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https://doi.org/10.31925/farmacia.2026.3.18 **ORIGINAL ARTICLE**

METAL HEALTH RISKS ASSESSMENT AND CRUDE POLYSACCHARIDE CHARACTERIZATION IN THREE *RUSSULA* SPECIES

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Abstract

Known in recent years as "vegetable meat" edible mushrooms offer numerous health benefits. Therefore, this study aimed to evaluate possible benefits and risks in three edible mushrooms collected from Romania: *Russula cyanoxantha* (Schaeff.) Fr. (RC), *Russula mustelina* Fr. (RM) and *Russula xerampelina* (Schaeff.) Fr. (RX) Metal content determination showed that through ingestion of analysed mushrooms, no possible non-carcinogenic risks of metal poisoning can occur to adults (for all mushrooms HI < 10), and also that they are a great source of micro- and macroelements. The crude polysaccharide physico-chemical characterization was performed using Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy, Gel Permeation Chromatography (GPC) and Thermogravimetric Analysis (TG). The by-products from the crude polysaccharide isolation process were evaluated for antioxidant and α-glucosidase inhibitory activities. The RC hydromethanolic extract showed good 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radical scavenging activity (EC_{50} = 97.37 ± 0.55 μg/mL) while the RM ethanolic extract presented good ferrous ion chelating activity (EC_{50} = 46.1 ± 0.2 μg/mL). All ethanolic extracts inhibited α-glucosidase, with the RC extract showing the strongest inhibitory activity (EC_{50} = 0.121 ± 0.001 μg/mL).

Rezumat

Cunoscute în ultimii ani sub denumirea de „carne vegetală", ciupercile comestibile prezintă numeroase beneficii pentru sănătate. Prin urmare, acest studiu și-a propus să evalueze posibilele beneficii și riscuri ale trei ciuperci comestibile colectate din România: *Russula cyanoxantha* (Schaeff.) Fr. (RC), *Russula mustelina* Fr. (RM) și *Russula xerampelina* (Schaeff.) Fr. (RX). Determinarea conținutului de metale a arătat că, prin ingerarea ciupercilor analizate, nu pot apărea riscuri non-cancerigene de intoxicație cu metale la adulți (pentru toate ciupercile HI < 10) și, de asemenea, că acestea reprezintă o sursă importantă de micro- și macroelemente. Caracterizarea fizico-chimică a polizaharidelor brute a fost realizată prin rezonanță magnetică nucleară și spectroscopie în infraroșu, cromatografie cu permeație în gel și analiză termogravimetrică. Extractele etanolice și hidrometanolice rezultate în urma procesului de izolare a polizaharidelor brute au fost evaluate pentru activitatea lor antioxidantă și pentru inhibarea α-glucozidazei. Extractul hidrometanolic RC a demonstrat o bună activitate de *scavenger* față de radicalul ABTS (EC_{50} = 97,37 ± 0,55 μg/mL), în timp ce extractul etanolic RM a prezentat o bună activitate de chelare a ionilor feroși (EC_{50} = 46,1 ± 0,2 μg/mL). Toate extractele etanolice au fost capabile să inhibe α-glucozidaza, extractul RC având cea mai bună capacitate de inhibare (EC_{50} = 0,121 ± 0,001 μg/mL).

Keywords: *Russula* species, metal content, risks of metal poisoning, crude polysaccharide characterization, antioxidant activity

Introduction

For more than 2000 years, wild mushrooms have been used for their nutritional value, organoleptic qualities and medicinal properties (1). Depending on several factors, such as species, cultivation conditions, environmental conditions, region of origin, stage of development, storage conditions, processing and mushroom part, they have the inherent ability to accumulate minerals, vitamins and various secondary metabolites (2). Chemical, biochemical and nutritional analyses have demonstrated that mushrooms are rich in bioactive compounds. With 2 - 6% lipid content (fats and related substances), 8 - 12% minerals and vitamins that usually represent 15 - 30% of the recommended daily dose and 50 - 70% carbohydrates, mushrooms represent an important part of a healthy diet (3-5). The varied content of bioactive compounds, such as polysaccharides, polyphenols, flavonoids, tocopherols, terpenoids (sesquiterpene and lactones), lecithin, alkaloids, sterols (ergosterol), glycoproteins, confer

mushrooms various biological properties: antioxidant, anticancer, immunomodulatory, antidiabetic, neuroprotective, antihypertensive, hepatoprotective, antifungal, antimicrobial, antiviral and antibacterial (3, 4, 6).

The genus *Russula*, belonging to the class *Agaricomycetes*, is an ectomycorrhizal macrofungal group that includes over 3,000 species worldwide. *Russula* species often occur in coniferous forests in the northern hemisphere with moderate temperatures, playing an important role in preserving the biodiversity of forest ecosystems (7). Symbiosis with other plants enhances nutrient, water and nitrogen uptake, especially under unfavourable conditions (8). Of the genus *Russula*, at least 78 species are edible and consumed for their nutritional properties, and 30 species have been used in traditional Chinese medicine for at least 440 years (9).

Russula cyanoxantha (RC), *Russula mustelina* (RM) and *Russula xerampelina* (RX), are wild edible mushrooms that can be easily found in deciduous and coniferous forests, in shrubs and meadows in Romania (10, 11). The population living in these areas collects and consumes these mushrooms, and for this reason, it is important to evaluate the benefits and risks of their consumption. To date, few studies on the above-mentioned *Russula* species have been conducted. Due to organoleptic properties, RC was the most studied.

In the fruiting bodies of RC, phenolic acids, fatty acids and carbohydrates have been identified (12-16). Also, the antioxidant activity of the extracts obtained from the fruiting bodies was evaluated (12, 13, 17). In the literature, studies have highlighted the metal content in the fruiting bodies of RC, RM and RX collected from different geographic areas (11, 16, 18-27).

The present research aimed to explore the potential therapeutic effects of RC, RM and RX collected from natural ecosystems in Romania. Therefore, the primary goal was to evaluate the metal content of the fruiting bodies to assess potential human health risks associated with their consumption. As mushrooms are a good source of carbohydrates, the crude polysaccharides were purified and characterized. In addition, the by-products of the purification process (ethanolic and hydromethanolic extracts) were evaluated regarding their antioxidant and antihyperglycemic potential.

Materials and Methods

Materials

Nitric acid (HNO_3) (65%), ethylenediaminetetraacetic acid (EDTA), acetone, potassium bromide, deuterium oxide (D_2O), sodium sulfate, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), gallic acid, dimethyl sulfoxide (DMSO), anhydrous sodium carbonate, phenol, ABTS, sodium and potassium tartrate, sodium hydroxide, disodium phosphate, potassium ferricyanide, iron (III) chloride, linoleic acid 99%, lipoxidase from soybean, sodium acetate, acetic acid, iron sulfate (II), ferrozine, α-glucosidase from *Saccharomyces cerevisiae*, monopotassium phosphate, glutathione (reduced form), p-nitrophenyl α-D-glucopyranoside (PNPG) and acarbose were purchased from Sigma Chemicals (Sigma-Aldrich Chemie GmbH, Stein-heim, Germania). Ethanol 96%, hydrogen peroxide (30%), sulfuric acid (98%), copper sulfate and absolute methanol were provided by Chemical Company (Bucharest, Romania). Metranal® 3, Strawberry Leaf was purchased from Analytika (Analytika, Prague, Czech Republic), bovine serum albumin (BSA) from Fluka (St. Louis, USA), D-glucose from Lach-Ner (Lach-Ner, Neratovice, Czech Republic), pullulan from Shodex Denko (Shodex Denko, Japan), Folin-Ciocâlteu's phenol reagent and monosodium phosphate from Merck (Darmstadt, Germany), quercetin dihydrate from Carl Roth GmbH (Karlsruhe, Germania), potassium persulfate and trichloroacetic acid from Riedel-de Haën (Seelze, Germany) and boric acid 99.8% from Carlo Erba (Milano, Italy).

Mushroom biomass

Samples of *Russula* species were collected in the Poiana Stampei area (Suceava County). Authentication was conducted in the Laboratory of Mycology and Phytopathology, Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, Romania. Immediately after harvesting, the mushrooms were cleaned of soil and leaf debris, without being washed, and then frozen at -18°C. For the chemical studies, mushrooms were previously thawed and air-dried at room temperature, protected from light.

