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Cellular Antioxidant and Antiproliferative Activities of *Morchella conica* Pers. Polyphenols *in vitro*

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Abstract This study analyzed the cellular antioxidant and antiproliferative activities, as well as the phenolic composition of three *Morchella conica* Pers. cultures. Results showed that the free phenolic contents of the three *Morchella conica* Pers. ranged from 4.928 to 6.157 mg GAE/g DW and their bound phenolic contents ranged from 0.188 to 0.250 mg GAE/g DW. Polyphenols in *M. conica* Pers. were dominated by phenolic acids, particularly for gallic acid. The free phenolic extracts exhibited higher cellular antioxidant activity than the bound phenolic extracts. Free phenolics in *M. conica* Pers. cultured from Yunnan China showed the highest antiproliferative activity against $HepG_2$ cells, whereas bound phenolics in *M. conica* cultured from Tibet China showed the highest antiproliferative activity. Results confirmed that *Morchella conica* Pers. (growing in Yunnan China especially) could be a new source of natural antioxidant and a potential inhibitor for the growth of $HepG_2$ cells.

Keywords: *Morchella conica Pers., polyphenols, antioxidation, antiproliferation*

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1. Introduction

Morchella conica Pers., belonging to the *Morchella* genus, is rich of nutrients such as proteins, polysaccharides, minerals, vitamins, amino acids, δ-carotene and ergosterol [1]. *Morchella conica* Pers. is also rich of bioactive compounds, such as polysaccharide, glutathione, etc [2,3,4]. Polysaccharide of *Morchella* importuna could significantly increase the viability of PC12 cells by enhancing the activity of antioxidant enzyme [5]. Studies also confirmed the antioxidant activity *in vivo* of *Morchella esculenta* polysaccharides activity [6].

Recently, a growing number of studies have indicated that wild fungus is a good source of phenolics [3,7]. The phenolic content of *M. conica* Pers. was 25.38 μg GAE (gallic acid equivalents)/mg extract [8]. Serbian *M. conica* Pers., compared with Portuguese *M. conica* Pers., had a higher level of phenolic compounds, which displayed a higher antioxidant capacity, while Serbian *M. conica* Pers. displayed a higher antibacterial capacity than Serbian *M. conica* Pers. [9]. Turkoglu *et al.* [10] found that the phenolic content of *M. conica* Pers. was 41.93 μg PE (pyrocatechol equivalents)/ mg extract, and the phenolic extract exhibited high DPPH· scavenging capacity, antioxidant capacity (β-carotene-linoleic acid system), and anti-inflammatory activity. The ethyl acetate extract of wild *Inonotus sanghuang* displayed potent antiproliferative, antioxidant, and antimicrobial activities [11]. The extracts of wild *Suillus bellinii* showed high reducing power, scavenging DPPH radicals capacity, and high antiproliferative activity against MCF7, NCI-H460 and HepG2 cells [12]. The aqueous-ethanolic extract of *M. esculenta* mycelium showed hepatoprotective activity *in vivo* [13]. Phenolic extracts of *Ganoderma lucidum* fruiting body even showed higher antioxidant capacity than its polysaccharidic extracts [14]. The researches mentioned above indicated that *Morchella conica* Pers. was also a potential source of antioxidative phenolic compounds.

Investigations on the genetic resources of *Morchella* reported that Yunnan, Tibet and Xinjiang were the major producing regions of wild *Morchella* in China [15,16,17]. It is necessary to screen the wild Mo*rchella conica* Pers. growing in Yunnan, Tibet and Xinjiang of China to provide the basic information serving for the breeding research.

To the best of our knowledge, studies on *Morchella* were mainly focused on its morphological and ultrastructural properties, genetics, and bioactive polysaccharides [3,18,19]. However, information on the composition, concentration, cellular antioxidant activity, and antiproliferative capacity against human hepatoma HepG2 cells of polyphenols extracted from wild *M. conica* Pers. is still poor. The objective of this study was to analyze the composition of polyphenols extracted from *M. conica* Pers. collected from Yunnan, Tibet, and Xinjiang, China. Human hepatoma HepG2 cells were used as a model to comprehensively evaluate the cellular antioxidant and antiproliferative

activities of polyphenols in the three *M. conica* Pers. species.

2. Materials and Methods

2.1. Materials

M. conica Pers. from Yunnan (*MCP-Y*), Tibet (*MCP-T*), and Xinjiang (*MCP-X*) were provided by the Kunming Institute of Edible Fungi. *Morchella* samples were oven-dried at 50°C to a constant weight, crushed with a pulverizer, passed through an 80-mesh sieve, sealed, and then stored in the dark at room temperature (20°C) in desiccators over silica gel-self indicator prior to analysis.

