JMF-2016-0176-ver9-Kolundzic_1P
Type: research-article

JOURNAL OF MEDICINAL FOOD
J Med Food 00 (0) 2017, 1–7

DOI: 10.1089/jmf.2016.0176

FULL COMMUNICATION

Cytotoxic and Antimicrobial Activities of *Cantharellus cibarius* Fr. (Cantarellaceae)

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ABSTRACT Antibacterial and cytotoxic activities of cyclohexane, dichloromethane, methanol, and aqueous extracts of *Cantharellus cibarius* were tested. Broth microdilution assay was performed against 10 bacterial strains (*Staphylococcus aureus, S. epidermidis, Micrococcus luteus, Bacillus subtilis, Enterococcus feacalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella abony*), with emphasis on *Helicobacter pylori*. Methanol extract was the most active against *H. pylori* strains with minimal inhibitory concentration values between 4 and 32 μg/mL. All extracts were active against antibiotic resistant *H. pylori*. Methanol and aqueous extracts had no cytotoxicity against tested cell lines, whereas cyclohexane and dichloromethane extracts were active against HeLa and N87 cells, but also against healthy MRC-5 cells (IC_{50} 39.26 ± 1.24–134.79 ± 0.01 μg/mL). The tested aqueous extracts have shown 68% of angiotensin-converting enzyme inhibitory activity in doses of 1.25 mg/mL. Chemical analysis has shown the presence of linoleic, *cis*-vaccenic, and oleic acids, sterols, β-glucans, and polyphenolic compounds.

KEYWORDS: • *antibacterial activity* • *ACE inhibitory activity* • *β-glucan* • *Cantharellus cibarius* • *cytotoxicity.*

INTRODUCTION

GROWING RESEARCH INTEREST in mushrooms have shown that edible mushrooms are not interesting only as tasty food rich in the important nutrients, but also as a source of primary and secondary metabolites with various biological activities.[1] The golden chanterelle, *Cantharellus cibarius* Fr. (Cantarellaceae), is an edible mushroom, yellow to orange with fruity and mildly peppery taste. It grows in deciduous and coniferous forests, in groups. Previous studies revealed nutrients and nutraceutical composition like phenols including flavonoids and phenolic acids, vitamins, volatile compounds, indols, sterols, and minerals.[2–8] Studied biological activities were antimicrobial, antioxidative, cytotoxic, and anti-inflammatory.[2,9–12]

This research covered different biological activities of *C. cibarius* extracts with an accent on anti-*Helicobacter pylori* activity and cytotoxicity against N87 gastric carcinoma cells. *H. pylori* is associated in the development of chronic gastritis and peptic ulcer disease, and is primarily involved in the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[13] Also, as the risk for cardiovascular diseases grows in our days, the natural products that inhibit angiotensin-converting enzyme (ACE) could be very important in preventing high blood pressure. In this context, one part of this work was *in vitro* testing of ACE inhibitory activity. In addition, nutritional value, β-glucan, and polyphenols content as well as mineral composition were determined.

MATERIAL AND METHODS

Reagents and chemicals

All organic solvents were HPLC grade and purchased from J.T. Baker (Deventer, The Netherlands). Coomassie Brilliant Blue G-250 was from Bio-Rad (Richmond, CA, USA) and mushroom and yeast beta-glucan assay kit was from Megazyme International Ireland; Mueller-Hinton broth, antibiotic supplement, was from Columbia Blood Agar Base (Difco, USA); Wilkins Chalgren broth with 10% horse serum was from Seromed, Biochrom KG, Berlin, Germany; glycerol was from Merck, USA; yeast extract was

Manuscript received 8 December 2016. Revision accepted 26 March 2017.

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from Difco, USA; RPMI-1640 medium was from Sigma, St. Louis, MO, USA; 10% heat-inactivated fetal bovine serum (FBS) was from GIBCO, Thermo Fisher Scientific, Life Technologies, USA; and ACE Kit-WST was from Dojindo Laboratories, Japan. All other reagents were from Sigma.