Metal contents

Metal content in the fruiting bodies was determined on a dry-weight basis using a previously described method (28, 29). Briefly, 1 g of dried mushroom powder was subjected to acid digestion using 5 mL of HNO_3 (65%) and 1 mL of H_2O_2. The mixture was maintained at 70°C for 4 h. After cooling to room temperature, 20 mL of ultrapure water was added, and the samples were reheated at 70°C for an additional 6 h. This digestion procedure was repeated twice to ensure complete mineralization. The digests were subsequently filtered through Whatman No. 42 filter paper and transferred into appropriate vessels. Before use, all glassware was soaked overnight in 10% (v/v) HNO_3 and thoroughly rinsed with ultrapure water. Elemental analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7700x system. The operating conditions for the ICP-MS were: radiofrequency power of 1550 W, carrier gas flow-rate of 1.01 L/min, plasma gas flow-rate of 15 L/min, auxiliary gas flow-rate of 0.70 L/min; integration time 0.3 s, with three repetitions. Internal standard (Rh) was added to compensate for acid effects and instrument drift. Metranal® 3 (Strawberry leaf) was employed as a certified reference material for quality control, with a recovery ranging between 80 - 105% for three replicates. Metal concentrations were expressed on a dry weight basis. Detection (LOD) and quantification limits (LOQ), expressed in mg/kg, for

each measured element were: B ($4.56 \times 10^{-6}/1.52 \times 10^{-5}$), Bi ($2.33 \times 10^{-3}/7.76 \times 10^{-3}$), Co ($8.25 \times 10^{-6}/2.75 \times 10^{-5}$), Cr ($9.83 \times 10^{-6}/3.28 \times 10^{-5}$), Cu ($2.03 \times 10^{-4}/6.76 \times 10^{-4}$), Fe ($3.96 \times 10^{-3}/1.32 \times 10^{-2}$), Li ($2.27 \times 10^{-7}/7.58 \times 10^{-7}$), Mn ($7.25 \times 10^{-5}/2.42 \times 10^{-4}$), Se ($1.04 \times 10^{-3}/3.48 \times 10^{-3}$), Zn ($1.44 \times 10^{-3}/4.81 \times 10^{-3}$), Ca ($6.82 \times 10^{-3}/2.27 \times 10^{-2}$), Mg ($4.54 \times 10^{-4}/1.51 \times 10^{-3}$), K ($3.5 \times 10^{-4}/1.05 \times 10^{-3}$), P ($1.2 \times 10^{-4}/3.0 \times 10^{-4}$), Na ($2.27 \times 10^{-4}/7.58 \times 10^{-4}$), Ag ($2.67 \times 10^{-7}/8.91 \times 10^{-7}$), As ($5.21 \times 10^{-4}/1.74 \times 10^{-3}$), Cd ($2.94 \times 10^{-7}/9.80 \times 10^{-7}$), Hg ($5.32 \times 10^{-5}/1.77 \times 10^{-4}$), Ni ($6.56 \times 10^{-6}/2.19 \times 10^{-5}$), Pb ($4.85 \times 10^{-3}/1.62 \times 10^{-2}$), Sr ($9.02 \times 10^{-6}/3.01 \times 10^{-5}$), Tl ($1.54 \times 10^{-4}/4.63 \times 10^{-4}$), U238 ($6.82 \times 10^{-8}/2.27 \times 10^{-7}$).

Health risk assessment

The health risk associated with metal intake through mushroom consumption was assessed by calculating the estimated daily intake (EDI) (eq. 1), target hazard quotient (THQ) (eq. 2) and hazard index (HI) (eq. 3) (16, 30):

$$EDI\left(\frac{mg}{kg \cdot day}\right) = \frac{C \cdot IR \cdot ED \cdot EF}{BW \cdot AT} \qquad \text{eq. 1,}$$

where, C – metal concentration in fruiting body (mg/kg); IR – ingestion rate of fruiting body (kg/person/day) (25.7 g/day) (dry weight); ED – exposure duration (adults = 30 years, children = 6 years); EF – exposure frequency (350 days/year); BW – body weight (adults = 70 kg, children = 15 kg); AT – average time exposure (EF × ED).

$$THQ = \frac{EDI}{R_fD} \qquad \text{eq. 2,}$$

where, R_fD – reference dose ($mg \cdot kg^{-1} \cdot day^{-1}$) (As = 3×10^{-4}; Cd = 1×10^{-3}; Co = 2×10^{-2}; Cr = 3×10^{-3}; Cu = 4×10^{-2}; Fe = 0.7; Hg = 3×10^{-4}; Mn = 1.4×10^{-1}; Ni = 2×10^{-2}; Pb = 3.5×10^{-3}; Zn = 3×10^{-1}) (31).

$$HI = \sum_{i=k}^{n} THQ \qquad \text{eq. 3.}$$

Isolation of crude polysaccharides

The method applied for the isolation of crude polysaccharides has been described elsewhere (28, 29). Briefly, the dried and finely powdered mushroom material was first extracted with 500 mL of 96% ethanol, then extracted with a methanol:water mixture (1:1, v/v). The resulting residue (20 g) was subsequently extracted with 400 mL of ultrapure water at room temperature for 6 h, and the procedure was repeated once under identical conditions. The combined aqueous extracts were concentrated under reduced pressure at 40°C to a final volume of 50 mL. The concentrated extract was then precipitated by adding 200 mL of ethanol and maintained at 4°C for 24 h. The precipitated fraction was collected by filtration, washed with acetone, and then lyophilized. The final product was stored at -18°C until further analysis. The procedure is illustrated in Figure 1.

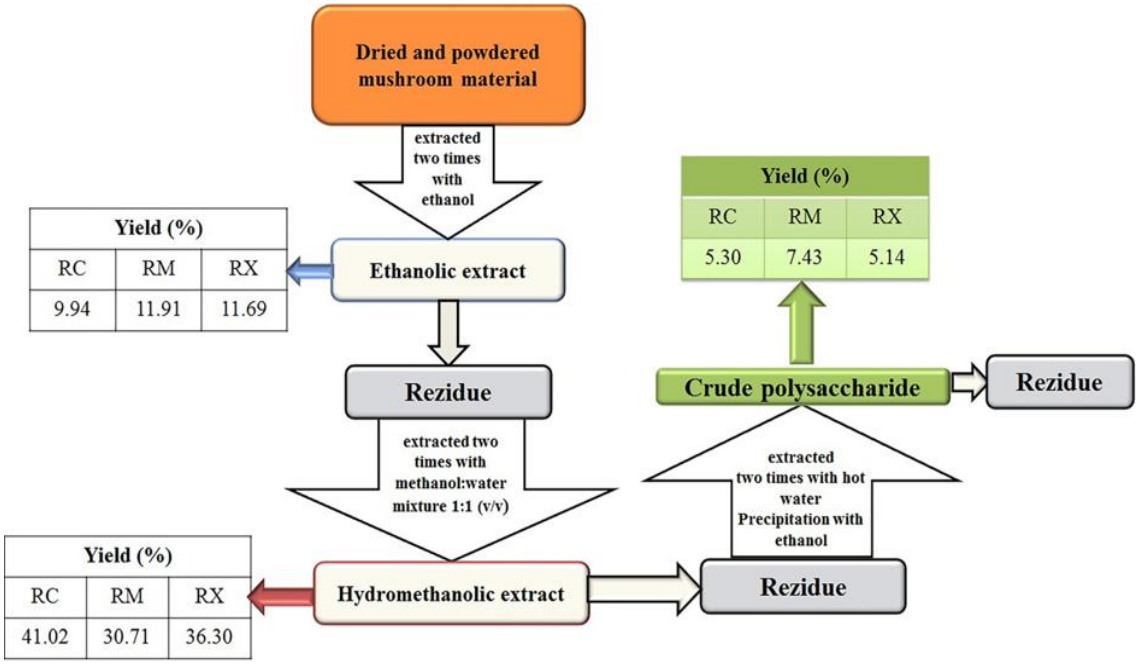

Figure 1.
Isolation of crude polysaccharides

Quantitative determination of protein and total carbohydrate content in crude polysaccharide

The quantitative analysis of total proteins from the isolated crude polysaccharide was made by the Hartree-Lowry method (32) using bovine serum albumin (BSA) as a standard. Total carbohydrate content was determined with Masuko *et al.*'s method (33), with slight modifications, using D-glucose as a standard (29).

Crude polysaccharide physico-chemical characterization

Crude polysaccharide physicochemical characterization was conducted using Fourier Transform Infrared

(FTIR) spectroscopy, NMR spectroscopy, GPC and TG. All methods have been previously described (29).

Total phenolic content in ethanolic and hydromethanolic extracts

The total phenolic content was determined by the Folin-Ciocâlteu method as previously described. The phenolic content was expressed as mg of gallic acid equivalents (GAE)/g of extract (34).

Antioxidant activity

DPPH radical-scavenging activity was assessed according to the method described by Wangensteen *et al*. (35), except that the reaction time was 60 min.

ABTS radical cation scavenging activity was evaluated according to the method of Re *et al*. (36). Scavenging activity was determined after 6 min – reaction time.

Reducing power assay was performed using the method described by Ferreira *et al*. (37).

Ferrous ion chelating activity was determined according to the method described by Venditti *et al*. (38) except that ethanol was used instead of acetate buffer.

The inhibitory capacity of 15-Lipoxygenase (15-LOX) was evaluated as previously described (35), except that the enzyme and the extract were incubated for 5 min. at 25°C.

Quercetin was used as a positive control in all antioxidant assays except the ferrous ion chelating assay, where EDTA was the positive control.

α-Glucosidase inhibitory activity

The α–glucosidase inhibitory activity was determined as described by Liu *et al*. (39), with minor modifications. The ethanolic extracts were dissolved in DMSO in concentrations ranging from 0.005 to 0.5 mg/mL. Hydromethanolic extracts were dissolved in a 1:1 (v/v) water:methanol mixture at concentrations ranging from 1 to 50 mg/mL.

α-Glucosidase from *Saccharomyces cerevisiae* was dissolved in phosphate buffer (67 mM, pH 6.8 at 37°C with 1 M NaOH) at a concentration of 0.86 unit/mL. 0.05 mL of each extract was added to a mixture consisting of 0.5 mL phosphate buffer, 0.02 mL glutathione (3 mM) (stabilizer) and 0.02 mL α-glucosidase. After vigorous shaking, the solutions were incubated for 5 min and at 37°C, followed by the addition of 0.05 mL of PNPG solution (10 mM). The solutions were vigorously shaken and incubated for 15 min. at 37°C. The reaction was quenched with 2.36 mL of sodium carbonate (100 mM). Absorbance was measured at λ = 400 nm against a blank containing no enzyme. Acarbose was used as a positive control.

