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St. George's mushroom, *Calocybe gambosa* (Fr.) Donk: A promising source of nutrients and biologically active compounds

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ABSTRACT

The composition of biologically active compounds of *Calocybe gambosa* (Lyophyllaceae) was analysed, and the antioxidant and cytotoxic activities were tested *in vitro*. *C. gambosa* was low in energy, fat, and carbohydrates, but rich in proteins and fibres. The total polyphenol content in the extracts was low (23.08 ± 0.67 in aqueous and 24.99 ± 4.25 μg GA mg^{-1} of extract in methanolic extract). The methanolic extract showed anti-DPPH radical activity with an IC_{50} of 626.10 ± 25.20 μg mL^{-1}. The sample of *C. gambosa* is rich in nucleotides and amino acids responsible for its pleasant taste. The nucleoside and 5′-monophosphates contents were 0.97 mg g^{-1} and 2.32 mg g^{-1} of dry mushroom, respectively. The contents of essential and non-essential amino acids were 18.41 ± 0.06 and 41.75 ± 0.30 mg g^{-1} d.w., respectively. The percentages of saturated and unsaturated fatty acids were 42.6% and 57.4%, respectively. The most abundant water-soluble and fat-soluble vitamins were B1 and E, respectively. Cytotoxic effect of the

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extracts was examined against different cancer cell lines, and the best cytotoxicity was showed by the peptide extract against colon cancer cell line LS174.

KEYWORDS

***Calocybe gambosa*, amino acids, nucleotides, *DPPH*, total polyphenols, cytotoxicity**

1. INTRODUCTION

Calocybe gambosa (Fr.) Donk (Lyophyllaceae) is an edible mycorrhizal fungus widespread throughout Europe growing from the end of April to July, often in circles. It forms a white or cream mat cap, distinctly fleshy with a characteristic odour of flour and cucumber (Flück, 2002). Phenolic compounds, flavonoids, ascorbic acid, β-carotene, tocopherols (α, β, and γ), and lycopene, as well as fatty acids, amino acids, and 5′-nucleotides have been identified in previous studies (Barros et al., 2008; Beluhan and Ranogajec, 2011; Vaz et al., 2011). Numerous volatile compounds contribute to its pleasant, *umami* taste (Kleofas et al., 2015). Several tribes in India used this mushroom to increase immunity (Vishwakarma et al., 2016). In Catalonia, it is used because of its antidiarrhoeal and intestinal antiseptic effect (Agelet and Vallès, 2003). *C. gambosa* possesses antimicrobial (Keller et al., 2002) and antioxidant activity (Palacios et al., 2011; Vaz et al., 2011).

The available data about this mushroom species is very rare concerning its biological activity and chemical composition, especially from the selected locality, which is rich in mushroom species, but unexplored. The aim of the study was to determine the antiradical and cytotoxic activities of *C. gambosa* extracts and peptide fraction, which has not been studied before, and the chemical composition that includes the content of total polyphenols, amino acids, nucleotides, fatty acids, and vitamins.

2. MATERIALS AND METHODS

2.1. Fungal material, extracts, and crude peptide

The fruiting bodies of *C. gambosa* were collected on the meadows around the village Odžak (44°09′28``N 16°48′31``E) in Glamoč, Bosnia and Herzegovina, in May 28, 2016, and deposited at the Faculty of Pharmacy, Department of Pharmacognosy (No. 31). The mushrooms were fleshy in mature stage, with thick and solid stalk. Before extraction, the fruiting bodies were cleaned from earth, washed, cut into stripes and dried at room temperature, pulverised in a laboratory mill, and stored at 4 °C.

