Black Trumpet, *Craterellus cornucopioides* (L.) Pers.: Culinary Mushroom with Angiotensin Converting Enzyme Inhibitory and Cytotoxic Activity

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Nutritional value and chemical composition, including the content of vitamins, fatty acids, 5'-nucleotides and nucleosides and amino acids, as well as biological activities, including antioxidant, angiotensin converting enzyme (ACE) inhibitory and cytotoxic activity of black trumpet (*Craterellus cornucopioides* (L.) Pers.) were tested *in vitro*. *C. cornucopioides* was low in energy, fat and carbohydrate contents, but rich in dietary fibre, especially β-glucan as well as niacin and α-tocopherol. The content of essential and non-essential free amino acids were 1.49 and 5.48 mg/g dry weight (dw). The nucleosides and 5'-nucleotides were determined at 1.84 and 3.99 mg/g dw, respectively. The share of unsaturated fatty acids (UFAs) was 75.92% with oleic acid as the major UFA. Cyclohexane and dichloromethane extracts expressed significant cytotoxic activity against selected cell lines, human epithelial cervical cancer cells (HeLa), adenocarcinomic human alveolar basal epithelial cells (A549), colorectal cancer cells (LS174) and normal MRC-5 human embryonic lung fibroblast cells (IC_{50} of 78.3–155.6 μg/mL). ACE inhibitory activity of the aqueous extract was strong with an IC_{50} of 0.74 μg/mL. It can be concluded that black trumpet is a good source of nutrients, such as vitamins, dietary fibres, amino acids, nucleotides and fatty acids, which contribute to the overall nutritional value of this fungus with potential for ACE inhibitory activity and use in anti-hypertensive diet.

INTRODUCTION

Black trumpet, *Craterellus cornucopioides* (L.) Pers. is a delicious edible mushroom from the family Cantharellaceae. It is black-brown or almost black mushroom without determined separation of fruiting body between stalk and cap. It looks like a trumpet at the bottom of the tank and slowly extends to the edges. This mushroom grows in groups, in deciduous forests especially on oak and beech [Wheeler & Jordan, 2007]. Primary metabolites (fatty acids, lipids, carbohydrates, amino acids, 5'-nucleotides) and secondary metabolites (phenolic compounds and sterols) were previously identified in *C. cornucopioides* [Beluhan & Ranogajec, 2011; Liu *et al*., 2012; Palacios *et al*., 2011]. Considerable amounts of vitamin C, lycopene, β-carotene, α- and γ-tocopherols, and β-glucan were determined as well [Gil-Ramirez *et al*., 2011; Liu *et al*., 2012; Vamanu & Nita, 2014; Watanabe *et al*., 2012]. It was found that the bioactive compounds of *C. cornucopioides* were responsible for several of its biological activities, inlcuding: anti-inflammatory, antimutagenic, cytotoxic, hypoglycemic and antioxidant ones [Kosanić *et al*., 2019; Liu *et al*., 2012; O'Callaghan *et al*., 2015; Palacios *et al*., 2011; Vamanu & Nita, 2014].

High blood pressure and cardiovascular disease are one of the risk factors for morbidity in both developed and underdeveloped countries. The main risk factors for hypertension that can be influenced are unhealthy diet (excessive salt intake, diet rich in fats and trans fats, low intake of fruits and vegetables), insufficient physical activity and sedentary lifestyle, smoking and alcohol abuse, overweight or obesity [World Health Organization Cardiovascular diseases factsheet, 2021, https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)]. According to the recommendations of the European Society of Cardiology and the British Society of Hypertension, therapy of hypertension begins with either angiotensin converting enzyme (ACE)

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inhibitors or angiotensin II receptor (AT_1) antagonists in patients who have normal or increased plasma renin, thiazide diuretics or calcium antagonists [Williams *et al*., 2018]. If an adequate antihypertensive effect is not achieved and the drug is well tolerated, an antihypertensive agent from another group is added (thiazide diuretics, Ca^{2+} antagonists, β-adrenoceptor antagonists, $α_1$-adrenoceptor antagonists) [Williams *et al*., 2018]. Previous studies have shown significant ACE inhibitory activity of different mushrooms, their extracts and different isolates *in vitro* and *in vivo* [Kundaković & Kolundžić, 2013], whereas clinical trials have confirmed the effects of individual mushrooms on lowering blood pressure [Khatun *et al*., 2007; Lo *et al*., 2012]. Mushrooms represent a significant source of bioactive peptides and other compounds that show ACE inhibitory activity. A large number of ACE inhibitory peptides have been isolated from edible mushrooms in the last twenty years, *e.g. Grifola frondosa* [Choi *et al*., 2001], *Mycoleptodonoides aitchisonii* [Sakamoto *et al*., 2001], *Tricholoma giganteum* [Lee *et al*., 2004], and *Ganoderma lucidum* [Wu *et al*., 2019]. In addition to peptides and oligopeptides, triterpenoids (ganoderic acid F) and D-mannitol of mushrooms have also been shown to elicit an inhibitory effect on ACE activity [Kundaković & Kolundžić, 2013].

Regarding antitumor activity, the effects have been shown for various mushrooms and their isolated compounds on different cell lines. For example, 3β,5α,9α-trihydroxy-ergosta-7,22-dien-6-one isolated from *Valvariella volvacea* showed significant antitumor activity against human liver cancer cell line (HepG-2) and human gastric cancer cell line (SGC-7901), while ergosterol and ergosterol peroxide expressed significant activity against human prostatic carcinoma cell line (PC-3M) [Chen *et al*., 2020]. Also, mushroom polysaccharides (*e.g.*, lentinan isolated from *Lentinula edodes*) have been reported to elicit the antitumor effect *via* different mechanisams including cancer-preventing activity, immuno-enhancing activity and direct tumor inhibition activity [Pandya *et al*., 2019]. Therefore, guided by the promising findings of previous research, we aimed to evaluate the ACE inhibitory activity of aqueous extracts of wild growing *C. cornucopioides* collected in Bosnia and Hercegovina and to test antitumor activity of its different extracts on several cell lines, in addition to the evaluation of nutritional value, chemical composition and radical scavenging activity.

MATERIAL AND METHODS

Materials and reagents

The following reagents were used: mushroom and yeast β-glucan assay kit K-YBGL and total dietary fibre assay kit K-TDFR obtained from Megazyme Int. (Wicklow, Ireland); ACE Kit-WST obtained from Dojindo Laboratories (Kumamoto, Japan). Analytical grade methanol was obtained from Macron Fine Chemicals (Avantor, Radnor, PA, USA); analytical grade cyclohexane, HPLC grade acetonitrile, methanol and ethanol, as well as sulfuric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Analytical grade dichloromethane, hexane and petroleum ether, tetrabuthylammonium bromide (TBAB), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·), Folin-Ciocalteu reagent, as well as analytical standards of vitamins (thiamine hydrochloride (B_1), riboflavin (B_2), nicotinamide (B_3) and pyridoxine hydrochloride (B_6)), amino acids and 5'-nucleotides and nucleoside analytical standards, RPMI-1640 medium, fetal bovine serum (FBS), L-glutamine, streptomycin, penicillin, HEPES buffer solution and *cis*-diaminedichloroplatinum (*cis*-DDP) were purchased form Sigma-Aldrich (St. Louis, MO, USA). Cholecalciferol (vitamin D_3), vitamin A palmitate and DL-α-tocopherol (vitamin E) analytical standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany); dimethylformamide and triethylamide were obtained from Acros Organics (Geel, Belgium); gallic acid and dimethyl sulfoxide from Carl Roth (Karlsruhe, Germany). Potassium dihydrogen phosphate, sodium bicarbonate, sodium carbonate and sodium phosphate were purchased form Centrohem (Stara Pazova, Serbia); and hydrochloric acid from Zorka Pharma (Šabac, Serbia). Fatty acid methyl esters (Supelco 37 Component FAME Mix) and dabsyl chloride solution were obtained from Supelco (Bellefonte, PA, USA). Formic acid was provided by Honeywell (Honeywell International, Inc., Morristown, NJ, USA) and ammonium formate by Agilent Technologies, Inc. (Santa Clara, CA, USA).

