al. 2014) for different *Ganoderma* species. Crude protein values in this investigation are in accordance with literature reports. Several factors affect the protein content of

Table 2 Mean values ($n = 3$) of proximate components (% on dry mass basis) and energy values (kcal/100 g and kJ/100 g) investigated in species of *Ganoderma*

Parameter	<i>G. applanatum</i>	<i>G. brownii</i>	<i>G. lucidum</i>	<i>G. philippii</i>
Ash (%)	8.02 ± 0.96 ^a	6.14 ± 0.74 ^b	8.32 ± 0.60 ^a	7.67 ± 0.95 ^{a,b}
Crude protein (%)	12.4 ± 1.09 ^a	11.6 ± 1.32 ^{a,b}	13.3 ± 0.99 ^a	9.29 ± 0.83 ^b
Crude fibre (%)	8.07 ± 0.34 ^a	4.92 ± 0.05 ^b	7.37 ± 0.13 ^a	5.04 ± 0.05 ^c
Crude fat (%)	1.70 ± 0.10 ^{a,c}	1.95 ± 0.05 ^a	2.87 ± 0.16 ^b	1.62 ± 0.25 ^c
Carbohydrate (%)	77.8 ± 1.39 ^a	80.3 ± 1.78 ^b	75.5 ± 0.82 ^a	81.4 ± 1.40 ^b
Energy (kcal/100 g)	376 ± 3.67 ^a	385 ± 3.26 ^b	381 ± 3.23 ^c	377 ± 4.30 ^a
Energy (kJ/100 g)	1598 ± 15.7 ^a	1635 ± 13.4 ^b	1616 ± 13.4 ^c	1602 ± 18.1 ^{a,c}

Results expressed with the mean ± standard error for each sample ($n = 3$)

Values in the same row with different superscript letters are significantly different ($p < 0.05$)

mushrooms including type and morphology of species, growth stage, a part used for analysis, nitrogen content, and the locality of sampling (Flegg and Maw 1977).

This study showed a mean concentration of crude fibre ranging from 4.92 to 8.07% which is in concomitant with the previous reports on mushrooms with values ranging from 2.80 to 8.42% (Ogbe and Obeka 2013; Stojković et al. 2014). Fibre consists of a group of non-digestible carbohydrate polymers and has many potential health benefits. It improves the function of the alimentary tract, lowers blood glucose and cholesterol levels, acts as a prebiotic, and supports the synthesis of short-chain fatty acids. Dietary fibre also improves sensory, texture, viscosity characteristics, oil and water holding properties, emulsion properties, and shelf life of foods (Elleuch et al. 2011). The crude fibre in mushrooms consists of major compounds such as chitin, β-glucans, and mannans (Cheung et al. 2013).

The fat content of the mushroom samples was generally low with *G. lucidum* having the highest value of 2.87%, while *G. applanatum* provided the lowest value of 1.62%. Fats or lipids are essential biomolecules that act as structural and functional components of membranes, hormone modulators, thermal insulators, and constituents of the myelin sheath. They can help the digestion process and serve as a source of metabolic energy (Tocher 2003). Therefore, lipids are vital components of the human body that must be supplied in small quantities in the diet. However, the intake of high quantities of lipids leads to the increased risk of some chronic diseases such as atherosclerosis, cardiovascular dysfunctions, hypertension, obesity, and diabetes (Eyre et al. 2004). Crude fat contents in the investigated species of *Ganoderma* are in agreement with those

estimated in previous reports (Ogbe and Obeka 2013; Stojković et al. 2014).

The total carbohydrate accounts for more than 70% of the proximate composition for all the mushrooms ranging between 70.3% in *G. brownii* and 81.4% in *G. philippii*, respectively. Carbohydrates in foods provide energy and constitute the prevailing component of fruiting bodies of mushrooms. The carbohydrate contents among the studied mushroom samples are in consistent with the earlier reported values for different *Ganoderma* species (Ogbe and Obeka 2013; Stojković et al. 2014).

The energy values of the studied mushroom samples were reported as 376.4–385.4 kcal/100 g by taking into consideration the percentage of proteins, fats, and carbohydrates present in 100 g of dry sample.

