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CHEMICAL COMPOSITION AND ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTICANCER EFFECTS OF EXTRACT FROM YUNZHI MUSHROOM (*CORIOLOPSIS ASPERA*) IN VIETNAM

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ABSTRACT

Introduction: *Coriolopsis* spp. are broadly used as traditional medicines in India, China, and Japan as antioxidant, antitumor, antiviral, antimicrobial, and immunomodulatory agents to treat many diseases. However, the bioactivities of yunzhi mushroom (*Coriolopsis aspera*) crude extract have not been studied yet. **Materials & Methods:** The aims of this study are the determination of chemical composition and antioxidant, anticancer, and anti-inflammatory effects of yunzhi mushroom (*Coriolopsis aspera*) extract in Vietnam. The chemical constituents of yunzhi mushroom extract were determined via MS, IR, and NMR spectrum analysis, and the antioxidant activity of the extract was evaluated by 1,1-diphenyl-2picryl-hydrazil (DPPH) radical scavenging assay. The anticancer activity of yunzhi mushroom against HeLa and HepG2 cancer cell lines was tested via the MTT assay. The anti-inflammatory effect of the extract was determined through the quantification of nitrite oxide production using the Griess reaction. **Results & Discussion:** We identified the presence of 9 bioactive compounds in CoAE extract, including trametenolic B, cerevisterol, ergosterol, ergosterol peroxide, trans-p-hydroxy coumaric acid, methyl ferulate, methyl (2-hydroxyphenyl) acetate, umbelliferone, and 8-hydroxy-3,4-dimethyl isocoumarin. Moreover, CoAE extract exhibited stronger antioxidant activity than another crude extract. Of note, anticancer and anti-inflammatory effects of CoAC were also higher than those of another crude extract. The data indicate yunzhi mushroom as potential functional food for treating inflammatory and cancer-related diseases. **Conclusion:** The data suggest *Coriolopsis aspera* as one of the promising functional foods for the treatment of inflammatory and cancer-related diseases.

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Introduction

In the past few years, much attention has been focused on natural products [1-3], because of the natural compounds they contain [4]. *Coriolopsis* is a genus of fungi in the family Polyporaceae. Most of them are distributed in tropical and subtropical Asia. Although the composition of the fruiting bodies of *Coriolopsis* has been reported [5-9], there are few studies concerning the secondary metabolites produced by fungi of the genus *Coriolopsis*. Today, *Coriolopsis* spp. are also broadly used as traditional medicines in India, China, and Japan as antioxidant, antitumor, antiviral, antimicrobial, and immunomodulatory agents to treat many diseases [10-12]. They also have numerous bioactive molecules, including terpenoids, steroids, phenols, glycoprotein derivatives, and polysaccharides [5-9]. They are also suggested as promising sources for screening new natural products. As a continued program aiming to search for new metabolites from the *Coriolopsis*, *C. aspera* was collected and studied for the first time. The aims of this study were determination of chemical composition and some bioactivities such as anti-oxidative, anticancer, anti-inflammatory effects of yunzhi mushroom extract, which provides more information to utilize this product as one of the potential functional foods in future.

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Materials and Methods

Chemicals and Materials

The fruiting bodies of *Coriolopsis aspera* (Jungh.) Teng was collected in Ky Son district of Nghean province, Vietnam in November 2018 and was identified by Dr. Van Hong Thien, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam. A voucher specimen (sample code COA102018FVN) was deposited at the herbarium of the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City.

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), Griess reagent, dimethyl sulfoxide (DMSO), Eagle's Minimum Essential Media (EMEM) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Dulbecco's Modified Eagle Media, L-glutamine, streptomycin, penicillin, and fetal bovine serum (FBS) were provided by Thermo Fisher Scientific Inc. (Waltham, MA, USA). All reagents were of analytical or cell culture grades.

Preparation of yunzhi mushroom extract

The dry powder fruiting bodies of *C. aspera* (1.0 kg) were extracted with ethanol 96% (EtOH) utilizing the Ultrasound-Assisted Extraction (UAE) technique (3 x 2L) at room temperature. The solvent extract was evaporated under low pressure to produce a crude ethanol extract (450 g). The crude ethanol extract was resolved in water and partitioned with hexane, ethyl acetate, chloroform to afford hexane (CoAH, 27 g), ethyl acetate (CoAE, 105 g), chloroform (CoAC, 37 g), and water-soluble (CoAW, 83 g) fractions, respectively (Figure 1).

