Genetics and Molecular Research

Antioxidant activity, anti-proliferative activity, and amino acid profiles of ethanolic extracts of edible mushrooms

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ABSTRACT. Biological activities of various mushrooms have recently been discovered, particularly, immunomodulatory and antitumor activities. Herein, three edible mushrooms, *Auricularia auricula-judae* (AA), *Pleurotus abalonus* (PA) and *Pleurotus sajor-caju* (PS) extracted using Soxhlet ethanol extraction were evaluated for their antioxidative, anti-proliferative effects on leukemia cells. Using the Folin-Ciocalteau method and Trolox equivalent antioxidant capacity assay, phenolics

and antioxidant activity were found in all sample mushrooms. Additionally, anti-proliferative activity of mushroom extracts against U937 leukemia cells was determined using a viability assay based on mitochondrial activity. PA (0.5 mg/mL) and AA (0.25-0.5 mg/mL) significantly reduced cell viability. Interestingly, PS caused a hormetic-like biphasic dose-response. Low doses (0-0.25 mg/L) of PS promoted cell proliferation up to 140% relative to control, whereas higher doses (0.50 mg/mL) inhibited cell proliferation. Against U937 cells, AA IC_{50} was 0.28 ± 0.04 mg/mL, which was lower than PS or PA IC_{50} (0.45 ± 0.01 and 0.49 ± 0.001 mg/mL, respectively). Furthermore, lactate dehydrogenase (LDH) leakage conferred cytotoxicity. PS and PA were not toxic to U937 cells at any tested concentration; AA (0.50 mg/mL) showed high LDH levels and caused 50% cytotoxicity. Additionally, UPLC-HRMS data indicated several phytochemicals known to support functional activities as either antioxidant or anti-proliferative. Glutamic acid was uniquely found in ethanolic extracts of AA, and was considered an anti-cancer amino acid with potent anti-proliferative effects on U937 cells. Collectively, all mushroom extracts exhibited antioxidant effects, but their anti-proliferative effects were dose-dependent. Nevertheless, the AA extract, with highest potency, is a promising candidate for future applications.

Key words: *Auricularia auricula-judae*; *Pleurotus abalonus*; *Pleurotus sajor-caju*; Antioxidation; Anti-proliferation; Amino acid profiles

INTRODUCTION

Several kinds of medicinal and edible mushrooms are found in different locations worldwide. Mushrooms contain various compounds such as polysaccharides, proteins, fats, glycosides, phenols, flavonoids, carotenoids, and organic acids (Patel and Goyal, 2012). A number of studies showed that many mushrooms exhibit biological and medicinal properties, including antitumor activities, stimulatory effects on the immune cells (Maiti et al., 2008), and antioxidant activities (Kanagasabapathy et al., 2011; Umeo et al., 2015). *Pleurotus sajor-caju* (PS), *Pleurotus abalonus* (PA), and *Auricularia auricula-judae* (AA) are common edible mushrooms known to possess several chemical properties. Several lines of evidence demonstrated that polysaccharides from PS, PA and AA could inhibit proliferation of tumor cells. For example, water-soluble extracts from PS displayed antitumor activity against Sarcoma 180 (Zhuang et al., 1993). Ren et al. (2015) demonstrated that polysaccharides from PA exert potent antioxidant and anti-proliferative activities against LoVo cancer cells. Other researchers showed that the polysaccharide-peptide complex from the fruit bodies of PA induced anti-proliferative activities against human hepatoma (HepG2) and human breast cancer cells (MCF-7) (Li et al., 2012). Anti-proliferative effects of AA on acinar cell carcinoma and sarcoma 180 cells were also described previously (Ma et al., 2010; Reza et al., 2011). However, the effect of these three mushrooms on human leukemic cells has not been investigated.