Elemental analysis

The concentrations of different mineral elements were identified and quantified by wavelength dispersive X-ray fluorescence (WDXRF) spectrometry. WDXRF chromatograms acquired from the quantification of elements in examined mushrooms are depicted in Figs. 1, 2, 3, and 4 (Supplementary Material). Element contents were estimated on the basis of dry biomass of mushrooms (mg/kg). The comparative element concentrations for the analysed mushroom samples are reported in Table 3. Potassium and calcium were detected in the maximum amount in the studied mushroom samples. The results showed varying element concentrations in mushrooms. The accumulation of elements in mushrooms has been found to be affected by environmental factors, such as the area of sample collection, distance

Table 3 Mean values ($n = 3$) of elements (mg/kg on dry mass basis) determined in species of *Ganoderma*

Element	<i>G. applanatum</i> (mean \pm SEM, $n = 3$)	<i>G. brownii</i> (mean \pm SEM, $n = 3$)	<i>G. lucidum</i> (mean \pm SEM, $n = 3$)	<i>G. philippii</i> (mean \pm SEM, $n = 3$)
Al	3.03 \pm 0.05 ^a	3.97 \pm 0.26 ^a	3.79 \pm 0.43 ^a	3.57 \pm 0.08 ^a
Ba	ND	ND	ND	7.26 \pm 0.05
Br	ND	ND	ND	6.34 \pm 0.04
Ca	5800 \pm 236 ^a	7200 \pm 289 ^b	5400 \pm 277 ^c	19,250 \pm 433 ^d
Cl	299 \pm 41.6 ^a	500 \pm 46.5 ^b	399 \pm 42.7 ^c	199 \pm 37.3 ^d
Cr	4.97 \pm 0.04 ^a	4.40 \pm 0.11 ^b	2.01 \pm 0.02 ^c	4.97 \pm 0.09 ^a
Cu	43.1 \pm 4.60 ^a	38.7 \pm 3.56 ^b	26.2 \pm 4.45 ^c	36.7 \pm 2.23 ^d
Fe	302 \pm 36.9 ^a	400 \pm 57.7 ^b	550 \pm 47.9 ^c	200 \pm 28.9 ^d
K	2901 \pm 114 ^a	4400 \pm 179 ^b	5601 \pm 236 ^c	2602 \pm 119 ^d
Mg	800 \pm 104 ^a	1103 \pm 110 ^b	1301 \pm 101 ^b	800 \pm 102 ^a
Mn	43.5 \pm 4.43 ^a	72.7 \pm 5.91 ^b	48.3 \pm 5.57 ^c	67.1 \pm 7.18 ^d
Mo	14.3 \pm 1.35 ^a	12.3 \pm 1.26 ^b	7.95 \pm 1.16 ^c	12.4 \pm 1.52 ^b
Na	300 \pm 43.4 ^d	401 \pm 54.8 ^b	500 \pm 48.1 ^c	200 \pm 43.8 ^d
Ni	6.36 \pm 0.15 ^a	12.3 \pm 0.14 ^b	7.23 \pm 0.11 ^c	6.46 \pm 0.15 ^a
P	800 \pm 98.7 ^a	1800 \pm 87.2 ^b	2101 \pm 107 ^c	802 \pm 70.1 ^a
Pb	ND	3.93 \pm 0.18 ^a	ND	4.67 \pm 0.36 ^a
Pd	ND	4.02 \pm 0.10 ^a	ND	4.51 \pm 0.15 ^a
Rb	4.00 \pm 0.01 ^a	2.51 \pm 0.01 ^b	5.57 \pm 0.15 ^c	ND
Ru	2.27 \pm 0.22 ^a	ND	5.10 \pm 0.20 ^b	5.15 \pm 0.18 ^b
S	1302 \pm 94.4 ^a	2000 \pm 123 ^b	1699 \pm 95.8 ^c	1798 \pm 69.2 ^d
Si	210 \pm 25.4 ^d	520 \pm 58.0 ^b	460 \pm 57.4 ^c	170 \pm 23.1 ^d
Sr	6.48 \pm 0.03 ^a	2.30 \pm 0.63 ^b	6.02 \pm 0.06 ^c	5.62 \pm 0.25 ^d
Ti	6.38 \pm 0.44 ^a	10.0 \pm 1.16 ^b	20.0 \pm 1.73 ^c	8.34 \pm 0.04 ^d
Zn	25.4 \pm 4.57 ^a	66.9 \pm 8.06 ^b	27.2 \pm 4.19 ^c	79.1 \pm 10.9 ^d
Zr	1.02 \pm 0.01 ^a	4.84 \pm 0.28 ^b	4.97 \pm 0.05 ^b	3.03 \pm 0.25 ^b

Results expressed with the mean \pm standard error for each sample ($n = 3$), ND- Not detected

Values in the same row with different superscript letters are significantly different ($p < 0.05$)

from polluted sites, geochemical characteristics of soil, host plant, and substrate in which the mushroom mycelium develops, and fungal factors such as species of mushroom, stages of growth, the morphology of the basidiocarp, and biochemical constituents affect the accumulation of elements in mushrooms (Kalač and Svaboda 2000; Dimitrijevic et al. 2016). Species of *Ganoderma* cultivated on different substrates under different conditions can be shown to affect the accumulation of elements in the mycelium and fruiting body. The overgrown substrate or soil has directly affected the bioaccumulation and concentration of elements in fruiting bodies of mushrooms (Marek et al. 2017). In the present study, *G. lucidum* generally accumulates higher contents of elements because it grows on roots and has direct contact with the soil, while *G.*

applanatum accumulates the least amounts of elements due to its association with the stem of the host plant. This is due to the limited availability of elements in the stem as compared with that in the roots.