Mushroom material

Fruiting bodies of mushroom *C. cibarius* were collected in the locality of Soko Banja (Serbia) in July 2013. Before extraction, the mushrooms were dried at room temperature and pulverized in a laboratory mill. A voucher specimen has been deposited at the Faculty of Pharmacy, Department of Pharmacognosy (No. 24).

Preparation of extracts

Dried and powdered fruiting bodies of *C. cibarius* were extracted with solvents of different polarity to obtain cyclohexane (CCC), dichloromethane (CCD), methanol (CCM), and aqueous (CCA) extracts. A total of 600 mL of cyclohexane were added in 120 g of powdered mushroom and extraction was carried out for 2 days including shaking at room temperature. The procedure was repeated with dichloromethane and methanol. Aqueous extract was prepared with 19 g of dried, powdered mushroom and 190 mL of distilled water. Extraction was carried out under heating in a water bath, 30 min at 100°C with occasional shaking. After filtration, solvents were evaporated under low pressure and stored at 4°C.

Determination of nutritional value

The sample of dried material was analyzed for the percentage of moisture, proteins, fats, carbohydrates, and ash. All values were calculated on a dry weight (d.w.) of mushroom. Nutritional value was determined by using the procedures described by the Association of Official Analytical Chemists.[14] The moisture and ash contents were estimated using gravimetric methods after drying on 105°C and incineration at 550°C, respectively. The crude protein content by the Kjeldahl method and the nitrogen factor used for protein calculation was 6.25, whereas the crude fats were determined using the extraction procedure on Soxhlet apparatus, using petroleum ether as a solvent, after treatment with HCl.

Dietary fiber was determined by the gravimetric method.[15] Total carbohydrates were calculated as the residual difference after subtracting protein, ash, moisture, total fiber, and crude fat content. Total energy was determined by the calculation of energy values of carbohydrate, fat, protein, and fiber. Energy, kcal = 9 (crude fat content, g) +4 (protein content, g + carbohydrate content, g) +2 (fiber content, g).

Determination of β-glucans

Enzymatic assay kit K-YBGL was used to determine content of β-glucans in dried samples, cooked samples, and in extracts. The assay was performed according to the instruction manual of the kit producer. The content of β-glucans was calculated indirectly from the difference in total glucans and α-glucans content. All glucans were hydrolyzed into their glucose monomers and measured spectrophotometrically (510 nm).

Determination of total dietary fiber

Total fibers were determined by the enzymatic-gravimetric method.[15] The method requires phosphate buffer, pH 6.0, and the following enzymes: heat stable α-amylase, protease, and amyloglucosidase. Heat-stable α-amylase depolymerizes starch, protease depolymerizes and dissolves proteins, whereas amyloglucosidase converts starch into glucose. One duplicate was analyzed for protein and the other was incubated at 525°C to determine ash. The total dietary fiber is the weight of the filtered and dried residue less the weight of the protein and ash.

Fatty acids composition

To study the chemical composition of prepared extracts, fatty acids and sterol composition of CCC were studied using GC-MS. Saponification was achieved by adding 50% KOH at 90°C for 60 min. The unsaponifiable fraction was separated using petrol ether and subjected to GC and GC-MS analysis. Fatty acids were esterified using 98% H_2SO_4/MeOH and to obtain fatty acid methyl esters (FAMEs). ◀AU9

The GC and GC-MS analyses were performed on an Agilent 6890N Gas Chromatograph equipped with a split/splitless injector (260°C), an FID detector and a capillary column (Agilent J&W HP-88, 100 m × 0.25 mm, 0.20 μm film thickness), and coupled with an Agilent 5975C MS Detector operating in the EI mode at 70 eV. The carrier gas was He, flow rate 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 MSD 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 (HPLC purity). All experiments were done in duplicate. ◀AU10 ◀AU11

The identification of the FAME was based on the comparison of their retention times (Rt) and mass spectra with those of the representative standards run under the same chromatographic conditions (Supelco™ 37 Component FAME Mix) and with those from the NIST/NBS 05 and Wiley (8th edition) libraries, and the literature.[16,17] Relative percentages of the compounds were calculated based on the peak areas from the FID data.

