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RESEARCH ARTICLE

# Targeted Phenolic Analysis in *Hericium erinaceum* and Its Antioxidant Activities

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**Abstract** The $ABTS^{+}$-radical-cation scavenging activity, DPPH radical scavenging activity, ferric reducing/antioxidant power, and nitrite scavenging activity of the methanol extract from *Hericium erinaceum* and its subfractions were assessed. Among the methanol extract subfractions tested, the chloroform subfraction exhibited the strongest antioxidant activity in the most experiments, except for the ferric reducing/antioxidant power. The Trolox equivalent antioxidant capacity (TEAC) of the chloroform subfraction was 378.89 μmol/g of sample. This subfraction also scavenged 35.80% of DPPH radicals at 500 μg/mL. The highest ferric reducing/antioxidant power was found in the *n*-hexane subfraction (174.82 μmol $FeSO_4 \cdot 7H_2O$/g). The chloroform, *n*-hexane, and *n*-butanol subfractions had high total phenolic compound content, with ferulic acid equivalents of 35.18, 19.08, and 11.23 mg/g, respectively. Flavonoids were found mostly in the chloroform subfraction, and the 4 phenolic compounds were identified in the same fraction as 4-hydroxybenzoic acid, syringic acid, 4-coumaric acid, and ferulic acid by electrospray ionization (ESI) LC-MS/MS analysis.

**Keywords:** mushroom, *Hericium erinaceum*, antioxidant activity, phenolic acid, electrospray ionization (ESI) LC-MS/MS

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## Introduction

*Hericium erinaceum* is a traditional edible mushroom that has been used as an herbal medicine in east Asia. The mushroom is called 'yamabushitake' in Japan and 'houtou' in China and is consumed in both countries. *H. erinaceum* can be found growing on old or dead broadleaf trees, and it has been successfully cultivated indoors on conifer sawdust. Various biological functions of *H. erinaceum* have been investigated in recent reports including its antimicrobial effect (1), anti-tumor activity (2), immunomodulatory effects (3), hypolipidemic effects, and promotion the nerve growth factor (NGF) synthesis (4). This mushroom has biologically active polysaccharides that have potent antitumor activity with immunomodulating properties. A number of bioactive molecules, including antitumor substances, have been identified in *H. erinaceum*. Galactoxyloglucan, xylan, and glucoxylan are antitumor and immunostimulating polysaccharides that have been extracted from *H. erinaceum* (5). Polysaccharides exert their antitumor effects primarily by activating various immune system responses in the host including complement system activation, macrophage-dependent immune system responses, and upregulation of interferon expression. These polysaccharides have various chemical compositions and belong primarily to the β-glucan group (6). *H. erinaceum* has not only been reported to have a hypoglycemic effect, but it is also able to reduce the rates of serum triglyceride and total cholesterol elevation when administered. The treatment of diabetes often involves medication to control blood glucose levels, although some of these medications have undesirable side-effects. Thus, *H. erinaceum* with

hypoglycemic effects can be safely used in the management of diabetes (7).

Mushrooms can be used in traditional medicines worldwide due to their high levels of various phenolic compounds that are widely recognized as excellent antioxidants (8). Generally the antioxidant activities of several edible mushrooms have been found to be correlated with their total phenolic and flavonoid contents (9). Phenolic compounds have been associated with the inhibition of atherosclerosis and cancer (10,11), and their bioactivity may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (12). Novel prenylated phenolics, asiaticusin A and B from an edible mushroom, *Boletinus asiaticus* (13), and 2 *p*-terphenyls from *Paxillus panuoides* were found to inhibit lipid peroxidation (14).

Several undesirable features of synthetic antioxidants used in the food industry have been discovered, including adverse side effects. This situation prompted some researchers to seek antioxidants from a natural source. So far, there have been few investigations of antioxidants derived from edible mushrooms that are cultivated artificially. These mushrooms have only recently become available on the market, and their health benefits, especially their antioxidant properties, have become the subject of intense research interest.

In the present study, the antioxidant properties of *H. erinaceum* were investigated and the major phenolic acid in this organism was identified. The antioxidant activities of various subfractions of the methanol extract from *H. erinaceum* were measured, and the profiles of major phenolic components in the subfractions were examined using electrospray ionization (ESI) LC-MS/MS.