Sample collection

Fruiting bodies of the mushroom *Craterellus cornucopioides* (L.) Pers. (Cantharellaceae family) were collected in Bosnia and Herzegovina, on the northern slopes of mountain Kozara (Kozarska Dubica) in July 2014 (GPS coordinates were: 45°05'25.7"N; 16°51'33.0"E), near the Monastery Moštanica.

Before extraction, the mushroom was dried at room temperature, pulverized in a sample laboratory mill (MRC SM-450, MRC-Laboratory Equipment, Essex, United Kingdom) and stored at 4°C. The voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Belgrade, Republic of Serbia (No 29).

Extract preparation

Extraction was carried out as described previously by Kolundžić *et al*. [2017] with solvents of different polarity including methanol, cyclohexane and dichloromethane. The dry powdered mushroom (20 g) was weighed and extracted once for two days with each mentioned solvent, at room temperature with a powder to solvent ratio of 1:10 (*w/v*). The organic solvents were evaporated under low pressure and dried to obtain the black-colored extracts: 0.34 g of cyclohexane extract (CCC), 0.11 g of dichloromethane extract (CCD) and 0.14 g of methanol extract (CCM). Aqueous extract was prepared with 1 g of dried, powdered mushroom mixed with 120 mL of distilled water, heated in a water bath for 30 min at 100°C with occasional shaking. After extraction, water was removed using an Alpha 1–4 LD plus freeze dryer (Martin Christ, Osterode am Harz, Germany) to obtain 0.45 g of dry extract (CCA).

Determination of nutritional value

The dried mushroom was analyzed for the contents of moisture, crude protein, crude fat, total dietary fibre and crude ash. Its nutritional value was determined by using the procedures described by the Association of Official

Analytical Chemists [AOAC, 1995]. Gravimetric method was deployed to determine the moisture and crude ash contents after drying at 105°C and incineration at 550°C, respectively. The crude protein content was determined by the Kjeldahl method using a nitrogen conversion factor of 4.38 for protein calculation. The crude fat content was determined using the Soxhlet extraction with petroleum ether as a solvent, after the treatment with HCl. Total carbohydrate content was calculated as the residual difference after subtracting crude protein, crude ash, moisture, total dietary fibre and crude fat content from 100. The content of total dietary fibre was determined according to the instruction manual of the assay kit (K-TDFR, Megazyme Int.). All results were expressed in g/100 g of dry weight (dw) of mushroom.

Determination of the content of glucans

The content of glucans (including α-glucan, β-glucan and total glucan) was determined according to the instruction manual of the mushroom and yeast β-glucan assay kit (K-YBGL) (Megazyme Int.), in dried, powdered mushroom (DM), in mushrooms after thermal treatment (cooking at 100°C for 30 min) (DMAC) and in CCA, CCM, CCC and CCD. Contents of total glucans and α-glucan were determined measuring absorbance at 510 nm on an Evolution 300 UV-Vis spectrophotometer (Thermo Scientific, Madison, WI, USA). The content of β-glucans was calculated indirectly from the difference between the content of total glucans and α-glucan. Results were expressed in g/100 g dw of mushroom or extract.

Determination of vitamin contents

The content of thiamine, riboflavin, niacin, pyridoxine, as water-soluble vitamins B_1, B_2, B_3 and B_6, respectively, was determined in CCA and CCM, whereas the content of retinol, cholecalciferol and α-tocopherol as fat-soluble vitamins A, D_3 and E, respectively, was determined in CCC. Before the analysis, methanol and aqueous extracts were diluted with water, whereas cyclohexane extract was diluted with hexane, followed by the filtration of solutions through a 0.45 μm filter. An Agilent 1200 series HPLC with diode aray detector (DAD) and a Zorbax Eclipse XDB-C18 (4.6×250 mm, 5 μm) reverse phase column (Agilent), thermostated at 25°C, was used for determinations.

Five microliters of polar extracts, used for the analysis of water-soluble vitamins, were injected into the column. The solvent A consisting of 0.1 M phosphate buffer, pH 2.8 and acetonitrile (90:10, *v/v*) was used as an eluent for 10 min at the flow rate of 1.0 mL/min. The absorbance for thiamine and niacin was measured at 254 nm, for riboflavin at 268 nm and for pyridoxine at 291 nm. For the analysis of fat-soluble vitamins, the non-polar extract was injected at the volume of 10 μL into the same column. The gradient solvent system, which consisted of mobile phase A and acetonitrile (B), was applied (1% of B for 4.5 min, and then 90% of B until 6 min) with the flow rate of 0.5 mL/min. The absorbances for retinol, cholecalciferol and α-tocopherol determination were measured at 326, 266 and 210 nm, respectively. Identification of vitamins in the samples was performed based on retention times (t_R) and UV spectra relative to t_R and UV spectra of the standards. Quantification was performed based on

peak areas using baseline construction method. All standard solutions were prepared with a range from 1 to 100 mg/L, except for α-tocopherol with a range of 5–200 mg/L. The content of vitamins was expressed in g/100 g of dry extract.

Determination of the contents of 5'-nucleotides and nucleosides

For analysing the content of 5'-nucleotides and nucleosides, the samples were prepared according to Beluhan & Ranogajec [2011], with slight modifications. Dried homogenized mushroom weighing 500 mg was mixed with 10 mL of deionized water in a round bottom flask. The suspension was heated on a water bath for 5 min and then stirred with a vortex stirrer for 30 s and cooled to room temperature. The samples were filtered through a 0.45 μm diameter filter before analysis. Stock standard solutions of single 5'-nucleotides (5'-adenosine monophosphate, 5'-AMP; 5'-citidine monophosphate, 5'-CMP; 5'-uridine monophosphate, 5'-UMP; and 5'-guanosine monophosphate, 5'-GMP) and nucleosides (adenosine, cytidine, uridine, guanosine) were prepared at a concentration of 0.5 mg/mL. A series of dilutions (0.25, 0.125, 0.0625, 0.0312 and 0.0156 mg/mL) were prepared from the stock standard solution for the construction of the calibration curves of each of the eight listed substances. All standard solutions were filtered through a 0.45 μm diameter filter. The stock standard solutions were stored at 4°C protected from light until the beginning of the analysis.

Identification and content determination of 5'-nucleotides and nucleosides was performed according to a modified method of Ranogajec *et al*. [2009] on an Agilent 1100 HPLC system with a binary pump, a G1315B DAD and a Zorbax Eclipse XDB-C18 column (4.6×250 mm, 5 μm). Twenty microliters of the test sample and standard solutions were injected. The solvent system was composed of two mobile phases: 0.1 M KH_2PO_4 and 4 mM tetrabuthylammonium bromide, pH 6 (A) and a mixture of mobile phase A with methanol (70:30, *v/v*), pH 7.2 (B). Elution started at the flow rate of 1.2 mL/min with 5% B until 9 min, 25% B at 15 min, 90% B at 17.5 min, 100% B at 19 min and 5% B at 24 min. The absorbance was recorded at 254 nm. Identification of 5'-nucleotides and nucleosides in the sample was performed based on t_R and UV spectra relative to t_R and UV spectra of the standards. Quantification was performed based on peak areas using baseline construction method. The content of 5'-nucleotides and nucleosides was expressed in mg/g dw of mushroom.

Analysis of free amino acid composition and umami potential

Samples of dried mushroom were prepared as described above for the analysis of the contents of 5'-nucleotides and nucleosides. An amino acid standard solution containing L-alanine, L-arginine, L-glutamic acid, L-cystine, L-aspartic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine was used to prepare stock standard solutions and their dilutions (250, 125, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.0678 μM).