The mean aluminium level of the mushroom samples ranged from 3.03 mg/kg in *G. applanatum* to 3.97 mg/kg in *G. brownii*. The statistical analysis revealed a lack of significant differences between the mean levels of aluminium among the studied mushrooms. The concentration of aluminium in wood-decaying mushrooms has been determined in the range of 130–640 mg/kg (Campos 2011) and 44.0–51.7 mg/kg (Lim et al. 2012). Aluminium content in the range of 10.0–1208 mg/kg has been observed in specimens of *Ganoderma* from China and Poland (Marek et al. 2017). Aluminium contents in our study are lower than those reported earlier.

The mean concentration of calcium ranged between 5400 and 19,250 mg/kg in the studied mushrooms. The average calcium content varied significantly ($p < 0.05$) among all the mushroom samples where *G. philippii* revealed the highest concentration, followed by *G. brownii*, *G. applanatum*, and *G. lucidum*, respectively. The calcium levels in *Ganoderma* estimated earlier ranged from 523 to 2398 mg/kg (Nguyen and Park 2007) and 568 to 2480 mg/kg (Obodai et al. 2017). The calcium levels in the present study are more than those reported previously.

Our study showed a mean concentration of chromium ranging from 2.01 to 4.97 mg/kg. Previous studies by Lim et al. (2012) recorded chromium in the range 0.09–0.22 mg/kg in specimens of *Phellinus*, while Yalcin et al. (2019) reported 9.50–172.2 mg/kg amount of chromium in *Ganoderma*.

The average levels of copper ranged from 26.2 mg/kg in *G. lucidum* to 43.1 mg/kg in *G. applanatum*. Significant differences ($p < 0.05$) have been observed between the analysed mushrooms for their copper content. Copper contents in our findings are more than those published previously for wood-rotten mushrooms (Campos 2011; Lim et al. 2012). Turkish *Ganoderma* species revealed higher levels of copper (277–291 mg/kg) according to the published findings of Yalcin et al. (2019).

The mean iron content in our mushroom samples ranged from 200 mg/kg in *G. philippii* to 550 mg/kg in *G. lucidum*. These values are in consistent with the earlier reported iron values, 24.0–5285 mg/kg (Marek et al. 2017) and 49.9–2990 mg/kg (Obodai et al. 2017) for wild *Ganoderma* species.

The magnesium content in the present study ranged from 800 to 1301 mg/kg. *G. lucidum* revealed significantly ($p < 0.05$) the highest magnesium concentration than the rest of the species. Magnesium levels in the earlier studies on wood-rotten fungi have been investigated in the range of 730–1340 mg/kg (Campos 2011) and 354–2537 mg/kg (Lim et al. 2012). Obodai et al. (2017) recorded values in the range of 430–2048 mg/kg in *Ganoderma* samples. Magnesium values in this investigation are in accordance with literature reports.

The mean potassium concentration ranged from 2602 mg/kg in *G. philippii* to 5601 mg/kg in *G. lucidum*, respectively. Similar findings for potassium in the range of 1000–6379 mg/kg were described for *Ganoderma* species from Ghana (Obodai et al. 2017).

Among the investigated mushroom species, *G. brownii* showed the maximum amount of sodium

(401 mg/kg), while *G. philippii* showed the minimum amount of sodium (200 mg/kg). Concentrations of sodium in the literature reports have been in the range of 110–640 mg/kg (Campos 2011) and 24.0–334 mg/kg (Marek et al. 2017), which are similar to our study.

G. brownii evinced significantly ($p < 0.05$) highest manganese content followed by *G. philippii*, *G. lucidum*, and *G. applanatum*. Manganese values of mushroom samples were found in the range of 43.5–72.7 mg/kg which are in accordance with those obtained earlier, 5.90–419 mg/kg (Lim et al. 2012) and 9.00–190 mg/kg (Marek et al. 2017).

The nickel contents of the mushrooms studied in the present work are more than the literature values ranging from 1.80 to 3.80 mg/kg (Campos 2011) and 0.08 to 1.60 mg/kg (Marek et al. 2017).