Acute myelocytic leukemia (AML) is a rare disease. It accounts for approximately 90% of all acute leukemias in adults, but is infrequent in children (Weinstein, 1999). The

survival rate of AML patients is the lowest among all leukemia patients, and poor prognosis is observed in older patients. (Sandler and Ross, 1997; Redaelli et al., 2003). The highest incidence of AML is found in the United States, Australia, and western Europe (Jemal et al., 2002). Cancer statistics in 2016 reported that new cases and deaths of AML are the highest among other leukemias in the United States (Siegel et al., 2016). Chemotherapy can cause many sides effects such as diarrhea, nausea, and vomiting. Therefore, natural products such as edible mushrooms could be an alternative treatment to reduce those side effects. This study aimed to examine the antioxidant and anti-proliferative properties of ethanolic extracts from PS, PA and AA on AML. The human acute myelocytic leukemia U937 cell line was used as the experimental model. In addition, metabolic compounds and amino acids were analyzed by ultra-high performance liquid chromatography-high-resolution mass spectroscopy (UPLC-HRMS). This is the first report which detailed that edible mushrooms suppressed human leukemia *in vitro*.

MATERIAL AND METHODS

Chemicals and reagents

Gallic acid, 2,20-azinobis (3-ethylbenzoline 6-sulfonate) (ABTS), trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, a water-soluble tocopherol analogue), campthothecin (CAM), Folin-Ciocalteu phenolic reagent, and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI)-1640 medium, fetal bovine serum (FBS), and phosphate-buffered saline (PBS) were purchased from Thermo Scientific HyClone (Logan, UT, USA). 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and lactate dehydrogenase (LDH) were manufactured by Promega (Madison, WI, USA), Bio Basic Inc. (East Markham, ON, Canada) and Roche Diagnostics (Mannheim, Germany), respectively. All other basic reagents were of analytical grade.

Extraction of PS, PA and AA fruit bodies

Fruit bodies of PS were collected from a mushroom farm in Bangkok, Thailand. Fruit bodies of PA and AA were collected from local mushroom farms in Nonthaburi and Bangkok, Thailand. Morphological characteristics the mushrooms are shown in Figure 1. Mushrooms from all farms were cultivated and used for daily consumption. The fruiting bodies were dried and homogenized, mushroom powders (20 g with 400 mL absolute ethanol) (Merck, Germany) were extracted using a Soxhlet extractor for 11 h. After ethanol was removed with a rotary evaporator, crude extracts were dried in a freeze-drier. The extraction yields of crude ethanolic extracts were calculated based on extracted materials using the formula below. The yields of ethanolic extracts of three mushrooms are shown in Table 1.

Yields (%) = [crude extract weight (g)/mushroom powder weight (g)] x 100 (Equation 1)

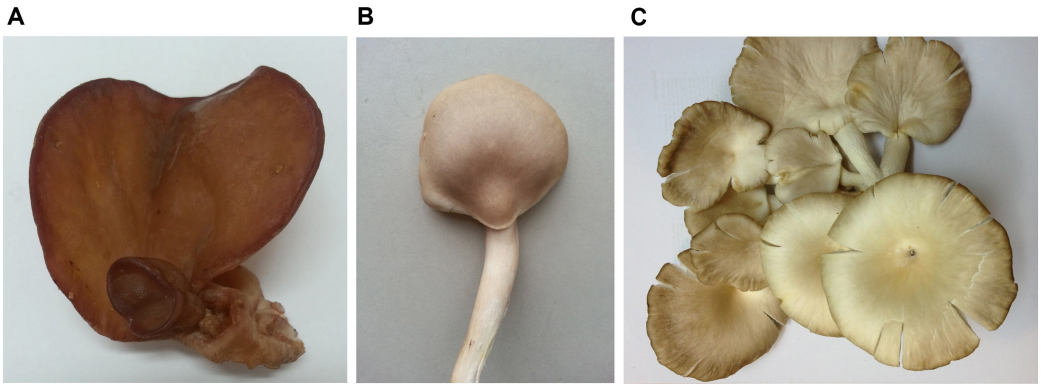

Figure 1. Morphological characteristic of *Auriculata auricula-judae* (**A**), *Pleutorus abalonus* (**B**) and *Pleutorus sajor-caju* (**C**). Fruiting bodies of mushrooms were collected from the local farms in Thailand.

Table 1. Characteristics and yields of crude mushroom ethanolic extracts.