## Materials and Methods

**Materials and chemical reagents** The fruit bodies of *H. erinaceum* were purchased from an open market in Seoul, Korea. Twelve phenolic standards (4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, 4-coumaric acid, ferulic acid, sinapic acid, caffeic acid, gallic acid, gentisic acid, protocatechuic acid, and α-resorcylic acid), DPPH, Folin-Ciocalteu phenol, quercetin, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Preparation of subfractions by systemic extraction** Freeze-dried mushrooms were ground into a powder using a food mixer (20 mesh), and 100 g of powder was extracted with 2 L of 80% methanol for 48 h at room temperature with shaking. After filtration through No. 4 Whatman filter paper, an equal volume of *n*-hexane was added to the filtrate, and the 80% methanol and *n*-hexane mixture would give upper and lower phase separation. An equal volume of chloroform was added to the lower phase, and the chloroform layer was separated from the partitioned solution. The same liquid-liquid partitioning procedure was used to obtain ethyl acetate, *n*-butanol, and the final aqueous subfraction. Five subfractions were evaporated separately with an evaporator (Eyela; Rikakikai Co., Tokyo, Japan) to dryness at 40°C under reduced pressure, and the yields were calculated (Table 1).

**Determination of total phenolic and total flavonoid contents** The total phenolic contents were measured using a modified version of Gutfinger's method (15). A small aliquot (0.1 mL) of sample extract was mixed with 0.05 mL of distilled water and 0.05 mL of Folin-Ciocalteu's reagent. After the addition of 0.2 mL $Na_2CO_3$ (10% aqueous solution) to the mixture, the mixture stood for 25 min at room temperature and subsequently centrifuged at 3,000×*g* for 20 min. The absorbance was then measured at 725 nm using a UV-visible spectrophotometer (Uvikon 933; Kontron Instruments Ltd., Milan, Italy) in order to determine the content of the phenolic compounds in each extract. Ferulic acid was used as a control to quantify the phenolic content in the sample (expressed in equivalent mg/g).

The flavonoid contents of the extracts were determined by the method described by Moreno *et al.* (16), with minor modifications. The extracts were dissolved in 80% methanol, and 0.1 mL of the sample was mixed with 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 μM potassium acetate, and 4.7 mL of 80% ethanol. After incubation for 40 min at room temperature, the absorbance of the solution was measured at 415 nm using a UV-visible spectrophotometer (Uvikon 933; Kontron Instruments Ltd.). Quercetin was used as a control flavonoid to calculate the standard curve. The antioxidant compounds were analyzed in triplicate, and the results are presented as mean± standard deviation (SD) values.

**Determination of $ABTS^+$-radical-cation scavenging activity** The $ABTS^+$ scavenging activity was measured using the method described by Re *et al.* (17), with minor modifications. The $ABTS^+$ reagent (7 mM ABTS with 2.45 mM $K_2S_2O_8$) was mixed with 10 μL of sample in methanol, and Trolox was prepared in a concentration range of 0.5-2 mM as the standard in order to generate a dose-response curve of the percent inhibition at different concentrations. The antioxidant activity of the samples was expressed as the Trolox equivalent antioxidant capacity (TEAC), which represents the mM concentration of Trolox having the same activity as 1 mg of sample. After the mixture was kept in a dark at room temperature for 12 min to allow for complete free-radical generation, the absorbance was measured at 734 nm using a spectrophotometer. The

percent inhibition of the samples was calculated as $(1-A_{sample}/A_{control})\times100$, where, $A_{sample}$ and $A_{control}$ represent the absorbance at 734 nm in the presence and absence of the sample, respectively.

**Ferric reducing/antioxidant power (FRAP) assay** The FRAP assay was performed according to the method reported by Bakar *et al.* (18). Briefly, 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution, and 20 mM $FeCl_3\cdot6H_2O$ were mixed at a 10:1:1 ratio, and the mixture was heated to 37°C in a water bath to generate the FRAP working solution. One-hundred μL of sample, 300 μL of distilled water, and 3 mL of FRAP reagent were mixed in a test tube, incubated at 37°C for 90 min, and then the absorbance was measured at 593 nm. Distilled water was used as a blank. $FeSO_4\cdot7H_2O$ was used as the standard at concentrations between 100 and 1,000 μM.