Methanolic (2.71 g) and cyclohexane (0.0549 g) extracts were prepared by shaking the powdered mushroom (26.70 g) for 2 days at room temperature with a ratio of 1:10. Aqueous extract (5.61 g) was prepared with 13.30 g of dried, powdered mushroom mixed with 150 mL of distilled water, heated in a water bath for 30 min at 100 °C with occasional shaking. Extracts were further used to determine vitamins, total polyphenol content, antioxidant and cytotoxic activity of the mushroom, while dry and powdered fruiting bodies were used for the analysis of

fatty, amino, and nucleic acids, as well as for determination of nutritional value, total fibre, and β-glucans.

Partially-purified peptide was prepared according to Prateep et al. (2017). Dried fruiting bodies were homogenised in tris-HCl buffer (pH 7.4) (3 mL g^{-1}) and centrifuged for 30 min at 12,000 *g* at 4 °C. Solid $(NH_4)_2SO_4$ was slowly added to the clear supernatant to reach saturation of 40–80% at 4 °C. The protein pellet was collected after 1 h by centrifuging at 12,000 *g*, 4 °C for 30 min. Crude peptide extract was kept at –20 °C until analysis. Chromatographic conditions for the analysis of vitamins, amino acids, 5′- nucleotides, and nucleosides are presented in Table 1.

2.2. Determination of nutritional value, total fibre, and β-glucans

The sample of dried, powdered mushroom was analysed for the percentage of moisture, crude proteins, crude fats, carbohydrates, and crude ash. Nutritional value was determined by using

Table 1. Chromatographic conditions for the analysis of vitamins, 5′- nucleotides and nucleosides, and amino acids

Conditions	Water soluble vitamins*	Fat-soluble vitamins*	5′- Nucleotides and nucleosides*	Amino acids*
Apparatus	HPLC-DAD (Agilent 1200 series)		HPLC (Agilent 1100 series), DAD (G1315B)	LC-MS-ESI system (Agilent 1260/6130)
Column	Zorbax Eclipse XDB-C18		Zorbax Eclipse XDB-C18,	Zorbax SB-Aq
(25 °C)	4.6 × 150 mm, 5 µm		4.6 × 250 mm, 5 µm	3.0 × 150 mm, 3.5 µm
Mobile phase	Phosphate buffer, pH 2.8: Acetonitrile (90:10 v/v)	Acetonitrile (90:10 v/v), pH 2.8: Acetonitrile	A: 0.1 M KH_2PO_4, 4 mM tetrabutylammonium bromide, pH 6 B: A: methanol (70:30, v/v), pH 7.2	A: 0.1% ammonium formate in water B: 0.1% formic acid in acetonitrile
Flow rate	1 mL min^{-1}	0.5 mL min^{-1}	1.2 mL min^{-1}	0.3 mL min^{-1}
Injection volume	5 µL	10 µL	20 µL	3 µL
DAD	254 nm (C, B_1, B_3); 268 nm (B_2); 291 nm (B_6).	326 nm (A); 266 nm (D_3); 210 nm (E).	254 nm	436 nm
Elution gradient	Isocratic, for 10 min	1% B until 4.5 min, 90% B until 6 min	5% B until 9 min, 25% B at 15 min, 90% B at 17.5 min, 100% B at 19 min and 5% B, for 24 min	15% B until 3 min, 85% B at 63 min and 15% B, for 74 min

*The identification of individual amino acids was performed by comparing the retention times (Rt) and mass spectra of the sample and corresponding standards, or UV spectra in the case of vitamins, 5′- nucleotides and nucleosides. Quantification was performed based on peak areas using baseline construction method.

the procedures described by the international standards (AOAC). The moisture and crude ash content was estimated gravimetrically (AOAC Method 930.04 and AOAC Method 930.05, respectively), the crude protein by the Kjeldahl method (AOAC Method 977.02), and the crude fats by Soxhlet extraction (AOAC Method 930.09). Total fibre was determined by enzymatic-gravimetric method (AOAC Method 991.43), described by Prosky et al. (1992), using total dietary fibre assay kit K-TDFR (Megazyme Int., Wicklow, Ireland), while the content of β-glucans was determined according to the instruction manual of the mushroom and yeast beta-glucan assay kit K-YBGL (Megazyme Int., Wicklow, Ireland).

