

Assessment of Mycochemical composition and biological activities of extracts from *Humphreya endertii*

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Abstract

This study presents the comprehensive analysis of the chemical composition and biological activities of methanol and ethanol extracts from *Humphreya endertii*, a recently discovered mushroom species in Vietnam. Gas chromatography/mass spectrometry identified 16 compounds in the methanol extract and 20 in the ethanol extract, with significant differences in composition between the two. The methanol extract contained 18.51% 5-Methoxypyrrolidin-2-one, 15.41% *cis*-Vaccenic acid, 14.03% 10(E),12(Z)-Conjugated linoleic acid, 13.95% *n*-Hexadecanoic acid, 8.25% 2,5-Furandione, dihydro-3-methylene and other compounds in smaller amounts (<8%). The ethanol extract was composed of 31.30% 17-Octadecynoic acid, 23.50% *cis*-Vaccenic acid, 16.32% *n*-Hexadecanoic acid, 10.59% Octadecanoic acid, 5.99% Ethyl α -D-glucopyranoside and other compounds in smaller amounts (<5%).

Both extracts exhibited antibacterial activity against ten bacterial pathogens including *S. aureus*, *E. hormachei*, *E. faecalis*, *B. cereus*, *S. sonnei*, *P. aeruginosa*, *S. typhimurium*, *S. saprophyticus*, *E. cloacae* and *S. enteritidis*. Antioxidant properties were also observed through DPPH radical scavenging activity, with similar IC₅₀ values of approximately 635 μ g/mL for both the methanol and ethanol extracts. These findings suggest that the methanol and ethanol extracts of *H. endertii* could be valuable for pharmaceutical applications and the development of medical resources in the future.

Keywords: Antibacterial, Antioxidant, Methanol extract, Ethanol extract, *Humphreya endertii*, Mycochemical composition.

Introduction

Medicinal mushrooms have been utilized for centuries, especially in Asian cultures, due to their health-promoting properties. These mushrooms are rich in bioactive compounds that exhibit a wide range of pharmacological activities²². Among the widely used medicinal mushrooms, the Ganodermataceae family is notable for its extensively studied medicinal value and applications in traditional

medicine. Since the 1970s, mushrooms from the Ganodermataceae family have been cultivated and commercialized. Reishi mushrooms, are traditionally used to treat cardiovascular diseases, diabetes, hepatitis and cancer^{23,36}. Numerous studies have highlighted their biological activities such as anti-inflammatory, antiviral, antibacterial, antioxidant, anti-aging and antitumor properties^{4,17,35}.

Various species of Reishi mushrooms, including *Ganoderma lucidum*, *G. sinensis*, *G. applanatum*, *G. tsugae*, *G. atrum* and *G. formosanum*, are recognized for their experimental pharmacological effects, as demonstrated in numerous studies¹². Research on the cultivation and medicinal value of Reishi mushrooms from Vietnam has also been conducted. For instance, Tsivileva et al³⁴ identified the chemical composition of the mycelium of five Reishi species, revealing the presence of free fatty acids and fatty alcohols. Other valuable bioactive compounds such as lucidenic acid N, ganoderic acid and ganodermanontriol have been found in *G. lucidum* strains from Tam Dao, Vietnam²⁴ while triterpenoids and steroids were detected in the fruiting bodies of *G. applanatum*¹⁴.

Despite the high medicinal value of Reishi mushrooms in Vietnam, comprehensive studies remain limited. Beyond *Ganoderma*, it is crucial to explore and develop new medicinal mushroom sources from the Ganodermataceae family for human health applications. Reports on the genus *Humphreya* within this family are still scarce, with *H. endertii* being one of the four recorded species¹³. In 2009, *H. endertii* was first reported in Cat Tien National Park, Dong Nai and Lam Dong provinces, Vietnam²¹. However, studies on the biological activities of *H. endertii* remain limited. In our previous work, we established the propagation conditions for cultivating *H. endertii* and discovered that 80°C-water extract from its fruiting bodies exhibited cytotoxicity against NCI-H460 lung cancer cells and HepG2 liver cancer cells^{25,26}.

Additionally, this extract showed antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Salmonella typhimurium*, while no effect was observed on *Escherichia coli*²⁶. Furthermore, endertiins A from *H. endertii* fruiting bodies exhibited cytotoxicity against human breast carcinoma MCF-7. In this study, the chemical composition, antibacterial and antioxidant activities of

ethanol and methanol extracts from *H. endertii* fruiting bodies were investigated to further elucidate the biological activities of this new medicinal mushroom species in Vietnam to contribute to the development of new medicinal resources.