**DPPH radical scavenging activity** The DPPH radical scavenging activity was measured according to the method described by Chung *et al.* (19), with slight modifications. Briefly, 100 μL of sample extracts resolved in methanol at various concentrations (2.5, 5, 10, and 15 mg/mL) were mixed with 400 μL of Tris-HCl buffer (100 mM, pH 7.4) and 500 μL of DPPH solution (100 μM DPPH in methanol). The mixture was shaken vigorously, and then left to stand for 30 min in the dark. The absorbance was measured at 517 nm. The DPPH radical scavenging activity (%) was calculated as a percentage according to $(1-A_{sample}/A_{control})\times100$, where, $A_{sample}$ and $A_{control}$ represent the absorbance in the presence and absence of the sample, respectively. All of the extracts were analyzed in triplicate.

**Nitrite scavenging activity** The nitrite scavenging activity was determined using the method reported by Kang *et al.* (20). The pH of 1 mL of sample mixed with 1 mL of 1 mM $NaNO_2$ was adjusted to 1.2 with 0.1 N HCl in a test tube, and the final volume was adjusted to 10 mL with distilled water. The mixture was incubated at 37°C for 1 h, and 1 mL of the mixture was mixed with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent. After the mixture was kept at room temperature for 15 min, the absorbance was measured at 520 nm. The blank was distilled water instead of Griess reagent. Ascorbic acid was used as the positive control.

**Identification and quantification of phenolic acids by ESI LC-MS/MS analysis** Antioxidant compounds were extracted according to the method reported by Krygier *et al.* (21), with minor modifications. Each subfraction was prepared as described in the method sections for the preparation of subfractions by systemic extraction. Dried subfractions were dissolved in 80% methanol, and then concentrated to 1/5 by evaporation under vacuum at 40°C (Eyela; Rikakikai Co.). The pH was adjusted to 2 with HCl solution (6 N), and the cloudy precipitate was removed by centrifugation, which contained fatty acids. The supernatant was collected and analyzed for the identification of phenolic compounds by ESI LC-MS/MS analysis.

The major phenolic components in the extracts were identified using an ESI mass spectrometry system (LTQ Velos; Thermo Scientific, Waltham, MA, USA) in negative mode, 12 phenolic standards including 4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, 4-coumaric acid, ferulic acid, sinapic acid, caffeic acid, gallic acid, gentisic acid, protocatechuic acid, and α-resorcylic acid. Sample (10 μL) was injected into an HPLC system and separated using a reverse-phase C18 column (100×2.0 mm, i.d., 3 μm, UK-C18; Union, Imtakt, Japan). The mobile phase consisted of 0.1% aqueous formic acid (solvent A), and acetonitrile solution containing 0.1% formic acid (solvent B). The non-linear gradient elution was as follows: 5% B at 0 min, 30% B at 12 min, 30% B at 30 min, 50% B at 32 min, 95% B at 34 min, 95% B at 36 min, 5% B at 38 min, and 5% B at 40 min. The flow rate was 200 μL/min and the injection volume was 10 μL. The capillary temperature was 250°C, and the capillary voltages were adjusted to −10 V for the negative mode. The level of phenolic acid was determined by ESI-MS/MS analysis using same ESI MS system (LTQ Velos; Thermo Scientific) in negative mode by comparison peak area of daughter ions of samples with those of standards using calibration curve.

**Statistical analysis** All experiments were performed in triplicate, and the values are expressed as means±standard deviations (SD). All of the data were analyzed with one-way analysis of variance (ANOVA) to compare the antioxidant activity of each sample ($p<0.05$).

## Results and Discussion

**Total phenolic and flavonoid contents** Extraction yields of all of the subfractions from the freeze-dried mushrooms are shown in Table 1. The total extract yield was about 22% in all of the methanol extract subfractions. The *n*-butanol and water subfractions showed high yields of 10.81 and 7.92%, respectively, followed by the ethyl acetate subfraction with a yield of 2.75%. This implies that most of the soluble components in the mushroom were highly polar. Previous reports suggested that the biological activity of several mushrooms was mainly attributable to polysaccharides, especially the high molecular weight glucans and polysaccharide protein complexes exhibiting