Before the analysis, derivatization of the standards and the amino acids of the test sample was performed

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according to Ribeiro *et al*. [2008]. Briefly, 20 μL of both the test solution and standard solutions was mixed with 180 μL of a reaction buffer (0.15 M NaHCO$_3$, pH 8.6), stirred, and 200 μL of dabsyl chloride solution (12.4 mM) was added. The vials were stirred again and heated to 70°C for 15 min. The reaction was ended by cooling on ice for 5 min. Then, 400 μL of a dilution buffer was added, containing 50 mL of acetonitrile, 25 mL of ethanol and 25 mL of an elution buffer (9 mM sodium phosphate, 4% dimethylformamide, 0.15% triethylamine, pH 6.5). The mixture was centrifuged for 5 min at 1,700×*g*. The supernatant was filtered and stored at –20°C until the analysis.

The analysis of amino acids was performed using the LC-MS-ESI system (Agilent 1260/6130) equipped in an Agilent Zorbax SB-Aq column (3.0×150 mm, 3.5 μm) thermostated at 25°C. The volume of injection was 3 μL. The mobile phase system was composed of two solvents: 0.1% (*v/v*) ammonium formate in water (A) and 0.1% (*v/v*) formic acid in acetonitrile (B). Elution started at the flow rate of 0.3 mL/min with 15% B until 3 min, 85% B at 63 min and 15% B at 74 min. Detection wavelengths were at 436 nm. Electrospray ionization (ESI) mass spectra were recorded in the positive ion mode in a range of 50–600 *m/z*; fragmentator voltage of 250, nitrogen flow of 10 mL/min at 350°C, under pressure of 40 psi and capillary voltage of 3500 V. Individual amino acids were identified in the mixture based on their t$_R$ and mass spectra in relation to the t$_R$ and mass spectra of the corresponding standards, analysed under the same chromatographic conditions. The amino acid contents were calculated based on the peak area using baseline construction method. The content of individual amino acids was expressed in mg/g dw of mushroom. Also, to evaluate the umami potential of our tested mushroom sample, the equivalent umami concentration (EUC) value was calculated according to the formula given by Yamaguchi *et al*. [1971]. The EUC value of 100% indicates that the umami intensity of fruit bodies or mycelia per 1 g dry weight is equivalent to the umami intensity given by 1 g of monosodium glutamate (MSG).

Determination of fatty acid composition

Fatty acid composition of CCC was analyzed according to the method described by Ušjak *et al*. [2019] using an Agilent 6890N gas chromatography (GC) system equipped with a split/splitless injector (260°C), a flame ionization detector (FID) and a capillary column (Agilent J&W HP-88, 100 m × 0.25 mm, 0.20 μm film thickness), coupled with an Agilent 5975C mass spectrometry (MS) detector operating in the electron ionization mode at 70 eV. Saponification was achieved with 50% KOH at 90°C for 1 h. Petroleum ether was used to separate unsaponifiable fractions. Fatty acid methyl esters (FAME) were obtained by esterification of fatty acids with 98% H$_2$SO$_4$/anhydrous MeOH. The carrier gas was He, used at the flow rate of 1.2 mL/min. The oven temperature was initially held at 140°C for 5 min, then increased linearly from 140°C to 240°C at 4°C/min, and finally held at 240°C for 10 min. The FID and MS transfer line temperatures were 260°C and 250°C, respectively. Split ratio was 1:25 and the injected volume was 1 μL of 1% solution of the FAME in dichloromethane.

The identification of the FAME was based on the comparison of their t$_R$ and mass spectra to those of the representative standards ran under the same chromatographic conditions and to those from the NIST/NBS 05 and Wiley (8th edition) libraries, and the literature. Relative percentages of the compounds were calculated based on the peak areas.

Determination of total phenolic content

The content of total phenolics in CCM and CCA was determined spectrophotometrically, as described previously by Kolundžić *et al*. [2017]. To this end, 100 μL of methanol solution of CCM (11 mg/mL) and CCA (12 mg/mL) was mixed with 750 μL of the diluted Folin-Ciocalteu reagent (1:1, *v/v*, in distilled water). After 5 min, 750 μL of 60 g/L Na$_2$CO$_3$ solution was added to each sample. Firstly, the mixtures were shaken and then left for 90 min in the dark at room temperature. Absorbance was measured at 725 nm using UV-Vis spectrophotometer Evolution 300 (Thermo Scientific). As a blank, 100 μL of the solvent was used. Series of gallic acid dilutions (1–10 mg/mL) were reacted in parallel, the absorbance was measured and the calibration curve was constructed. The content of total phenolics was expressed as mg of the gallic acid equivalent (GA)/g of dry extract.

Determination of DPPH radical scavenging activity

The ability of CCM and CCA to scavenge free radicals was measured in a DPPH test [Kolundžić *et al*., 2017]. Briefly, six volumes (10, 30, 50, 70, 90, 100 μL) of stock methanol solutions of extracts (CCM 60 mg/mL; CCA 16 mg/mL) were mixed with methanol, so that the total volumen became 2 mL, and 0.5 mL of 0.5 mM DPPH radical solution in methanol was added. The concentration range was 240, 720, 1200, 1680, 2160 and 2400 μg/mL for CCM and 64, 192, 320, 448, 576 and 640 μg/mL for CCA. The mixtures were left in the dark at room temperature for 30 min. The absorbance of the solution was measured at 517 nm using an Evolution 300 UV-Vis spectrophotometer (Thermo Scientific) and the percent of inhibition of DPPH radical was calculated. The results were expressed as a IC$_{50}$ value, *i.e.* concentration of the extract that reduced 50% of the DPPH radicals.

Determination of ACE inhibitory activity

The ACE Kit-WST (Dojindo Laboratories) was used to determine ACE inhibiting activity of CCA. In the assay, 3-hydroxybutyric acid (3HB), which is released from 3-hydryoxy-butyryl-Gly-Gly-Gly (3HB-GGG), was enzymatically detected. The concentrations ranging between 0.1 and 20 mg/mL of the aqueous extract were used and the procedure of the manufacturer was completely followed. The experiment was done in the 96-well microtiter plate. The absorbance of the solutions was measured on an EL×800 absorbance microplate reader (BioTek Instruments, Winooski, VT, USA) at 450 nm and the extract concentration needed to inhibit 50% of ACE activity (IC$_{50}$) was calculated.

Cytotoxicity assay

Treatment of cell lines and determination of cell survival rate were done according to the procedure described by Kolundžić *et al*. [2017]. Human epithelial cervical cancer

cells HeLa, adenocarcinomic human alveolar basal epithelial cells A549, colorectal cancer cells LS174 and normal MRC-5 human embryonic lung fibroblast cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). All cancer cell lines were maintained in the recommended RPMI-1640 medium supplemented with 10% heat--inactivated (56°C) FBS, L-glutamine (3 mM), streptomycin (100 mg/mL), penicillin (100 IU/mL), and 25 mM HEPES buffer and adjusted to pH 7.2 by bicarbonate solution. Cells were grown in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Stock solutions (100 mg/mL) of four extracts were made in dimethyl sulfoxide and dissolved in corresponding medium to the required working concentrations. Final concentrations achieved in the treated wells were 12.5, 25, 50, 100 and 200 μg/mL. The effects on cancer cell survival were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) test, 72 h upon extract addition, as described previously [Kolundžić *et al*., 2017]. The number of viable cells in each well was proportional to the intensity of the absorbance of light, which was then read in an ELISA plate reader (Thermo Labsystem Multiskan EX, Thermo Fisher scientific, Waltham, MA, USA) at 570 nm. IC_{50} was defined as the concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. Cisplatin (*cis*-DDP) was used as a positive control.

