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Amino Acid, Phytochemical Compositions and Antioxidant Activity of Inky Cap Mushroom (*Coprinus radiata*)

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Evaluation of the amino acid composition, phytochemical properties and antioxidant activity of *Coprinus radiata* mushrooms was conducted. Free amino acid composition of fresh mushrooms was analyzed by LC-MS/MS. The phytochemical properties analysis included total phenolic compound content and total flavonoid content of these mushrooms. Furthermore, antioxidant activity assays were performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). Based on the results shown that a high concentration of non-essential amino acids, especially those of arginine and glutamic acid, were observed. However, isoleucine and leucine were major essential amino acids in these mushrooms. The ethanolic extract of *C. radiata* showed higher phytochemical contents and antioxidant activity than those of the water extract. It suggested that the *C. radiata* is a natural source containing some essential amino acids, a high amount of phytochemical properties and high antioxidant activity, potentially having medicinal and nutritional properties that provide health benefits.

Keywords: *Coprinus radiata*, Amino acid, Phytochemical compounds, Antioxidant activity

INTRODUCTION

Edible mushrooms are widely used as food, and there has been increasing interest in the investigation of their biological effects, such as those of antioxidant components in mushrooms, and they are also abundant components of proteins and amino acids [1]. In recent years, anticancer and antifungal properties of mushrooms are reported [2,3]. Various edible mushrooms contain bioactive phytochemicals with antitumour effects [3]. Moreover, mushrooms are good sources of antioxidants [3].

The content of free amino acid compositions of mushrooms is important because of their effects on the flavours and health benefits of mushrooms [4]. In recent years, several techniques for analyzing amino acids have been described, an example of that are high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) [5]. These techniques were developed principally for the determination of amino acids. Typically, reversed-phase HPLC is a popular technique to determine amino acids in plants, but the relatively low-polarity compounds are not adequately retained and amino acids require derivatization prior to analysis. The advantage of LC-MS technique is that the analytes often do not require any derivatization [6].

Coprinus radiata mushroom, also known as the inky cap mushroom, is the most widely consumed mushroom, has a delicious taste, and is easily cultivated [7]. This mushroom is important for the economy and has properties to aid digestion [8]. The study of *C. radiata* has been less reported, especially regarding its nutritional value and phytochemical properties. Moreover, the amino acid profile of *C. radiata*, including its content of all protein and non-protein amino acids, has not been

well investigated. The aim of this study is to determine the types and contents of amino acids and phytochemical compounds and the antioxidant properties of *Coprinus radiata* cultivated in Thailand.

EXPERIMENTAL

Individual standards were purchased from Sigma-Aldrich. Catechin was obtained from Fluka (Germany). Folin-Ciocalteu reagent, DPPH, 2,4,6-tris(2-pyridylstriazine) and gallic acid were obtained from Sigma-Aldrich (USA). All laboratory chemicals and reagents employed were of analytical grade.

Total amino acid analysis: Sample preparation with a 100 mg sample was weighed and ground to a fine powder under liquid nitrogen. A volume of 0.5 mL of 0.05 M aqueous HCl-ethanol (1:1, v/v) was added and vortexed for 5 min followed by centrifuged at 4 ºC for 15 min. The clear supernatant was analyzed by LC-MS/MS method.

LC-MS/MS analysis by amino acids were analyzed following a published method [9]. The analysis was operated using an LCMS-8030 couples the power of a triple-quadrupole mass spectrometer (Shimadzu, Japan) operated in ESI mode and a Shimadzu LC-20AC series HPLC system (Shimadzu, Japan). A 10 µL sample was injected and separated under the following conditions: the carried solvent flow rate, 0.2 mL/min and set column oven temperature, 38 ºC. The mobile phase was based on (I) 50:50 methanol/water consisting of 0.1% formic acid (v/v) and (II) water with 0.1% formic acid (v/v). The MS/MS parameters were as follows: multiple reaction monitoring (MRM) mode; capillary voltage, 4.5 kV (positive mode; ESI (+)); cone voltage, 1.72 kV; and ion source temperature, 400 ºC. The results of the auto-optimizations are summarized in Table-1.

Preparation of mushroom in ethanolic and aqueous extracts: For the preparation of ethanolic and aqueous extracts was conducted according to Wang *et al*. [10] method with slight modifications. Dried powdered sample (25 g) was soaked overnight in 250 mL of 95% ethanol, while another sample (25 g) was boiled in water (250 mL) for 2 h. Both solution extracts were filtered and evaporated in a rotary evaporator at 50 ºC. Stored all extracts at 4 ºC for the analysis of phytochemical compositions and antioxidant activity.

Total phenolic contents (TPCs): The content of TPCs in the extracts was analyzed as reported by Yawadio *et al*. [11]. Gallic acid was applied as standard for determining TPC and the results are reported as mg gallic acid equivalents (GAE).

Total flavonoid content (TFC): The content of TFC in the extracts was analyzed by colorimetric analysis technique as described earler [12]. The TFC results are reported as mg catechin equivalents (CA).