The α-glucosidase inhibition capacity (%) was calculated using the equation:

$$Inhibition\ capacity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100,$$

where, $A_{control}$ – represents the absorbance of the control; A_{sample} – represents the absorbance of the sample.

The EC_{50} was calculated by linear interpolation of the values of the activities immediately above and immediately below the 50% activity.

Statistical analysis

All assays were performed in triplicate. The results were expressed as mean value ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post-hoc test. Differences were considered statistically significant at $p < 0.05$. The EC_{50} values (concentration of sample producing 50% activity) were calculated by linear interpolation between values above and below 50% activity. The Pearson correlation coefficient (r) was applied to assess the relationship between total phenolic content and antioxidant activity.

Results and Discussion

Metal contents

Mushrooms can accumulate a number of metals, micro- and macro-elements, including toxic metals, and for this reason, it is important to determine the metal content in edible mushrooms from natural ecosystems (40, 41). In the fruiting bodies of the three mushroom species studied, 24 metals were identified and quantified (Table I).

The most abundant microelement found in the fruiting bodies of mushrooms was Zn. This microelement is often considered deficient in human nutrition (41). The studied mushrooms showed Zn concentrations ranging from 154.61 ± 0.50 mg/kg for RX, to 188.59 ± 1.83 mg/kg for RC (Table I). For RC the value obtained (188.59 ± 1.83 mg/kg) is higher than those reported for the caps of specimens collected from the Târgoviște area, Romania (86.0 ± 22.1 mg/kg) (22) and the fruiting bodies of the samples collected from Cluj-Napoca area, Romania (74.5 - 108 mg/kg) (11), Czech Republic (39 - 97 mg/kg) (25) and North-Western Romania (119 ± 20.5 mg/kg) (16). The value obtained for RM (157.86 ± 0.54) is comparable to or lower than the values obtained for samples collected from the Czech Republic (80.4 - 793 mg/kg) (25). Samples of RX collected from the Czech Republic and Poland presented lower amounts of Zn than those determined in this study (39.7 - 127, 92.3 ± 17.5 mg/kg *vs*. 154.61 ± 0.50 mg/kg) (25, 26).

The analysed mushrooms showed Cu levels ranging from 75.35 ± 0.30 mg/kg in RX, to 85.45 ± 0.21 mg/kg in RM. According to Kalač (42), in wild-growing mushrooms, the usual Cu contents range from 10 to 75 mg/kg. Our values are comparable with the ones reported in literature for RX, 74.1 ± 17.2 mg/kg (26) and bigger than the ones reported for RC, 18.1 ± 18.0 mg/kg (22), 37.1 - 55.0 mg/kg (11) and 36.1 ± 8.6 mg/kg (16).

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Table I

Metal content of mushrooms fruiting bodies (mg/kg, dry weight basis, means ± standard deviations)

Element	RC	RM	RX
Microelements content			
B	16.4 ± 0.15[b]	21.46 ± 0.57[a]	14.39 ± 0.35[c]
Bi	0.016 ± 0.001	0.016 ± 0.000	0.016 ± 0.001
Co	0.049 ± 0.001[b]	0.163 ± 0.002[a]	0.046 ± 0.000[b]
Cr	b.l.d.	0.07 ± 0.00	0.16 ± 0.00
Cu	82.42 ± 0.50[a]	85.45 ± 0.21[a]	75.35 ± 0.30[b]
Fe	47.24 ± 0.43[b]	81.94 ± 1.62[a]	30.72 ± 0.52[c]
Li	0.008 ± 0.000[a]	0.005 ± 0.000[b]	0.008 ± 0.000[a]
Mn	35.63 ± 0.20[a]	29.56 ± 1.05[b]	18.52 ± 0.25[c]
Se	0.229 ± 0.001[b]	0.257 ± 0.001[b]	0.617 ± 0.001[a]
Zn	188.59 ± 1.83[a]	157.86 ± 0.54[b]	154.61 ± 0.50[b]
Macroelements content			
Ca	871.08 ± 2.07[a]	112.54 ± 0.50[b]	57.35 ± 0.35[c]
Mg	527.45 ± 1.09[a]	503.46 ± 3.01[a]	353.23 ± 1.01[b]
K	13320 ± 800	12880 ± 2200	12520 ± 1100
P	4620 ±100	4470 ± 300	3910 ± 90
Na	2370 ± 100	2350 ± 100	2240 ± 90
Toxic and radioactive metals			
Ag	0.99 ± 0.00[a]	0.75 ± 0.00[b]	0.29 ± 0.00[c]
As	0.25 ± 0.02[a]	0.25 ± 0.03[a]	0.25 ± 0.00[a]
Cd	0.10 ± 0.00[b]	1.12 ± 0.09[a]	0.05 ± 0.00[c]
Hg	0.350 ± 0.001[a]	0.053 ± 0.000[b]	0.014 ± 0.000[c]
Ni	0.45 ± 0.00	b.l.d.	b.l.d.
Pb	0.442 ± 0.002[b]	0.737 ± 0.003[a]	0.494 ± 0.001[b]
Sr	b.l.d.	0.15 ± 0.00	b.l.d.
Tl	0.025 ± 0.000	0.025 ± 0.000	0.025 ± 0.000
U238	0.024 ± 0.000	0.024 ± 0.000	0.024 ± 0.000

Trace level: < 0.01 mg/100 g dry weight. Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate statistically significant differences between mushroom species according to one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$). Identical letters indicate no significant differences. b.l.d. = below the limit of detection

Fe is an essential element that contributes to the transport of oxygen and nutrients in the human body. The highest Fe content was observed in the fruiting body of RM (81.94 ± 1.62 mg/kg) and was almost two times higher than the values obtained for RC and RX (Table I). Previous studies reported values between 22.6 - 69.1 mg/kg (11) and 69.3 ± 17.0 mg/kg (16) in the fruiting body and 308.0 ± 70.2 mg/kg in the cap of RC (22) and 115 ± 63.2 mg/kg in the fruiting body of RX (26).

In wild-growing mushrooms, Mn content is usually below 25 mg/kg (42). In the cap of specimens of RC collected from the Târgoviște area, Romania, a Mn content of 35.9 ± 31.2 mg/kg was determined, a value comparable to that reported in this study (35.63 ± 0.20 mg/kg) (22), while samples collected from Cluj-Napoca area and around Jibou town, Romania, presented values between 10.9 - 19.1 mg/kg (11) and 9.21 ± 2.10 mg/kg, respectively (16). For RX, the obtained value (18.52 ± 0.25 mg/kg) is lower than the one found in samples collected in Poland (24.1 ± 9.52 mg/kg) (26).

The recommended daily dose of Se in adults varies between 55 and 70 µg/day. Se is an essential trace element and a component of many enzymes involved in antioxidant defense mechanisms (42). Thus, a low intake of Se is associated with depression, fatigue, anxiety and others. In wild-growing mushrooms, the usual Se content is below 0.5 - 5 mg/kg. The values obtained in this study are lower than those reported in RC (0.229 ± 0.001 *vs*. 0.635 ± 0.067 mg/kg) collected from northern Portugal (43).

Among the analysed mushrooms, K was the most abundant macroelement, with values ranging from 12520 ± 1100 to 13320 ± 800 mg/kg. According to Kalač (42), the usual contents are between 10 and 35 g/kg. The high K content reported in mushrooms is comparable to that found in vegetables, making mushrooms a good source of this major mineral. The obtained values are lower than the ones reported in literature for RC (13320 ± 800 mg/kg *vs*. 15800 - 21400 mg/kg and 20900 ± 4120 mg/kg) (11,16) and for RX (12520 ± 1100 mg/kg *vs.* 35000 ± 13000 mg/kg) (26). The data reported in this study for Ca and Mg are in agreement with the average contents

reported by Kalač (42): 0.05 - 0.75 g/kg for Ca and 0.8 - 1.8 g/kg for Mg.

In the scientific literature, there are few data on the heavy-metal content of RC, RM and RX. These metals must be analysed because, in large quantities, they can harm the health of the consumer. For RC and RX, the obtained values for cadmium (0.10 ± 0.00 mg/kg and 0.05 ± 0.00 mg/kg) are lower than those reported in the literature (11, 16, 19, 21). In the studied mushrooms, nickel concentrations range from below the detection limit in RM and RX to 0.45 ± 0.00 mg/kg in RC (Table I). For samples of RC collected from the Cluj-Napoca area and around Jibou town, Romania, Ni concentration was reported to vary between 1.07 - 2.49 mg/kg (11) and 0.87 ± 0.44 mg/kg (16), respectively. Pb concentrations in the RC and RX samples were smaller or comparable with the ones reported in literature for the same mushrooms harvested from different areas (16, 19, 21, 44). Regarding Hg concentration, for RC, the obtained value (0.350 ± 0.001 mg/kg) was smaller than the one reported for samples harvested from North-Western Romania (1.15 ± 0.39 mg/kg) (16), Spain (2.0 mg/kg) (20), Czech Republic (0,8 mg/kg) (21) and Switzerland (5.61 ± 1.75 mg/kg) (23).

According to our findings, the studied mushrooms represent an important source of Ca, Mg and K, while showing low heavy metal contents.

Health risk assessment

The metal health risks from mushroom ingestion were evaluated for As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn. The health risk assessment, by means of EDI, THQ and HI, was calculated for adults and children (Table II and Table III). Values for THQ < 1 and HI < 10 indicate no-carcinogenic effects in humans (16).