Statistical analysis

All measurements were done in three analytical repetitions. Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Data were statistically analysed using Student's *t*-test (for two groups) or one-way analysis of variance (ANOVA) with post-hoc Tukey's test (for three or more groups), where appropriate. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Nutritional value, contents of total dietary fibre and glucans

Fruiting bodies of *C. cornucopioides* were low in crude fat (5.02±0.16 g/100 g dw), total carbohydrates (11.97±0.87 g/100 g dw) and crude proteins (11.51±0.16 g/100 g dw), which resulted in a low energy value (248.03±9.84 kcal/100 g dw; 1037.63±31.00 kJ/100 g dw). The moisture content was 6.84± 0.10 g/100 g dw, and crude ash was 10.20±0.12 g/100 g dw. Results obtained in this study are in correlation with previous results in terms of the contents of crude ash and crude fat, but the protein content was far lower than 47.21%, 69.45% and 50.10% reported by Beluhan & Ranogajec [2011], Barros *et al*. [2008] and Colak *et al*. [2009], respectively. *C. cornucopioides* was rich in total dietary fibre (54.46±0.90 g/100 g dw). β-Glucan content in our sample (dried mushroom) was 15.65 g/100 g dw, which accounted for approximately 98% of total glucans (Table 1) and one third of the total dietary fibre. A similar result (16.20 g/100 g dw) has been reported for black trumpet growing in Poland of [Mirończuk-Chodakowska *et al*.,2017]. Cooking has been reported to influence the proximate glucan content in mushrooms [Kolundžić *et al*., 2017].

TABLE 1. The content of total, α- and β-glucan (g/100 g dw) in dried *Craterellus cornucopioides* before and after cooking and in extracts of mushrooms.

	Total glucan	α-Glucan	β-Glucan
Dried mushroom	15.96±0.07[a]	0.31±0.01[a]	15.65±0.06[a]
Dried mushroom after cooking	1.53±0.02[d]	0.08±0.01[c]	1.44±0.03[c]
CCA	2.54±0.10[b]	0.15±0.01[b]	2.39±0.10[b]
CCM	1.61±0.04[c]	0.13±0.01[b]	1.47±0.04[c]

Results are expressed as mean ± standard deviation (*n*=3). Values with different superscript letters in column differ significantly at $p < 0.05$. CC, *C. cornucopioides* extracts (A, aqueous; M, methanol); dw, dry weight.

In the present study, the content of total glucans decreased significantly ($p < 0.05$) after cooking to 1.53 g/100 g dw, indicating approximately 90% reduction (Table 1). The content of glucans was also determined in extracts. The presence of glucans was detected in polar (aqueous and methanol) but not in non-polar extracts (cyclohexane and dichloromethane). The significantly ($p < 0.05$) highest content of total glucans, α- and β-glucan was detected in dried mushrooms compared to extracts. In turn, CCA had a significantly ($p < 0.05$) higher content of all three glucan types (total glucans, α- and β-glucan) compared to DMCA and CCM, except for α-glucan in CCM. Therefore, it can be concluded that glucan contents in mushrooms depend not only on the cooking process, but also on the extraction method as well as the choice of solvent used for extraction.

Vitamins

The contents of water-soluble vitamins B_1, B_2, B_3 and B_6 were determined in polar extracts (CCA and CCM), while those of fat-soluble vitamins A, D_3 and E were determined in the non-polar extract (CCC), (Table 2). The most abundant water-soluble vitamin was B_3 – niacin with the content of 249.0 mg/100 g dry CCA and 367.0 mg/100 g dry CCM, followed by vitamin B_2 – riboflavin, vitamin B_1 – thiamine and vitamin B_6 – pyridoxine. ANOVA showed a significantly higher vitamin B_1 content in CCA compared to CCM ($p < 0.05$), and significantly higher contents of vitamin B_3 and B_6 in CCM compared to CCA ($p < 0.05$). So far, the content of B-group vitamins in *C. cornucopioides* has not been determined extensively. Çağlarırmak [2011] reported thiamine, riboflavin, pyridoxine and niacin contents of 0.17––0.63, 0.26–0.90, 0.14–0.56 and 6.38–8.37 mg/100 g dw, respectively, in dried cultivated shiitake, white and brown button mushrooms and oyster mushroom.

α-Tocopherol is the most active form of vitamin E in humans and as a free radical scavenger it protects our bodies against degenerative diseases [Tucker *et al.,* 2005]. α-Tocopherol was found as the most abundant fat-soluble vitamin in the cyclohexane extract of *C. cornucopioides*, with the content of 2816 mg/100 g dry extract corresponding to 47.90 mg/100 g dw of mushroom. The content of α-tocopherol previously reproted in *C. cornucopioides* was 0.115 mg/100 g dw [Liu *et al*., 2012], which is much lower than the value reported in our study. In turn, contents of total tocopherols have

TABLE 2. The content of vitamins in extracts of *Craterellus cornucopioides* (mg/100 g dry extract).

Vitamin	CCC	CCM	CCA
B_1	–	19.4 ± 1.4^b	54.7 ± 2.9^a
B_2	–	81.2 ± 8.2^a	76.3 ± 6.2^a
B_3	–	367.0 ± 4.5^a	249.0 ± 5.0^b
B_6	–	17.0 ± 1.7^a	8.3 ± 1.0^b
E	2816 ± 36	–	–
D_3	89.3 ± 8.9	–	–
A	16.1 ± 1.8	–	–

Results are expressed as mean ± standard deviation ($n=3$). Values with different superscript letters in row differ significantly at $p<0.05$. CC, *C. cornucopioides* extracts (A, aqueous; M, methanol; C, cyclohexane).

been determined in mushrooms of *Boletus* species, revealing 2533 μg/100 g dw in *B. reticulatus* [Heleno *et al*., 2011].

Group-D vitamins occurred in two forms: ergocalciferol – vitamin D_2 being the major form of vitamin D found in edible mushrooms, and cholecalciferol – vitamin D_3 found in lesser amounts [Cardwell *et al*., 2018]. Also, ergosterol (one vitamin D precursors) is commonly present in fungi [Venditti *et al.*, 2017]. Considerable amount of vitamin D_3 has been found in the cyclohexane extract of *C. cornucopioides*, *i.e.* 89.3 mg/100 g dry extract which corresponded to 1.52 mg/100 g dw of mushroom. There is no literature data that support this founding. So far, only vitamin D_2 has been detected in the wild growing species of the genus Cantharellaceae, *i.e.* at 10.70 mg/100 g dw in *Cantharellus cibarius* and 21.10 mg/100 g dw in *C. tubaeformis* [Teichmann *et al*., 2007]. In the case of vitamin A, it was detected in the CCC at 16.1 mg/100 g dw (0.27 mg/100 g dw of mushroom), and here likewise, there is no literature data to compare this value.

5'-Mononucleotides and nucleosides

Nucleosides, being precursor molecules for DNA and RNA synthesis, are vital for cellular function. Also, both 5'-nucleotides and nucleosides were reported to exert various positive impacts on brain function, immune system, fatty acid metabolism, and gastrointestinal tract [Phan *et al*., 2018]. The most common nucleotides found in mushrooms are 5'-adenosine monophosphate (5'-AMP), 5'-cytidine monophosphate (5'-CMP), 5'-guanosine monophosphate (5'-GMP), 5'-inosine monophosphate (5'-IMP), 5'-uridine monophosphate (5'-UMP) and 5'-xantosine monophosphate (5'-XMP) [Phan *et al*., 2018].

The total content of 5'-nucleotides in dried body of *C. cornucopioides* was 3.99 mg/g dw, while the content of nucleosides was 1.84 mg/g dw. The 5'-nucleotide content could be ordered as follows: 5'-UMP > 5'-AMP > 5'-GMP > 5'-CMP (1.55 ± 0.03, 0.87 ± 0.47, 0.83 ± 0.15 and 0.74 ± 0.13 mg/g dw, respectively). In turn, the content of nucleosides decreased in the following order: uridine > adenosine = guanosine > cytidine (0.50 ± 0.01, 0.47 ± 0.07, 0.47 ± 0.03 and 0.40 ± 0.04 mg/g dw, respectively). The findings

obtained from this research are different than the results published before. Ranogajec *et al*. [2009] reported lower contents of the same 5'-nucleosides and nucleotides in *C. cornucopioides* sample, *i.e.* 0.30 and 0.91 mg/g dw, respectively, while the content of 5'-nucleosides in *C. cornucopioides*, reported later by the same research group, was much higher, reaching 10.48 mg/g dw [Beluhan & Ranogajec, 2011]. These differences in the results are due to the influence of different factors on 5'-nucleotide content, such as the use of different parts of the fungus, the stage of fungal development, and collection time, as shown in the example of *Tricholoma matsutake* where the contents of 5'-AMP and 5'-GMP were higher in its pileus than stalk [Cho *et al*., 2010].