Sterols

GC analysis of the CCC extract was performed on an Agilent 6890N GC system equipped with 5975 MSD and FID, using a HP-5 MS column (30 m × 0.25 mm × 0.25 μm). Injection volume was 1 μL and injector temperature 280°C with a 10:1 split ratio. Helium was a carrier gas (1.0 mL/min; constant flow mode). Column temperature was linearly

programmed in the range 60–280°C at a rate of 3°C/min and held at 280°C for 20 min. The transfer line was heated at 250°C. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in the *m/z* range 35–550. The identification of the compounds was based on the comparison of mass spectra from databases and the literature.

Determination of macro- and microelements

Preparation of the sample for determining heavy metal concentration was carried out by wet digestion using nitric acid. The weighed mass of dry sample (0.01 g) was topped with 1 mL of concentrated nitric acid and allowed to stand for 24 h. Thereafter, the mixture was heated at acid-boiling temperature until brown vapors of nitrogen oxide stopped forming. After digestion, the sample was filtered and the filtrate was transferred to a volumetric flask and filled with distilled water up to 10 mL.

All measurements were done on ICP-OES (*Inductively Coupled Plasma - Optical Emission Spectrometry*, ARCOS FHE12, SPECTRO, Germany) according to the instruction for the quantitative analysis given by the manufacturer in triplicate. Argon 5.0 (purity 99.999%) was used as a carrier gas. Except the sample for the analysis, the solution for the calibration curve construction was prepared using Multistandard IV—multielement standard solution, which contained Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Se, Tl, V, and Zn, and also specific standard solution of Si, As, P, Mo, and Sb in the concentration of 1000 ppm.

Total phenolic content

The content of total phenols in CCM and CCA extracts was determined spectrophotometrically.[18] Absorbance was measured at 725 nm. Series of gallic acid (GA) dilutions (1–10 mg/mL) were made, the absorbance was measured and the calibration curve was constructed. The content of polyphenols was expressed as mass (mg) of the standard substance GA per mass (g) of extract.

AU12 ▶ *DPPH test*

The ability of the methanol and aqueous extracts to scavenge free radicals was measured using the DPPH test.[18,19] The absorbance of the solution was measured at 517 nm and the percent of inhibition of DPPH radical was calculated. Each measurement was done in duplicate. The results are expressed as a concentration of the test sample that reduced 50% of the DPPH radicals (EC_{50} value).

ACE inhibitory activity

Natural products that block ACE have received attention for their use in preventing high blood pressure. The ACE Kit-WST was used for the determination of ACE inhibition activity. ACE inhibition assay kit enzymatically detects 3-hydroxybutyric acid (3HB), which is made from 3-hydryoxybutyryl-gly-gly-gly. The concentration ranged between 0.3125 and 5 mg/mL of aqueous extract was used and the procedure of the manufacturer was completely followed. The experiment was done in duplicate in the 96-wells microtiter plates. The absorbance of the solutions was measured on a microplate reader at 450 nm.

Antibacterial activity

For antimicrobial testing, CCC, CCD, CCM, and CCA extracts were used. Antimicrobial activity was evaluated using nine different laboratory strains of bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 9341), *Bacillus subtilis* (ATCC 6633), *Enterococcus feacalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCIMB 9111), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella abony* (ATCC 13076). The broth microdilution method was used to determine minimal inhibitory concentrations (MICs) of tested extracts according to Clinical and Laboratory Standards Institute.[20] Bacterial strains were suspended in Müller–Hinton broth to give a final density of 2.0×10^6 cfu/mL for bacteria. Samples of extracts were dissolved in 1% solution of dimethylsulfoxide (DMSO) in concentrations of 1.0 mg/mL. Serial dilutions of the stock solutions in broth medium were prepared in a microtiter plate (96 wells). The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth. All microbial tests were performed in duplicate and two positive growth controls were included. One percent solution of DMSO did not show an apparent effect on bacterial growth. The MICs of ampicillin and amikacin were determined in parallel experiments.