The minimum and maximum values of phosphorus were 800 mg/kg and 2101 mg/kg in *G. applanatum* and *G. lucidum*. Phosphorus contents of mushroom samples in the literature have been reported to be in the range of 925–10,961 mg/kg (Marek et al. 2017) and 377–1936 mg/kg (Obodai et al. 2017). Phosphorus contents found in this study are parallel to those stated in the literature.

The mean lead contents in the present study varied from 3.93 to 4.67 mg/kg. The reported lead values in the literature for *Ganoderma* by Marek et al. (2017) were in the range of 0.20–7.40 mg/kg, while Yalcin et al. (2019) reported a range of 4.60–6.96 mg/kg. The lead values in this study are in concomitant with those mentioned in the literature.

The average content of zinc ranged from 25.4 mg/kg in *G. applanatum* to 79.1 mg/kg in *G. philippii*. Values are in correlation with the literature reports ranging from 17.1 to 140.4 mg/kg (Campos 2011) and 0.7 to 147.5 mg/kg (Lim et al. 2012).

The average levels of chlorine, molybdenum, palladium, rubidium, ruthenium, sulphur, silicon, strontium, titanium, and zirconium of the studied mushroom samples ranged from 199 to 500, 7.95 to 14.3, 4.02 to 4.51, 2.51 to 5.57, 2.27 to 5.15, 1302 to 2000, 170 to 520, 2.30 to 6.48, 6.38 to 20.0, and 1.02 to 4.97 in mg/kg, respectively. Barium (7.26 mg/kg) and bromine (6.34 mg/kg) were detected only in *G. philippii* among the studied mushroom samples.

Correlation analysis quantifies the relationship between the two variables and is measured by correlation coefficient r . The value of r ranges from -1 to $+1$. The positive correlation indicates an increase or decrease in values of one variable with respect to the other variable.

The negative correlation occurs between pairs of uncorrelated variables where an increase in the value of one variable causes a decrease to the other variable. As is evident from Table 4, very high significant correlation ($r \geq \pm 0.90$) occurs between Al and S, Al and Zr, Cl and Si, Cr and Fe, Cr and K, Cr and Mg, Cr and Mo, Cr and Ti, Cu and Mo, Fe and K, Fe and Mg, Fe and Na, Fe and P, K and Mg, K and Na, K and P, K and Ti, Mg and Na, Mg and P, Mg and Ti, Mn and Zn, Mo and Ti, P and Na, Ni and Sr, and P and Si, respectively. For other pairs of elements, positive or negative correlations are high ($r \pm 0.7$ to ± 0.9), moderate ($r \pm 0.5$ to ± 0.7), low ($r \pm 0.3$ to ± 0.5), or almost negligible (r 0 to ± 0.2).

Friedman rank-sum test evinced a lack of significant differences among the studied *Ganoderma* species regarding the amount of all 19 elements jointly in particular mushroom species (Friedman chi-squared, $\chi_F^2 = 8.33$, p value = 0.40). Concerning the amount of 13 trace elements (Al, Cl, Cr, Cu, Fe, Mn, Mo, Ni, Si, Sr, Ti, Zn, and Zr) jointly in the basidiocarps of mushrooms ($\chi_F^2 = 4.62$, $p = 0.20$), no significant differences have been observed between them. Similarly, a comparison of the analysed mushroom species with respect to the amount of all 6 macroelements (Ca, K, Mg, Na, P, and S) evinced no significant differences ($\chi_F^2 = 6.97$, $p = 0.07$). The highest level of all 19 and 13 trace elements has been confirmed in *G. brownii*, while *G. lucidum* revealed the highest content of 6 macroelements jointly.

Elements in mushrooms and human safety

Ideally, the fruiting bodies of mushrooms consumption should be supplying the relevant essential element contents and lowest possible concentration of toxic elements. *G. lucidum* achieves both these requirements of being the mushroom with the highest rank with respect to the amount of essential macroelements. The extreme lowest and highest values of elements are presented on a logarithmic scale (Fig. 1) to show the clear-cut distinction of the amount of the elements in the studied mushrooms. Using the logarithmic scale, the levels of elements depicted in ranges (shown in red lines), along with the names of species marked by their corresponding values. Such a way of data presentation gives clarity regarding which mushroom is most beneficial and provides direct information on the potential risk to the health of consumers due to the consumption of these mushrooms (Mleczek et al. 2018).

Measuring the values (differences in the levels of elements among studied species), the most heterogeneous distribution has been observed for Zr. Among all analysed mushrooms, the lowest maximum amount was observed in fruiting bodies of *G. applanatum* (Al, Mg, Mn, Ni, P, Ru, S, Ti, Zn, and Zr), whereas the highest maximum amount was found in *G. lucidum* (Fe, K, Mg, Na, P, Rb, Ti, and Zr). The values indicated comprehensible differences in the amount of nutritionally important essential and non-essential toxic elements.