(a) **(b)** **(c)** **(d)**

Fig. 1. Four types of extracts from four different solvents.
(a): hexane extract; (b): ethyl acetate extract
(c): chloroform extract; (d): aqueous extract

Cytotoxicity Assay.

The cancer cell lines (Hela cells and HepG2) were kept in Dulbecco′s D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 µg/mL), and gentamicin (10 µg/mL). Stock solutions of compounds were set in DMSO–H2O (1:9), and cytotoxicity assays were performed in 96-well microtiter plates against Hela and HepG2 cancer cell lines (3 × 103 cells/mL) utilizing a modification of the published technique [13]. After 72 h incubation at 37°C in the air–CO_2 (95:5) with or without test compounds, the cell growth was assessed utilizing a colorimetric measurement of stained living cells by neutral red. Optical density was measured at 540 nm with a Titertek Multiscan photometer. The IC_{50} value was expressed as the concentration of the sample essential to inhibit the cell growth to 50% of the control. Ellipticine was utilized as a reference compound.

Determination of the antioxidant activity of yunzhi mushroom extract

The antioxidant activity of CoAE, CoAW, CoAC, and CoAH were measured via the 1,1-diphenyl-2picryl-hydrazil (DPPH) radical scavenging assay using the method of Kumar et al. with some modifications [14]. Briefly, the 0.1 mM DPPH solution was prepared in ethanol, and extracts were diluted in DMSO to obtain the 4 mg/mL solution. Then 0.1 mM DPPH solution was mixed with a volume of crude extract to obtain the final extract concentration of about 200 µg/mL. The mixture was shaken vigorously and stand at room temperature for 30 minutes, after which the absorbance of the sample was measured at 515 nm using a microplate reader (Infinite 50, Tecan, Switzerland). The mixture without crude extract was used as the control and ascorbic acid solution (final concentration 44 µg/mL) was utilized as the positive control. DPPH radical scavenging activity was determined as the following formula: DPPH radical scavenging activity (%) = $\frac{Ao-A1}{Ao}$ ×100%; in which, A_0 is the absorbance of control at 515 nm, and A_1 is the absorbance of the sample at 515 nm.

Determination of the anti-inflammatory activity of yunzhi mushroom extract

The anti-inflammatory activity of the extract was estimated via quantification of nitrite oxide production utilizing the Griess reaction [15]. In brief, mouse macrophage cells (Raw 264.7) were sub-cultured into 96-well plate with a density of about 2.5

x 10^4 cells/well for 24 hours and subsequently incubated with or without yunzhi mushroom extracts (30-100 µg/mL) for 2 hours. Cardamonin (0.3-3.0 µM) was utilized as a positive control. Then cells were stimulated with 2 µL LPS (0.1 mg/mL) for 24 hours. The cell culture media was incubated with Griess reagent (ratio 1:1) and kept at room temperature for 10 min. The absorbance of the sample was recorded at 540 nm via a microplate reader (Infinite 50, Tecan, Switzerland). The nitrite concentration in each sample was calculated utilizing a serial dilution standard calibration curve prepared with sodium nitrate.

Cell viability of macrophages was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide MTT assay [16]. Briefly, 264.7 cells after treated with extracts or the reagent were incubated with MTT solution (5 mg/mL in phosphate-buffered saline) at 37ºC in a 5% CO_2 humidified atmosphere for 4 hours. The formazan crystal was dissolved by adding 100 µL of DMSO (Sigma-Aldrich, St. Louis, MO, USA) and absorbance of the solution was recorded at 540 nm via a microplate reader (Infinite 50, Tecan, Switzerland). The cell viability and inhibition of nitrite oxide production were calculated and expressed as the average percentage from three analyses.