2.3. Fatty acids analysis

The fatty acid composition of dry mushroom was determined by gas chromatography after fatty acid glycerides were converted into methyl esters (FAMEs) by esterification from 1 g of powdered fruiting bodies. FAMEs were identified by comparing retention times with a reference mixture of FAMEs (Supelco 37 component FAME mix) obtained from Supelco (Bellefonte, PA, USA). The chromatographic conditions were previously described in details by Kolundžić et al. (2017).

2.4. Analysis of vitamins

The water-soluble vitamins (C, B1, B2, B3, and B6) contents were determined in aqueous and methanolic extracts, while the contents of fat-soluble vitamins (A, D3, and E) were determined in cyclohexane extracts. Standard solutions for calibration curve were prepared with a range from 1 to 100 mg L^{-1}, except for vitamin E with a range of 5–200 mg L^{-1}. For vitamin analysis, the concentrations of methanolic and aqueous extracts were 20 mg $mL^{-1,}$ and for cyclohexane extracts 10 mg mL^{-1}.

2.5. Analysis of 5′- nucleotides and nucleosides

The contents of 5′- nucleotides and nucleosides in the sample were determined by the modified method of Beluhan and Ranogajec (2011). The suspension made of 500 mg of dried homogenised mushroom and 10 mL of deionised water was heated in a water bath for 5 min, then stirred with a Vortex stirrer for 30 s, cooled to room temperature, and filtered through a 0.45 µm diameter filter before analysis. 5′-AMP-sodium, 5′-CMP-sodium, 5′-UMP-disodium, 5′-GMP-sodium, adenosine, cytidine, uridine, and guanosine were used as the standard substances (Sigma-Aldrich, St. Louis, MO, USA). A series of double dilutions of standard solutions (0.0156–0.5 mg mL^{-1}) were prepared for the construction of the calibration curves.

2.6. Amino acids analysis

Amino acid standard solution (Sigma-Aldrich, St. Louis, MO, USA) was used for the preparation of initial standard solutions and dilutions (0.0678–250 µmol L^{-1}). The derivatisation of the standards and the test sample was performed according to Ribeiro et al. (2008) and the analysis was performed using LC-MS. For the analysis of amino acids the same amount, 500 mg in 10 mL of water, was used. The procedure for sample preparation was the same as in section 2.5. The identification of individual amino acids was performed by comparing the retention times (Rt) and mass spectra of the sample and corresponding standards. The amino acid contents were calculated based on the peak area using baseline construction method.

2.7. Total phenolics content and DPPH radical scavenging activity

The content of polyphenols in aqueous and methanolic extracts were expressed as % of the standard substance (gallic acid, GA), while the percentage of inhibition of DPPH (2,2 diphenyl-1-picrylhydrazyl) radical of aqueous and methanolic extracts was expressed as IC_{50} value, determined according to Kolundžić et al. (2017).

2.8. Cytotoxicity assay

The cytotoxic effect of examined extracts was determined using MTT test as described previously (Kolundžić et al., 2017). Human epithelial cervix adenocarcinoma cells (HeLa), human epithelial lung carcinoma cells (A549), human colon adenocarcinoma cells (LS174), and human normal lung fibroblasts (MRC-5) were obtained from the American Type Culture Collection (Manassas, VA, USA). Selected cells were exposed to aqueous, methanolic, and crude peptide extracts in series of different concentrations (12.5–200 $\mu g\,mL^{-1}$). IC_{50} concentration is the concentration of an agent inhibiting cell survival by 50%, compared to a vehicle-treated control. Cisplatin (cis-DDP) was used as positive control.

2.9. Data analysis

All measurements were done in triplicate. Values were expressed as mean ± SD. Data analyses were performed using the EXCEL and SPSS software packages.