Radical scavenging activity: The radical scavenging activity was determined using DPPH assay [13]. The radical scavenging activity was calculated as % scavenging activity using the according to equation:

$$\text{Scavenging activity } (\%) = \left(1 - \frac{A_c}{A_d}\right) \times 100$$

where A_c is the absorbance of the sample extract added with the DPPH solution and A_d is the absorbance of control only DPPH solution.

Ferric reducing antioxidant potential (FRAP) assay: The FRAP assay was used to determine the antioxidant activity according to Benzie & Strain method [14] with some modifications. The FRAP assay of the sample extract were reported as mmol $FeSO_4$/100 g dry weight.

Statistical analysis: The analysis of experimental results was investigated in triplicate. The results were reported as mean value and standard deviation, and a statistical significance test was conducted using the independent t-test, where p-values less than 0.05 is statistically significant.

RESULTS AND DISCUSSION

Amino acids: About 14 amino acids in inky cap mushrooms (*C. radiata*) were analyzed using LC-MS/MS and the results are shown in Table-2. The amino acids include eight essential and non-essential amino acids. However, cystine, which is an essential amino acid, was not found. The content of 14 amino acids were ranged between 1.73 and 850.63 µg/g in the cultivated mushrooms. Among the 14 amino acids found, arginine was the most abundant amino acid, followed by glutamic acid and leucine. The amino acid profiles were similar

TABLE-1
CONDITIONS APPLIED DURING LC-MS/MS ANALYSIS

No.	Amino acid	Retention time (min)	Precursor ion $[M+H]^+$ (*m/z*)	Product ion (*m/z*)	Q1 Pre Bias (V)	Collision energy (V)	Q3 Pre Bias (V)
1	Asparic acid	2.145	134.00	74.00	-15.00	-15.00	-16.00
2	Glutamic acid	2.176	148.00	84.00	-17.00	-18.00	-18.00
3	Threonine	6.107	132.10	86.00	-15.00	-14.00	-20.00
4	Tyrosine	2.797	182.00	136.00	-21.00	-13.00	-16.00
5	Alanine	2.128	90.00	44.00	-10.00	-13.00	-18.00
6	Methionine	3.748	150.05	104.00	-20.00	-18.00	-29.00
7	Valine	11.105	205.00	188.00	-18.00	-13.00	-14.00
8	Phenylalanine	5.658	132.10	68.00	-14.00	-14.00	-18.00
9	Leucine	2.163	120.00	74.00	-14.00	-10.00	-18.00
10	Isoleucine	2.006	175.05	70.00	-26.00	-27.00	-29.00
11	Tryptophan	8.942	166.05	120.05	-12.00	-12.00	-14.00
12	Histidine	1.967	156.05	10.05	-12.00	-13.00	-23.00
13	Lysine	3.177	118.05	72.05	-13.00	-13.00	-15.00
14	Arginine	1.843	147.05	84.01	-17.00	-17.00	-18.00

TABLE-2
AMINO ACID CONTENT OF FRESH INKY CAP MUSHROOM

Amino acid	Content (µg/g fresh weight)	Amino acid	Content (µg/g fresh weight)
Alanine	13.67 ± 1.42	Lysine*	15.07 ± 1.7
Arginine	850.63 ± 26.44	Methionine*	2.30 ± 0.78
Aspartic acid	8.33 ± 0.93	Phenylalanine*	21.87 ± 1.78
Glutamic acid	56.67 ± 1.07	Threonine	5.3 ± 0.26
Histidine*	1.73 ± 0.06	Tryptophan*	17.1 ± 1.15
Isoleucine*	22.73 ± 1.17	Tyrosine	5.77 ± 0.50
Leucine*	32.10 ± 4.80	Valine*	2.87 ± 0.29

*Essential amino acids

in the family of *Coprinus* mushrooms, in which the basic amino acids found in these mushrooms are glutamic acid, leucine and arginine [15]. The content of sulfur-containing amino acids arginine is much higher in fungi than in plants [16]. The second most abundant amino acid evaluated in the study was glutamic acid, which is a well-established excitatory neurotransmitter in the central nervous system [17]. In mushrooms, the resulting taste of glutamic acid is umami, which is an important factor in the taste of mushrooms [18].

Phytochemical properties: The TPC content in ethanolic extract (32.27 ± 2.23 mg GAE/g dry weight) was higher than in the aqueous extract (19.30 ± 1.60 mg GAE/g dry weight). A higher TFC content was found in the ethanolic extract (19.06 ± 1.89 mg CA/g dry weight) than in the aqueous extract (10.96 ± 0.23 mg CA/g dry weight). All extracts with a relatively high TFC also had a high TPC.

The results showed that solvent polarity influenced the TPC and TFC contents of *C. radiata* mushroom extracts. Phytochemical compounds in mushrooms include polysaccharides and tocopherols, among others, especially phenolic compounds and flavonoids, which are found in fruiting bodies and mycelia [19]. Polyphenols include a large number of phenolic compounds, including flavonoids [20]. It is interesting to note that flavonoids exhibit antioxidant properties by scavenging free radicals [21]. Thus, the TPC and TFC contents of *C. radiata* mushroom extract might play a role in the antioxidant activity.