Antihelicobacter pylori activity

Ten clinical strains of *H. pylori*, isolated from patients with duodenal ulcer or gastritis, were tested with known resistance patterns. Three were resistant to clarithromycin (CLR) (MIC >1 μg/mL), one was resistant to metronidazole (MNZ) (MIC >16 μg/mL), two were resistant to both MNZ and CLR, and four were CLR and MNZ susceptible; *H. pylori* ATCC43504 was used as control in daily assay sessions. *H. pylori* bacteria were grown on Columbia agar base supplemented with 10% horse serum and 0.25% bacto yeast extract under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) in 85% humidity in an incubator at 37°C. Fresh plates were started from glycerol stocks and subcultured every 48 h.

MICs were determined by broth microdilution test performed using Mega-Cell™ RPMI-1640 medium containing 3% fetal calf serum, as previously described.[21] Twofold dilutions of tested antimicrobial extracts were prepared in 96-well microtiter plates. An inoculum equivalent to one McFarland standard was prepared in Wilkins Chalgren broth and diluted in MegaCell RPMI-1640 medium. Each well was inoculated with *H. pylori* at a final concentration of $\sim 5 \times 10^5$ cfu/well. The plates were incubated at 37°C under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2 in a

gas incubator). The plates were examined visually after 72 h of incubation.

Cytotoxicity assay

Human epithelial cervical cancer cells HeLa, NCI-N87 human gastric carcinoma cells, 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 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. Treatment of cell lines and determination of cell survival were done according to the procedure described by Kolundžić *et al.*[18] Stock solutions (30 mg/mL) of the extracts were made with DMSO and dissolved in corresponding medium to the required working concentrations. Final concentrations achieved in treated wells were 200, 100, 50, 25, and 12.5 μg/mL. The effects on cancer cells survival were determined by MTT test, 72 h upon addition of the extracts, as described previously.[22,23]

The number of viable cells in each well was proportional to the intensity of the absorbance of light (A), which was then read in an ELISA plate reader at 570 nm. IC_{50} concentration is defined as the concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. All experiments were done in triplicate. The *cis*-DDP was used as positive control.

RESULTS AND DISCUSSION

Nutritional value and β-glucans content

C. cibarius is edible mushroom rich in the nutrients with valuable biological activity. The nutritional parameters were the total carbohydrates 8.15 ± 1.52 g/100 g d.w., fats only 4.12 ± 0.15 g/100 g d.w., and proteins 13.28 ± 0.18 g/100 g d.w. Energy value was 237.04 ± 9.71 kcal (990.08 ± 40.59 kJ)/100 g of mushroom. The tested sample was very rich in total fibers (57.12 ± 0.78 g/100 g).

One of the fractions of dietary fibers is β-glucan fraction. Content of β-glucans in dried sample of *C. cibarius* was 14.90 ± 0.38 mg/g. The content of β-glucan decreased rapidly after thermal treatment, which is usual during the process of cooking for 80%. Amount of β-glucans in polar extracts was reduced, whereas their presence in nonpolar extracts was not detected (Table 1).

Two polysaccharides were isolated from the fruiting bodies of *C. cibarius*, one composed of a main chain consisting of α-(1 → 6)-Glcp units with β-(1 → 4)-linked branches every third glucose residue, and the second glucan-type carbohydrate composed of β-(1 → 3)-linked glucose units with branches at O-6 every third sugar residue.[24]

Polyphenolic content in CCM and CCA was 10.96 ± 0.30 mg GA/g and 9.98 ± 0.17 mg GA/g, respectively. The methanol extract exhibited moderate DPPH scavenging activity with an

Table 1. The Content of α-, β-, and Total Glucan in *Cantharellus cibarius* Samples

Samples	*α-Glucan*	*β-Glucan*	*Total glucan*
Dried	3.21 ± 0.06[a]	14.90 ± 0.38	18.11 ± 0.44
Cooked	0.23 ± 0.01	3.44 ± 0.07	3.67 ± 0.07
CCC[b]	n.d.[c]	n.d.	n.d.
CCD[b]	n.d.	n.d.	n.d.
CCM[b]	1.60 ± 0.10	5.38 ± 0.21	6.99 ± 0.12
CCA[b]	2.28 ± 0.08	5.90 ± 0.20	8.18 ± 0.12

[a]The results represent the mean ± standard deviation of the analysis performed in duplicate.