3. RESULTS AND DISCUSSION

3.1. Nutritional value, dietary fibre, and β-glucans

The nutritive analysis of *C. gambosa* has shown high content of crude proteins (33.95 g/100 g d.w.) and total fibre (23.39 g/100 g d.w.), and low content of crude fat (1.54 g/100 g d.w.) (Table 2). Comparing with previous studies conducted on samples obtained from India and

Table 2. Nutritional value of dried mushroom *Calocybe gambosa*, expressed in g/100 g d.w. as mean ± SD

Parameter	*C. gambosa*
Moisture	7.34 ± 0.11
Crude ash	8.44 ± 0.10
Crude fat	1.54 ± 0.15
Crude proteins as Nx4.38	33.95 ± 0.17
Carbohydrates	25.34 ± 1.85
Total fibre	23.39 ± 1.32
Insoluble fibre	18.83 ± 0.90
Soluble fibre	4.56 ± 0.43
β-glucan	5.04 ± 0.15
Energy (kcal/100 g)	297.80 ± 12.07
Energy (kJ/100 g)	1252.03 ± 50.45

Data are presented as the mean of three independent determinations.
d.w.: dry weight.

Portugal, our sample have shown higher contents of proteins (20.22 and 15.46 g/100 g d.w., respectively), but lower contents of carbohydrates (65.61 and 69.83 g/100 g d.w., respectively) (Vaz et al., 2011; Mridu and Atri, 2017). The content of β-glucan was also low (5.04 g/100 g d.w.). In previous studies, three polysaccharides of glucan-like structure were isolated from *C. gambosa,* but with different, linear β (1→6) and side β (1→4), linkages than in β-glucans (Villares, 2013).

3.2. Fatty acids analysis

The amounts of saturated (42.60%) and unsaturated (57.40%) fatty acids were determined from the fruiting bodies of the mushroom (Table 3). Polyunsaturated essential fatty acids, including ω-6, linoleic (40.50%) and ω-3, linolenic acid (5.90%) were identified. Among saturated fatty acids, palmitic acid was present in the highest percent (29.30%). The results obtained in this paper are consistent with the previously obtained results concerning the identified major fatty acids, but with different quantities. The oleic and linoleic acids were present in slightly lower amounts in our samples, while the content of other identified fatty acids was higher. Also, the content of MUFA (monosaturated fatty acids) (Bragança: 33.38%; commercial sample: 19.05%) and PUFA (polyunsaturated fatty acids) (commercial sample: 58.42%) was higher, and SFU (saturated fatty acids) (Bragança: 21.54%; commercial sample: 22.51%) lower in the samples from Portugal than in our study (MUFA: 11.00%; PUFA: 46.40%; SFA: 42.60%) (Barros et al., 2008; Vaz et al., 2011). The calculated Σ PUFA/Σ SFA ratio was higher than 0.45 (1.09), which is the recommended ratio in human diet as being beneficial to prevention of some chronic diseases, such as cancer and cardiovascular diseases (Wołoszyn et al., 2020).

3.3. Analysis of vitamins

The results of the analysis of vitamins are presented in Table 3. The most abundant water-soluble vitamin was B1 (292.15 ± 4.62 mg/100 g in methanolic and 317.26 ± 6.15 mg/100 g in aqueous extracts), followed by vitamin B3 (206.64 ± 5.22 mg/100 g in methanolic and 178. 56 ± 8.51 mg/100 g in aqueous extracts) and B2 (77.82 ± 5.16 mg/100 g in methanolic and 76.51 ± 6.43 mg/100 g in aqueous extracts). Vitamin B6 and vitamin C were detected only in aqueous

Table 3. The content of vitamins (mg/100 g of extract), total polyphenols (μg GA mg^{-1} of extract), and anti-DPPH activity (μg mL^{-1})