**Table 1. Extraction yields, total phenolic, and flavonoid contents, ABTS$^+$ radical scavenging activity, and ferric reducing/ antioxidant powder of various subfractions of the methanol extract from *H. erinaceum***

| Solvent | Yield (%)[1)] | Total phenolics (mg FAE/g extract) | Total flavonoids (mg QE/g extract) | TEAC (μmol Trolox/g extract) | FRAP (μmol $FeSO_4 \cdot 7H_2O$/g extract) |
|---|---|---|---|---|---|
| *n*-Hexane | 0.33±0.07$^{a2)}$ | 19.08±1.06$^{d}$ | 0.62±0.12$^{b}$ | 111.12±7.60$^{bc}$ | 174.82±14.07$^{c}$ |
| Chloroform | 0.48±0.26$^{a}$ | 35.18±0.71$^{e}$ | 1.46±0.24$^{d}$ | 378.89±6.99$^{d}$ | 10.66±1.03$^{a}$ |
| Ethyl acetate | 2.75±2.00$^{ab}$ | 6.37±0.32$^{b}$ | 1.16±0.05$^{c}$ | 98.70±9.90$^{b}$ | ND |
| *n*-Butanol | 10.81±3.43$^{c}$ | 11.23±0.32$^{c}$ | 0.38±0.03$^{b}$ | 126.32±4.94$^{c}$ | 111.12±8.23$^{b}$ |
| Water | 7.92±1.47$^{bc}$ | 4.36±0.29$^{a}$ | 0.07±0.06$^{a}$ | 45.54±3.08$^{a}$ | 19.46±1.05$^{a}$ |

[1)]Based on total dry mushroom weight; FAE, ferulic acid equivalents; QE, quercetin equivalents; TEAC, Trolox equivalent antioxidant capacity; FRAP, ferric reducing/antioxidant power
[2)]Values are averages±SD of 5 different fractions analyzed in triplicate; Different superscripts within a column denote statistically significant differences ($p<0.05$); ND, not detected

anticancer properties (22), as well as polyphenols and flavonoids, which are responsible for the anticytotoxic and antioxidant activities (23).

The methanol extract including the *n*-hexane and chloroform subfractions contained larger amounts of total phenolics, which might be related to the antioxidant activities of these hydrophobic subfractions. The total phenolic and flavonoid contents were evaluated, as shown in Table 1. Hydrophobic subfractions such as *n*-hexane and chloroform exhibited high levels of phenolic compounds at 19.08 (extract) and 35.18 mg/g (extract), respectively. The *n*-butanol subfraction contained a phenolic content of 11.23 mg/g (extract) and a flavonoid content of 0.38 mg/g (extract). The level of flavonoids in the chloroform subfraction was 1.46 mg/g (extract). The lowest phenolic and flavonoid contents were observed in the water subfraction at 4.36 (extract) and 0.07 mg/g (extract), respectively. Gursoy *et al.* (24) reported that a methanol extract from *Morchella rotunda* contained a total phenolic content of 16.98 mg/g (extract) and a flavonoid content of 0.59 mg/g (extract). They also reported that the total phenolic and flavonoid contents in *M. deliciosa* were 12.36 (extract) and 0.15 mg/g (extract), respectively.