2.2. Chemicals

Folin-Ciocalteu reagent and quercetin were purchased from Sigma, Inc.. 2,2'-azobis (2-amidinopropane) dihydrochloride (ABAP), 2′,7′-dichlorofluorescin diacetate (DCFH-DA) and William's medium E were purchased from Wako Chemicals. Antibiotics, trypsin, fetal bovine serum, Hank's balanced salt solutions Dulbecco's modified Eagle's medium, hydrocortisone, antibacterial-antifungal agents, and phenanthroline (biochemical reagent) were purchased from Invitrogen. Methanol and acetonitrile (HPLC grade) were purchased from the Tianjin Shield Fine Chemical Product Co., Ltd. Other reagents were all of analytical grade. Human hepatoma HepG2 cells were provided by the American Type Culture Collection.

2.3. Methods

2.3.1. Extraction Phenolic Compounds

Extraction was performed following the methods reported by Okarter *et al*. [20] with slight modifications. Briefly, 2.00 g of sample was accurately weighed into a 100 mL centrifuge tube, and 50 mL of chilled acetone (80%, *v/v*) solution was added. The mixture was homogenized for 2 min and then stirred for 10 min. The sample was centrifuged at 2500×*g* for 10 min, and the supernatant was collected. The residue was re-extracted, and the supernatants were combined, suction filtered, and concentrated by evaporation at 45°C. The sample was adjusted to a constant volume of 25 mL with double-distilled water to obtain the free phenolic extract, which was stored at -80 °C until use within two weeks.

The residue was collected after the extraction of free phenolic compounds, and 20 mL of NaOH (2 mol/L, *w/v*) solution was added. The mixture was digested by oscillation for 1.5 hours and then adjusted to a pH of 2 using concentrated hydrochloric acid. Subsequently, 25mL of *n*-hexane was added to remove the fat layer. Then, 20 mL of ethyl acetate was added and fully stirred for 10 min. The mixture was centrifuged at 2500×*g*, and the supernatant was collected. The extraction procedure was repeated five times, with the centrifugation rate increasing by 500×*g* every time, and the supernatants were combined. After suction filtration, the extract was rotary evaporated at 45°C and adjusted to a constant volume of 10 mL with double-distilled water to obtain the bound phenolic extract. The extract was stored at –80°C until use within two weeks.

2.3.2. Measurement of the Total Phenolic Content (TPC)

TPC was detected using the Folin-Ciocalteu method [21]. Briefly, 200 µL of extract was absorbed, followed by the successive addition of 800 µL of deionized water and 200 µL of Folin-Ciocalteu agent. The sample was thoroughly mixed by shaking the tube, and then, it was placed in the dark for 6 min. Then, 2 mL of 7% Na_2CO_3 solution and 1.6 mL of deionized water were added, and the sample was placed in the dark for another 90 min before the absorbance was measured at 760 nm. Gallic acid was used as the reference standard to plot the standard curve over a concentration range of 0-400 µg/mL. The results are expressed as mg gallic acid equivalents/g dry-weight basis (mg GAE/g DW).

2.3.3. HPLC Analysis of Phenolic Compounds

The method previously reported by Liang *et al*. [22] was used with slight modifications. Briefly, the polyphenol extracts and 0.1 mg/mL standards (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, catechin, and chlorogenic acid) were filtered through a 0.45 µm organic membrane filter and analyzed using an LC-20A HPLC (Shimadzu Corp., Japan) equipped with a Shimadzu LC-20AD pump, a Shimadzu SIL-20A Autosampler, and a Shimadzu SPD-M20A diode array detector (DAD). A Thermo BDS C_{18} reverse-phase column (250 × 4.6 mm i.d., 5 µm grain size) was applied. Mobile phase A was 0.2% (*v/v*) formic acid, and mobile phase B was pure acetonitrile. The gradient elution program was as follows: 0-5 min, 10% B; 5-25 min, 10-40% B; 25-35 min, 40-90% B; 35-40 min, 90% B; 40-45 min, 90-10% B; and 45-50 min, 10% B. The other conditions were as follows: flow velocity, 0.7 mL/min; injection volume, 8 µL; column temperature, 40°C; and detection wavelength, 280 nm. Date was analyzed by LCsolution Version 1.25.

2.3.4. Cell Culture

HepG2 cells were maintained in the growth medium CM (William's Medium E, WEM) containing 5% FBS, 2 mmol/L glutamate, 10 mmol/L HEPES, 5 µg/mL insulin, 0.05 µg/mL hydrocortisone, 50 units/mL penicillin, 50 µg/mL streptomycin, and 100 µg/mL gentamicin. The cells were incubated at 37°C and 5% CO_2 [23,24]. The number of cell passages was 12-35.