Table II

The metal health risk for adults through mushroom ingestion collected from Romania

	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
	RC										
EDI (mg/kg/day)	9.18×10^{-5}	3.67×10^{-5}	1.8×10^{-5}	-	0.03	0.02	12.8×10^{-5}	0.01	16.5×10^{-5}	16.2×10^{-5}	0.07
THQ	0.3	0.04	9×10^{-4}	-	0.75	0.03	0.43	0.07	0.008	0.05	0.24
HI	1.92										
	RM										
EDI (mg/kg/day)	9.18×10^{-5}	41.1×10^{-5}	5.98×10^{-5}	2.57×10^{-5}	0.03	0.03	1.95×10^{-5}	0.01	-	27×10^{-5}	0.06
THQ	0.3	0.41	3×10^{-3}	8.6×10^{-3}	0.75	0.04	0.07	0.07	-	0.08	0.2
HI	1.93										
	RX										
EDI (mg/kg/day)	9.18×10^{-5}	1.83×10^{-5}	1.39×10^{-5}	5.87×10^{-5}	0.03	0.01	0.51×10^{-5}	0.007	-	260×10^{-5}	0.06
THQ	0.3	0.02	7×10^{-4}	0.02	0.75	0.01	0.02	0.05	-	0.7	0.2
HI	2.1										

(EDI – Estimated Daily Intake, THQ – target hazard quotient, HI – hazard index)

Table III

The metal health risk for children through mushroom ingestion collected from Romania

	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
	RC										
EDI (mg/kg/day)	4.28×10^{-4}	1.71×10^{-4}	0.8×10^{-4}	-	0.14	0.09	5.97×10^{-4}	0.05	7.7×10^{-4}	7.5×10^{-4}	0.33
THQ	1.43	0.17	0.004	-	3.5	0.13	2	0.36	0.04	0.2	1.1
HI	8.9										
	RM										
EDI (mg/kg/day)	4.28×10^{-4}	19.2×10^{-4}	2.8×10^{-4}	1.2×10^{-4}	0.14	0.14	0.91×10^{-4}	0.05	-	12.6×10^{-4}	0.28
THQ	1.43	1.92	0.014	0.04	3.5	0.2	0.3	0.36	-	0.36	0.93
HI	9.05										
	RX										
EDI (mg/kg/day)	4.28×10^{-4}	0.85×10^{-4}	0.65×10^{-4}	2.74×10^{-4}	0.14	0.05	0.24×10^{-4}	0.03	-	0.012	0.28
THQ	1.43	0.09	0.003	0.09	3.5	0.07	0.08	0.2	-	3.4	0.93
HI	9.8										

(EDI – Estimated Daily Intake, THQ – target hazard quotient, HI – hazard index)

The highest EDI values in adults were determined for As (9.18×10^{-5} mg/kg) in RC, RM and RX, Cd (41.1×10^{-5} mg/kg) in RM, Co (5.98×10^{-5} mg/kg) in RM, Cr (5.87×10^{-5} mg/kg) in RX, Cu (0.03 mg/kg) in RC, RM and RX, Fe (0.03 mg/kg) in RM, Hg (12.8×10^{-5} mg/kg) in RC, Mn (0.01 mg/kg) in RC and RM, Ni (16.5×10^{-5} mg/kg) in RC, Pb (260×10^{-5} mg/kg in RX) and Zn (0.07 mg/kg) in RC. The highest THQ value (0.75) was obtained for all analysed mushrooms for Cu. According to our results,

the THQ and HI scores in adults show that no possible non-carcinogenic risks of metal poisoning can occur through the ingestion of the analysed mushrooms.

The biggest EDI values in children were determined for (As 4.28 × 10^{-4} mg/kg) in RC, RM and RX, Cd (19.2 × 10^{-4} mg/kg) in RM, Co (2.8 × 10^{-4} mg/kg) in RM, Cr (2.74 × 10^{-4} mg/kg) in RX, Cu (0.14 mg/kg) in RC, RM and RX, Fe (0.14 mg/kg) in RM, Hg (5.97 × 10^{-4} mg/kg) in RC, Mn (0.05 mg/kg) in RC and RM, Ni (7.7 × 10^{-4} mg/kg) in RC, Pb (0.012 mg/kg) in RX and Zn (0.33 mg/kg) in RC. THQ values above 1 were obtained for As in all analysed mushrooms (1.43), for Cu in all analysed mushrooms (3.5), for Pb in RX (3.4) and for Zn in RC (1.1). Even though the HI value remains below 10, the THQ values suggest a potentially notable non-carcinogenic health risk associated with the consumption of these mushrooms in children.

In 2011 and 2019, the FAO/WHO organization established the maximum values for the intake of As (2 - 7 μg/kg bw/day), Cd (25 μg/kg bw/month), Cu (0.05 - 0.5 mg/kg bw/day), Fe (0.8 mg/kg bw/day), Hg (4 μg/kg bw/week), Pb (0.3 mg/kg for fresh farmed mushrooms) and Zn (0.3 - 1 mg/kg bw/day) (45, 46). Considering that a consumer with a body weight of 70 kg ingests on average 25.7 g of dry weight mushrooms *per* day, the maximum recommended doses for As, Cu, Fe, Hg and Zn are not exceeded, and even they are far below the established limits (Table II) (16). For Pb, the FAO/WHO organization withdrew, since 2010, the value of 25 μg/kg bw/week without setting a new value, as there are areas where the population is exposed to high concentrations of Pb from the atmosphere, and because the absorption of Pb through ingestion can vary between 3 and 80%.

Quantitative determination of protein and total carbohydrate content in crude polysaccharide

The nutritional value of mushrooms is given primarily by their protein and carbohydrate content. In mushrooms, carbohydrates are the main component of the fruiting bodies, and proteins are the second major component, with average contents of 12 - 75% and 12 - 60% of dry matter, respectively (47, 48). In Table IV, the crude polysaccharides, total carbohydrate and protein contents are presented. The isolated crude polysaccharides show high amounts of total carbohydrates. The proximate composition of mushrooms varies from species to species, as well as within the same species harvested from different regions, and also depends on their stage of development, the part of the mushroom harvested, and the location of harvest (49). In a study conducted on samples of RC collected from Thailand, Srikram and Supapvanich (50) reported values of 49.20 ± 23.61% dry wt for crude protein and 9.56% dry wt for carbohydrate content, which differ from those in Table IV. Similar values for carbohydrate content in RC samples (61.29 g/100g dw) were reported by Senila *et al*. (16).

Table IV

Crude polysaccharides protein and total carbohydrate content

Crude polysaccharides	Total carbohydrate (% w/w)	Total protein (% w/w)
RC	63.9 ± 0.8	17.9 ± 0.2
RM	39.8 ± 1.1	10.5 ± 0.6
RX	80.6 ± 0.5	14.9 ± 0.4

Values are means ± SD of data from triplicate analysis

Crude polysaccharide physico-chemical characterization

NMR, FT-IR, GPC and TG characterized the crude polysaccharides.

NMR spectroscopy revealed the general and specific structural characteristics of each isolated crude polysaccharide (Figure 2 and Figure 3). The polysaccharides isolated from *Russula* species are composed of sugar units that have both α and β configurations. They differ from each other by the number of signals in the anomeric region. Thus, for the crude polysaccharide isolated from RC, 6 signals in the anomeric region of the proton NMR spectrum (suggesting the presence of 6 carbohydrate units) and 4 signals in the anomeric region of the carbon NMR spectrum were recorded. The crude polysaccharide isolated from RM showed five signals in the anomeric region of the proton NMR spectrum (suggesting the presence of 5 carbohydrate units) and 3 signals in the anomeric region of the carbon NMR spectrum. The crude polysaccharide isolated from RX showed five signals in the anomeric region of the proton NMR spectrum (suggesting the presence of 5 carbohydrate units) and 4 signals in the anomeric region of the carbon NMR spectrum. The signal observed around 175 ppm in all ^{13}C NMR spectra, except for the one recorded for RM, corresponds to C-6 (COO-R) of the β-D-glucuronic acid units (51).

Crude polysaccharides showed signals in the anomeric region of the ^{1}H-NMR spectrum (4.5 - 5.4 ppm) that can be attributed to different carbohydrate units. Thus, the ^{1}H-NMR spectra of the crude polysaccharides isolated from RM and RX showed five signals (5.4, 5.23, 5.0, 4.65 and 4.54 ppm and 5.4, 5.23, 5.11, 5.0 and 4.54 ppm, respectively), while for RC six signals are observed (5.4, 5.23, 5.0, 4.65, 4.63 and 4.54 ppm). The signals in the 5.0 - 5.4 ppm region belong to the α-type configurations, and those

in the 4.54 - 4.65 ppm region to the β-type configurations (52, 53). The crude polysaccharides present both α-glycosidic and β-glycosidic units (52). The signal at 4.8 ppm in all ^{1}H-NMR spectra is assigned to the D_2O solvent.