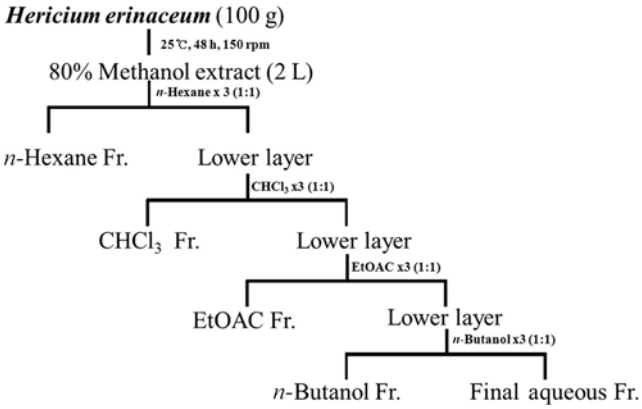


**Fig. 1. Preparation of *H. erinaceum* subfractions by systemic extraction.**

**FRAP and ABTS$^+$ radical scavenging activity** The ability of the subfractions to chelate ferrous ions are expressed as μmol of $FeSO_4 \cdot 7H_2O$/g (extract). In some cases, food may deteriorate with lipid peroxidation which induced by metal ions. Ferrous ions are the most effective pro-oxidants, and are commonly found in food systems (25). FRAP refers to the antioxidant effect exerted by the donation of a hydrogen atom and subsequent breakage of the free radical chain. The *n*-hexane subfraction showed the highest FRAP of 174.82 $FeSO_4 \cdot 7H_2O$/g (extract), and the *n*-butanol subfraction exhibited a 111.12 μmol $FeSO_4 \cdot 7H_2O$/g (extract) FRAP. Other antioxidant activity, the chloroform subfraction exhibited the lowest FRAP with 10.66 μmol $FeSO_4 \cdot 7H_2O$/g (extract) among tested subfractions. Gursoy *et al.* (24) reported that methanolic extracts of *M. rotunda* mushroom showed similar reducing power concentration dependently. At 4.5 mg/mL concentration, the reducing power was higher than 0.50 for the all extracts. The mushroom (*Morchella conica*) exhibited an absorbance value of 1.055 at 700 nm. Reducing power of butylated hydroxyltoluene (BHT), butylated hydroxyl anisole (BHA), and quercetin at 0.02 mg/mL were 0.163, 0.321, and 0.459, respectively.

In the ABTS$^+$ radical scavenging activity expressed by TEAC value, the higher value means stronger antioxidant activity. The ABTS$^+$ is generated by reaction of ABTS with potassium persulfate and the radical was decolorized when it reacted with hydrogen-donating antioxidant. In our experiment, the chloroform subfraction of the methanol extract from *H. erinaceum* showed the highest ABTS$^+$ radical scavenging activity of 378.89 mg/g (extract), and the water subfraction showed the lowest at 45.54 μmol Trolox/g (extract). The *n*-hexane and *n*-butanol subfractions exhibited 111.12 (extract) and 126.32 μmol Trolox/g (extract), respectively. The methanol extract of *M. conica* exhibited high radical scavenging activity (78.66%) at 40 μg/mL and similar activity was observed in the methanol extracts of *M. esculenta* var. *umbrina* and

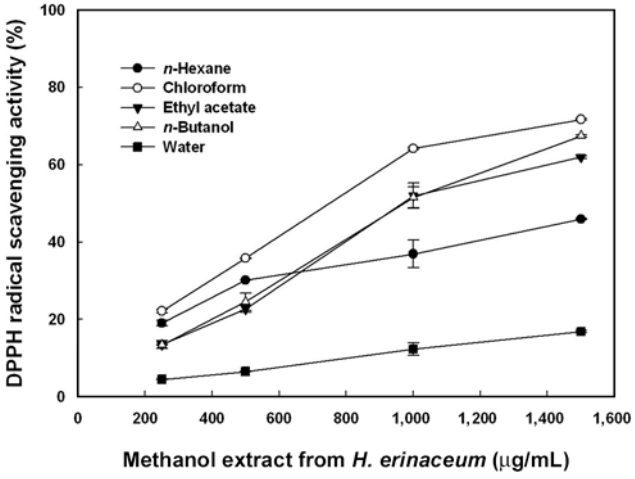


**Fig. 2. DPPH radical scavenging effects of the various fractions of the methanol extract from *H. erinaceum*.**

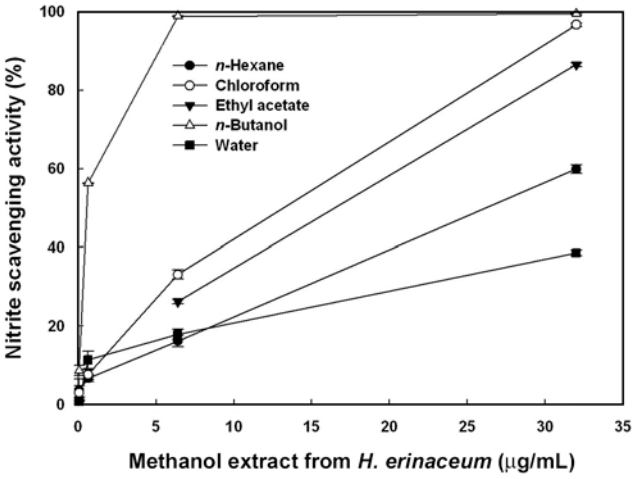


**Fig. 3. Nitrite scavenging activities of the various fractions of the methanol extract from *H. erinaceum*.**

*Morchella rotunda* (24).