2.3.5. Cellular Antioxidant Activity (CAA) Assay

According to the method reported by Wolfe, & Liu [25], the HepG2 cells (6×10^4 cells/well) were seeded into a 96-well plate containing 100 µL of growth medium per well. The cell culture was incubated at 37 °C and 5% CO_2 for 24 h. The medium was removed, and the plate was washed with 100 µL of phosphate-buffered saline (PBS) per well. Subsequently, 100 µL/well of different concentrations of quercetin reference standard (control group), polyphenol extracts (treatment group), or water (blank group) containing a 2',7'-dichlorofluorescin diacetate (DCFH-DA) working solution was added. All groups were cultured under the same conditions for another hour, and each well was then washed with 100 µL of PBS. Then, either the PBS wash

protocol between antioxidant and ABAP treatment (PBS wash protocol) was performed or not (no PBS wash protocol). Then, 100 μL of ABAP working solution was added. The blank group also received 100 μL of oxidant-treated medium. The 96-well plate was immediately placed on a microplate reader (Fluoroskan Ascent FL) and read at 37°C. The measurement was performed for 60 min at 5 min intervals (emission 538 nm; excitation 485 nm).

After subtraction of the blank from the fluorescence readings of the control and treatment groups, the integral of the curve of fluorescence over time was used to calculate the CAA value of reference standards or polyphenol extracts at each concentration as follows:

$$\text{CAA unit} = 100 - \left(\int SA / \int CA\right) \times 100$$

Where ∫SA is the integral of the curve of fluorescence for the sample and ∫CA is the integral of the curve of fluorescence for the control. The half-maximal effective concentrations (EC_{50}) of the reference standards and polyphenol extracts were calculated according to the linear relationship between log (fa/fu) versus log (dose), where fa is the affected portion after treatment (CAA unit) and fu is the unaffected portion (1-CAA unit). Quercetin was used as the reference standard. The CAA values, expressed as μmol quercetin equivalents (QE)/100 g DW, were converted from the EC_{50} values of quercetin and the samples.

2.3.6. Cytotoxicity

Following the methods reported by Yoon *et al*. [26] with slight modifications, the HepG2 cells were seeded into a 96-well plate (4×10^4 cells/well) and incubated at 37°C and 5% CO_2 for 24 h. Then, the plate was washed with PBS (100 μL/well). Subsequently, medium containing different concentrations of free polyphenol extract (25, 50, 75, 100, 125, 150, 175, 200, 225, 250 μg/mL) and bound polyphenol extract (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg/mL) was added (100 μL/well), followed by incubation at 37°C for another 24 h. The medium was then removed, and the plate was washed with PBS (100 μL/well). Thereafter, 50 μL/well of methylene blue solution (prepared with 0.6% methylene blue, 0.67% glutaraldehyde, and 98% HBSS) was added for staining. Cells were incubated at 37°C for one hour before the staining solution was removed. The plate was washed with deionized water until the water was clear. Next, 100 μL/well of elution solution (prepared with 1% acetic acid, 49% PBS, and 50% ethanol) was added. The plate was rotary oscillated for 20 min. Then, the 96-well plate was placed on a microplate reader to measure the absorbance at 570 nm. Compared with the blank wells, when the decrease in the ratio of the absorbance in the treatment wells was greater than 10%, the solution was considered to be cytotoxic.

2.3.7. Measurement of Cell Proliferation

Following the method reported by Yang *et al*. [27], HepG2 cells were seeded into a 96-well plate, and 100 μL of growth medium was added to each well. The cells (2.5×10^4 cells/per well) were incubated at 37°C under 5% CO_2 for 4 hours. The medium was then removed, and the plate was washed with 100 μL of PBS. Next, 100 μL of medium containing different concentrations of polyphenol extracts was added per well, and cells were incubated at 37°C for another 72 h. The same procedure was performed for the cytotoxicity test. The cell proliferation rate was calculated as the ratio of the absorbance in the treatment well to the absorbance in the blank well.

2.4. Statistical Analyses

The results are expressed as the mean ± SD (n=3) and statistically analyzed using SPSS 19.0 software. The figures were generated using Sigma Plot 12.0. Significant differences were evaluated at the $p<0.05$ level.