5'-Nucleotides, such as 5'-GMP, 5'-IMP,5'-XMP and 5'-AMP, in synergy with glutamic and aspartic acid contribute to the specific umami taste of mushrooms, which is the taste induced by monosodium glutamate (MSG) [Phat *et al*., 2016]. Among these compounds, 5'-GMP is the one responsible for the meaty taste of mushrooms, and it is a stronger flavor enhancer than MSG [Beluhan & Ranogajec, 2011]. The umami intensity of the mentioned compounds is discussed below in the Free amino acids section.

Free amino acids

The contents of individual free amino acids in dried *C. cornucopioides* are shown in Table 3. All amino acids, except histidine and cystine, were identified in the sample. The total amino acid content was 6.97 mg/g dw. According to the literature, the content of free amino acids in mushrooms is low and varies from 7.14 mg/g to 12.3 mg/g in dried edible mushrooms, with glutamic acid (21.7–23.7%) and alanine (17.7–17.9%) found as major ones [Cheung, 2010]. The content of amino acids in *C. cornucopioides* has been previously examined, where higher values were obtained for amino acids (67.48 mg/g dw), with glutamic acid as the most major amino acid [Beluhan & Ranogajec, 2011].

The content of essential amino acids in *C. cornucopioides* was 1.49 mg/g dw, while non-essential amino acids were present in a higher content of 5.48 mg/g dw (Table 3). The calculated ratio between essential and non-essential amino acids was 0.27, which was below the value of 0.6 recommended by FAO/WHO [Wang *et al*., 2014].

Another classification of amino acids can be made according to their characteristic taste [Beluhan & Ranogajec, 2011]. Among the *C. cornucopioides* amino acids were those with sweet taste, including alanine, glycine, serine and threonine; bitter taste, including arginine, isoleucine, leucine, methionine, phenylalanine and valine; tasteless, including lysine and tyrosine; and monosodium glutamate-like (MSG-like) taste, including glutamic and aspartic acids (Table 3). The predominant amino acids were those with bitter taste (3.14 mg/g dw) followed by MSG-like tasting (1.83 mg/g dw) and tasteless (1.00 mg/g dw). The low content of sweet-tasting amino acids (0.68 mg/g dw) was not surprising and it was in accordance with the low content of carbohydrates. Nevertheless, it has been found that sweet and MSG-like amino acids are so-called taste-active amino acids in mushrooms, unlike the bitter-testing ones, and so bitter taste might be suppressed by the sweet components [Mau, 2005].

TABLE 3. Free amino acid content in dried *Craterellus cornucopioides* and taste of amino acids.

Type of amino acid	Amino acid	Taste*	Content (mg/g dw)
Essential amino acid	Histidine	Bitter	nd
	Lysine	Tasteless	0.38±0.02
	Threonine	Sweet	0.21±0.01
	Methionine	Bitter	0.01±0.00
	Valine	Bitter	0.04±0.01
	Leucine/isoleucine	Bitter	0.68±0.04
	Phenylalanine	Bitter	0.17±0.05
Non-essential amino acid	Cystine	/	nd
	Arginine	Bitter	2.24±0.01
	Serine	Sweet	0.15±0.06
	Aspartic acid	MSG-like	0.43±0.11
	Glutamic acid	MSG-like	1.40±0.10
	Glycine	Sweet	0.22±0.03
	Alanine	Sweet	0.10±0.03
	Tyrosine	Tasteless	0.62±0.01
	Proline	/	0.32±0.01

*according Beluhan & Ranogajec [2011].
Results are expressed as mean ± standard deviation (*n*=3). MSG-like, monosodium glutamate-like; nd, not detected; dw, dry weight.

TABLE 4. Fatty acid composition (% total fatty acids) of *Craterellus cornucopioides* cyclohexane extract.

Fatty acid	Content
C 15:0	0.33±0.05
C 16:0	9.96±0.25
C 16:1	0.34±0.20
C 17:0	0.19±0.01
C 18:0	12.43±0.37
C 18:1*n*9c	60.83±1.46
C 18:2*n*6c	10.85±0.10
C 20:0	0.41±0.03
C 20:5*n*3	0.70±0.03
C 22:0	0.36±0.01
C 22:6*n*3	2.93±0.21
C 24:0	0.41±0.08
C 24:1*n*15c	0.26±0.06
SFAs	24.08±0.79
MUFAs	61.44±1.73
PUFAs	14.48±0.34

Results are expressed as mean ± standard deviation (*n*=3). SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

As mentioned before, the synergistic effect of umami 5'-nucleotides (5'-AMP, 5'-IMP, 5'-GMP, and 5'-XMP) and umami amino acids (glutamic and aspartic acid) may enhance the umami taste of mushrooms. Hence, the EUC value was calculated to evaluate the umami potential of the mushroom sample. The EUC values are grouped into four levels: >1000% (>10 g MSG/g dw); 100–1000% (1–10 g MSG/g dw); 10–100% (0.1–1 g MSG/g dw) and 10% (<0.1 g MSG/g dw) [Mau, 2005]. The EUC value for the tested *C. cornucopioides* was 36.20%, which classifies it as the mushroom with EUC values at the third level. According to Mau [2005], mushrooms with EUC values at the second and third levels are those that do not contribute to umami taste but are still used for culinary purposes for their flavor, consistency and texture.

Fatty acids

The fatty acid composition of the cyclohexane extract of *C. cornucopioides* is shown in Table 4. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) have been identified and quantified. The most abundant group of fatty acids were MUFAs (61.44%), followed by SFAs (24.08%) and PUFAs (14.48%). This is considered to be a positive ratio since the SFAs are recognized to be associated with cardiovascular disorders and atherosclerosis, while the high intake of PUFAs and MUFAs is considered to prevent these disorders [Silva Figueiredo *et al*., 2017]. The obtained data are in accordance with findings from previously conducted studies where MUFAs were also detected as the most represented class of fatty acids in *C. cornucopioides* (59.85%), followed by PUFAs (23.79%) and SFAs (16.36%) [Barros *et al*., 2008].

The main fatty acid in the tested mushroom was oleic acid (Table 4). This monounsaturated but not an essential fatty acid is effective in lowering cholesterol level, which affects high blood pressure [Abugri *et al*., 2016]. Essential fatty acids include linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3c). Since the human organism is not able to synthesize these fatty acids, they must be provided with diet. In our study, only linoleic acid was found in *C. cornucopioides* with a relative content of 10.85% (Table 4). In turn, stearic acid was the most abundant (12.43%) among the identified saturated fatty acids. However, it has no impact on increasing serum low density lipoprotein (LDL) cholesterol level, due to its rapid conversion into oleic acid in the organism [Grundy, 2013]. Nevertheless, there was a higher content of unsaturated than saturated fatty acids in the tested *C. cornucopioides*, which can be classified as a healthy food.

Total phenolic content and DPPH radical scavenging activity

Phenolic compounds are very good antioxidants and possess very good radical scavenging ability [Zhao *et al.,* 2014]. The content of total phenolics in the methanol and aqueous extracts of *C. cornucopioides* is shown in Table 5. The methanol extract contained almost twice less total phenolics than the

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TABLE 5. The total phenolic content, DPPH radical scavenging activity and angiotensin converting enzyme (ACE) inhibitory activity of *Craterellus cornucopioides* extracts.