Mushrooms	Colors of crude extracts	Yields (% w/w)
P. sajor-caju	Light yellow	3.70
P. abalonus	Dark yellow	6.40
A. auricula-judae	Pink	2.40

Measurement of phenolic content

Total phenolic phytochemical content in all mushroom extracts was assessed by Folin-Ciocalteau's method, the standard method for determining antioxidant properties (Singleton et al., 1999). In the present study, total phenolic compounds from three edible mushrooms extracted by ethanol using Soxhlet extraction was expressed as millimolar gallic acid equivalent (GAE) per kilogram of dried fruit material.

Trolox equivalent antioxidant capacity (TEAC) assay

The antioxidant activities in all mushroom extracts were detected by TEAC (Pietta et al., 2000). Standard TEAC assay was used to assess the total radical scavenging ability of the compound of interest, which was measured by its ability to scavenge the stable ABTS radical in 6 min. The result was given in relation to the activity of trolox, a water-soluble vitamin E analogue. Therefore, TEAC was the concentration of trolox with the same antioxidant activity as 1 mM solution of the sample.

Preparation of U937 cells

The U937 cell line, a human cell line established from a diffuse histiocytic lymphoma (Clement and Lehmeyer, 1983), was a generous gift from Asst. Prof. Dr. Yaneenart Suwanwong (Department of Clinical Microscopy, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand). Cells were maintained in RPMI-1640 supplemented with 10% FBS at 37°C with 5% CO_2.

Determination of cell viability by MTS and MTT assays

The mitochondrial dependent reduction of either MTS or MTT was determined colorimetrically in order to measure cell respiration, which was an indicator of cell viability (Mosmann, 1983; Goodwin et al., 1995). Briefly, U937 cells were seeded on 96-well plates (5 x 10^3 cells/well), and incubated with each crude extract at various concentrations (0.008-0.5 mg/mL) for 24-72 h at 37°C with 5% CO_2. CAM, a chemotherapeutic drug, served as the positive control of topoisomerase I inhibitor, and was used as the reference drug (Wu et al., 2010). DMSO was used as control solvent. Cell viability after PA and PS treatments was determined by MTS assay following the manufacturer instructions. After treatments, 20 µL MTS solution was added to all wells. Plates were incubated at 37°C in 5% CO_2 for 4 h. In the case of AA extract, as its natural color was found to interfere with the resulting color following MTS reaction, cell viability was determined by MTT assay instead. Supernatants were collected and absorbance was measured at 490 and 570 nm for MTS and MTT, respectively, using a Beckman Coulter DTX880 Multimode Detector (Fullerton, CA, USA). Percent cell viabilities and IC_{50} values were calculated as follows:

Cell viability (% of control) = (Absorbance sample / Absorbance control) x 100 (Equation 2)

Determination of cytotoxicity by LDH leakage

Cytotoxicity induced by crude extracts was determined by LDH leakage into the culture medium. The activity of LDH in the medium was detected using the LDH assay kit commercially available from Roche Diagnostics (Mannheim, Germany). The percentages of cytotoxicity were calculated using the formula below:

Cytotoxicity (%) = [(experimental value - low control)/(high control-low control)] x 100 (Equation 3)

UPLC-HRMS analysis

All mushroom extracts were prepared and sent to the Institute of Chemical Technology, Hyderabad, India, for UPLC-HRMS analysis. The molecular formula of compounds was detected by HRMS via comparison between theoretical and observed mass. We also compared the mass values obtained with those found on existing databases such as the knapsack family databases (Afendi et al., 2012), the Metabolomics Workbench (http://www.metabolomicsworkbench.org), and the Human Metabolome Database (http://www.hmdb.ca). Unidentified metabolites were then matched using other general chemical databases such as Pubchem (http://pubchem.ncbi.nlm.nih.gov).

Statistical analysis

All values are reported as means ± standard deviation, and one-way analysis of variance (ANOVA) was applied using the SAS program. The GraphPad Prism5 program was used to obtain the IC_{50} values. P values < 0.05 were considered significant.

RESULTS

Total phenolic content and antioxidant activity of PS, PA, and AA ethanolic extracts

Assays for total phenolic content and antioxidant activity of PS, PA and AA ethanolic extracts were carried out; results are presented in Figure 2.