3. Results and Discussion

3.1. Total Phenolic Content in *Morchella conica* Pers

The free, bound, and total phenolic contents of *M. conica* Pers. from the three species were summarized in Table 1. With regards to the free phenolics, *M. conica* Pers. from Yunnan (*MCP-Y*) contained the highest concentration (6.157 mg GAE/g DW), followed by *M. conica* Pers. from Xinjiang (*MCP-X*, 6.145 mg GAE/g DW), and *M. conica* Pers. from Tibet (*MCP-T*, 4.928 mg GAE/g DW). With regards to bound phenolics, *MCP-Y* contained the highest concentration (0.250 mg GAE/g DW), followed by *MCP-X* (0.206 mg GAE/g DW) and *MCP-T* (0.188 mg GAE/g DW). Free phenolics comprised approximately 96% of the total phenolic content. Other dietary fungi varieties, such as *Lentinula edodes* [28] and *Pleurotus ostreatus* [29], also contained large proportions of free phenolics. The total phenolic contents of *M. conica* Pers. were higher than those of *Pleurotus ostreatus* (1.44 mg GAE/g DW), *Cantharellus cibarius* (0.77 mg GAE/g DW) [30], *Agaricus arvensis* (2.83 mg GAE/g DW) and *Saarcodon imbricatum* (3.76 mg GAE/g DW) [31], but lower than the ethanol extracts of *Portabella* (10.65 mg GAE/g DW) and *Crimini* (9.89 mg GAE/g DW) [32].

Table 1. Phenolic contents of free, bound and total phenolics extracted from *M. conica* Pers. growing in three different habits. (mean ± SD, n=3)

M. conica Pers.	Phenolics (mg GAE/g DW)		
	Free	Bound	Total (free+bound)
M. conica Pers. From Yunnan (MCP-Y)	6.157±0.157[a] (96.10%)	0.250±0.018[a] (3.90%)	6.407±0.171[a] (100%)
M. conica Pers. From Tibet (MCP-T)	4.928±0.045[b] (96.33%)	0.188±0.026[b] (3.67%)	5.116±0.045[b] (100%)
M. conica Pers. From Xinjiang (MCP-X)	6.145±0.192[a] (96.77%)	0.206±0.018[b] (3.23%)	6.350±0.187[a] (100%)

Note: Values with different superscripts letters in each column are significantly different at $p<0.05$ level. Values in parentheses indicate percentage contribution to the total.

Table 2. Composition and content (μg/g) of detected polyphenolics extracted from *M. Conica* Pers. growing in three different habits. (mean±SD, n=3)

Phenolics	*M. conica* Pers.	gallic acid	protocatechuic acid	*p*-hydroxybenzoic acid	chlorogenic acid	catechin
Free	*MCP-Y*	1043.49±20.35^{B}	795.06±15.68^{A}	777.60±16.74^{A}	827.22±15.46^{A}	555.69±10.24^{A}
	MCP-T	951.26±18.58^{C}	505.26±10.24^{C}	599.28±15.46^{B}	517.85±10.29^{B}	396.51±5.64^{C}
	MCP-X	1124.13±15.27^{A}	707.52±11.36^{B}	615.67±14.31^{B}	510.30±8.47^{B}	518.30±5.41^{B}
Bound	*MCP-Y*	11.41±0.31^{a}	9.02±0.23^{a}	nd	20.21±0.41^{a}	nd
	MCP-T	8.83±0.15^{c}	8.65±0.19^{b}	nd	13.01±0.33^{b}	nd
	MCP-X	10.96±0.21^{b}	7.11±0.12^{c}	nd	13.18±0.30^{b}	nd

Note: A-C, different uppercase letters indicate significant differences of free phenolics in the same column (p<0.05); a–c, different lowercase letters indicate significant differences of bound phenolics in the same column (p<0.05). "nd" not detected.

3.2. Characterization of the Phenolic Compounds

The compositions of free and bound phenolic compounds from the three varieties were identified by RP-HPLC-DAD. As shown in Table 2, gallic acid was the major free phenolic compound in the three *M. conica* Pers. varieties, and the contents of free gallic acid in the three varieties followed the order: *MCP-T* (951.20 μg/g) < *MCP-Y* (1,043.49 μg/g) < *MCP-X* (1,124.13 μg/g) (p<0.05). The bound gallic acid contents were: *MCP-Y* (11.41 μg/g), *MCP-X* (10.96 μg/g), and *MCP-T* (8.83 μg/g) (p<0.05).

The free protocatechuic acid content was the highest (p<0.05) in *MCP-Y* (795.06 μg/g), followed by *MCP-X* (707.52 μg/g) and *MCP-T* (505.26 μg/g). The bound protocatechuic acid contents in the three *M. conica* Pers. were as follows: *MCP-X* (7.11 μg/g) <*MCP-T* (8.65 μg/g) <*MCP-Y* (9.02 μg/g).

The free *p*-hydroxybenzoic acid content in *MCP-Y* (777.60 μg/g) was significantly higher (p<0.05) than in *MCP-X* (615.67 μg/g) and *MCP-T* (599.28 μg/g). However, no *p*-hydroxybenzoic acid was detected in the bound extracts of the *M. conica* Pers. varieties.