[b]CC, *Cantarellus cibarius* extracts (C, cyclohexane; D, dichloromethane; M, methanol; A, aqueous).

[c]n.d. *nondetected*.

EC_{50} = 689.64 ± 29.79 μg/mL, whereas aqueous extract had EC_{50} = 580.39 ± 7.02 μg/mL. Antioxidant activity of mushroom was not always correlated with total phenolics, which was shown by the other authors.[3]

High selenium was determined in our sample that could be important because of the role of selenoproteins in the antioxidant activity, and therefore, in cancer chemoprevention, heart disease, and immunity.[25] The content of Se was higher (63 μg/g d.w.) than in Boletus species from China and Portugal, which were known for selenium accumulation (10–48.5 mg kg d.w.). Anyway, the quantity of Se in the mushroom depends on the surface soil content.[26]

Fatty acids and sterols composition

The results of fatty acids analysis of CCC, the composition of total saturated fatty acids, monounsaturated fatty acids

Table 2. Fatty Acids Composition of *Cantharellus cibarius* Cyclohexane Extract

Fatty acids	*Percent*[a]
C14:0	0.17 ± 0.00
C15:0	0.19 ± 0.00
C16:0	10.78 ± 0.25
C16:1	0.93 ± 0.16
C17:0	0.16 ± 0.06
C 18:0	6.42 ± 0.16
C 18:1n9c	12.11 ± 0.20
C 18:1n11c	31.54 ± 0.46
C 18:2n6c	31.80 ± 0.50
C 20:0	0.60 ± 0.02
C 18:3n3	0.51 ± 0.04
C 20:1	0.85 ± 0.04
C 20:2	1.98 ± 0.03
C 22:0	0.96 ± 0.14
C 24:0	1.01 ± 0.08
SFA	20.29 ± 0.73
MUFA	45.43 ± 0.86
PUFA	34.28 ± 0.57

[a]The results represent the mean ± standard deviation of the analysis performed in duplicate.

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

AU19 ▶ TABLE 3. MINERAL COMPOSITION OF THE SAMPLE OF *CANTHARELLUS CIBARIUS*

	Mineral composition (μg/g)[a]										
C. cibarius	Al	As	Ba	Bi	Ca	Cu	Fe	Ge	In	K	Li
	396	12	86	24	92892	60	234	66	24	18168	5
	Mg	Mn	Mo	Na	P	Pb	Pt	Si	Sr	Zn	Se
	1426	41	26	2431	8095	2	406	799	12	94	63

[a]All measurements were done in triplicate.

(MUFA), and polyunsaturated fatty acids (PUFA) are presented in Table 2. CCC was rich in MUFA (45.43% ± 0.86%) with *cis*-vaccenic (18:1n11c) and oleic acid (C18:1n9c) as major fatty acids, followed by PUFA (33.77% ± 0.53%) with linoleic acid as the major fatty acid in CCC (31.80% ± 0.50%).

In continuation of lipid analysis, several ergosterol derivatives were identified in CCC using GC/MS: ergosta-5,7,9 (11),22-tetraen-3β-ol; 5,6-dihydroergosterol; ergosta-5,7-dien-3-ol; ergostenol; ergosta-4,6,8 (14),22-tetraen-3-one; ergosterol acetate, as well as lanosterol and euphorbol.

Micro- and macroelements

The mushrooms are a very good source of minerals, but also could be enriched with Se and Li.[27–29] The most abundant mineral in our sample of *C. cibarius* was Ca, followed by K and P, which are common macroelements in mushrooms.[26] Ag, B, Cd, Co, Cr, Ga, Ni, Sb, and Tl had not been detected. Lithium was detected in small amount, 5 μg/g. Considerable amount of Pb (2.0 μg/g d.w.) and As (12.0 μg/g d.w.) was detected and concentration of Pb was above maximal levels allowed by the European Commission (2001). The JECFA provisional tolerable daily intake for inorganic arsenic is 0.002 mg/kg b.w., equivalent to 0.12 mg/day for a 60 kg adult. The quantity of Se was high, 63 μg/g d.w., which was important concerning recommended daily allowance of 50 μg.