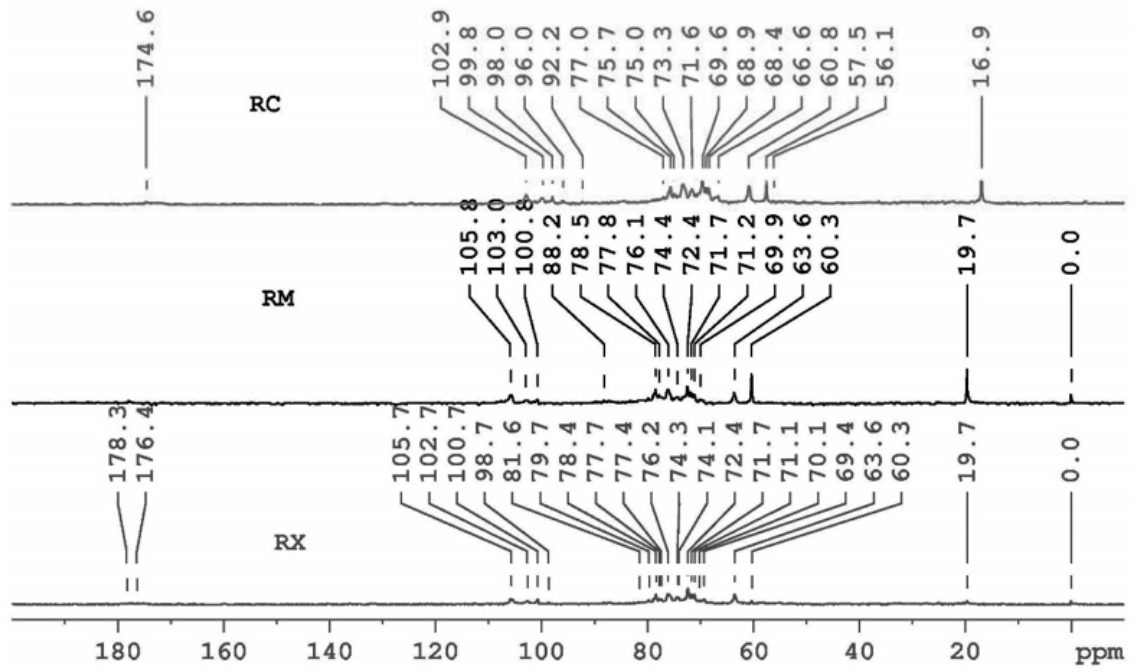

Figure 2.
^{13}C-NMR spectra of crude polysaccharide isolated from RC, RM and RX

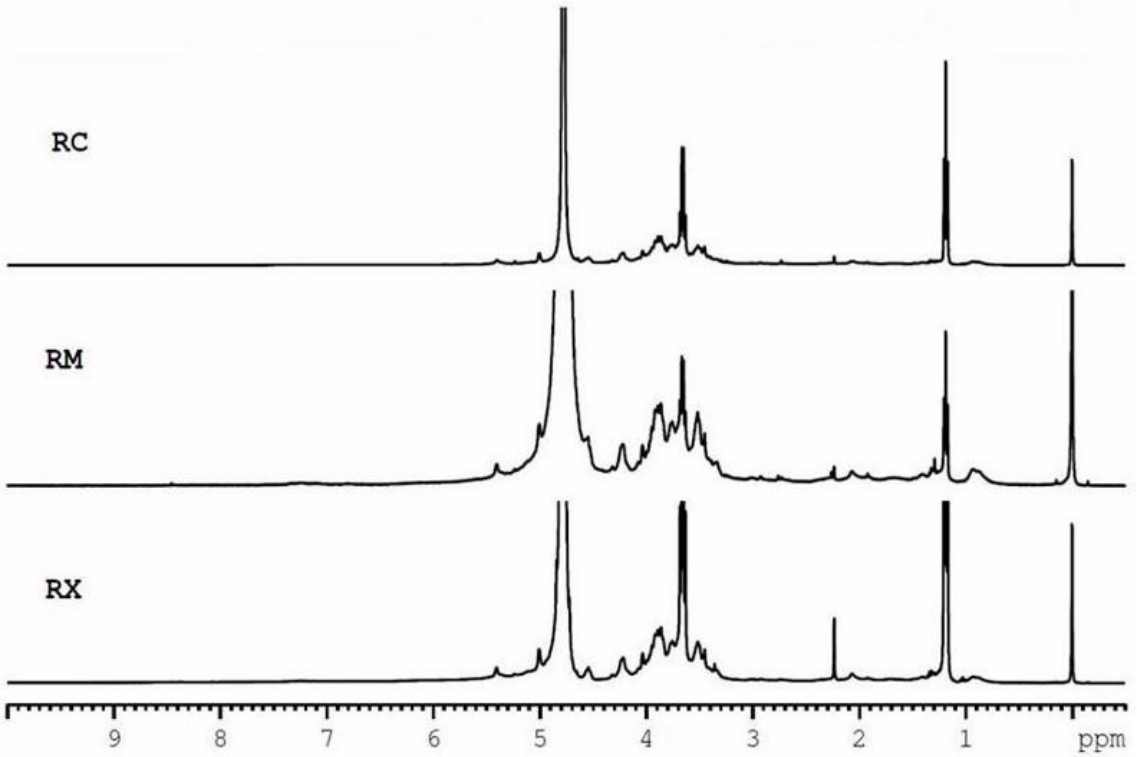

Figure 3.
^{1}H-NMR spectra of crude polysaccharide isolated from RC, RM and RX

The signals in the anomeric region of the ^{1}H-NMR spectrum can be correlated with those in the anomeric region of the ^{13}C-NMR spectrum (96 - 106 ppm). In this region, crude polysaccharides isolated from RC and RX showed four signals (102.94, 99.75, 97.97 and 96.0 ppm) and, respectively, 105.72, 102.68, 100.71

and 98.69 ppm, and RM showed three signals (105.84, 103.0 and 100.79 ppm). According to the literature, signals in the range of 97 - 101 ppm correspond to β-glycosidic bonds, and those in the range of 102 - 106 ppm correspond to α-glycosidic bonds (52, 54). In the ^{13}C-NMR spectra, numerous signals appear in the range of 60 - 70 ppm, suggesting the pyranose structure of the crude polysaccharide constituents (54). Thus, the isolated crude polysaccharides exhibit α- and β-glucopyranose and α- and β-galactopyranose linkages (55). Signals in the anomeric region of the spectra indicate the presence of several carbohydrate units, including rhamnose, glucose, arabinose, xylose, fucose, or mannose (51).

The FT-IR spectra of crude polysaccharides are presented in Figure 4. Crude polysaccharides showed a strong absorption band in the range of 3428 - 3420 cm^{-1}, corresponding to the stretching vibrations of the O–H groups specific to polysaccharides (52). The band from the 2924 cm^{-1} and the shoulder from the 2822 cm^{-1} correspond to the asymmetric and symmetric C–H stretching vibrations of the –CH_2 and –CH_3 groups in the carbohydrate structure (56). In the range 2139 - 2136 cm^{-1}, the obtained spectra show a low-intensity absorption band that can be attributed to the valence vibration of the C–H bond, characteristic of aliphatic compounds (51). The presence of protein structures is suggested by a high-intensity band at 1654 cm^{-1}, characteristic of the –NH–CO– deformation vibration (57, 5). The bands specific for C–H bond stretching vibration (1425 - 1375 cm^{-1}) and the asymmetric COO– stretching vibration of carboxylic groups (1556 - 1545 cm^{-1}) indicate the presence of uronic acids (51). In the spectra recorded for the crude polysaccharides, the band corresponding to the pyranose ring appears at 1158 cm^{-1} (58). The absorption band also suggests the presence of carbohydrates in the isolated crude polysaccharides at 1042 cm^{-1}, which corresponds to the valence vibration of the C–O–C group (52). These spectral data are consistent with those reported in other studies of aqueous extracts (in hot water) from different mushroom species (52, 53).

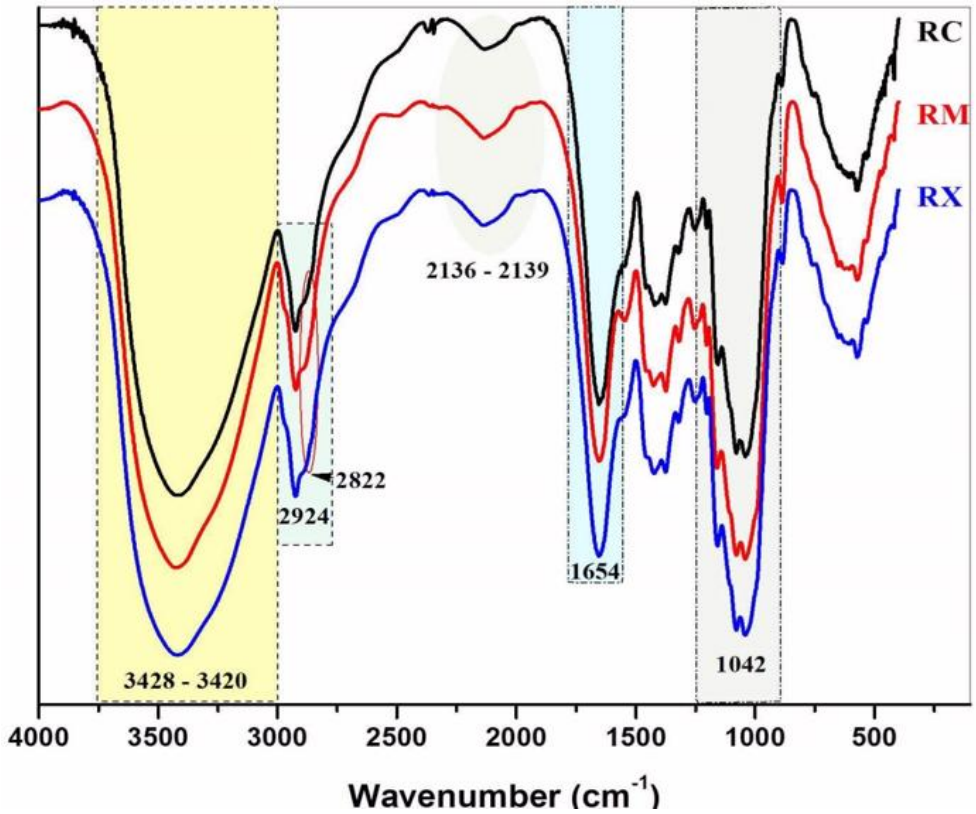

Figure 4.
FT-IR spectra of crude polysaccharide isolated from RC, RM and RX

Molecular weight is an extremely important variable because it influences a polymer's physical properties and its concentration in solution. To determine the molecular weight of a polymer, reference chromatograms of polymers with known average molecular weights, obtained under the same conditions (solvent, volume and temperature), are used. Table V presents the molecular mass distributions of the polysaccharide fractions in the composition of crude polysaccharides isolated from the mushrooms under study.