The free chlorogenic acid content in *MCP-Y* was the highest (827.22 μg/g), followed by *MCP-T* (517.85 μg/g) and *MCP-X* (510.30 μg/g) (p<0.05). The content of bound chlorogenic acid was relatively high in *MCP-Y* (20.21 μg/g), followed by *MCP-X* (13.18 μg/g) and *MCP-T* (13.01 μg/g).

The free catechin content was the highest in *MCP-Y* (555.69 μg/g), followed by *MCP-X* (518.30 μg/g) and (*MCP-T*) 396.51 μg/g (p<0.05). No catechin was detected in the bound of the three *M. conica* Pers. varieties.

Accordingly, the polyphenol compositions of the three varieties of *M. conica* Pers. were similar, but the contents varied significantly (p<0.05). It was found that the content or composition of phenolics was influenced by the breading environment and varieties [33]. We found that gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, catechin, and chlorogenic acid were the major free phenolics in the three *M. conica* Pers., whereas gallic acid, protocatechuic acid, and chlorogenic acid were the major bound phenolics. Gallic acid, protocatechuic acid, chlorogenic acid and *p*-hydroxybenzoic acid were associated with the antioxidant acitivity of foods [34,35,36,37]. Catechin showed cellular antioxidant activity [38]. In another side, gallic acid, catechin and chlorogenic acid could inhibit the proliferation of HepG2 cells [39,40,41]. Therefore, it could be inferred that these phenolic compounds identifed in *M. conica* Pers might be responsible for its bioactive activities.

3.3. Cellular Antioxidant Activities

The kinetic curve of DCFH oxidation induced by peroxyl radicals produced by ABAP in HepG2 cells was illustrated in Figure 1. Results showed that both the quercetin standard (Figure 1 A,B) and the polyphenol extracts of *M. conica* Pers. (Figure 1 C,D,E,F) significantly inhibited the production of DCF, as reflected by the increase in the fluorescence value.

The EC_{50} and CAA values for the cellular antioxidant activity of polyphenols from the three varieties of *M. conica* Pers. and the CC_{50} values for the cytotoxicities of the samples were listed in Table 3. With the PBS wash, the EC_{50} values of free phenolic extracts ranged from 112.60 to 582.03 mg/mL, and the CAA values ranged from 2.24 to 7.09 μmol QE/100 g. Without the PBS wash, the EC_{50} values ranged from 24.51 to 71.00 mg/mL, and the CCA values ranged from 13.04 to 32.10 μmol QE/100 g. These results indicated that within the nontoxic concentration (< 250 mg/mL), the free phenolic extracts exhibited antioxidant activity on the cell membrane surface (no PBS wash), and the antioxidant activity was enhanced with increasing phenolic contents. However, in the interior of the cells (PBS wash), only the free phenolic extract of *MCP-Y* showed cellular antioxidant activity. The free phenolic extract of *MCP-Y* produced the lowest EC_{50} (112.60 mg/mL and 24.51 mg/mL) and highest CAA (7.09 μmol QE/100 g and 32.10 μmol QE/100 g), when the protocols of no PBS wash and PBS wash were performed. The free phenolic extract of *MCP-X* produced the highest EC_{50} (582.03 mg/mL and 71.00 mg/mL) and lowest CAA (0.26 μmol QE/100 g and 0.47 μmol QE/100 g). These data indicated the great potential of the free phenolic extract of *MCP-Y* in scavenging cellular reactive oxgen species (ROS). In both the protocols of PBS wash ad no PBS wash, the CAA values of free phenolic extracts were significantly higher than those of bound phenolic extracts, and the polyphenol extract of *MCP-Y* showed the best cellular antioxidant capacity.

Generally, the polyphenol extracts of *Morchella* showed significantly higher capacity to inhibit the DCF production in the protocol of PBS wash than in the PBS wash protocol. It means that the *Morchella* polyphenols took more advantage in scavenging ROS on/ in cell membrane than intracellular ROS. Similar results were

found in the polyphenols of *Semen coicis* [42] and polyphenols of *M. umbrina* Boud [43]. The macromolecules in the polyphenol extracts of *Morchella* might prevent phenolic compounds from penetrating through the cell membranes, resulting in relatively high levels of extracellular antioxidant activity. While, when the PBS wash protocol was performed, both the macromolecules and phenolic compounds were washed off, and the capacity to inhibit DCF production was lower than that without the PBS wash.