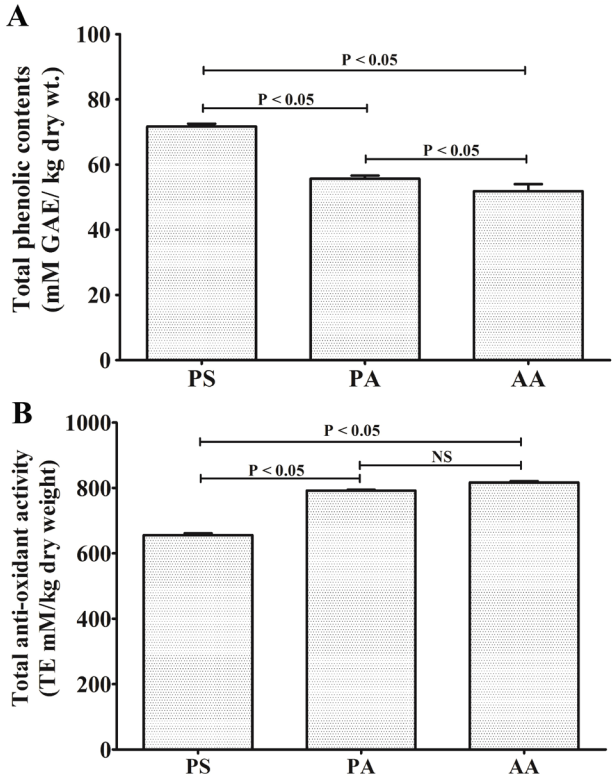

Figure 2. Total phenolic content (**A**) and antioxidant activity (**B**) of ethanolic extracts from PS, PA and AA. Data are reported as means ± SD. All experimental data were from three independent experiments. P values < 0.05 were considered significant. NS = non-significant. GAE = gallic acid equivalent. TE = trolox equivalent.

The values of phenolic compounds found in PS, PA, and AA extracts were 71.77 ± 0.83, 55.70 ± 0.93, and 51.83 ± 2.19 GAE mM/kg dried weight, respectively (Figure 2A). Total phenolic content of PS extract was significantly greater than that of PA and AA. Among all three mushroom extracts, AA extract contained the lowest total phenolic content. On the contrary, AA extract demonstrated the highest antioxidant activity (816.75 ± 3.95 TE mM/kg dried weight), followed by PA extract (792.25 ± 2.41 TE mM/kg dried weight) and PS extract (655.92 ± 5.25 TE mM/kg dried weight), as shown in Figure 2B.

In vitro anti-proliferative effect of ethanolic extracts on U937 cells

We evaluated the effects of varied concentrations of each mushroom ethanolic extract on U937 cell viability with different lengths of treatment (24, 48, and 72 h) via MTS and MTT assays. As shown in Figure 3A, we found that U937 cells treated with 0.031-0.5 mg/mL PA extract for 24 h and 0.063-0.5 mg/mL for 48 h showed significant improvement in cell viability, which was dose-dependent. In contrast, a significant reduction of cell viability was observed after U937 cells were treated with 0.5 mg/mL PA extract for 72 h; no change in cell viability with 0.008-0.25 mg/mL PA.