Dietary intakes of elements

The combination of essential and toxic element levels obtained from this study and the information on the average consumption of mushrooms allow the estimation of dietary exposure of elements in adult population groups. A 30-g/day portion of dried mushroom is assumed for intake calculations based on the recommendations of the European Scientific Committee for Food Adult Weight parameter (Kalač and Svaboda 2000; Çayir et al. 2010). Estimates are for adult men and women with an average weight of 60 kg.

Dietary intakes of essential elements

Recommended dietary intake (RDI) is the intake level of essential nutrients on average daily basis considered to be sufficient to meet the known nutrient requirements of all healthy individuals in each life stage and sex group which should be fulfilled by including a variety of nutrient-dense foods in the diet (van Heerden and Smith 2013). The average estimated dietary intakes (EDIs) by a normal (60 kg) adult person in mg/day of essential metals from mushroom consumption and their contribution to RDI reference values are given in Table 5.

The most important source of calcium is *G. philippii* (577 mg/day) which contributed 96.3% of RDI, followed by *G. brownii* (36%), *G. applanatum* (29%), and *G. lucidum* (27%), respectively. The most important source of calcium is *G. philippii* (577 mg/day) which contributed 96.3% of RDI, followed by *G. brownii* (36%), *G. applanatum* (29%), and *G. lucidum* (27%), respectively. Calcium is the predominant macroelement present in the human body which is the main component of bones and teeth. Calcium plays a vital role in the production and release of

Table 4 Correlation matrix for values of the elements quantified in species of *Ganoderma*

	Al	Ca	Cl	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	S	Si	Sr	Ti	Zn	Zr	
Al	1																			
Ca	0.02	1																		
Cl	0.62	-0.71	1																	
Cr	-0.47	0.47	-0.43	1																
Cu	-0.74	-0.05	-0.24	0.83	1															
Fe	0.48	-0.75	0.73	-0.91*	-0.62	1														
K	0.65	-0.62	0.75	-0.92*	-0.74	0.98*	1													
Mg	0.71	-0.55	0.74	-0.91*	-0.73	0.95*	0.99*	1												
Mn	0.67	0.53	0.20	0.33	0.14	-0.29	-0.11	0.08	1											
Mo	-0.62	0.21	-0.33	0.95*	0.96*	-0.79	-0.85	-0.81	0.11	1										
Na	0.51	-0.78	0.80	-0.86	-0.57	0.99*	0.97*	0.95*	-0.24	-0.74	1									
Ni	0.70	0.28	0.84	0.01	-0.06	0.29	0.38	0.54	0.67	-0.01	0.38	1								
P	0.77	-0.55	0.82	-0.83	-0.78	0.93*	0.98*	0.99*	-0.03	-0.87	0.94*	0.40	1							
S	0.93*	0.30	0.44	-0.13	-0.54	0.15	0.34	0.42	0.89	-0.34	0.18	0.72	0.50	1						
Si	0.78	-0.60	0.96	-0.61	-0.51	0.82	0.88	0.88	0.24	-0.57	0.87	0.77	0.94*	0.57	1					
Sr	-0.71	0.07	-0.71	-0.14	0.05	-0.12	-0.24	0.86	-0.81	-0.06	-0.21	-0.98*	-0.42	-0.80	0.66	1				
Ti	0.55	-0.36	0.40	-0.99*	-0.90	0.87	0.90*	0.91*	-0.23	-0.99*	0.82	0.01	0.84	0.24	0.61	0.09	1			
Zn	0.45	0.79	-0.16	0.49	-0.06	-0.56	-0.38	-0.21	0.93*	0.22	-0.53	0.37	-0.38	-0.75	-0.10	-0.55	-0.37	1		
Zr	0.97*	-0.12	0.65	-0.67	-0.84	0.66	0.81	0.86	0.47	-0.78	0.67	0.60	0.89	0.88	0.84	-0.57	0.73	0.24	1	