**DPPH radical scavenging activity** Free radicals generated in biological systems via metabolic reactions can cause chronic arterial, immune, neuronal, and ischemic heart diseases. The DPPH radical is stable at 517 nm, and it was used to study the radical scavenging activity of each sample in this study. The DPPH radical scavenging activities of all of the subfractions are shown in Fig. 2. The chloroform subfraction exhibited the highest DPPH radical scavenging activity among the tested subfractions, while the water subfraction showed the lowest scavenging activity. The DPPH radical scavenging activities exhibited by the *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and water subfractions at 1.5 mg/mL were 45.91, 71.70, 61.97, 67.46, and 16.83%, respectively. The DPPH radical scavenging activities of all subfractions were increased in a concentration-dependent manner. The half maximal scavenging concentration ($IC_{50}$) can be determined by constructing a dose-response curve and examining the effect of different concentrations of the sample on antioxidant activity. The $IC_{50}$ values of the *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and water subfractions were 1.6, 0.7, 1.2, 1.1, and 4.8 mg/mL, respectively, indicating that the hydrophobic subfractions exhibited stronger DPPH radical scavenging activities than the hydrophilic subfractions. Gursoy *et al*. (24) reported that the radical scavenging activities of methanol extracts from *M. rotunda, M. crassipes, M. esculenta* var. *umbrina, M. deliciosa, M. lata, M. conica,* and *M. angusticeps* at 2 mg/mL were 33.94, 34.82, 33.68, 19.24, 22.54, 43.27, and 22.90%, respectively. Barros *et al*. (26) reported that the methanol extracts from *Agaricus arvensis* and *Cantharellus cibarius* exhibited $IC_{50}$ values of 15.85 and 19.65 mg/mL, respectively. On the other hand, the methanol extracts from *Hypholoma fasciculare* and *Leucopaxillus giganteus* exhibited $IC_{50}$ values of 1.13 and 1.44 mg/mL, respectively. The chloroform subfraction of *H. erinaceum* showed relatively good antioxidant activity compared to the extracts described in these previous reports.

**Nitrite scavenging activity** Nitroamines are produced by nitrites and secondary or tertiary amine reactions can be carcinogenic. The consumption of nitrites contained in food can result in the formation of nitrosamines that can attack bodily tissues and result in adverse effects. Many substances such as phenolic compounds, organic acids, and vitamins have been reported to scavenge nitrites (27). The nitrite scavenging activities of all of the subfractions from the methanol extract of *H. erinaceum* are shown in Fig. 3. The nitrite scavenging activities of the subfractions exhibited dose-dependent increases. The highest scavenging activity was observed in the *n*-butanol subfraction, while the water subfraction showed the lowest scavenging activity. The *n*-butanol subfraction exhibited 56.33% scavenging activity at a concentration of 0.64 mg/mL; however, the chloroform subfraction exhibited only 7.64% scavenging activity at the same concentration. The nitrite scavenging activities of the *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and water subfractions at 32 mg/mL were 59.87, 96.70, 86.51, 99.47, and 38.64%, respectively. The nitrite scavenging activity of ascorbic acid, the positive control, was much stronger than that of the subfractions from the methanol extract, at 10.25, 36.83, 53.56, and 98.98%, for concentrations of 0.064, 0.32, 0.64, and 3.2 mg/mL, respectively. The nitrite scavenging activities of the subfractions from the methanol extract of *H. erinaceum* were relatively high compared to that of the ethanol extract from *Gardenia jasminoides Ellis*, which scavenged 56.8% of the nitrite present at a concentration of 2.0 mg/mL. The distilled water extract from this fruit showed a scavenging activity of 68.5% (28).