	Total phenolic content (mg GA/g extract)	DPPH scavenging activity (IC$_{50}$; μg/mL)	ACE inhibitory activity (IC$_{50}$; mg/mL)
CCA	16.96±0.22[a]	473±5[b]	0.74±0.07
CCM	8.95±0.06[b]	887±15[a]	–

Results are expressed as mean ± standard deviation (*n*=3). Values with different superscript letters in column differ significantly at *p*<0.05. CC, *C. cornucopioides* extracts (A, aqueous; M, methanol); GA, gallic acid equivalent.

aqueous extract and thus exhibited lower DPPH radical scavenging activity (IC$_{50}$ of 887 μg/mL for CCM and 473 μg/mL for CCA). Previous results of Liu *et al.* [2012] have shown significant, but lower than the results in this study, DPPH radical scavenging activity of *C. cornucopioides* extracts with the IC$_{50}$ value of 40.30 mg/mL for ethanol extract and 26.37 mg/mL for aqueous extract and with quercetin as a major compound in the ethanol extract. Vamanu & Nita [2014] have used a different extraction method with a fluidized bed extractor, but the extract produced also had a lower total phenolic content (88.4 mg GA/100 g of extract) than in this study, whereas DPPH radical scavenging activity was about 60% for the extract concentration of 1 mg/mL. Different extraction procedure leads to the extraction of other compounds with antioxidative power as ascorbic acid, lycopene, β-carotene and tocopherols [Vamanu & Nita, 2014], but in our study, lipophilic compounds could accumulate in non--polar, cyclohexane extract which was not suitable for antioxidant activity estimation using the DPPH method.

ACE inhibitory activity

The results indicated dose-dependent ACE inhibitory activity of the *C. cornucopioides* aqueous extract with an IC$_{50}$ of 0.74 mg/mL (Table 5). According to the manufacturer of the ACE kit (Dojindo Laboratories), IC$_{50}$ of captopril – well-known ACE inhibitor – was 2.14 nM (0.465 ng/mL), in the same test [https://dojindo.com/product/ace-kit-wst-a502/]. The results of Kolundžić *et al.* [2017] study with *Cantharellus cibarius* from the same family Cantharellaceae showed 68% ACE inhibitory activity at a dose of 1.25 mg/mL. In turn, Jang *et al.* [2011] isolated and characterized two ACE

inhibitors with IC$_{50}$ values of 0.46 and 1.14 mg/mL from fruiting bodies of *Pleurotus cornucopiae*, and demonstrated an antihypertensive effect *in vivo* of an aqueous extract of the same fungus. Aqueous and ethanolic extracts of haesongi mushroom (*Hypsizigus marmoreus*) showed dose-dependent ACE inhibitory activity *in vitro* (aqueous extract had strong activity: 95.34% at a concentration of 40 mg/mL) [Jung *et al.*, 2009]. Lee *et al.* [2004] showed ACE inhibitory activity of an aqueous extract of *T. giganteum* with an IC$_{50}$ of 0.31 mg/mL and of an isolated peptide consisting of proline, glutamic acid and glycine (IC$_{50}$ of 0.04 mg/mL), as well as different ACE inhibitory activities for aqueous, ethanolic and methanolic extracts of several mushrooms: *Pleurotus sajor-caju*, *P. ostreatus*, *Flammulina velutipes*, *T. giganteum*, *Agaricus bisporus*, *Poria cocos*, *Grifolia umbellata*, and *Lentinus edodes*, but the best activity was shown for the aqueous extract of *T. giganteum*.

Cytotoxic activity

The cytotoxic effect of four *C. cornucopioides* extracts was tested against human epithelial carcinoma cells, human lung carcinoma cells, human colon carcinoma cells, as well as on normal cell line. The respective results are presented in Table 6. From the obtained data, it can be concluded that all four extracts of *C. cornucopioides* expressed moderate cytotoxic activity against the tested cell lines. Non-polar extracts (cyclohexane and dichloromethane) were significantly (*p*<0.05) more active against the tested cell lines, including normal ones. The highest activity was observed in the cyclohexane extract against human epithelial carcinoma cells (IC$_{50}$ of 78.3 μg/mL). Polar extracts (aqueous and methanol) showed almost no activity against the tested cell lines.

The anti-tumor effect of some non-polar compounds has been confirmed before, including fatty acids [Sagar *et al.*, 1992]. Linoleic acid exhibited antimicrobial activity and cytotoxic activity against the HeLa tumor cell line. This fatty acid found as abundant fatty acid in the cyclohexane extract (Table 4) could be responsible for its high cytotoxic activity. Seven illudin sesquiterpenoids, *i.e.* craterellin A, B and C, illudin F, M, T and illudalenol, and one gymnomitrane sesquiterpenoid, gymnomitr-3-en-10β,15-diol, have been isolated by Guo *et al.* [2017a] from an ethyl acetate extract of *C. cornucopioides* as colorless oils and tested against several cancer cell lines (breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1 and lung cancer A-549). Only craterellin C (sesquiterpenoid) isolated from

TABLE 6. Cytotoxic activity (IC$_{50}$, μg/mL) of *Craterellus cornucopioides* extracts.

	CCA	CCM	CCD	CCC	*cis*-DDP*
HeLa	>200	>200	135.6±9.7[a]	78.3±0.3[b]	2.37±0.28
LS174	191.5±7.2[a]	186.6±19.0[a]	135.7±9.9[b]	139.1±9.9[b]	4.83±0.35
A549	>200	>200	153.2±7.0[a]	141.9±8.0[a]	11.6±1.6
MRC5	189.2±9.4[a]	>200	148.5±3.6[b]	155.6±4.9[b]	14.3±1.3

Results are expressed as mean ± standard deviation (*n*=3). Values for extracts with different superscript letters in row differ significantly at *p*<0.05. CC, *C. cornucopioides* extracts (A, aqueous; M, methanol; C, cyclohexane; D, dichloromethane); HeLa, human epithelial cervical cancer cells; LS174, colorectal cancer cells; A549, adenocarcinomic human alveolar basal epithelial cells; MRC5, human embryonic lung fibroblast cells; *cis*-DDP, *cis*-diaminedichloroplatinum. **cis*-DDP was used as positive control.

the ethyl acetate extract of black trumpet, expressed moderate cytotoxity against A-549 (21.0 μM). Craterellin D and E, as well as menthane monoterpene, 4-hydroxy-4-isopropenyl-cyclohexanemethanol acetate, have also been isolated from the ethyl acetate extract of *C. cornucopioides*, and tested against the same cell cultures, but none of the compounds exhibited cytotoxic activity [Guo *et al.*, 2017b]. In turn, methanolic extracts of twenty nine mushroom species, including *C. cornucopioides*, were tested by MTT assay against human lung adenocarcinoma A549 [Vasdekis *et al.*, 2018]. Black trumpet extract was among the extracts with the most prominent activity with IC_{50} <1 mg/mL. Piceatannol, the natural stilbene with antioxidant, anticancer and anti-inflammatory activities, was identified in all tested extracts with promising cytotoxic activity. Similar research has recently been conducted by Kosanić *et al.* [2019]. This research group tested the cytotoxic potential of an acetone extract of *C. cornucopioides* against the same cell lines as in our research. The tested extract showed a moderate effect, and HeLa were the most sensitive. These authors concluded that this effect might have been due to the presence of polyphenols. The non-polar extract analyzed in our study (CCC) was also the most active against HeLa cell line.

CONCLUSION

Based on the results of this study, it can be concluded that black trumpet was a good source of nutrients, suitable for daily diet, primarily due to its low fat content and high content of dietary fibre, especially β-glucan. The nutritional potential of this fungus was also ascribed to the presence fatty acids, especially oleic acid and linoleic acid, which would be very important for people with hypertension or metabolic syndrome. The most promising result was the inhibitory effect of the aqueous extract on ACE. This was the first time that ACE inhibitory activity of black trumpet extract was tested. Concerning cytotoxicity, the cyclohexane extract, which was rich in fatty acids and vitamin E, exhibited the greatest potential.

In terms of the content of other nutrients and bioactive compounds, this mushroom was rich in vitamins and, to the best of our knowledge, this is the first time that their content was determined in extracts of different polarities, according to the solubility of the vitamins themselves, and not in fresh mushrooms. The content of amino acids, nucleotides and fatty acids, was obviously lower than in previous studies, but it was still correlated with the overall nutritional potential of this fungus. As expected, the content of total phenolics was also low, which resulted in poor antioxidant activity. Black trumpet should be analyzed in more detail for identification and isolation of specific compounds that contribute to the ACE inhibitory and cytotoxic activity.