Table V

The molecular weight of the separated polysaccharide fractions

Crude polysaccharide	Polysaccharide fractions	Number average molecular weight (Mn) (g/mol)	Weight average molecular mass (Mw) (g/mol)	Polydispersity index
RC	I	1.83×10^7	1.05×10^8	5.77
	II	3.20×10^4	4.60×10^4	1.43
	III	324	379	1.16
RM	I	2.13×10^7	1.13×10^8	5.32
	II	3.47×10^4	4.73×10^4	1.36
RX	I	2.86×10^7	2.82×10^8	9.84
	II	4.43×10^4	6.78×10^4	1.52

In the isolated crude polysaccharides, three polysaccharide fractions were identified (Figure 5, Figure 6 and Figure 7). For the first polysaccharide fractions, the high polydispersity index (5.77, 5.32 and 9.82 for RC, RM and RX, respectively) suggests the presence of a mixture of polysaccharides whose cumulative numerical molecular mass is 1.83×10^7 g/mol, 2.13×10^7 and 2.86×10^7 for the polysaccharides isolated from RC, RM and respectively RX. The values obtained for the second fraction can be compared with those presented in the literature for polysaccharide fractions isolated from *Boletus edulis* species collected from China: 3.20×10^4 g/mol for RC, 3.47×10^4 g/mol for RM and 4.43×10^4 g/mol for RX *vs*. 2.76×10^4 g/mol (59), 2.5×10^4 g/mol (60) and 1.08×10^4 g/mol (61). The molecular masses of the third polysaccharide fractions isolated from RM and RX could not be estimated.

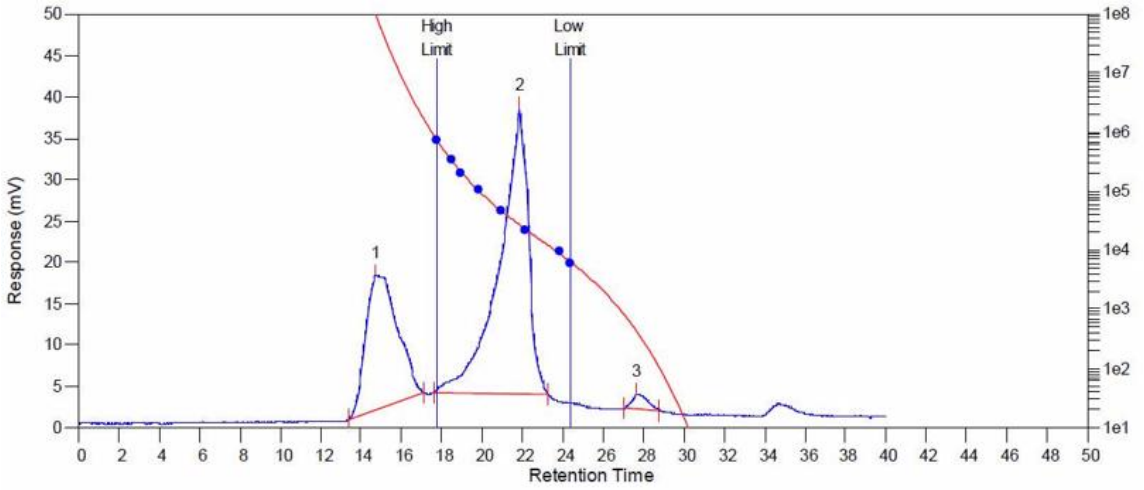

Figure 5.

Distribution plot for RC crude polysaccharide and calibration curve of pullulan

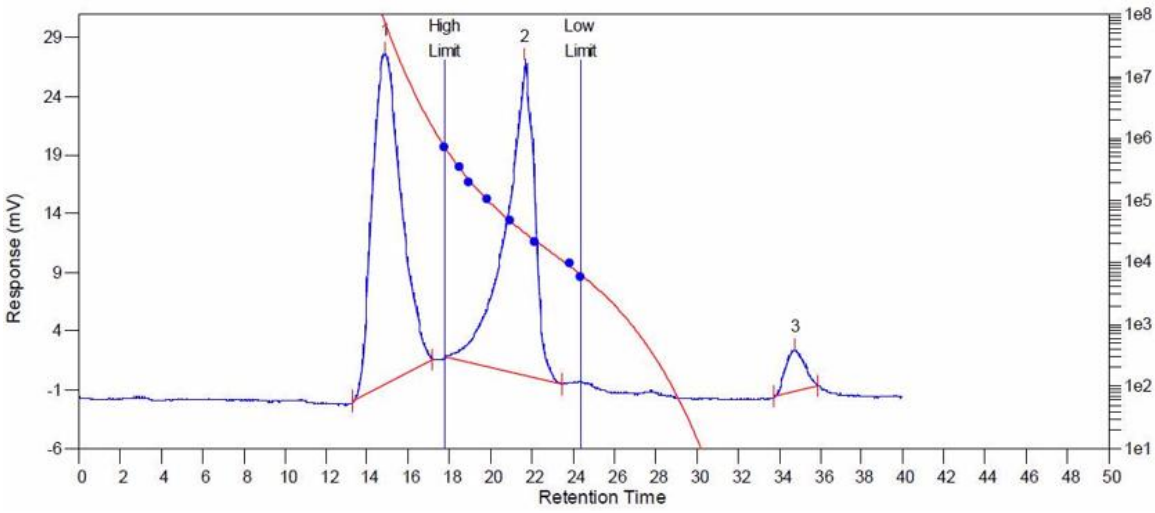

Figure 6.

Distribution plot for RM crude polysaccharide and calibration curve of pullulan

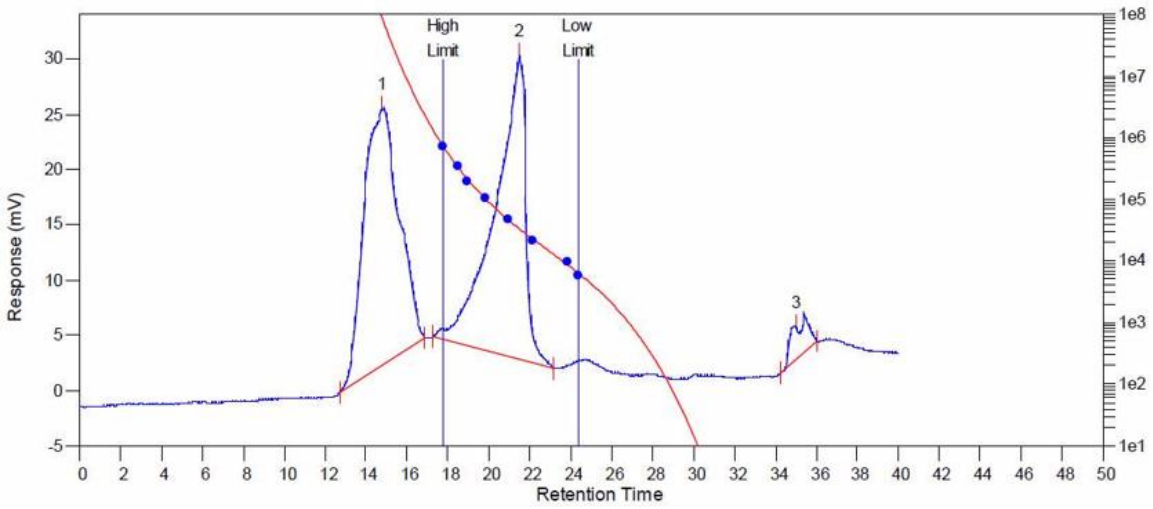

Figure 7.
Distribution plot for RX crude polysaccharide and calibration curve of pullulan

To characterize the thermal behaviour and chemical changes that may occur at elevated temperatures in the isolated crude polysaccharides, the following techniques were used: TG, differential thermal analysis (DTA) and derivative thermogravimetric analysis (DTG). The thermal characteristics of the polysaccharides are presented in Figure 8, Figure 9, Figure 10 and Table VI.