Material and Methods

Mushroom sample collection and preparation: *H. endertii* samples were collected from Phuoc Binh National Park, Ninh Thuan province, Vietnam (Figure 1). Fruiting bodies were dried at 50°C until constant weights were obtained. The mushroom samples were then ground into powder and subsequently used for extraction with methanol and ethanol solutions.



Figure 1: Samples of *H. endertii* fruiting body

Bacterial strains and culture condition: Fourteen strains from American Type Culture Collection were used to determine the antibacterial activity of mushroom extracts. These strains include four Gram-positive bacteria such as *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Staphylococcus saprophyticus* ATCC BAA-750 and ten Gram-negative bacteria such as *Escherichia coli* ATCC 25922, *Enterobacter hormaechei* ATCC 700323, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 13311, *Shigella sonnei* ATCC 25931, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 700232, *Salmonella enteritidis* ATCC13072, *Listeria monocytogenes* ATCC 19111 and *Shigella flexneri* ATCC 9199. All strains were stored at -80°C and pre-cultured overnight in Luria-Bertani broth at 37°C before use.

Preparation of *H. endertii* methanol and ethanol extracts: Twenty-five grams of dried mushroom powder were soaked in 1000 mL of 80% methanol or 80% ethanol (Thermo Fisher Scientific, USA) for 72 hours at room temperature. The extracts were then filtered three times through Whatmann papers and concentrated using a rotary evaporator under reduced pressure at 50°C to obtain solvent-free, concentrated brown extracts²⁰. These two extracts were subsequently used for analyzing mycochemical composition, antibacterial activity and antioxidant activity.

Analysis of mycochemical profile: The chemical composition of the mushroom extracts was analyzed using a

TRACE 1310 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an ISQ 7000 single quadrupole mass spectrometer. The separation was performed on a DB-5MS column (30 m × 0.25 mm × 0.25 μm), with helium as the carrier gas at a constant flow rate of 1.2 mL/min. Sample injections were conducted with a split ratio of 30:1, with a splitless time of 1 minute and a flow rate of 36 mL/min at an injection temperature of 250°C. Electron impact ionization was employed at 70 eV and the filament source temperature was maintained at 250°C. The oven temperature program began at 80°C for 5 minutes followed by an increase of 20°C/min to 280°C where it was held for 10 minutes. The mass spectrometer operated in a 2 scans per second scanning mode, covering a mass range of 29-650 m/z. Identification of chemical constituents was achieved by comparing the obtained mass spectra with those in the NIST 2017 library.

Antibacterial assay: The antibacterial activity of the mushroom extracts was determined using the disc diffusion method (CLSI, 2016)⁶. Bacterial strains were cultured in Luria-Bertani medium at 37°C until the culture turbidity reached 0.5 McFarland standard, then strains were spread on Mueller Hinton agar plates. Mushroom extracts (10 μL) were loaded onto the discs and the plates were incubated at 37°C for 16-18 hours. Antimicrobial activity against 14 bacterial strains was assessed based on the zones of growth inhibition. Gentamicin (10 μg, Nam Khoa BioTek, Vietnam) served as the positive control.

DPPH radical scavenging assay: The free radical scavenging activity of the mushroom extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Sample solutions of the extracts and DPPH were dissolved in methanol to prepare the test solutions. One milliliter of each extract, at varying concentrations, was mixed with 4 mL of DPPH solution (0.076 mM) and incubated in the dark for 30 minutes at room temperature. Following incubation, the absorbance was measured at 516 nm using a UV-Vis spectrophotometer (UVS 2800, Labome, USA).

The DPPH radical scavenging activity (DPPH_{RSA}) of the extracts was calculated using the formula:

$$\text{DPPH}_{\text{RSA}} (\%) = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}} \times 100\%$$

where $\text{Abs}_{\text{control}}$ represents the absorbance of the DPPH solution in methanol and $\text{Abs}_{\text{sample}}$ corresponds to the absorbance of the mixture of DPPH solution and methanol extract. The IC₅₀ value was determined from the concentration-response curve of the antioxidant activity of the extracts. The results were compared to ascorbic acid which served as the reference standard²⁹.

Data processing and statistical analyses: The experimental results represent the mean of three replicates. Data were processed and visualized using Microsoft Excel 2013 while

statistical analysis was performed using ANOVA through Statgraphics Centurion 18 software.