*Highly significant correlated at $p \leq 0.05$

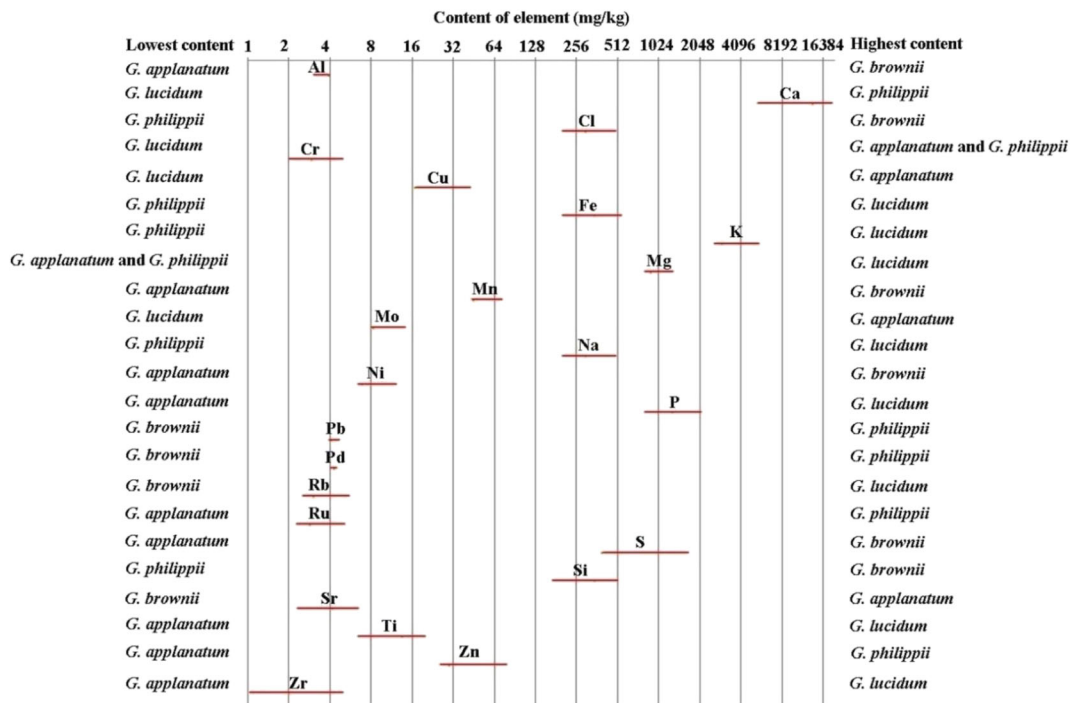


Fig. 1 Characteristics of the values of particular element contents calculated on the basis of highest and lowest content of these elements (mg/kg on dry mass basis) in particular mushroom species

enzymes and hormones, neuromuscular function, and blood clotting (McDowell 1992).

The mean percentage realisation of copper met between 58.2 and 95.8% of the daily requirement of males and females. Copper is required for major biochemical reactions as a vital component of enzymes associated with redox reactions and hemocyanin. It is essential for cellular metabolism, development of connective tissue, mitochondrial function, and the assimilation, storage, and metabolism of iron (McDowell 1992).

The species that contributed most to the intake of iron is *G. lucidum* accounting for 97.1% and 78.6% followed by *G. brownii* (70.6%, 57.1%), *G. applanatum* (53.4%, 43.2%), and *G. philippii* (35.3%, 28.6%) of the total dietary intake of this element for men and women. Iron is an essential trace element that is a component of several heme enzymes and performs a crucial role in the electron transport chain, synthesis of nucleic acids, and transport of oxygen. A sufficient amount of iron in the diet helps in decreasing the occurrence of anaemia (McDowell 1992). It has been demonstrated that the utilisation rate of iron in mushrooms is very high, so about 90% of the iron obtained from mushrooms can be consumed by the human body (Kalač and Svaboda

2000). Therefore, the studied mushrooms can be considered an interesting source of this trace element.

The average percentage contributions of manganese ranged from 37.3 to 62.3% among the studied mushrooms. Manganese is an essential trace element required for several enzyme reactions as a co-factor, bone growth, carbohydrate, and lipid metabolism (McDowell 1992).

For sodium and potassium, the studied mushrooms represent low percentage values of RDI. Insufficient potassium in the diet leads to high blood pressure, stroke, and weakened reflexes (Dimitrijevic et al. 2016). However, in comparison to potassium, the amount of sodium is significantly lower in examined mushrooms which are appraised to be favourable from the nutritional perspective as consumption of a diet rich in sodium with a high Na/K ratio leads to health issues. According to earlier reports, the Na/K ratio often ranged from 0.01 to 0.2 (Kalač 2009). The Na/K ratio varied between 0.08 and 0.1 in the present study.