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CONFLICT OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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REFERENCES

1. Abugri, D., McElhenney, W., William, K. (2016). Fatty acid profiling in selected cultivated edible and wild medicinal mushrooms in Southern United States. *Journal of Experimental Food Chemistry*, *2*(1), art. no. 100108.
https://doi.org/10.4172/2472-0542.1000108

2. AOAC. (1995). Official Methods of Analysis (16th ed.). Arlighton, VA: Association of Official Analytical Chemists.

3. Barros, L., Cruz, T., Baptista, P., Estevinho, L., Ferreira, I. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemical Toxicology*, *46*(8), 2742–2747.
https://doi.org/10.1016/j.fct.2008.04.030

4. Beluhan, S., Ranogajec, A. (2011). Chemical composition and non-volatile components of Croatian wild edible mushrooms. *Food Chemistry*, *124*(3), 1076–1082.
https://doi.org/10.1016/j.foodchem.2010.07.081

5. Cağlarirmak, N. (2011). Chemical composition and nutrition value of dried cultivated culinary–medicinal mushrooms from Turkey. *International Journal of Medicinal Mushrooms*, *13*, 351–356.
https://doi.org/10.1615/IntJMedMushr.v13.i4.50

6. Cardwell, G., Bornman, J.F., James, A.P., Black, L.J. (2018). A review of mushrooms as a potential source of dietary vitamin D. *Nutrients*, *10*(10), art. no. 1498.
https://doi.org/10.3390/nu10101498

7. Chen, P., Qin, H.J., Li, Y.W., Ma, G.X., Yang, J.S., Wang, Q. (2020). Study on chemical constituents of an edible mushroom *Volvariella volvacea* and their antitumor activity *in vitro*. *Natural Product Research*, *34*(10), 1417–1422.
https://doi.org/10.1080/14786419.2018.1509324

8. Cheung, P.C.K. (2010). The nutritional and health benefits of mushrooms. *Nutrition Bulletin*, *35*(4), 292–299.
https://doi.org/10.1111/j.1467-3010.2010.01859.x

9. Cho, I.H., Choi, H.K., Kim, Y. (2010). Comparison of umami-taste active components in the pileus and stipe of pine-mushrooms (*Tricholoma matsutake* Sing.) of different grades. *Food Chemistry*, *118*(3), 804–807.
https://doi.org/10.1016/j.foodchem.2009.05.084

10. Choi, H.S., Cho, H.Y., Yang, H.C., Ra, K.S., Suh, H.J. (2001). Angiotensin I-converting enzyme inhibitor from *Grifola frondosa*. *Food Research International*, *34*(2–3), 177–182.
https://doi.org/10.1016/S0963-9969(00)00149-6

11. Colak, A., Faiz, Ö., Sesli, E. (2009). Nutritional composition of some wild edible mushrooms. *Turkish Journal of Biochemistry*, *34*(1), 25–31.

12. Gil-Ramirez, A., Clavijo, C., Palanisamy, M., Soler-Rivas, C., Ruiz-Rodrigez, A., Martin, F., Reglero, G., Perez, M. (2011). Edible mushrooms as potential sources of new hypocholesterolemic compounds. (2011). *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products* (ICMBMP7), 4–7 October 2011, Arcachon, France, 110–119.

13. Grundy, S.M. (2013). Cholesterol: Factors determining blood levels. In B. Caballero (ed.), *Encyclopedia of Human Nutrition* (3rd edition). London, United Kingdom: Academic Press, pp. 335–340.
https://doi.org/10.1016/B978-0-12-375083-9.00051-9

14. Guo, H., Diao, Q., Hou, D., Li, Z., Zhou, Z., Feng, T., Liu, J. (2017a). Sesquiterpenoids from cultures of the edible mushroom *Craterellus cornucopioides*. *Phytochemistry Letters*, *21*, 114–117.
https://doi.org/10.1016/j.phytol.2017.06.007

15. Guo, H., Diao, Q.P., Zhang, B., Feng, T. (2017b). Two new illudane sesquiterpenoids and one new menthane monoterpene from cultures of *Craterellus cornucopioides*. *Journal of Asian Natural Products Research*, *21*(2), 123–128.
https://doi.org/10.1080/10286020.2017.1394293

16. Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., Santos-Buelga, C., Ferrerira, I.C.F.R. (2011). Targeted metabolites analysis in wild *Boletus* species. *LWT – Food Science and Technology*, *44*(6), 1343–1348.
https://doi.org/10.1016/j.lwt.2011.01.017

17. https://dojindo.com/product/ace-kit-wst-a502/ [last visited 15.4. 2022]

18. Jang, J.H., Jeong, S.C., Kim, J.H., Lee, Y.H., Ju, Y.C., Lee, J.S. (2011). Characterisation of a new antihypertensive angiotensin I-converting enzyme inhibitory peptide from *Pleurotus cornucopiae*. *Food Chemistry*, *127*(2), 412–418.
https://doi.org/10.1016/j.foodchem.2011.01.010

19. Jung, E.B., Jo, J.H., Cho, S.M. (2009). Hypoglycemic and angiotension converting enzyme inhibitory effect of water and ethanol extracts from Haesongi mushroom (*Hypsizigus marmoreus*). *Food Science and Biotechology*, *18*(2), 541–545.
https://www.earticle.net/Article/A105510

20. Khatun, K., Mahtab, H., Khanam, P.A., Sayeed, M.A., Khan, K.A. (2007). Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects. *Mymensingh Medical Journal*, *16*(1), 94–99.
https://doi.org/10.3329/mmj.v16i1.261

21. Kolundžić, M., Stanojković, T., Radović, J., Tačić, A., Dodevska, M., Milenković, M., Sisto, F., Masia, C., Farronato, G., Nikolić, V., Kundaković, T. (2017). Cytotoxic and antimicrobial activities of *Cantharellus cibarius* Fr. (Cantarellaceae). *Journal of Medicinal Food*, *20*(8), 790–796.
https://doi.org/10.1089/jmf.2016.0176

22. Kosanić, M., Ranković, B., Stanojković, T., Radović-Jakovljević, M., Ćirić, A., Grujičić, D., Milošević-Djordjević, O. (2019). *Craterellus cornucopioides* edible mushroom as source of biologically active compounds. *Natural Product Communications*, *14*(5), 1–6.
https://doi.org/10.1177/1934578X19843610

23. Kundaković, T., Kolundžić, M. (2013). Therapeutic properties of mushrooms in managing adverse effects in the metabolic syndrome. *Current Topics in Medicinal Chemistry*, *13*(21), 2734–2744.
https://doi.org/10.2174/15680266113136660196

24. Lee, D.H., Kim, J.H., Park, J.S., Choi, Y.J., Lee, J.S. (2004). Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom *Tricholoma giganteum*. *Peptides*, *25*(4), 621–627.
https://doi.org/10.1016/j.peptides.2004.01.015

25. Liu, Y.T., Sun, J., Luo, Z.Y., Rao, S.Q., Su, Y.J., Xu, R.R., Yang, Y.J. (2012). Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity. *Food and Chemical Toxicology*, *50*(5), 1238–1244.
https://doi.org/10.1016/j.fct.2012.01.023

26. Lo, H.C., Hsu, T.H., Lin, F.Y., Wasser, S.P., Chen, Y.H., Lee, C.H. (2012). Effects of yellow brain culinary-medicinal mushroom, *Tremella mesenterica* Ritz.:Fr. (Higher basidiomycetes), on immune function in normal and type 1 diabetic rats. *International Journal of Medicinal Mushrooms*, *14*(5), 447–457.
https://doi.org/10.1615/IntJMedMushr.v14.i5.20

27. Mau, J.L. (2005). The umami taste of edible and medicinal mushrooms. *International Journal of Medicinal Mushrooms*, *7*(1–2), 119–126.
https://doi.org/10.1615/IntJMedMushr.v7.i12.120

28. Mirończuk-Chodakowska, I., Witkowska, A.M., Zujko, M.E., Terlikowska, K.M. (2017). Quantitative evaluation of 1,3,1,6 β-D--glucan contents in wild-growing species of edible Polish mushrooms. *Roczniki Państwowego Zakładu Higieny*, *68*(3), 281–290.