Vitamin	CCC	CCM	CCA
B1 (mg/100 g)	–	292.15 ± 4.62	317.26 ± 6.15
B2 (mg/100 g)	–	77.82 ± 5.16	76.51 ± 6.43
B3 (mg/100 g)	–	206.64 ± 5.22	178.56 ± 8.51
B6 (mg/100 g)	–	–	24.77 ± 2.07
C (mg/100 g)	–	–	71.32 ± 3.94
E (mg/100 g)	4080.76 ± 65.98	–	–
D (mg/100 g)	469.23 ± 14.35	–	–
Total polyphenols (μg GA mg^{-1})	–	24.99 ± 4.25	23.08 ± 0.67
DPPH (μg L^{-1})	–	626.10 ± 25.20	1145.91 ± 88.60

Results are expressed in a dry weight basis.

extracts (24.77 ± 2.07 mg/100 g and 71.32 ± 3.94 mg/100 g, respectively). α-Tocopherol was found as the most abundant fat-soluble vitamin in cyclohexane extract (4080.76 ± 65.98 mg/100 g). Considerable amount of vitamin D has been found in cyclohexane extracts (469.23 ± 14.35 mg/100 g extract). In general, cultivated mushrooms are considered as a good source of vitamin B2, B3, and folates. Vitamin C and B1 were detected in lower concentrations and vitamin B12 and D were found in traces (Gupta et al., 2019). Nevertheless, some wild mushroom (*Cantharellus cibarius* and *C. tubaeformis*) showed significantly higher contents of vitamin D compared to cultivated *Agaricus bisporus*, and recently, it is concluded that mushrooms produce high amounts of vitamin D after exposition to UV light (Mattila et al., 1994; Rathore et al., 2017). Rathore et al. (2020) concluded that higher levels of vitamin D were detected in *Calocybe indica* samples exposed to UVB light (14.06 mg/100 g d.w.) than in the samples exposed to sunlight (7.83 mg/100 g d.w.), but these were far less amounts of vitamin D than in our sample.

Additionally, in this paper higher contents of vitamin C and vitamin E, namely 1.03 and 0.4 mg/100 g; and 280 mg/100 g and 80 mg/100 g, respectively, in *C. gambosa* extracts were detected in comparison to previously conducted studies on fresh and dry samples of *C. indica*, while vitamin A was detected in concentration of 0.35 mg g^{-1} (fresh sample) and 0.275 mg g^{-1} (dry sample) by Subbiath and Balan (2015), unlike our study, where vitamin A was not detected.

Also, contents of vitamin B complex were higher in both aqueous (597.1 mg/100 g) and methanolic (576.61 mg/100 g) extracts compared to extracts of *C. indica* previously obtained after EDTA/TCA extraction (195 mg/100 g) (Sumathy et al., 2015).

3.4. Amino acids analysis

The total amino acids content was 60.16 mg g^{-1} d.w., while major amino acids were aspartic acid (12.50 mg g^{-1} d.w.), glutamic acid (8.78 mg g^{-1} d.w.), and arginine (6.38 mg g^{-1} d.w). The tested *C. gambosa* was extremely rich in essential amino acids with their total contents 18.41 ± 0.06 mg g^{-1} d.w. It also contained significant amounts of non-essential amino acids (alanine, arginine, glycine, glutamic acid, aspartic acid, proline, serine, and tyrosine), and their total contents were 41.75 ± 0.30 mg g^{-1} d.w. The amino acid cysteine was not detected (Table 4).

Amino acids in edible mushrooms are divided into four groups based on their taste, monosodium glutamate-like (MSG-like), sweet, bitter, and tasteless. Taste active amino acids in mushrooms are sweet (alanine, glycine, serine, and threonine) and MSG-like amino acids (aspartic and glutamic acids). Their contents in our samples were high, 14.04 and 21.25 mg g^{-1} d.w., respectively. Although the content of the bitter amino acids were found to be 12.05 mg g^{-1} d.w., they are considered as taste inactive, and their taste can be suppressed by sweet amino acids and sugars in mushrooms (Mau, 2005). Previously conducted study showed similar results with MSG-like and sweet taste amino acids as most abundant. Croatian samples of *C. gambosa* showed lower levels of total amino acids content (50.78 mg g^{-1} d.w.) with main amino acids being glutamic acid, threonine, and alanine. Differently, cystine was detected, while isoleucine not (Beluhan and Ranogajec, 2011).