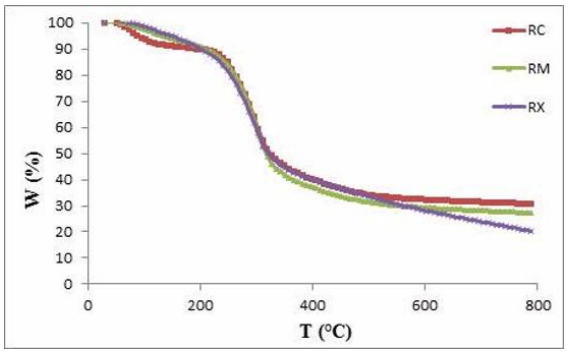

Figure 8.
TGA curves for RC, RM and RX crude polysaccharides

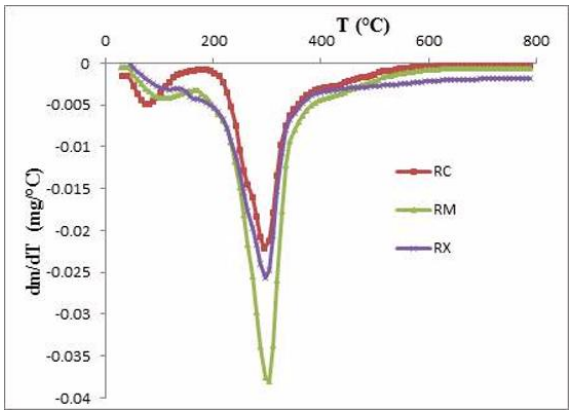

Figure 9.
DTA curves for RC, RM and RX crude polysaccharides

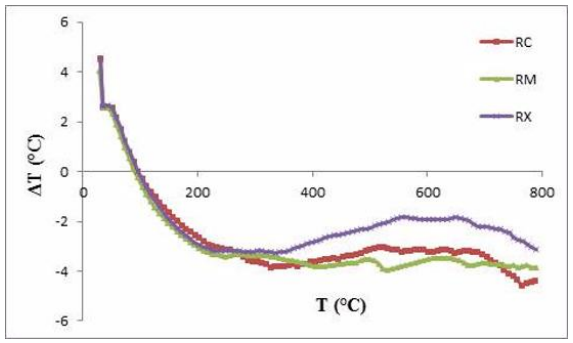

Figure 10.
DTG curves for RC, RM and RX crude polysaccharides

All crude polysaccharides exhibit a first thermal degradation stage which, according to the DTA curve, is endothermic. In this stage, the sample is dehydrated (thus indicating the presence of hydrophilic groups) and any impurities involved in the isolation process are removed (62, 63). According to the TG curve, the first stage begins, at the earliest, at 53°C for RC and ends, at the latest, at 78°C for RX. The crude polysaccharide with the lowest content of water and impurities proved to be the one isolated from RM which, according to the DTG curve, presented a percentage of mass loss of 8.72%. (Table VI).
The DTA curve indicates that the second degradation stage is exothermic, and the temperature at which the degradation rate is maximum is approximately 300ºC (TG curve). According to the DTG curve, this is the main thermal decomposition stage of crude polysaccharides, with the percentage mass of sample lost being the highest. Thus, at this stage, the isolated crude polysaccharides lose approximately 60% of their mass. The depolymerization and decomposition of polysaccharides take place in the second stage of thermal degradation, which ends at a temperature between 326 - 402ºC (62, 63).

Table VI

Thermogravimetric characteristics of RC, RM and RX crude polysaccharides

Crude polysaccharide	Thermal degradation step	T_{onset} °C	T_{peak} °C	T_{endset} °C	W %	Residue %	DTA characteristic
RC	I	53	84	120	9.95	30.16	Endothermic
	II	240	297	402	59.89		Exothermic
RM	I	69	100	141	8.72	26.87	Endothermic
	II	250	302	326	64.41		Exothermic
RX	I	78	130	142	12.48	19.36	Endothermic
	II	235	300	327	68.16		Exothermic

T_{onset} – initial temperature at which thermal decomposition starts, T_{peak} – temperature at which maximal degradation is registered, T_{endset} – temperature at which investigated process ends, W – the percentage of mass loss in each step

DPPH radical scavenging activity of the crude polysaccharides

The antioxidant activity of the crude polysaccharide was assessed by DPPH radical scavenging activity (Figure 11). The crude polysaccharides obtained from RC, RM and RX could not be tested at concentrations higher than 416.67 µg/mL, because the samples gelled; at this concentration, the scavenging activity of the three polysaccharides was 34.87 ± 0.28%, 21.27 ± 0.50% and 40.62 ± 0.40%, respectively. Until this point, the activity increased with concentration (Figure 11). For the aqueous extracts obtained from different samples of RC collected in Portugal, Ribeiro *et al*. (17) reported scavenging activities of 7 - 11.5% at a concentration of 500 µg/mL.

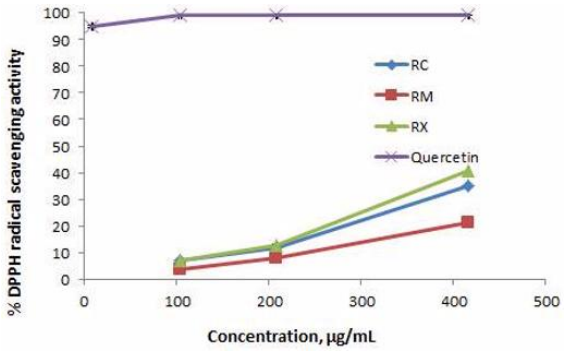

Figure 11.

DPPH radical scavenging activity of RC, RM and RX crude polysaccharides

Total phenolic content in ethanolic and hydromethanolic extracts

Phenolic compounds show numerous biological effects (antioxidant, antimicrobial, anti-inflammatory, antitumour) (64). Since the solvent used for the extraction influences the content in polyphenols and thus the biological effects of the extracts, the fruiting bodies of the studied mushrooms were successively extracted with 96% ethanol and a methanol:water mixture (1:1, v/v). The content of total phenols in the ethanolic and hydromethanolic extracts is graphically represented in Figure 12. The ethanolic extracts showed a lower total phenol content than the hydromethanolic extracts. Also, the values determined in this study differ from those reported in the literature for the same species. These differences might be attributed to the composition of the substrate on which the mushrooms grew, pedoclimatic conditions and the time of harvesting and/or storage until processing (65, 66).

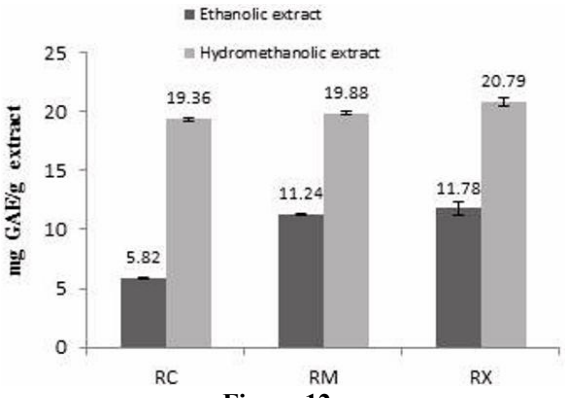

Figure 12.

Total phenolic content of ethanolic and hydromethanolic extracts obtained from the RC, RM and RX

For the methanolic extract of RC harvested from Bragança, Portugal, a higher total phenolic content than that determined in the present study was reported (approx. 32 mg GAE/g *vs*. 19.36 ± 0.13 mg GAE/g in the hydromethanolic extract) (13). Also, the values obtained in this study were different from those reported by Srikram and Supapvanich (50) for samples collected in Thailand (10.66 ± 0.03 mg GAE/100g).

The analysis of total phenolic content conducted on the ethanolic extract of RX collected from Brasil showed a value of 1.851 mg GAE/g, which is lower than the value obtained in this study (11.78 ± 0.57 mg GAE/g) (66).

Antioxidant activity of ethanolic and hydromethanolic extracts

For the ethanolic and hydromethanolic extracts obtained from RC, RM and RX, the scavenging effects against the DPPH radical are comparable. Thus, at 833.34 µg/mL, the ethanolic extracts of RC, RM and RX inactivated the DPPH radical in a proportion of 14.81 ± 0.06%, 18.75 ± 0.21% and 17.58 ± 0.31%, respectively, while the hydromethanolic extracts inactivated the DPPH radical in a proportion of 56.53 ± 0.2%, 59.75 ± 0.2% and 58.89 ± 0.12%,

respectively. The EC_{50} values obtained are much higher than those determined for the positive controls (Table VII). Grangeia *et al.* (13) reported an EC_{50} of 0.69 ± 0.03 mg/mL for the hydromethanolic extract of RC collected in Portugal. EC_{50} values of 262.08 and 86.27 µg/mL were reported for the methanolic and acetonic extracts of RC samples collected in Serbia (12).

Table VII

Antioxidant activities of RC, RM and RX ethanolic and hydromethanolic extracts

Extract/ positive control		EC_{50} (µg/mL)				
		DPPH scavenging activity	**ABTS scavenging activity**	**Reducing power**	**Ferrous ion chelating activity**	**15-Lipoxygenase inhibitory activity**
RC	Ethanolic extract	> 833.34	336.93 ± 4.60	893.14 ± 7.63	112 ± 1	263.34 ± 1.35
	Hydromethanolic extract	692.9 ± 3.6	97.37 ± 0.55	142.24 ± 1.13	5414.47 ± 36.40	> 833.34
RM	Ethanolic extract	> 833.34	165.3 ± 1.9	370.62 ± 1.86	46.1 ± 0.2	202.8 ± 1.3
	Hydromethanolic extract	652.2 ± 2.8	100.33 ± 1.65	148.24 ± 0.20	3736.4 ± 20.4	> 833.34
RX	Ethanolic extract	> 833.34	176.57 ± 3.50	507.61± 1.80	86.4 ± 0.4	215.77 ± 1.05
	Hydromethanolic extract	653.34 ± 1.55	102.23 ± 1.95	219.94 ± 1.65	1096.27 ± 1.95	> 833.34
Pearson correlation coefficient (r)*		-	- 0.93	- 0.93	+ 0.7	-
Quercetin		2.33 ± 0.06	1.07 ± 0.06	3.58 ± 0.07	-	19.34 ± 1.05
EDTA		-	-	-	6.34 ± 0.06	-

Values represent the mean of three determinations ± SD

* The analysis was carried out only for those assays and samples for which complete numerical datasets were available. It included both ethanolic and hydromethanolic extracts, each of them being considered as independent samples due to differences in extraction solvent and composition

The hydromethanolic extract of RC proved to be 3.4 times more efficient than the ethanolic extract in scavenging the ABTS radical cation (EC_{50} = 97.37 ± 0.55 *vs*. 336.93 ± 4.60 µg/mL) (Table VII). The ethanolic extracts obtained from RM and RX showed comparable EC_{50} values (165.3 ± 1.9 and 176.57 ± 3.50 µg/mL, respectively). At 250 µg/mL, the two extracts inactivated the ABTS cation radical by 64.99 ± 0.30% and 60.58 ± 0.76%, respectively. In the case of the hydromethanolic extracts of RC, RM and RX, the EC50 values were also comparable (97.37 ± 0.55, 100.33 ± 1.65 and 102.23 ± 1.95 µg/mL, respectively). At 250 µg/mL, the extracts showed similar scavenging effects against the ABTS cation radical: 91.10 ± 0.44%, 89.18 ± 0.70 and 93.22 ± 0.36%, respectively.