Chlorogenic acid and gallic acid contributed the most to the antioxidant capacity [44,45]. As gallic acid and chlorogenic acid showed high levels in *M. conica* Pers. (Table 2). Therefore, it could be inferred that gallic acid and chlorogenic acid might be the major contributors to the antioxidant activities of *M. conica* Pers. varieties.

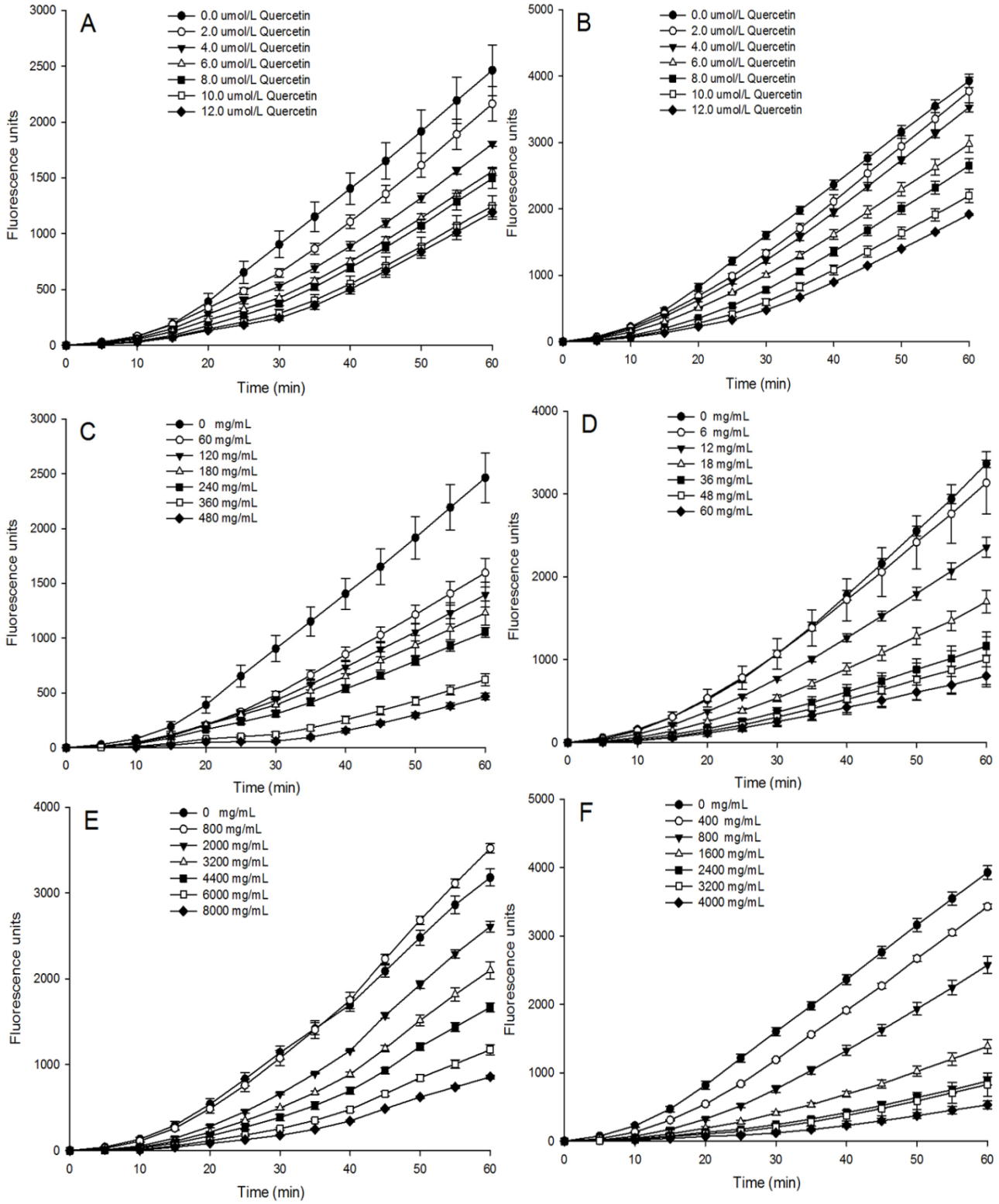

Figure 1. Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by quercetin (A, B) and free phenolic (C, D), as well as bound phenolic (E, F) extracts of *M. Conica* Pers. over time. A, C, and D provided the results of the PBS wash protocol; B, D, and F provide the results of the no PBS wash protocol

Table 3. Cellular antioxidant activities and cytotoxicity of polyphenols extracted from *M. Conica* Pers. growing in three different habits. (mean±SD, n=3)