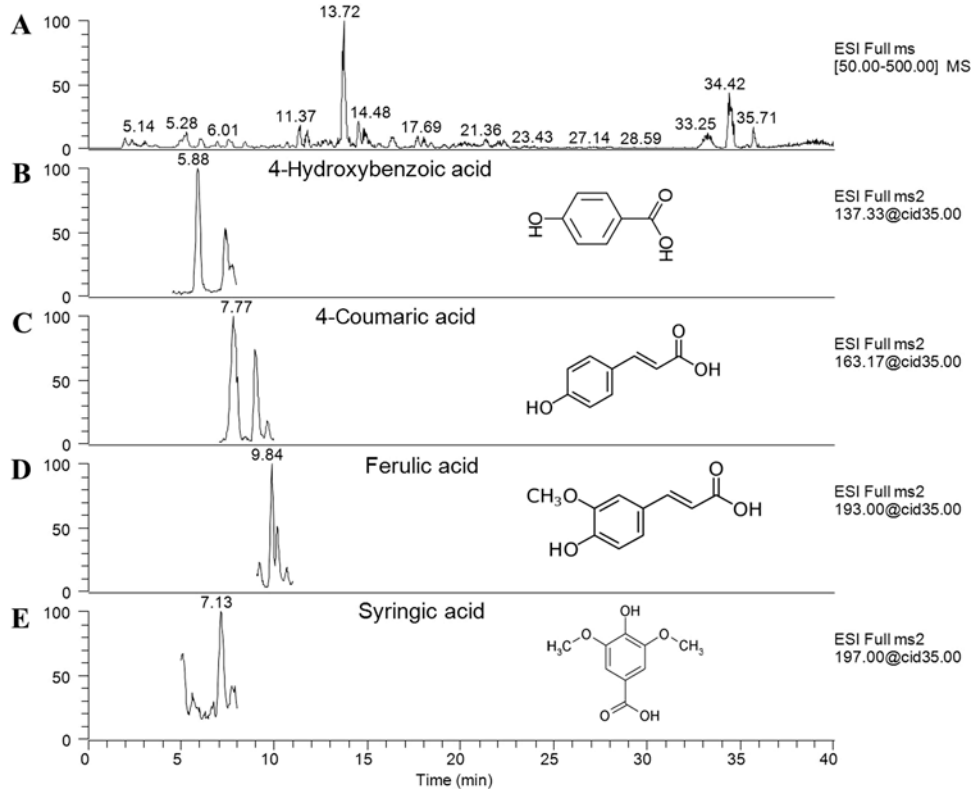


**Fig. 4. Full mass spectrum of the chloroform extract (A) and MS/MS analysis of phenolic compounds in the same sample; 4-hydroxybenzoic acid (B), 4-coumaric acid (C), ferulic acid (D), and syringic acid (E).**