3.5. Nucleotide analysis

The results of the analysis of the composition of 5'-nucleosides and nucleotides, as well as their quantities are given in Table 4. The amount of total nucleosides was 0.97 mg g^{-1} d.w., and their monophosphates 2.32 mg g^{-1} d.w. Based on the amount of 5'-nucleotides present, the tested

Table 4. Composition of fatty acids (%), amino acids (mg g^{-1} d.w.), nucleosides and nucleotides (mg g^{-1} d.w.) in *C. gambosa*

Fatty acid		Amino acid		Nucleotides and nucleosides	
	Content (%)		Content (mg g^{-1} d.w.)		Content (mg g^{-1} d.w.)
C16:0	29.30	Asp	12.50 ± 0.18	Adenosine	Not detected
C18:0	10.80	Glu	8.78 ± 0.05	Guanosine	0.01 ± 0.00
C18:1n9	11.00	Ala	5.92 ± 0.04	Cytidine	0.38 ± 0.00
C18:2n6	40.50	Gly	0.82 ± 0.01	Uridine	0.58 ± 0.00
C20:0	1.20	Ser	5.45 ± 0.01	5'- AMP	0.44 ± 0.00
C18:3n3	5.90	Thr	1.85 ± 0.00	5'- GMP	1.06 ± 0.00
C22:0	1.30	Arg	6.38 ± 0.13	5'- CMP	0.34 ± 0.00
SFA	42.60	His	1.08 ± 0.04	5'- UMP	0.46 ± 0.00
MUFA	11.00	Ile/Leu	1.45 ± 0.02		
PUFA	46.40	Met	0.11 ± 0.01		
UFA	57.40	Phe	1.59 ± 0.07		
PUFA/SFA	1.09	Val	1.44 ± 0.07		
		Lys	10.88 ± 0.03		
		Tyr	1.00 ± 0.01		
		Pro	0.91 ± 0.09		

Results are expressed in a dry weight basis.

mushroom is classified in the mushrooms with middle level of nucleotides (1–5 mg g^{-1}) (Yang et al., 2001).

In addition to MSG-like amino acids, some 5′-nucleotides (5′-GMP, 5′-AMP, 5′-IMP, and 5′-XMP) are responsible for the pleasant, *umami* taste of mushrooms. This property is mostly attributed to 5′-GMP, which was present in the sample in the amount of 1.06 mg g^{-1} d.w. This was higher content compared to previous results obtained in Croatia, in which the amount of GMP was 0.60 mg g^{-1} d.w. of mushroom. The amounts of uridine (0.30 mg g^{-1} d.w.) and cytidine (0.12 mg g^{-1} d.w.) in the tested sample were found in slightly higher concentration compared to the results of Beluhan and Ranogajec (2011).

3.6. Total polyphenols and antiradical activity

Polyphenolic compounds show significant antioxidant activity, inhibiting the chain reaction of free radicals and inactivating metal ions. The following polyphenolic compounds have been identified in *C. gambosa* previously: caffeic, chlorogenic, ferulic, gallic, homogentisic, protocatechuic, *p*-hydroxybenzoic, coumaric, and cinnamic acids and myristicin (Palacios et al., 2011; Vaz et al., 2011). The contents of total polyphenols in the aqueous and methanolic extracts were 23.08 ± 0.67 and 24.99 ± 4.25 μg GA mg^{-1} extract, respectively.