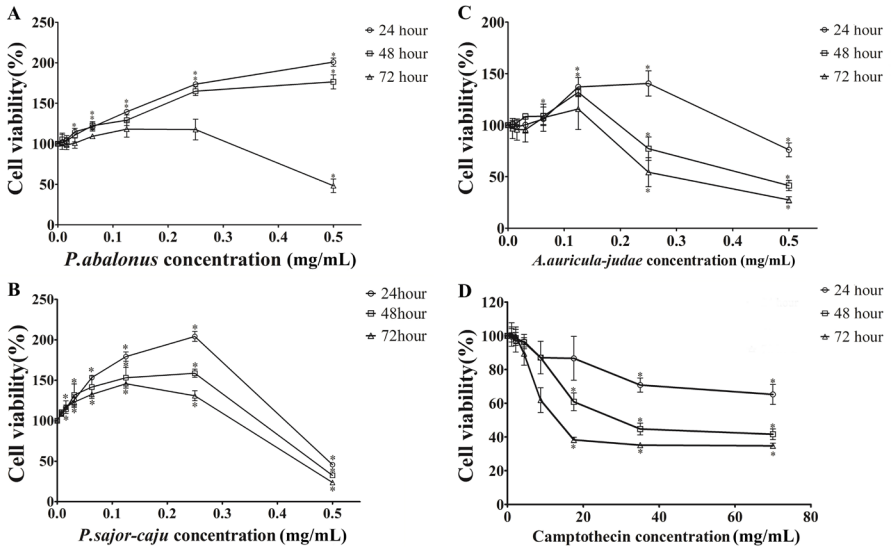

Figure 3. Effects of PS, PA, and AA ethanolic extracts on proliferation of U937 cells. After cell seeding, extracts (0.008-0.5 mg/mL) were added to the cell culture for 24, 48 and 72 h. Control cells were treated with 0.5% DMSO and camptothecin, a chemotherapeutic drug used as a positive control. Cell viability of PS and PA was determined by the MTS cell proliferation assay. Viability of AA treated cells were determined by MTT assay. Each data point represents the mean ± SD of at least 3 independent experiments and each experiment was performed in triplicates. (*P < 0.05).

As shown in Figure 3B, treatment of U937 cells with PS extract at lower doses (ranging from 0.016 to 0.25 mg/mL) resulted in a significant increase in cell viability. However, PS extract at 0.5 mg/mL significantly reduced cell viability by more than 50% at 24 h, and its potency was increased in a time-dependent manner.

As shown in Figure 3C, increased cell viability was detected with at 0.125-0.25 mg/mL AA extract for 24 h, and 0.25 mg/mL for 48 h. Treatment with at 0.5 mg/mL AA extract resulted in a significant drop in cell viability at all three time points; low concentration of AA extract treatment (0.25 mg/mL) was found to cause a significant decline of cell viability starting from 48 h of treatment.

CAM was used as a positive control for our cell viability assay, as shown in Figure 3D. When it was used at an ng/mL concentration range, a significant reduction in cell viability was observed in a time- and dose-dependent manner. Accordingly, as presented in Table 2, its

IC_{50} value calculated from 72 h treatment was 11.45 ± 2.12 ng/mL. We found that the IC_{50} of AA was 0.28 ± 0.04 mg/mL, which was lower than that of PS (0.45 ± 0.01 mg/mL) and PA (0.49 ± 0.001 mg/mL).

Table 2. IC_{50} values of mushroom ethanolic extracts and camptothecin

Mushrooms	IC_{50}
Auricularia auricula-judae	0.28 ± 0.04 mg/mL
Pleutorus sajor-caju	0.45 ± 0.01 mg/mL
Pleutorus abalonus	0.49 ± 0.001 mg/mL
Camptothecin	11.45 ± 2.12 ng/mL

Each value represents the mean ± SD of at least 3 replicates, and each experiment was independently performed 3 times. The GraphPad Prism5 program was used to obtain the IC_{50} values.

In vitro cytotoxic effect of ethanolic extracts on U937

Cytotoxic effect of mushroom extracts on U937 cells was determined by LDH assay. As shown in Figure 4, crude PS and PA ethanolic extracts had no effect on U937 cells at any of the tested concentrations as compared with untreated cells. On the contrary, AA extract at 0.25 and 0.5 mg/mL induced the cytotoxicity of 6.74 ± 0.63% and 49.95 ± 3.28%, respectively.

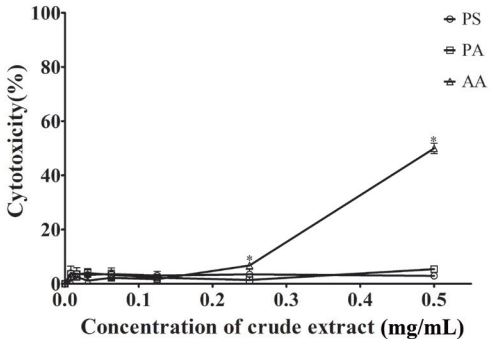

Figure 4. Cytotoxicity of PS, PA, and AA. Cell death was determined by the LDH release assay, which was carried out 24 h following PS, PA, and AA treatments. Data are reported as percentage of cytotoxicity. Each data point represents the mean ± SD of at least 3 independent experiments, and each experiment was performed in triplicates. *P < 0.05.