Phenolics	*M. conica* Pers.	PBS wash		No PBS wash		Cytotoxicity
		EC_{50} (mg/mL)	CAA (μmol QE/100g)	EC_{50} (mg/mL)	CAA (μmol QE/100g)	CC_{50} (mg/mL)
Free	*MCP-Y*	112.60±10.50^{C}	7.09±0.60^{A}	24.51±2.16^{B}	32.10±2.99^{A}	>250
	MCP-T	263.55±16.43^{B}	2.59±0.22^{B}	60.20±2.90^{A}	13.04±1.07^{B}	>250
	MCP-X	582.03±9.68^{A}	2.24±0.07^{B}	71.00±4.27^{A}	13.47±0.69^{B}	>250
Bound	*MCP-Y*	4009.82±57.24^{a}	0.24±0.01^{b}	968.28±26.76^{b}	0.10±0.00^{c}	96.31±4.36
	MCP-T	2643.37±18.42^{b}	0.26±0.01^{b}	1854.45±144.22^{a}	0.47±0.05^{b}	>100
	MCP-X	2109.91±75.43^{b}	0.32±0.02^{a}	1086.63±37.59^{b}	0.80±0.05^{a}	82.37±4.05

Note: A-C, different uppercase letters indicate significant differences of free phenolics in the same column (p<0.05); a–c, different lowercase letters indicate significant differences of bound phenolics in the same column (p<0.05).