Previously obtained results, in which the polyphenol content in the methanolic extract was about 2 mg GA g^{-1} extract, showed inhibition of lipid peroxidation. This property was mostly derived from homogentisic acid, which was predominantly present in the sample, among other polyphenolic compounds. The selectivity of extraction of polyphenolic compounds and the extraction method itself can affect extract yield and thus, the final result. Sugars, ascorbic acid, and amino acids (tyrosine and tryptophan) can be oxidised, disabling the reaction with Folin-

Ciocalteu reagent, and providing false positive results (Palacios et al., 2011). The DPPH test showed antiradical activity of the aqueous extract with IC_{50} 1145.91 ± 88.60 μg mL^{-1}, as well as of the methanolic extract with IC_{50} 626.10 ± 25.20 μg mL^{-1}. Demonstrated activity was weaker than previously tested standard antioxidants vitamin C (IC_{50} 3.8 μg mL^{-1}) and quercetin (IC_{50} 2.75 μg mL^{-1}), but higher than the results obtained by other authors. Queirós et al. (2009) obtained lower anti-DPPH activity with the concentration of IC_{50} 7.14 mg mL^{-1}, while Vaz et al. (2011) had EC_{50} values of 34.60 mg mL^{-1} for ethanolic extract and of 7.06 mg mL^{-1} for water-soluble polysaccharidic fraction. Also, ethyl acetate extract of related species *C. indica* showed good antiradical activity with IC_{50} 70.12 ± 10.56 μg mL^{-1} and total polyphenols content (10.05 ± 2.45 mg GAE/g extract) (Ghosh et al., 2020). Similar to our results, *C. indica* aqueous extract at concentration 1000 μg mL^{-1} led to 47.06% of scavenging activity of free DPPH radicals, while the same concentration of extract caused 49.03 ± 0.2% and 49.4 ± 0.3% scavenging of NO_2 and H_2O_2, respectively (Bains and Tripathi, 2017).

Definitely, the method of extraction is crucial for the extraction of active ingredients with antioxidant activity, and concerning antioxidant activity, compounds with high molecular weight (e.g. polysaccharides) are less active than compounds with low molecular weights (e.g. phenolic compounds such as phenolcarboxylic acids, flavonoids, vitamin C).

3.7. Cytotoxicity

Recently, Rezvani et al. (2020) have shown that peptides from mushrooms were active against cancer cells, so beside the methanolic and aqueous extracts, which have shown good anti-radical scavenging activity, the peptide fraction was tested as well. Methanolic and aqueous extracts did not show cytotoxicity against either cancer or healthy cells (IC_{50} >200 μg mL^{-1}). Peptide extract was cytotoxic against colorectal cancer cells LS174 with an IC_{50} 168.87 ± 9.72 μg mL^{-1}, while against non-cancer MRC-5 cells showed weak cytotoxicity with IC_{50} 197.86 ± 3.70 μg mL^{-1} Vasdekis et al. (2018) in their research confirmed that methanolic extract of *C. gambosa* did not have cytotoxic effect with IC_{50} 1.6 mg mL^{-1} and 53.4% of cell death. Jose and Geetha (2019) confirmed that ethanolic extract of cultivated *C. gambosa* from India, at concentration of 200 μg mL^{-1}, decreased viability of HeLa cells to 60.9%, and showed anti-proliferative effect (26.75%) on healthy human Chang liver cells. Related species *C. indica* extracts had low cytotoxic or anticancer effect against different malignantly transformed cell lines (Ghosh et al., 2020).

4. CONCLUSIONS

Studied *C. gambosa* is very rich in nutrients, especially proteins, with the dominant essential fatty acids linoleic acid, and lysine as the most abundant essential amino acid. It is also rich in MSG-like amino acids (aspartic and glutamic acid) and GMP, which contribute to the taste of this mushroom and its wide use in the diet. The contents of total polyphenols, compounds with antiradical activity, in both aqueous and methanolic extracts were high without anti-proliferative effect against healthy cell line. Cyclohexane is a good solvent for the extraction of vitamin D3, while methanol and water were good solvents for the extraction of antioxidants. Especially interesting results were obtained for peptide fraction and cytotoxicity, which should be subjected for further purification and isolation of active peptides from St. George's mushroom.

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