G. lucidum is the best source of magnesium and phosphorus covering more than 10% RDI for different gender groups. *G. applanatum*, *G. brownii*, and *G. philippii* are not significant sources of magnesium

Table 5 Estimated dietary intake (EDI, mg/day) values and contribution (%) to recommended dietary intake (RDI) values of essential mineral elements for adults (60 kg) through consumption of 30-g species of *Ganoderma* on dry weight basis

Element	Group	RDI	<i>G. applanatum</i>		<i>G. brownii</i>		<i>G. lucidum</i>		<i>G. philippii</i>	
			EDI	% RDI	EDI	% RDI	EDI	% RDI	EDI	% RDI
Ca	Men	600	174	29.0	216	36.0	162	27.0	578	96.3
	Women	600	174	29.0	216	36.0	162	27.0	578	96.3
Cu	Men	1.35	1.29	95.8	1.16	85.8	0.79	58.2	1.10	81.6
	Women	1.35	1.29	95.8	1.16	85.9	0.79	58.2	1.10	81.6
Fe	Men	17.0	9.07	53.3	12.0	70.6	16.5	97.1	6.00	35.3
	Women	21.0	9.07	43.2	12.0	57.1	16.5	78.6	6.00	28.6
K	Men	3750	87.0	2.32	132	3.52	168	4.48	78.1	2.08
	Women	3225	87.0	2.69	132	4.09	168	5.21	78.1	2.42
Mg	Men	340	24.0	7.06	33.1	9.73	39.0	11.5	24.0	7.06
	Women	310	24.0	7.74	33.1	10.7	39.0	12.6	24.0	7.74
Mn	Men	3.50	1.30	37.3	2.18	62.3	1.45	41.4	2.01	57.5
	Women	3.50	1.30	37.3	2.18	62.3	1.45	41.4	2.01	57.5
Na	Men	2092	9.01	0.43	12.0	0.57	15.0	0.72	6.01	0.29
	Women	1902	9.01	0.47	12.0	0.63	15.0	0.79	6.01	0.32
P	Men	600	24.0	4.00	54.0	9.00	63.0	10.5	24.1	4.01
	Women	600	24.0	4.00	54.0	9.00	63.0	10.5	24.1	4.01
Zn	Men	12.0	0.76	6.35	2.01	16.7	0.82	6.81	2.37	19.8
	Women	10.0	0.76	7.65	2.01	20.1	0.82	8.17	2.37	23.7

EDI estimated dietary intake; RDI recommended dietary intake

and phosphorus contributing less than 10% RDI for both groups. *G. philippii* represented the basic source of zinc among the studied mushroom samples, providing 19.8% of RDI for men and contributing 23.7% of the total intake of this element in women, followed by *G. brownii*, *G. lucidum*, and *G. philippii*, respectively.

The estimated dietary intakes calculated for different elements show a significant contribution of the studied mushroom species in accordance with the reference daily requirements for calcium, copper, iron, magnesium, manganese, and zinc.

Dietary intakes of toxic elements

Toxic metals present in functional food may exhibit harmful effects on the health of consumers (Oostdam et al. 2005). The ‘safe’ levels of intake can be determined in terms of ‘tolerable intake’, either daily (TDI or tolerable daily intake) or weekly (TWI or tolerable weekly intake). The tolerable intakes of heavy metals as PTWI (provisional tolerable weekly intake) are laid by the Food and Agriculture Organization/World Health

Organization (FAO/WHO) and Joint Expert Committee on Food Additives (JECFA). PTWI is the acceptable level of a contaminant that can be ingested by a person on a weekly basis over a lifetime without appreciable risk to health.

The average estimated dietary intakes (EDIs) by a normal (60 kg) adult person in mg/day of toxic metals from mushroom consumption are given in Table 6. The results are as per FAO/WHO standards for Al and Pb (toxic metals). For aluminium, provisional tolerable weekly intake (PTWI) is 1 mg per week (JECFA 2007). At high levels, aluminium may have serious effects on the nervous system. There have been several investigations connecting an accumulation of aluminium in the brain and Alzheimer’s disease but a causal relationship has not yet been established (Frisardi et al. 2010). As indicated by the FAO/WHO guidelines, the tolerable intake of Pb is 1.5 mg per week (0.21 mg per day) for an adult with 60-kg average body weight. At higher concentrations, lead diminishes intellectual improvement in kids and enhances the risk of cardiovascular disease in adults. Lead causes health

Table 6 Risk induction of toxic elements in terms of PTWI (provisional tolerable weekly intake, mg/day) and PTDI (provisional tolerable daily intake, mg/week) for adults (60 kg) through consumption of 30-g species of *Ganoderma* on dry weight basis

Species	Element	EDI	EWI
<i>G. applanatum</i>	Al	0.09	0.63
	Pb	ND	ND
<i>G. brownii</i>	Al	0.12	0.84
	Pb	0.12	0.84
<i>G. lucidum</i>	Al	0.11	0.77
	Pb	ND	ND
<i>G. philippii</i>	Al	0.11	0.77
	Pb	0.14	0.98
WHO/FAO, JECFA (PTDI and PTWI reference values)	Al	0.14	1
	Pb	0.21	1.5

EDI estimated dietary intake; EWI estimated weekly intake; WHO/FAO World Health Organization/Food and Agriculture Organization; JECFA Joint Expert Committee on Food Additives; PTDI provisional tolerable daily intake; PTWI provisional tolerable weekly intake

problems such as insomnia, loss of hearing, weakness, and weight loss (Rankin et al. 2005). From the estimated daily intake of the studied toxic metals through the

consumption of mushrooms, it can be suggested that the studied species can be regarded as safe from a health perspective as the values observed comply with the threshold limits for toxic metal intake based on the body weight of an average adult (60 kg body weight).