Results and Discussion

Chemical composition of *H. endertii* fruiting body: The extracts from *H. endertii* fruiting body revealed a chemical profile consisting of 16 distinct compounds in the methanol extract and 20 compounds in the ethanol extract. The methanol extract primarily contained 5-Methoxypyrrolidin-2-one (18.51%), cis-Vaccenic acid (15.41%), 10(E),12(Z)-Conjugated linoleic acid (14.03%), n-Hexadecanoic acid (13.95%), 2,5-Furandione, dihydro-3-methylene (8.25%), Cyclohexanone, 5-methyl-2-(1-methylethyl)-, O-methyloxime, (2S-trans)- (4.92%), But-3-enyl (E)-2-methylbut-2-enoate (4.59%) and 1H-Pyrazol-3-amine (4.29%). The remaining compounds constituted less than 3% of the total composition. In contrast, the ethanol extract was

dominated by 17-Octadecynoic acid (31.30%), cis-Vaccenic acid (23.50%), n-Hexadecanoic acid (16.32%), Octadecanoic acid (10.59%) and Ethyl α -d-glucopyranoside (5.99%) as the major components (Table 1).

Both *H. endertii* extracts contain significant amounts of *n*-hexadecanoic acid and cis-Vaccenic acid. The biological activities of *n*-hexadecanoic acid extracted from plant species, such as its antibacterial, antioxidant and anti-inflammatory properties, have been documented in prior studies^{2,11,28}. Additionally, cis-vaccenic acid may offer health benefits for humans as studies have found that higher levels of cis-vaccenic acid in red blood cell membranes correlate with a lower risk of heart failure among people who have previously experienced coronary heart disease¹⁰. Higher levels of cis-vaccenic acid in phospholipids have been associated with improved insulin sensitivity and β -cell function over a six-year period¹⁶.

Table 1
Chemical constituents of *H. endertii* fruiting bodie extracts

S.N.	RT (min)	Compounds	Methanol extract (%)	Ethanol extract (%)
1	3.56	2,5-Furandione, 3-methyl	-	0.46
2	3.59	2,5-Furandione, dihydro-3-methylene	8.25	-
3	4.79	1H-Pyrazol-3-amine	4.29	0.35
4	6.34	2-Pyrrolidinone	1.60	0.13
5	6.7	But-3-enyl (E)-2-methylbut-2-enoate	4.59	-
6	6.73	Pyridine, 2,3,4,5-tetrahydro-	-	0.37
7	7.36	Succinimide	1.81	-
8	7.95	5-Methoxypyrrolidin-2-one	18.51	-
9	8.57	Nicofuranose	-	0.20
10	8.7	l-Glutamic acid, 5-O-benzyl-1-O-ethyl(ester)	-	1.12
11	9.86	DL-Proline, 5-oxo-, methyl ester	2.69	-
12	9.99	3-Acetoxy-2(1H)-pyridone	1.32	0.10
13	11.53	9-Hexadecenoic acid	2.68	-
14	11.77	Ethyl α -d-glucopyranoside	-	5.99
15	12.53	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, O-methyloxime, (2S-trans)-	4.92	-
16	12.89	Pentadecanoic acid	-	0.97
17	13.46	n-Hexadecanoic acid	13.95	16.32
18	13.6	Hexadecanoic acid, ethyl ester	-	1.61
19	13.92	Heptadecanoic acid	-	0.39
20	14.11	8,11-Octadecadienoic acid, methyl ester	1.34	-
21	14.13	10-Octadecenoic acid, methyl ester	1.88	-
22	14.28	10(E),12(Z)-Conjugated linoleic acid	14.03	-
23	14.34	17-Octadecynoic acid	-	31.30
24	14.36	cis-Vaccenic acid	15.41	23.50
25	14.4	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	2.03	-
26	14.44	Octadecanoic acid	-	10.59
27	14.56	Ethyl 14-methyl-hexadecanoate	-	0.54
28	16.2	Phthalic acid, di(2-propylpentyl) ester	-	1.09
29	16.33	9-Hexadecenoic acid	-	0.15
30	17.78	Ethyl iso-allocholate	-	0.23
31	17.92	cis-13-Eicosenoic acid	-	0.10
Total			99.29	95.5