ACE inhibitory activity

Plasma renin is responsible for the conversion of angiotensinogen released by the liver into angiotensin I, which subsequently undergoes proteolytic cleavage. ACE catalyzes the removal of C-terminal histidylleucine from angiotensin I to form angiotensin II. The hormone angiotensin II is a vasoconstrictor and increases blood pressure. ACE also degrades bradykinin that has vasodilatation properties. Natural products that inhibit ACE could be very useful in managing high blood pressure. The tested aqueous extract has shown 68% of ACE inhibitory activity in doses of 1.25 mg/mL.

These benefits in hypertension could be connected with very favorable Na/K ratio. In patients with hypertension, lower sodium intake is recommended.[30] Na/K ratio for our sample was also very low, 0.13. Concerning the effects on blood pressure, the low concentration of sodium and the presence of a large amount of potassium (1.82 g/100 g) supports the utilization of mushroom within an antihypertensive diet.

Antibacterial activity

The results of antibacterial activity of tested extracts are shown in Table 4. Based on the results, MIC values were in range from 62.5 to 250 μg/mL. CCD was significantly active against *S. aureus* with an MIC of 62.5 μg/mL, but other extracts had moderate activity. Our extracts possessed very strong antibacterial activity against *H. pylori*. The results of anti-*H. pylori* activity are presented in Table 5. The values of MICs ranged from 4 to 256 μg/mL. In this case, CCM and CCA were more active than nonpolar extracts. CCM was very active with low MIC values from 4 to 32 μg/mL. MNZ-

TABLE 4. ANTIBACTERIAL ACTIVITY OF *CANTHARELLUS CIBARIUS* EXTRACTS (MINIMAL INHIBITORY CONCENTRATIONS, μg/mL)

	MIC (μg/mL)[a]					
Bacterial strains	*CCC*[b]	*CCD*[b]	*CCM*[b]	*CCA*[b]	*Ampicillin*	*Amikacin*
Staphylococcus aureus (ATCC 25923)	250	62.5	125	125	0.5	n.t.
S. epidermidis (ATCC 12228)	250	250	125	250	1.5	n.t.
Micrococcus luteus (ATCC 9341)	250	125	125	250	2.0	n.t.
Bacillus subtilis (ATCC 6633)	250	125	125	250	1.8	n.t.
Enterococcus feacalis (ATCC 29212)	250	125	125	250	n.t.	n.t.
Escherichia coli (ATCC 25922)	250	125	125	125	2.0	1.5
Klebsiella pneumoniae (NCIMB 9111)	125	125	125	125	2.8	2.0
Pseudomonas aeruginosa (ATCC 27853)	125	125	125	125	n.t.	2.5
Salmonella abony (ATCC 13076)	250	250	125	250	n.t.	n.t.

[b]CC, *Cantarellus cibarius* extracts (C, cyclohexane; D, dichloromethane; M, methanol; A, aqueous).

AU21 ▶ [c]All measurements were done in duplicate.

MIC, minimal inhibitory concentrations; n.t., not tested.

Table 5. Anti-*Helicobacter Pylori* Activity of *Cantharellus cibarius* Extracts (MIC, μg/mL)

Helicobacter pylori strains	*MIC (μg/mL)*[a]					
	CCC[b]	*CCD*[b]	*CCM*[b]	*CCA*[b]	*MNZ*[c]	*CLR*[d]
ATCC 43504	128	128	16	128	R	S
1.	64	128	4	64	S	R
2.	128	256	32	128	S	S
3.	64	64	4	64	S	S
4.	64	128	4	64	S	R
5.	128	256	4	128	R	S
6.	128	256	16	128	S	S
7.	64	256	4	64	S	R
8.	64	64	4	64	R	R
9.	256	256	32	256	R	R
10.	256	256	8	256	S	S

[a]All measurements were done in duplicate.
[b]CC, *C. cibarius* extracts (C, cyclohexane; D, dichloromethane; M, methanol; A, aqueous).
[c]MNZ, metronidazole.
[d]CLR, clarithromycin; S-sensitive; R-resistant.

and CLR-resistant strains of *H. pylori* (8 and 9) and CLR-resistant strains (1, 4, 7) were very sensitive to the methanol extract (MIC 4 μg/mL). Both MNZ- and CLR-resistant strains (8 and 9) were very sensitive to the methanol extract with an MIC value of 4–32 μg/mL. All extracts were active against MNZ- and CLR-resistant strain (8) with an MIC value of 4–64 μg/mL.