Metabolite profiling of PS, PA, and AA, as analysed by UPLC-HRMS

Although qualitative screening of chemical compounds found in mushrooms have been conducted using different analytical techniques (Petrova et al., 2007; Kanagasabapathy et al., 2011; Lau et al., 2013; Mohd Jamil et al., 2013), our metabolite profile study of these three edible mushrooms was performed for the first time via UPLC-HRMS. Chromatograms for the positive modes of all mushroom extracts studied are compared in Figure 5. Several metabolites were found in the extracts, including phenols and derivatives, flavonoids, heterocyclic compounds, organic compounds, carbohydrates and sugar acids and derivatives, amino acids, nucleotides and nucleotides, benzenoids, lipids and fatty acids, steroids, and sterol lipids (Chuchawankul S, Panthong S and Boonsathorn N, unpublished results).

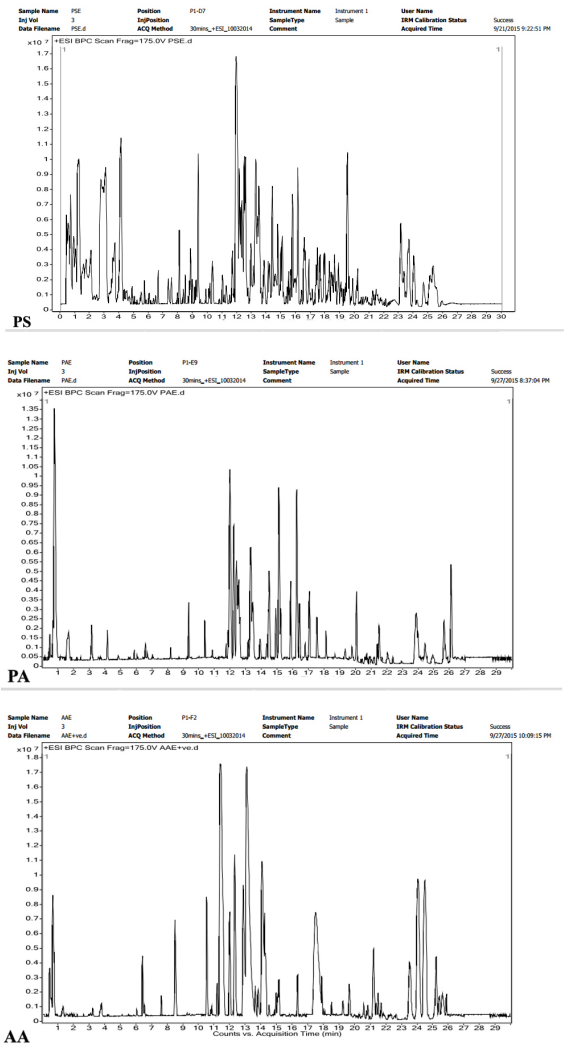

Figure 5. Full UPLC-HRMS spectrum scan of PS, PA, and AA ethanol extracts in the positive mode.

The phenols and flavonoids as well as their derivatives found in PS, PA and AA are shown in Tables 3 and 4.

Table 3. Phenol, phenol ethers and derivatives in PS, PA, and AA crude ethanolic extracts.

RT (min)	Formula	Mass	Metabolite	Mushroom
Phenol and derivatives				
1.324	$C_9H_{13}NO_3$	183.09	Normetanephrine	AA
8.209, 13.667, 13.756	$C_{18}H_{29}NO_3$	307.21	Betaxolol	PA, PS
11.421	$C_{13}H_{11}NO_3$	229.07	Fenamisal	AA
18.121	$C_8H_{10}O_2$	138.07	4-Hydroxyphenylethanol	PS
Phenol ether				
8.129	$C_{18}H_{29}NO_3$	307.21	4-Hydroxypenbutolol	PS

Table 4. Phenylpyruvic acid, phenylpropanoid and polyketides, flavonoids, terpenoids, and porphyrins in PS, PA and AA crude ethanolic extracts.