Antioxidant activity: The scavenging activity of *C. radiata* mushroom extracts is shown in Fig. 1, which different concentrations, expressed as % scavenging activity, increased with rising extract concentration. The scavenging activity (%) of the ethanolic extract was significantly higher than that of the water extract of *C. radiata* mushrooms ($p < 0.05$). The TPC and TFC contents were consistent with the % scavenging activity, and the higher the TPC and TFC contents were, better the % scavenging activity.

Measurement of FRAP assay was determined by the reducing power of *C. radiata* mushroom extracts by reducing Fe^{3+}-TPTZ to a blue coloured Fe^{2+}-TPTZ [22]. The extracts of *C. radiata* mushrooms expressed increasing antioxidant activity with increasing concentration (Fig. 1). Importantly, the FRAP values of the extracts of were similar to the % scavenging activity in both the ethanolic and water extracts. The FRAP values were higher in the ethanolic extract ($p < 0.05$) than in the water extract, also the result correspond with previous study [23].

Plant phenolic compounds are important for antioxidant effects by acting as free radical inhibitors, peroxide decomposers, oxygen scavengers or metal inactivators [24,25]. Importantly, all mushrooms contain phenolic compounds, such as caffeic acid, quercetin and catechin, among others [25,26]. In addition, the methanolic extract of edible mushrooms showed antioxidant activity due to a relatively high content of phenolic compounds, followed by that of flavonoids, which have antioxidant mechanisms by directly scavenging free radicals [20,27].

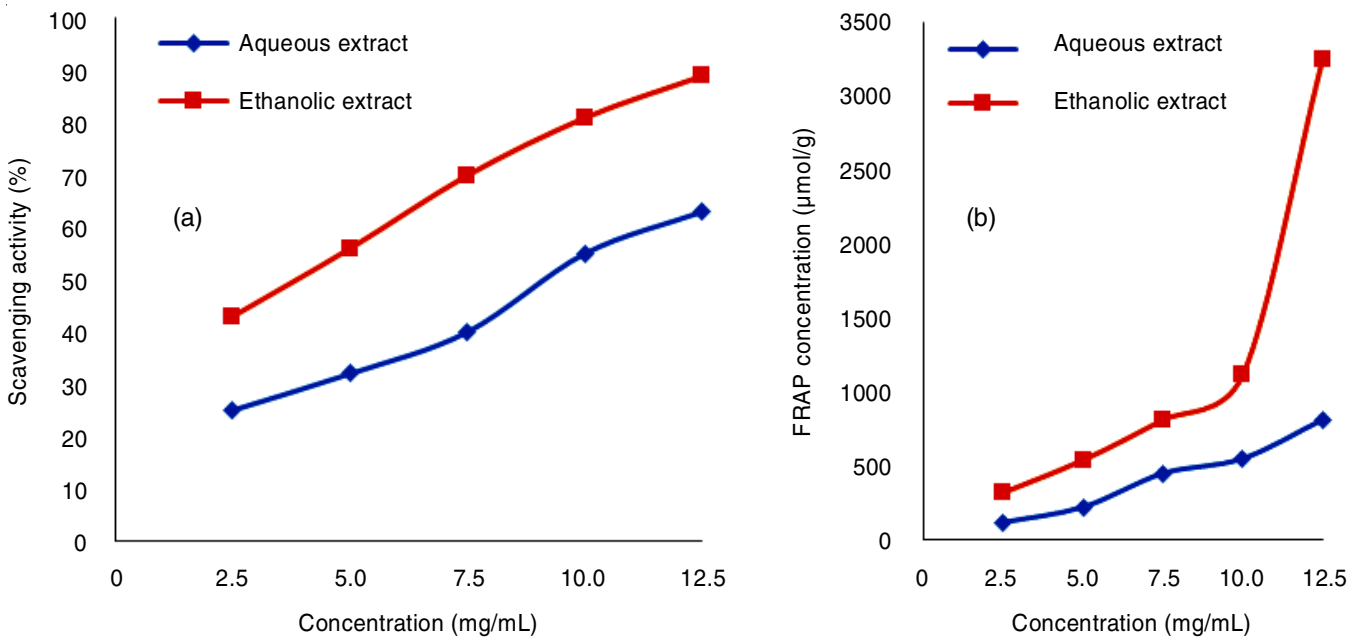

Fig. 1. Antioxidant activity of *C. radiata* mushroom (a) on DPPH assay shown in % scavenging activity (b) on FRAP assay shown in FRAP concentration (µmol/g)

Conclusion

Mushrooms are an abundant source of nutrient especially protein, and antioxidants are important for human health. The amino acid profiles in *C. radiata* mushrooms were investigated for the first time. The extracts from *C. radiata* mushrooms showed strong antioxidant activity because they contained high concentrations of phytochemical properties with particularly high amounts of phenolic compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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