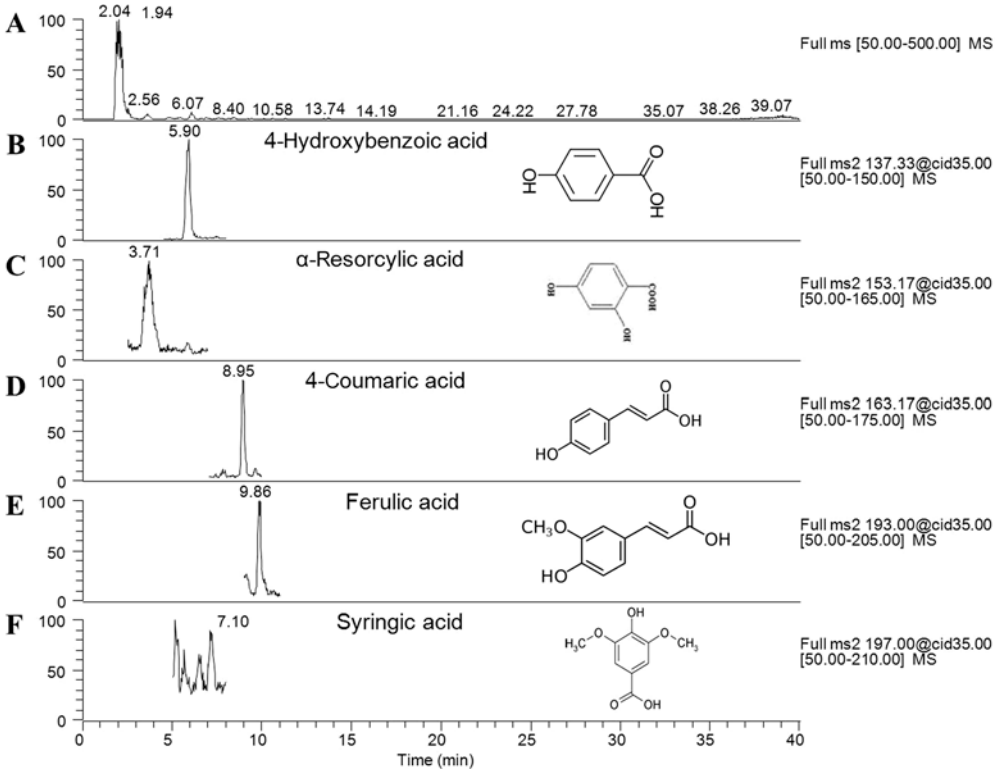


**Fig. 5. Full mass spectrum of the ethyl acetate extract (A) and MS/MS analysis of phenolic compounds in the same sample; 4-hydroxybenzoic acid (B), α-resorcylic acid (C), 4-coumaric acid (D), ferulic acid (E), and syringic acid (F).**

**Table 2. Phenolic acids identified by a comparison of ESI LC-MS/MS spectra to the standards**

| Compound | $CHCl_3$[1)] (μg/g extract) | EtOAC (μg/g extract) | Dry weight (μg/g extract) | Retention time | MS(*m/z*) (M-H) | MS/MS (*m/z*) |
|---|---|---|---|---|---|---|
| 4-Hydroxybenzoic acid | 10.88 | 24.73 | 0.73 | 5.83 | 137 | 93 |
| α-Resorcylic acid | ND | 8.73 | 0.24 | 3.71 | 153 | 109 |
| 4-Coumaric acid | 2.98 | 2.00 | 0.069 | 8.87 | 163 | 119 |
| Ferulic acid | 245.83 | 169.82 | 5.85 | 9.77 | 193 | 178, 149, 134 |
| Syringic acid | 189.58 | 33.91 | 1.82 | 7.12 | 197 | 182, 153, 135 |

[1)]$CHCl_3$, chloroform fraction; EtOAC, ethyl acetate fraction; Dry weight, (mg/kg d.w. of mushroom)=total (chloroform+ethyl acetate)

**Identification and quantification of phenolic acids by ESI LC-MS/MS analysis** Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes, and steroids. Among these secondary metabolites, phenolic compounds from *Ganoderma lucidum* were reported to be correlated with free radical scavenging activity, reducing power, and lipid peroxidation (29). In the present study, major phenolic acids in the chloroform subfraction exhibiting maximum antioxidant activity were profiled by a comparison of their ESI LC-MS/MS spectra to the standards (Fig. 4). Some phenolic acids, such as 4-hydroxybenzoic acid, 4-coumaric acid, ferulic acid, and syringic acid were identified in the chloroform extract of *H. erinaceum*. In addition, α-resorcylic acid could be identified in ethyl acetate subfraction with other four phenolic acids as shown in Table 2. The levels of ferulic acid, syringic acid, 4-hydroxybenzoic acid, and 4-coumaric acid in chloroform subfraction were 245.83, 189.58, 10.88, and 2.98 μg/g (extract), respectively. The level of α-resorcylic acid in ethyl acetate subfraction was 8.73 μg/g (extract) and the contents of 4-hydroxybenzoic acid was higher (24.73 μg/g extract) than in chloroform subfraction. There was no difference in concentration of 4-coumaric acid between chloroform and ethyl acetate subfractions and lower levels of ferulic acid (169.82 μg/g extract) and syringic acid (33.9 μg/g extract) was found in ethyl acetate subfraction. No phenolic acids were detected in other subfrations including *n*-hexane, *n*-butanol, and water subfractions (Table 2).

Total levels of phenolic acid in fruiting body of *H. erinaceum* could be calculated from these results as 0.73, 0.24, 0.069, 5.85, and 1.82 mg/g fruiting bodies for 4-hydroxybenzoic acid, α-resorcylic acid, 4-coumaric acid, ferulic acid, and syringic acid, respectively. Heleno *et al*. (30) reported that protocatechuic acid (2.02 mg/kg d.w.), 4-hydroxybenzoic acid (6.55 mg/kg d.w.), and 4-coumaric acid (1.17 mg/kg d.w.) were present in the wild *Boletus* species. The profile and levels of phenolic acids of *H. erinaceum* were quite different from the result by Heleno *et al.* (30). Further study including identification of other phenolic acids will be performed to explain the antioxidant activity of this mushroom.

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