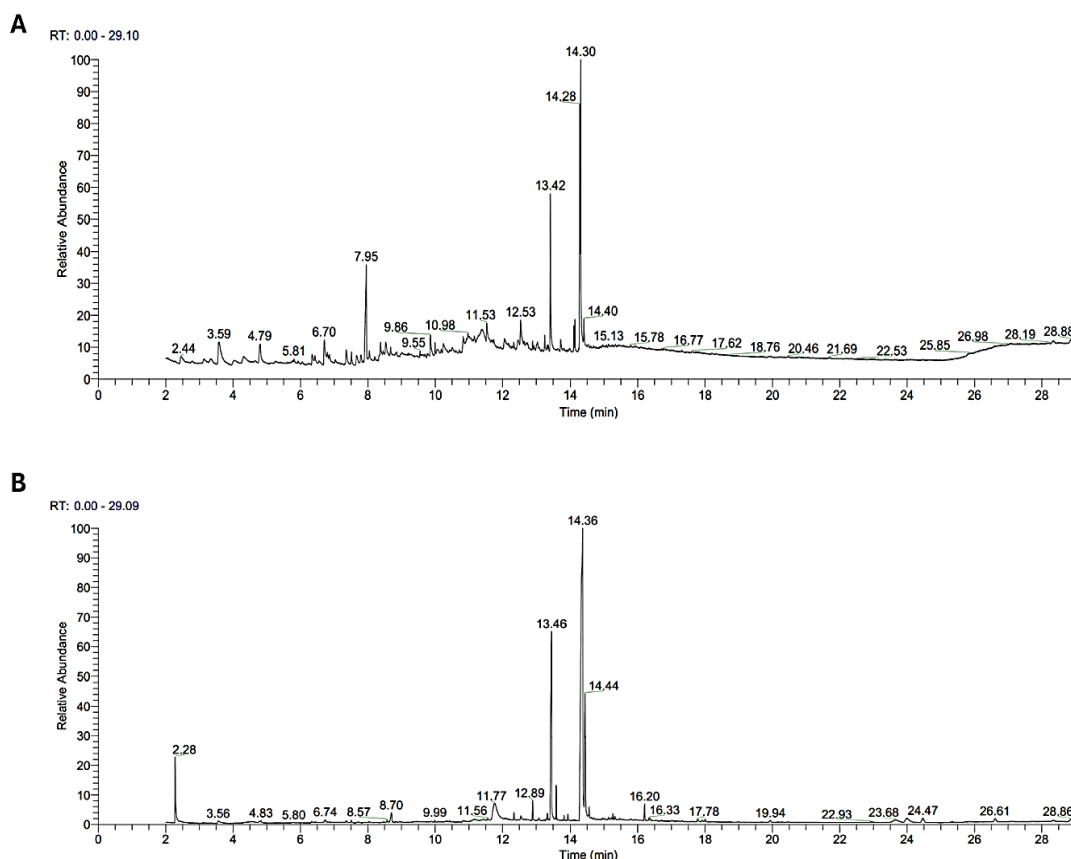


Figure 2: Gas chromatogram of methanol extract (A) and ethanol extract (B) from *H. endertii*

Table 2
Antibacterial activity of *H. endertii* extracts

Bacteria	Inhibition zone (mm)	
	Methanol extract	Ethanol extract
<i>S. aureus</i> ATCC 29213	8.3±0.6	8.0±0.0
<i>E. hormachei</i> ATCC 700323	7.0±0.0	7.0±0.0
<i>E. faecalis</i> ATCC 29212	9.0±1.4	9.7±1.2
<i>B. cereus</i> ATCC 11778	11.0±0.0	11.0±0.0
<i>S. sonnei</i> ATCC 25931	8.3±0.6	8.0±0.0
<i>P. aeruginosa</i> ATCC 27853	8.7±0.5	8.0±1.0
<i>S. typhimurium</i> ATCC 13311	7.0±0.0	7.0±0.0
<i>S. saprophyticus</i> ATCC BAA750	7.0±1.0	7.3±0.6
<i>E. coli</i> ATCC 25922	-	-
<i>E. cloacae</i> ATCC 700232	7.3±0.6	7.7±1.2
<i>S. flexneri</i> ATCC 9199	-	-
<i>S. enteritidis</i>	7.3±0.6	7.0±1.0
<i>L. monocytogenes</i>	-	-
<i>K. pneumoniae</i>	-	-

Table 3
Antioxidant activity of ethanol and methanol extracts from *H. endertii*

	Methanol extract	Ethanol extract	Ascorbic acid
IC ₅₀ (µg/mL)	634.78±12.86	639.38±7.89	13.92±0.14

The ethanol extract of *H. endertii* contains a significant amount of 17-octadecynoic acid (31.30%), a compound that inhibits cytochrome P450 enzymes and thus influences inflammatory processes, cardiovascular homeostasis and

related health conditions^{1,15,37}. Additionally, the methanol extract contains 5-Methoxypyrrolidin-2-one (18.51%), a compound with antifungal properties⁸ and 10(E),12(Z)-Conjugated linoleic acid (14.03%), which impairs adipocyte

triglyceride storage by promoting fatty acid oxidation, lipolysis and the generation of mitochondrial reactive oxygen species⁹.