Nutritional quality index (NQI) of essential elements

The nutritional quality index (NQI) is the amount of nutrients supplied from 100-g powdered samples as a function of the RDI in relation to the energy supplied. The more nutrients present in mushrooms with a limited amount of energy, the higher is the nutrient density. Numerous nutrients such as protein, minerals, vitamins, and fatty acids are present in sufficient proportions in nutrient-dense foods (Whitney and Rolfes 2002). Based on recommended intakes, consumers can achieve good health by consuming a variety of nutrient-dense foods. A value exceeding 1 indicates a positive contribution of an essential nutrient. NQI values of elements are calculated using the energy values and RDI values. NQI values of different elements through the consumption of mushrooms are depicted in Table 7. All the studied mushrooms supply significant quantities of calcium, copper, manganese, iron,

Table 7 Nutritional quality index (NQI) values of the elements for consuming species of *Ganoderma*

Element	Group	<i>G. applanatum</i>	<i>G. brownii</i>	<i>G. lucidum</i>	<i>G. philippii</i>
Ca	Men	7.01	8.50	6.45	23.2
	Women	5.73	6.95	5.26	19.0
Cu	Men	23.2	20.3	13.9	19.7
	Women	18.9	16.6	11.4	16.1
P	Men	0.97	2.12	2.51	0.97
	Women	0.79	1.73	2.05	0.79
Mg	Men	1.71	2.29	2.74	1.70
	Women	1.53	2.06	2.45	1.52
Mn	Men	9.01	14.72	9.89	13.9
	Women	7.36	12.02	8.08	11.3
Na	Men	0.10	0.13	0.17	0.07
	Women	0.09	0.12	0.15	0.06
K	Men	0.56	0.83	1.07	0.50
	Women	0.53	0.79	1.02	0.48
Fe	Men	12.9	16.7	23.2	8.51
	Women	8.53	11.0	15.3	5.63
Zn	Men	1.53	3.95	1.63	4.77
	Women	1.50	3.87	1.59	4.67

and zinc with lower values for sodium and potassium for a little amount of calories. This is evident from Table 7 that *G. applanatum* evinced the highest NQI of copper in comparison to the rest of the species. NQI of manganese was highest in *G. brownii* followed by *G. philippii*, *G. lucidum*, and *G. applanatum*. *G. lucidum* revealed the highest NQI of iron, magnesium, and phosphorus, whereas the NQI values of calcium and zinc are higher in *G. philippii* among the studied mushrooms.

Conclusions

The detailed information on proximate and elemental composition is crucial for estimating dietary intakes, the safety of food consumed, and sharing relevant information regarding nutrients to the consumers which may be a basis for future monitoring studies. It is evident from this study that *Ganoderma applanatum*, *G. brownii*, *G. lucidum*, and *G. philippii* contain appreciable amounts of carbohydrate proteins, fibre, and essential elements for maintaining good health. The concentrations of different elements varied widely among the studied mushroom samples which mainly depend on the mushroom species, accumulation capacity, environment, and species-environment interactions. From the public health perspective, the studied mushrooms can serve as a dietary source of calcium, copper, iron, magnesium, manganese, and zinc, and therefore, makes a remarkable contribution to dietary intakes for humans. Potassium contributions are inadequate in comparison to the reference intake values. Thus, supplementation of potassium is recommended before the formulation of *Ganoderma* powder in various dietary supplements. Further, there is a need to screen out more species for a higher uptake of potassium and sodium to decrease the deficiency of these essential elements in humans. Moreover, consumption of these mushrooms does not represent a toxicological health risk for humans as mean exposure estimates of toxic elements such as aluminium and lead were found to be below the toxicological reference values.

Acknowledgements The authors are grateful to the University Grants Commission (UGC), New Delhi, India, for awarding Senior Research Fellowship to Ranjeet Singh. The authors also express their sincere thanks to the Department of Botany and Department of Pharmaceutical Sciences and Drug Research, Punjab University, Patiala, for providing laboratory infrastructure and support to carry out the research work. The authors also extend

sincere thanks to the Sophisticated Analytical Instrumentation Facility (SAIF) centre, Panjab University, Chandigarh, for providing the WDXRF facility.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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