Statistical analysis

All determinations were performed in triplicate. The experimental results were expressed as mean ± standard deviation, and the Statgraphics Centurion XVI software (Statpoint Technologies Inc., Warrenton, Virginia, USA) was used to conduct statistical analysis. Differences between different treated groups were analyzed using ANOVA variance analysis and multiple range tests with the criterion of statistical significance less than 0.05 (p<0.05).

Results and Discussions

Chemical constituents of yunzhi mushroom extract

To determine the chemical constituents of *C. aspera*, we wish to report the characterization of nine compounds (**1**-**9**), which was isolated from CoAE of the fruiting bodies of *C. aspera* for the first time. The structures of all compounds were characterized by comparison with the spectral data reported in the literature. Their structures were identified as trametenolic B (**1**) [17], cerevisterol (**2**) [18], ergosterol (**3**) [19], ergosterol peroxide (**4**) [20], trans-p-hydroxy coumaric acid (**5**) [21], methyl ferulate (**6**) [22], methyl (2-hydroxy phenyl) acetate (**7**) [23], umbelliferone (**8**) [24], and 8-hydroxy-3,4-dimethyl isocoumarin (**9**) [25].

Table 1. Phytochemical composition of ethanol extract of yunzhi mushroom.

No	Compounds	Bioactivities
1	trametenolic B	Anti-inflammatory, anti-oxidative, anti-cancer effects
2	cerevisterol	Anti-inflammatory, anti-oxidative, anti-cancer effects
3	ergosterol	Anti-oxidative, anti-cancer effects
4	ergosterol peroxit	Anti-oxidative, anti-cancer effects
5	trans-p-hydroxycoumaric acid	Anti-oxidative, anti-cancer effects
6	methyl ferulate	Anti-oxidative, anti-inflammatory, anticancer, anti-diabetic effects
7	methyl (2-hidroxyphenyl) acetate	Anti-inflammatory, anti-oxidative effects
8	umbelliferone	Anti-inflammatory, anti-oxidative effects
9	8-hydroxy-3,4-dimethylisocoumarin	Anti-inflammatory, anti-oxidative, anti-cancer effects

DPPH radical scavenging activity

The antioxidant activity of yunzhi mushroom extract was evaluated via DPPH radical scavenging activity. As shown in Table 2, ascorbic acid exhibited the strongest antioxidant activity with IC_{50} value of 0.019, next coming with CoAE, CoAW, CoAC, CoAH with IC_{50} value of 0.072, 0.194, 0.570, and 0.720, respectively. The antioxidant activity of yunzhi mushroom extract may be explained through the existence of some antioxidant compounds including trans-p-hydroxy coumaric acid (5), methyl ferulate (6), methyl (2-hydroxy phenyl) acetate (7), umbelliferone (8), 8-hydroxy-3,4-dimethyl isocoumarin (9). Of note, the antioxidant activity of CoAE extract exhibited the strongest antioxidant activity than another yunzhi mushroom extract, which provides the evidence of chemical composition as well as bioactivity of yunzhi mushroom.

Table 2. Antioxidant activity of yunzhi extract was determined via DPPH radical scavenging activity

Sample	DPPH radical scavenging activity (SC,%)	Conc. (mg/ml)	IC_{50} (mg/mL)
Control (+) [Acid ascorbic]	93.82 ± 2.95	0.035	0.019
Control (-) [DPPH/EtOH + DMSO]	0.0	-	-
CoAE	65.64^a ±1.48	0.1	0.072
CoAW	31.21^b ±1.36	0.1	0.194
CoAC	9.75^c ±1.05	0.1	0.570
CoAH	16.23^d ±1.26	0.1	0.720

IC_{50}: effective concentration at which 50% of DPPH radicals are scavenged.