Mushrooms could have valuable role in the prevention or treatment of *H. pylori* infection because of the presence of both group of compounds: polyphenols and polysaccharides. Polysaccharides from algae and plants have anti-*H. pylori* activity because of their antiadhesive and preventing properties.[31] They prevent *H. pylori* from binding to porcine gastric mucin, but without direct bactericidal effect. Polyphenolic compounds could be active against *H. pylori* and other bacterial strains, but the presence of β-glucans in the methanol extract is very important.[32] For the moment, only one study considered anti-*H. pylori* activity of water-soluble melanin–glucan complex and showed antimicrobial effect identical to erythromycin and stronger than other tested antibiotics.[33] A very recent report suggested that some fatty acids had bactericidal activity *in vitro* against *H. pylori*, especially polyunsaturated fatty acids from which some were present in our extract (18:2n6c; 18:3n3; 20:2).[34]

Table 6. Cytotoxicity of *Cantharellus cibarius* Extracts (IC_{50}, μg/mL ± SD)

Compounds	*IC_{50} (μg/mL ± SD)*[a]		
	HeLa	*N87*	*MRC-5*
CCC[b]	57.40 ± 2.06	69.35 ± 0.43	86.98 ± 0.01
CCD[b]	39.26 ± 1.24	63.46 ± 0.01	134.79 ± 0.01
CCM[b]	>200	>200	>200
CCA[b]	>200	>200	>200
cis-DDP[c]	3.18 ± 0.29	—	—

[a]IC_{50} values are expressed as the mean ± standard deviation determined from the results of MTT assay in two independent experiments. The compounds were incubated with cells for 72 h.
[b]CC, *Cantarellus cibarius* extracts (C, cyclohexane; D, dichloromethane; M, methanol; A, aqueous).
[c]Used as a positive control.

Cytotoxic activity

All extracts were tested against two malignant and one healthy cell line. The results are presented in Table 6. On the contrary to anti-*H. pylori activity*, nonpolar extracts were more active against tested cell lines. CCD was significantly active against the tested HeLa and N87 cell lines with low IC_{50} between 39.26 ± 1.24 μg/mL and 63.46 ± 0.01 μg/mL, but also against healthy MRC-5. Similar activity exhibited CCC. Both CCM and CCA were not active against tested cancer cell lines (IC_{50} > 200 μg/mL), which could be important, especially in the context of antibacterial activity against *H. pylori*. In very low doses, methanol extract showed anti-*H. pylori* activity, which were not cytotoxic.

The effect against this Gram-negative bacteria could not be connected with antiproliferative effect against gastric carcinoma cells N87. Important findings came from Choi *et al.* (2012) concerning multidrug resistance reversal activity of methanol extracts from Basidiomycete mushrooms in cancer cells.[10] *C. cibarius* methanol extract had minor antiproliferative effect on cancer cells, but it could be very useful in reversal activities of anticancer drugs resistance in cancer cells. Also, ergosterol peroxide was the most active constituent of *Gomphus clavatus* against MCF-7 and PC-3 cells, suggesting that the cytotoxic activity could be at least partly attributed to the presence of ergostane derivatives, which were also detected in cyclohexane extract.[35]

SUMMARY

Important and promising anti-*H. pylori* activity, cytotoxicity against tested cancer cell lines, and ACE inhibitory activity of *C. cibarius* extracts would be subjected to further research of preventive role *in vivo* and to chemical structure determination of active compounds, especially in dichloromethane and methanol extracts.

ACKNOWLEDGMENT

The authors are grateful to the Ministry of Education, Science and Technological Development of Serbia for financial support (Grant No. 173021 and TR. 34012).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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