Antibacterial activity of *H. endertii* extracts: Our findings demonstrate promising antibacterial activity against ten out of fourteen tested bacterial strains including both Gram-negative and Gram-positive bacteria. Specifically, both extracts exhibited antibacterial effects against *S. aureus* ATCC 29213, *E. hormachei* ATCC 700323, *E. faecalis* ATCC 29212, *B. cereus* ATCC 11778, *S. sonnei* ATCC 25931, *P. aeruginosa* ATCC 27853, *S. typhimurium* ATCC 13311, *S. saprophyticus* ATCC BAA750, *E. cloacae* ATCC 700232 and *S. enteritidis* ATCC 13072, with inhibition zone diameters ranging from 7.0 mm to 11 mm (Table 2). Notably, these effects are consistent with the results from the previous study on *H. endertii* aqueous extract which exhibited antibacterial activity against *B. cereus*, *S. aureus*, *P. aeruginosa*, *S. enteritidis* and *S. typhimurium*, with inhibition zones ranging from 6.0 mm to 18.0 mm.

The antibacterial effects of the methanol and ethanol extracts against most tested bacteria were similar, though the inhibition of *S. typhimurium* was slightly lower compared to the aqueous extract²⁶. Additionally, the ethanol extract from *H. endertii* displayed comparable antibacterial inhibition zones to those of the ethanol extract from *G. lucidum* against *P. aeruginosa* and *S. aureus* while the methanol extract showed lower inhibition zones than its counterpart from *G. lucidum*³⁰.

Antioxidant activity of extracts from *H. endertii*: While the antioxidant activity of medicinal mushrooms in the Ganodermataceae family is well-documented, this study reported on the antioxidant activity of extracts from *H. endertii* fruiting bodies. Both methanol and ethanol extracts from *H. endertii* demonstrated DPPH radical scavenging activity, with IC₅₀ values of 634.78±12.86 µg/mL and 639.38±7.89 µg/mL respectively (Table 3). Within the Ganodermataceae family, *H. endertii* extracts show lower DPPH radical scavenging activity (IC₅₀ ≈ 635 µg/mL) compared to ethanol extracts of *Ganoderma* spp. from the Balkans (2.55-10.11 µg/mL)³¹ and *G. adspersum* from Turkey (48.00 µg/mL)³², as well as methanol extracts of *G. adspersum* from Greece (21.45 µg/mL)⁵ and *G. lucidum* from Turkey (47.6 µg/mL)³³.

It is worth noting that although the DPPH radical scavenging activity of *H. endertii* extracts is lower compared to a few long-established medicinal mushrooms species, these extracts demonstrate higher antioxidant activity than several others. For instance, the *H. endertii* extracts show much stronger antioxidant properties than those of methanol extracts from *Pleurotus ostreatus* (321-3,486 µg/mL)³ and *Phlebopus portentosus* (2110-3650 µg/mL)¹⁸ as well as ethanol extracts from *Pleurotus flabellatus* (2130-3150 µg/mL)²⁷ and various other mushrooms including *Agaricus bisporus* var *brunnescens*, *Pleurotus ferulae*, *Lentinula*

edodes, *Hericium coralloides*, *Pholiota nameko*, *Flammulina velutipes* var. white and *P. ostreatus*, which have IC₅₀ values ranging from 640 µg/mL to 3400 µg/mL¹⁹.

Conclusion

The Ganodermataceae family has been widely used in health food and pharmaceutical products. This study presents the detailed chemical profile and biological activity analysis of methanolic and ethanolic extracts from *H. endertii*, a recently discovered species in Vietnam. Both extracts contain n-hexadecanoic acid and cis-vaccenic acid as major compounds but differ significantly in their overall chemical composition and concentrations. Both extracts demonstrated broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria, as well as antioxidant properties through DPPH radical scavenging, suggesting potential applications for *H. endertii* extracts in the pharmaceutical industry and related fields.

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