^{a,b,c,d} *Different letters in the same column indicate significant differences among different groups (p<0.05).*

Anti-inflammatory effect of yunzhi mushroom extract

The anti-inflammatory effect of yunzhi mushroom extract was measured via inhibition of nitric oxide (NO) production in Raw 264.7 cells. We observed that the elevation of inhibition action of NO production by LPS-stimulated macrophages was associated with the increase of concentration of yunzhi mushroom extract (CoAE and CoAC) and Cardamonin. Moreover, the capacity of CoAC extract to regulate the NO production was higher than that of CoAE extract at the same concentration (Table 3). For example, CoAC in the concentration of 100 µg/mL could inhibit 34.67 ± 0.54% of the amount of NO production while CoAE only inhibited 14.39 ± 0.44% of the same concentration. On the other hand, both yunzhi mushroom extracts (CoAE and CoAC) exhibited low toxicity to macrophages (cell viability over 85%), and cell viability of CoAC was not significantly different from reference drug (Cardamonin, 0.3-3.0 µM). Of note, the cell viability of CoAC in the concentration of 30 µg/mL was not remarkably different with that of CoAE in the concentration of 100 µg/mL but the anti-inflammatory effect of CoAC was higher than CoAE effect (17.84 ± 0.37% and 14.39 ± 0.44%, respectively), which implied CoAC as a natural anti-inflammatory reagent.

Table 3. Anti-inflammatory effect of yunzhi mushroom extract

		Inhibition of NO production (%)	Cell viability (%)
Cardamonin	0.3 µM	17.03 ± 0.85^{a}	87.39 ± 1.24^{c}
	3.0 µM	96.29 ± 0.51^{b}	86.58 ± 1.09^{c}
CoAE	30 µg/mL	9.76 ± 0.21^{c}	90.07 ± 1.18^{a}
	100 µg/mL	14.39 ± 0.44^{d}	89.72 ± 1.14^{ab}
CoAC	30 µg/mL	17.84 ± 0.37^{e}	87.74 ± 1.27^{bc}
	100 µg/mL	34.67 ± 0.54^{f}	86.52 ± 1.32^{c}

^{a,b,c,d,e,f} *Different letters in same column indicate significant differences among different groups ($p<0.05$).*

Anti-cancer effect of yunzhi mushroom extract

The anti-cancer effects of black shallot extract and fresh shallot extract were evaluated via MTT assay and the results were presented in Table 4. The anti-cancer activities of CoAC extract against Hela cells and HepG2 (58,3 ±2,8% and 61,9 ±1,4%, respectively) were higher than those of another crude yunzhi mushroom extract (13.65 ± 0.42% and 14.85 ± 0.49%, respectively). To the best of our knowledge, this study is the first work that proves the anticancer effect of CoAC and compares the efficiency of CoAW, CoAH, and CoAE against cancer cells. These data provide the scientific basis for further application of black shallot for the treatment of cancer-related diseases.

Table 4. Anti-cancer effect of yunzhi mushroom extract

Sample	Conc.	HeLa		Hep-G2	
		Inhibition rate on Hela cells (%)	IC50	Inhibition rate on HepG2 cells (%)	IC_{50}
Control (-) [DMSO]	0.1%	0	-	0	-
Control (+) [Ellipticine]	5µg/ml	89.6±2,4	3.63µM	81.2±1,6	3.98µM
CoAE	100µg/ml	15.2^{a} ±0,4	>100µg/ml	23.1^{a} ±2,1	>100µg/ml
CoAW	100µg/ml	18.3^{a} ±1,5	>100µg/ml	30.8^{b} ±1,7	>100µg/ml
CoAC	100µg/ml	58.3^{b} ±2,8	67.5 µg/ml	61.9^{c} ±1,4	69,2 µg/ml
CoAH	100µg/ml	6.3^{c} ±0,8	>100µg/ml	9.4^{d} ±1,2	>100µg/ml

^{a,b,c} *Different letters in the same column indicate significant differences among different groups ($p<0.05$).*

Conclusion

In this study, the presence of 9 compounds in CoAE extract was identified, including trametenolic B, cerevisterol, ergosterol, ergosterol peroxide, trans-p-hydroxy coumaric acid, methyl ferulate, methyl (2-hydroxyphenyl) acetate, umbelliferone, and 8-hydroxy-3,4-dimethyl isocoumarin. Moreover, CoAE extract exhibited stronger antioxidant activity than another crude extract. Of note, anticancer and anti-inflammatory effects of CoAC were also higher than those of another crude extract. The data indicate yunzhi mushroom as the potential functional foods for treating inflammatory and cancer-related diseases.

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Conflict-of- interest Notification Page:

The authors declare there is no conflict of interest.

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