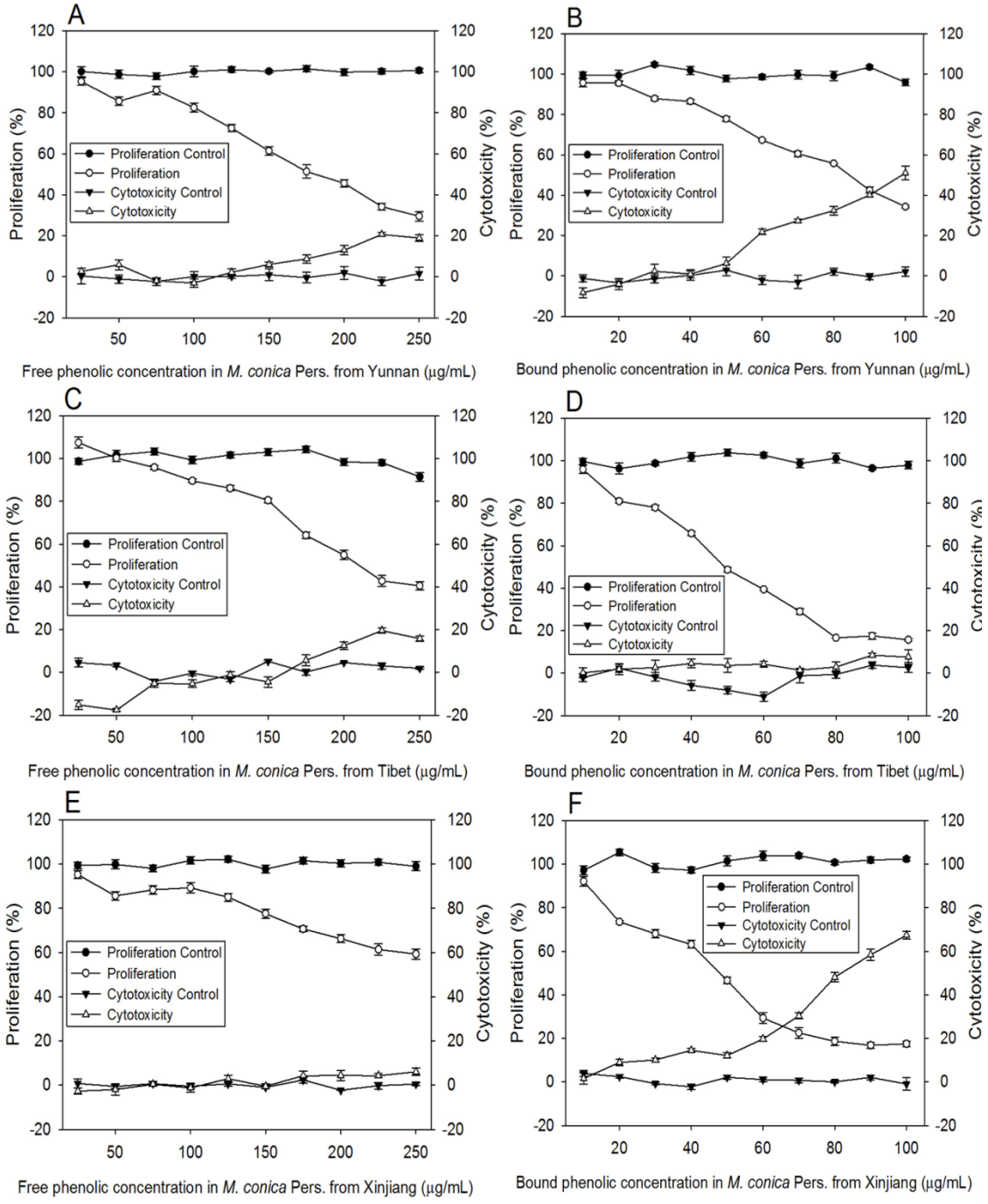

Figure 2. The inhibited proliferation and cytotoxicity of HepG2 human liver cancer cells treated by *M. Conica* Pers. polyphenol extracts. *M. Conica* Pers. from Yunnan (A, B), *M. Conica* Pers. from Tibet (C, D), and *M. Conica* Pers. from Xinjiang (E, F); free phenolics (A, C, E) and bound phenolics (B, D, F)

3.4. Antiproliferative Effect on HepG2 Cells

The antiproliferative capacity and cytotoxicity of polyphenol extracts of *M. conica* Pers. against HepG2 cells were shown in Figure 2. The cell proliferation rate was approximately 100% in the control group. At the 200 μg/mL of free phenolic extracts of *M. conica* Pers., the proliferation inhibition rate increased from 33.80% (*MCP-X*) to 54.42% (*MCP-Y*). As 200 μg/mL was a non-cytotoxic concentration (Table 4), it indicated that the inhibitory effect of free phenolic extracts on HepG2 cells was primarily induced by the inherent anti-tumor effect rather than the cytotoxicity. Within the non-cytotoxic concentrations, free phenolic extracts of *MCP-Y* showed the highest antiproliferative capacity against HepG2 cells, whereas free phenolic extracts of *MCP-X* exhibited the lowest antiproliferative capacity. The antiproliferative capacity increased in a dose- dependent manner.

At the concentration of 100 μg/mL (non-cytotoxic concentration, Table 4), the bound phenolic extract of *MCP-T*, inhibited the proliferation of HepG2 cells by 83.53%, suggesting that bound phenolic extracts of *MCP-T* had higher capacity to inhibit HepG2 cell proliferation than the three of free phenolic extracts. For the bound phenolic extracts of *MCP-Y* and *MCP-X*, their highest proliferation inhibition rates were 22.23% and 29.17%, respectively, which were much weaker than *MCP-T*.

HepG2 cells have been extensively used in the research of cancer mechanisms, genetics and nutriology [42,46]. In this study, the proliferation of HepG2 cells was inhibited in a dose-depended manner by *M. conica* Pers. polyphenols. Within the non-cytotoxic concentrations, the EC_{50} values of free phenolic extracts ranged from 186.43 to 271.38 μg/mL. Specifically, *MCP-Y* showed the highest antiproliferative capacity with a 54.42% of the inhibiting rate against HepG2 cells. For bound phenolic extracts, *MCP-T* showed the highest antiproliferative capacity against HepG2 cells, with an inhibition rate of 83.53% and an EC_{50} value of 49.84 μg/mL. However, the other two varieties showed almost no inhibition of HepG2 cell proliferation.

Table 4. Antiproliferation activity (EC_{50}) and cytotoxicity (CC_{50}) of polyphenols extracted from three *M. Conica* Pers. on HepG2 cells (mean±SD, n=3)

Phenolics	*M. conica* Pers.	Anti-proliferation activity	Cytotoxicity
		EC_{50}(μg/mL)	CC_{50}(μg/mL)
Free	*MCP-Y*	186.43±8.21^{C}	>250
	MCP-T	215.82±8.00^{B}	>250
	MCP-X	271.38±4.39^{A}	>250
Bound	*MCP-Y*	82.28±4.75^{a}	96.31±4.36
	MCP-T	49.88±2.32^{b}	>100
	MCP-X	49.84±1.43^{b}	82.37±4.05

Note: A-C, different uppercase letters indicate significant differences of free phenolics in the same column (p<0.05); a–c, different lowercase letters indicate significant differences of bound phenolics in the same column (p<0.05).

4. Conclusions

Above all, a total of five phenolic compounds were identified in the wild *M. conica* Pers. Gallic acid and chlorogenic acid represented the major free and bound phenolic compound, respectively. The free phenolic extract exhibited high CAA, whereas the bound phenolic extract showed nearly no CAA when incubated within non-cytotoxic concentrations. The wild *M. conica* Pers. growing in Yunnan, China showed the highest CAA values owing to its highest concentrations of free and bound polyphenols. This study confirmed the antioxidant and antitumor activities of wild *Morchella* polyphenols. Particularly, the wild *Morchella* growing in Yunnan, China might be a good choice for breeding. Our results might also contribute to the comprehensive utilization of *Morchella.*

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