The hydromethanolic extract of RC showed a 6.2 times lower reducing capacity than the ethanolic extract (EC_{50} = 142.24 ± 1.13 *vs*. 893.14 ± 7.63 µg/mL) (Table VII). For the methanolic extract of RC collected in Portugal, an EC_{50} value of 2.26 ± 0.00 mg/mL was reported (13). Methanolic and acetonic extracts of RC samples collected from Serbia exhibited low absorbance values at 1000 µg/mL (0.0109 and 0.0072, respectively) (12). The hydromethanolic extracts of RM and RX were much more active than the corresponding ethanolic extracts, showing a 2.5 times lower reducing capacity than the ethanolic extract (EC_{50} = 148.24 ± 0.20 *vs*. 370.62 ± 1.86 µg/mL and 219.94 ± 1.65 *vs*. 507.61 ± 1.80 µg/mL, respectively). The hydromethanolic extracts, with a higher content in total phenols, were found to be more active in terms of reducing capacity than the ethanolic extracts.

Regarding ferrous ion chelating activity, at 576 µg/mL, the ethanolic extracts obtained from the fruiting bodies of RC, RM and RX showed comparable values: 93.49 ± 0.20%, 95.29 ± 0.12% and 94.67 ± 0.29%, respectively. The EC_{50} calculation revealed significant differences in activity that varied as follows: RM (46.1 ± 0.2 µg/mL) > RX (86.4 ± 0.4 µg/mL) > RC (112.0 ± 1.0 µg/mL) (Table VII). For the hydromethanolic extracts, the highest ferrous ion chelating capacity was determined for RX, followed by RM and RC (EC_{50} = 1096.27 ± 1.95, 3736.40 ± 20.40 and 5414,47 ± 36.40 µg/mL, respectively). It should be noted that the ethanolic extract of RM showed a remarkable ferrous ion chelating capacity, only 7.2 times lower than that of the positive control, EDTA (EC_{50} = 46.1 ± 0.2 µg/mL *vs*. 6.34 ± 0.06 µg/mL). It seems that the phenolic compounds are not responsible for the ferrous ion chelating activity. Kalogeropoulos *et al.* (67) reported no close correlation between polyphenol content and ferrous-ion chelating capacity. The ability of plants to chelate ferrous ions can be mostly attributed to triterpenic acids, carbohydrates and proteins.

The 15-LOX inhibitory capacities of the extracts obtained from RC, RM and RX were comparable. Thus, at 833.34 µg/mL, the ethanolic extract from RC showed a 15-LOX inhibitory capacity of 98.01 ± 0.25%, and those obtained from RM and RX of 100 ± 0%. Considering the EC_{50} values, the 15-LOX inhibition of the ethanolic extracts obtained from the three *Russula* species varied as follows: RM > RX > RC

(Table VII). In the literature, few studies report the ability of mushroom extracts to inhibit LOX. A study conducted on 335 samples collected from Korea reported that methanolic extracts obtained from *Collybia maculata*, *Tylopilus neofelleus*, *Strobilomyces confusus*, *Phellinus gilvus*, *P. linteus*, *P. baumii* and *Inonotus mikadoi* can inhibit soybean lipoxygenase in proportions of 73.3%, 51.6%, 52.4%, 66.7%, 59.5%, 100% and 85.2%, respectively, at the concentration of 100 µg/mL (68). The hydromethanolic extracts, with higher total phenol content, show no activity against 15-LOX (Table VII).

Pearson correlation analysis was performed to evaluate the relationship between total phenolic content and antioxidant activity, expressed as EC_{50} values, for the investigated mushroom extracts. The analysis was conducted on all samples (n = 6), including both ethanolic and hydromethanolic extracts, which were treated as independent due to differences in extraction solvent and composition. As presented in Table VII, strong negative correlations were observed between total phenolic content and ABTS radical scavenging activity (r = - 0.93), as well as reducing power (r = - 0.93). These results indicate that extracts with higher phenolic content tend to exhibit lower EC_{50} values, corresponding to enhanced antioxidant activity (69). In contrast, ferrous ion chelating activity showed a moderate positive correlation with total phenolic content (r = 0.70), suggesting a different trend compared to the other antioxidant assays. Correlation analysis was not performed for DPPH and lipoxygenase inhibitory activities due to the presence of non-numerical EC_{50} values (> 833.34 µg/mL), which precluded reliable statistical evaluation.

α-Glucosidase inhibitory activity of the ethanolic and hydromethanolic extracts

Diabetes mellitus is a chronic disease whose incidence has increased by 4 - 5% each year. The efficacy of antidiabetic drugs can be enhanced by α-glucosidase inhibitors (70). For all ethanolic extracts, the α-glucosidase inhibition increased with concentration. The ethanolic extract obtained from RC was the most active (EC_{50} = 0.121 ± 0.001 µg/mL). At a concentration of 0.34 µg/mL, the extract showed an α-glucosidase inhibition of 90.77 ± 0.39%. The hydromethanolic extract was inactive: at the maximum concentration tested (833.34 µg/mL), it showed an activity of 1.44 ± 0.23%. Ethanolic extracts obtained from RM and RX showed comparable activities: 99.14 ± 0.18% and 99.06 ± 0.4%, respectively, at a concentration of 83.34 µg/mL. Their effects were superior to the positive control, acarbose (EC_{50} = 2.424 ± 0.004 and 2.508 ± 0.003 *vs*. 72.2 ± 0.3 µg/mL) (Table VIII).

Table VIII

α-Glucosidase inhibitory activity of the ethanolic and hydromethanolic extracts

Extract/positive control	EC_{50} for ethanolic extract (µg/mL)	Hydromethanolic extract activity	
		Concentration (µg/mL)	% Inhibitory activity
RC	0.121 ± 0.001	833.34	1.44 ± 0.23
RM	2.424 ± 0.004	833.34	0.37 ± 0.22
RX	2.508 ± 0.003	833.34	14.40 ± 0.28
Acarbose	72.2 ± 0.3	-	-

Values represent the mean of three determinations ± SD

Antidiabetic effects of mushroom extracts have been reported in the literature. Analysing the potential antidiabetic activity of the ethanolic and aqueous extracts obtained from five mushroom species (*Clitocybe maxima*, *Catathelasma ventricosum*, *Stropharia rugosoannulata*, *Craterellus cornucopioides* and *Laccaria amethystea*) collected from China, Liu *et al*. (39) reported EC_{50} values ranging from 2.12 ± 0.13 µg/mL for the aqueous extract of *C. ventricosum* to 274.38 ± 21.2 µg/mL for the aqueous extract of *C. maxima*. Studies have shown that the source of α-glucosidase is very important, as the analysed extracts showed greater inhibition of α-glucosidase from *Saccharomyces cerevisiae* than of α-glucosidase from rat intestinal mucosa (71).

Conclusions

Determination of metal content demonstrated that RC, RM and RX mushrooms represent an important source of Ca, Mg, Se, Zn, P, K, Mn and Fe. The calculation of the daily intake of metals when consuming these mushrooms indicated that the maximum doses recommended by the FAO/WHO (2011) for As, Cu, Fe, Hg and Zn are not exceeded. Two extracts (ethanolic and hydromethanolic) were obtained from the fruiting bodies of each mushroom; the crude polysaccharides were further isolated from the fruiting body residues. For all samples, the methanol:water (1:1) mixture was more efficient for extraction yield. The antioxidant activities of the ethanolic and hydromethanolic extracts (ABTS radical-scavenging activity and reducing power) were correlated with their phenolic content. In these tests, the most active were the hydromethanolic extracts, which showed a high phenolic content. The ethanolic extracts were more active as 15-LOX inhibitors and ferrous ion chelators. All ethanolic extracts significantly inhibited α-glucosidase. The ethanolic extract of RC exhibited remarkable inhibitory activity, being much more active than the positive control, acarbose.

The crude polysaccharide isolated from RX, which had the highest carbohydrate content, exhibited the

highest DPPH radical-scavenging activity at the tested concentration, based on % inhibition values, among the isolated crude polysaccharides. Physicochemical analysis of the crude polysaccharides revealed the presence of three polysaccharide fractions in each mushroom species, all of which are thermostable over a wide temperature range. The NMR and FT-IR spectra revealed the presence of several sugar units linked through α- and β-glycosidic bonds.

In accordance with the results presented, we conclude that RC, RM and RX mushrooms have good nutritional value, serving as an important source of micro- and macronutrients and bioactive compounds.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

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Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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