RT (min)	Formula	Mass	Metabolite	Extract
Phenylpyruvic acid and derivatives				
4.567	$C_9H_8O_4$	180.04	3-(4-Hydroxyphenyl) pyruvic acid	PS
Phenylpropanoid and polyketides				
9.929	$C_{35}H_{45}NO_{10}$	639.31	Protorifamycin I	PS
8.81	$C_{28}H_{47}NO_8$	525.32	Pikromycin	PS
3.687	$C_{10}H_7NO_3$	189.04	Alpha-cyano-3-hydroxycinnamic acid	PS
4.084	$C_{18}H_{18}O_2$	266.13	Dienestrol	PA
11.421	$C_9H_{10}FNO_2$	183.07	p-Fluorophenylalanine	AA
Flavonoids				
0.708	$C_{21}H_{24}O_{10}$	436.13	Phloridzin	PA
Terpenoids				
9.4	$C_{32}H_{40}O_9$	568.27	Swietenine	AA
Porphyrins				
11.415	$C_{39}H_{38}N_4O_{12}$	754.23	Heptacarboxylporphyrin III	AA

In terms of individual amino acids analyzed by UPLC-HRMS, we found essential and non-essential amino acids from three mushrooms, as demonstrated in Table 5. Eleven amino acids were found in all three ethanolic extracts, including alanine, arginine, asparagine, cysteine, glycine, isoleucine, lysine, phenylalanine, proline, tryptophan, and tyrosine. Interestingly, methionine, threonine and glutamic acid were only found in AA, but not in PA and PS. Moreover, serine was the only amino acid found in PA.

Table 5. Amino acid profiles of ethanolic extracts analyzed by UPLC-HRMS.

Amino acid	Mushroom species	
	Found	Not found
Alanine	PS, PA, AA	-
Arginine	PS, PA, AA	-
Asparagine	PS, PA, AA	-
Aspartic acid	PS, AA	PA
Cysteine	PS, PA, AA	-
Glutamic acid (Glu)	AA	PS, PA
Glutamine (Gln)	PA, AA	PS
Glycine	PS, PA, AA	-
Histidine*	-	PS, PA, AA
Isoleucine*	PS, PA, AA	-
Leucine*	PS, PA	AA
Lysine *	PS, PA, AA	-
Methionine*	AA	PS, PA
Phenylalanine*	PS, PA, AA	-
Proline	PS, PA, AA	-
Serine	PA	PS, AA
Threonine*	AA	PS, PA
Tryptophan*	PS, PA, AA	-
Tyrosine	PS, PA, AA	-
Valine*	PA, AA	PS

*Essential amino acids.

DISCUSSION

Mushrooms are gradually gaining popularity due to their medicinal properties such as antitumor activities, stimulatory effects on the immune cells (Maiti et al., 2008) and

antioxidant activities (Kanagasabapathy et al., 2011). Detrimental effects of oxidative stress can lead to many disorders in humans, which can be prevented by consumption of antioxidant substances (Ghosh et al., 2008; Ognjanović et al., 2008). It has long been known that natural antioxidants show fewer side effects than synthetic antioxidants. Edible mushrooms may be excellent sources of natural antioxidants and antitumor compounds.

In this study, we found that all mushroom extracts have phenolic content and demonstrated antioxidant activities. However, levels of total phenolic content found did not correlate with antioxidant activities, suggesting that non-phenolic compounds in mushrooms may also contribute to antioxidant properties. In particular, AA, with the lowest total phenolic content, was revealed to have the greatest antioxidant activity. However, we did not quantify the amount of flavonoids and other phytochemicals.