29. O'Callaghan, Y.C., O'Brien, N.M., Kenny, O., Harrington, T., Brunton, N., Smyth, T.J. (2015). Anti-inflammatory effects of wild Irish mushroom extracts in RAW264.7 mouse macrophage cells. *Journal of Medicinal Food*, *18*(2), 202–207.
https://doi.org/10.1089/jmf.2014.0012

30. Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M.A., Martínez, J.A., García-Lafuente, A., Guillamón, E., Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry*, *128*(3), 674–678.
https://doi.org/10.1016/j.foodchem.2011.03.085

31. Pandya, U., Dhuldhaj, U., Sahay, N.S. (2019). Bioactive mushroom polysaccharides as antitumor: an overview. *Natural Product Research, 33*(18), 2668–2680.
https://doi.org/10.1080/14786419.2018.1466129

32. Phan, C., Wang, J., Cheah, S., Naidu, M., David, P., Sabarantam, V. (2018). A review on the nucleic acid constituents in mushrooms: nucleobases, nucleosides and nucleotide. *Critical Reviews in Biotechnology*, *38*(5), 762–777.
https://doi.org/10.1080/07388551.2017.1399102

33. Phat, C., Moon, B., Lee, C. (2016). Evaluation of umami taste in mushroom extracts by chemical analysis, sensory evaluation, and an electronictongue system. *Food Chemisty*, *192*, 1068–1077.
https://doi.org/10.1016/j.foodchem.2015.07.113

34. Ranogajec, A., Beluhan, S., Šmit, Z. (2009). Analysis of nucleosides and monophosphate nucleotides from mushrooms with reversed-phase HPLC. *Journal of Separation Science*, *33*(8), 1024–1033.
https://doi.org/10.1002/jssc.200900516

35. Ribeiro, B., Andrade, P., Silva, B., Baptista, P., Seabra, R., Valentão, P. (2008). Comparative study on free amino acid composition of wild edible mushroom species. *Journal of Agricultural and Food Chemistry*, *56*(22), 10973–10979.
https://doi.org/10.1021/jf802076p

36. Sagar, P.S., Das, U.N., Koratkar, R., Ramesh, G., Padma, M., Kumar, G.S. (1992). Cytotoxic action of *cis*-unsaturated fatty acids on human cervical carcinoma (HeLa) cells: relationship to free radicals and lipid peroxidation and its modulation by calmodulin antagonists. *Cancer Letters*, *63*(3), 189–198. https://doi.org/10.1016/0304-3835(92)90260-3

37. Sakamoto, Y., Takeuchi, A., Sato, T., Obara, K., Takai, K., Fujino, K., Hirose, T., Inagaki, Y. (2001). Identification of antihypertensive substance in an aqueous extract from fruit body of *Mycoleptodonoides aitchisonii*. Oyo Yakuri/*Pharmacometrics*, *61*, 221–229.

38. Silva Figueiredo, P., Carla Inada, A., Marcelino, G., Maiara Lopes Cardozo, C., de Cássia Freitas, K., de Cássia Avellaneda Guimarães, R., Pereira de Castro, A., Aragão do Nascimento, V., Aiko Hiane, P. (2017). Fatty acids consumption: the role metabolic aspects involved in obesity and its associated disorders. *Nutrients*, *9*(10), art. no. 1158. https://doi.org/10.3390/nu9101158

39. Teichmann, A., Dutta, P.C., Staffas, A., Jägerstad, M. (2007). Sterol and vitamin D_2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. *LWT – Food Science and Technology*, *40*(5), 815–822. https://doi.org/10.1016/j.lwt.2006.04.003

40. Tucker, J.M., Townsend, D.M. (2005). Alpha-tocopherol: roles in prevention and therapy of human disease. *Biomedicine & Pharmacotherapy,* 59(7), 380–387. https://doi.org/10.1016/j.biopha.2005.06.005

41. Ušjak, L., Sofrenić, I., Tešević, V., Drobac, M., Niketić, M., Petrović, S. (2019). Fatty acids, sterols, and triterpenes of the fruits of 8 *Heracleum* taxa. *Natural Product Communications, 14*(6). https://doi.org/10.1177/1934578X19856788

42. Vamanu, E., Nita, S. (2014). Biological activity of fluidized bed ethanol extracts from several edible mushrooms. *Food Science and Biotechnology*, *23*(5) 1483–1490. https://doi.org/10.1007/s10068-014-0203-4

43. Vasdekis, E.P., Karkabounas, A., Giannakopoulos, I., Savvas, D., Lekka, M. (2018). Screening of mushrooms bioactivity: piceatannol was identified as a bioactive ingredient in the order *Cantharellales*. *European Food Research and Technology*, *244*, 861–871. https://doi.org/10.1007/s00217-017-3007-y

44. Venditti, A., Frezza, C., Sciubba, F., Serafini, M., Bianco, A. (2017). Primary and secondary metabolites of an European edible mushroom and its nutraceutical value: *Suillusbellinii* (Inzenga) Kuntze. *Natural Product Research, 31*(16), 1910–1919. https://doi.org/10.1080/14786419.2016.1267731

45. Wang, X.M., Zhang, J., Wu, L.H., Zhao, Y.L., Li, T., Li, J.Q., Wang, Y.Z., Liu, H.G. (2014). A mini-review of chemical composition and nutritional value of edible wild-grown mushroom from China. *Food Chemistry*, *151*, 279–285. https://doi.org/10.1016/j.foodchem.2013.11.062

46. Watanabe, F., Schwarz, J., Takenaka, S., Miyamoto, E., Ohishi, N., Nelle, E., Hochstrasser, R., Yabuta, Y. (2012). Characterization of vitamin B12 compounds in the wild edible mushrooms black trumpet (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*). *Journal of Nutritional Science and Vitaminology*, *58*(6), 438–441. https://doi.org/10.3177/jnsv.58.438

47. Wheeler, S., Jordan, P. (2007). A Practical Encyclopedia of Mushrooms (Praktična enciklopedija pečuraka). JRJ: Zemun-Belgrade, Serbia, p. 54.

48. Williams, B., Mancia, G., Spiering, W., Rosei, E.A., Azizi, M., Burnier, M., Clement, D.L., Coca, A., De Simone, G., Dominiczak, A., Kahan, T., Mahfoud, F., Redon, J., Ruilope, L., Zanchetti, A., Kerins, M., Kjeldsen, S.E., Kreutz, R., Laurent, S., Lip, G.Y.H., McManus, R., Narkiewicz, K., Ruschitzka, F., Schmieder, R.E., Shlyakhto, E., Tsioufis, C., Aboyans, V., Desormais, I. (2018). ESC Scientific Document Group. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). *European Heart Journal*, *39*(33), 3021–3104. https://doi.org/10.1093/eurheartj/ehy339

49. World Health Organization Cardiovascular diseases factsheet, 11 June 2021. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) [last visited 20.5.2022].

50. Wu, Q., Li, Y., Peng, K., Wang, X. L., Ding, Z., Liu, L., Xu, P., Liu, G.Q. (2019). Isolation and characterization of three anti-hypertension peptides from the mycelia of *Ganoderma lucidum* (Agaricomycetes). *Journal of Agricultural and Food Chemistry*, *67*(29), 8149–8159. https://doi.org/10.1021/acs.jafc.9b02276

51. Yamaguchi, S., Yoshikawa, T., Iikeda, S., Ninomiya, T. (1971). Measurement of the relative taste intensity of some L-amino acids and 5'-nucleotides. *Journal of Food Science*, *36*(6) 846–849. https://doi.org/10.1111/j.1365-2621.1971.tb15541.x

52. Zhao, H., Zhang, H., Yang, S. (2014). Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. *Food Science and Human Wellness,* 3(3–4), 183–190. https://doi.org/10.1016/j.fshw.2014.12.005