Based on the UPLC-HRMS data, a flavonoid, phloridzin, was detected in the PA extract. Phloridzin was previously found in apples and processed apple foods such as ciders and juice. This particular compound derived from apples was demonstrated to exhibit antioxidant activity (Boyer and Liu, 2004). Unique to the AA extracts, swietenine was also detected in this study. Swietenine found in the seed of the sky fruit, *Swietenia macrophylla*, has been reported to display anti-inflammatory, anti-mutagenicity, and antitumor activities (Dewanjee et al., 2009). Our study also detected tyrosol, or 4-hydroxyphenylethanol, in the PS extract. This specific constituent was quantitatively determined in five wide mushrooms from Greece, and was associated with free radical scavenging capacity, reducing power, and chelating ability (Kalogeropoulos et al., 2013).

The current investigation found betaxolol in both PS and PA extracts. Intriguingly, betaxolol, b-adrenergic antagonists (b-blockers), was revealed to induce antioxidative stress in rat retinal ganglion cells (Yu et al., 2007). Indeed, betaxolol is a common pharmaceutical active compounds found in the environment (Bosun, 2014). In this study, we found betaxolol in both PS and PA extracts from different farms. In addition, dienestrol, recognized as a phenolic environmental estrogen, was detected in PA extracts. Our results therefore suggested that the mushrooms were grown in an environment with a potential dienestrol contamination.

The anti-proliferative results (Figure 3B) suggested that PS extracts may either prevent or promote U937 cell growth depending on the dose applied, as it exhibited a biphasic effect on cell growth. This is a common phenomenon; more than a hundred antitumor agents enhance proliferation of human tumor cells at low doses and inhibit cell growth at high doses (Calabrese, 2005). Well-known antioxidant compounds include resveratrol, epigallocatechin gallate, curcumin, quercetin, allicin, capsaicin, carnosic acid, and sulforaphane have been classified as dietary hormetic compounds (Son et al., 2008; Calabrese et al., 2010; Vargas and Burd, 2010; Pietsch et al., 2011).

Our present data suggested that AA extract possess the highest potency with regard to anti-proliferative properties. Accordingly, previous studies have demonstrated that ethanolic extract from AA at 1 mg/mL showed an inhibitory activity against Sarcoma 180 cells (Reza et al., 2011; Reza et al., 2014). Mushrooms contain various compounds such as polysaccharides, proteins, fats, glycosides, phenols, flavonoids, carotenoids, organic acids, and minerals (Patel and Goyal, 2012; Yokota et al., 2016). Common active components in mushrooms such as polysaccharide, b-glucan, and protein-polysaccharide complex have been found to promote antitumor activities (Ooi and Liu, 2000). However, in terms of amino acids, methionine, threonine, and glutamic acid were found only in AA ethanolic extracts, but not in the ethanolic extracts of PA and PS. In addition, serine levels were undetectable in the ethanolic extracts

of AA and PS, but were detected in those of PA. Nonetheless, we found that all the amino acids that were undetectable in the ethanolic extracts were present in the water extracts of the respective mushrooms. Furthermore, histidine, leucine, and serine, which were undetectable in AA ethanolic extracts, were present in the AA water extracts (Chuchawankul S, Panthong S and Boonsathorn N, unpublished results).

Glutamic acid, an essential amino acid, is the most abundant amino acid found in various mushroom species. Our results confirmed the presence of glutamic acid in AA ethanolic extracts. However, we did not quantify its exact concentration in this study. As expected, we found glutamic acid in the water extracts of all three mushrooms (Chuchawankul S, Panthong S and Boonsathorn N, unpublished results).

All nine essential amino acids were previously found in the three mushrooms investigated in this study (Chirinang and Intarapichet, 2009; Li et al., 2012; Kadnikova et al., 2015), thus supporting the amino acid profiles detected by UPLC-HRMS. Interestingly, glutamic acid was found only in ethanolic extracts of AA, but not in that of PS and PA. The strong anti-proliferative effect of AA on U937 cells may be contributed by glutamic acid, as it was previously identified as an anticancer agent (Dutta et al., 2013).

In conclusion, this is the first study that not only demonstrated the *in vitro* anti-proliferative activity of ethanolic extracts derived from fruiting bodies of edible mushrooms (PS, PA, and AA) on U937 cells, but also displayed their metabolite profile by UPLC-HRMS. Our metabolite profile data supported the hypothesis that mushroom extracts may exert antioxidant and anti-proliferative effects.

Conflicts of interest

The authors declare no conflict of interest.

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