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Total Phenolics Content, Free Radical Scavenging Capacity and Tyrosinase Inhibition Activity of Various Edible Mushroom Extracts

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Introduction

Over the last decade, rising demands of skin care products because of the climatic conditions that encourages the growth of the market for cosmetics. Free radicals play a major responsible for skin aging and related degenerative processes, particularly, cancer and cardiovascular diseases[1]. Moreover, UV exposure caused damage to the skin attributable to the UV-induced the melanin synthesis pathway that generating many highly reactive intermediate substances, such as hydrogen peroxide and hydroxyl radicals. To protect cells against oxidative damage, free radicals are neutralized by cellular defence systems but, these systems are not sufficient to completely prevent[2]. Therefore, antioxidants and tyrosinase inhibitors are very attractive as beneficial substances that can serve as protecting agents for skin aging and skin disorder based on their antioxidative properties and anti-tyrosinase activity[3]. Changing lifestyle preference towards natural, herbal and organic beauty products raises the growth of the cosmetics market. Various natural sources of active compound particularly, plant, microorganism and animal, have been reported. Edible mushrooms have been widely consumed by humans for centuries. Recently, Mushrooms are not only food or functional food but also become attractive as a cosmetic ingredient due to its physiologically beneficial[4]. They are ubiquitous in bioactive compounds that contain a variety of secondary metabolites including phenolic compounds, polyketides, terpenes and steroids[5]. Phenolic compounds have been reported to have multiple biological effects, especially antioxidant activity[6]. Therefore, the objectives of this study were to determine the total phenolic content of extracts from three edible mushroom species, including *Agaricus blazei Murill*, *Cordyceps militaris* and *Ganoderma lucidum* and to evaluate the antioxidant activity and tyrosinase inhibition activity. Furthermore, the effect of extractants i.e. water and 95% ethanol on the phenolic content and their activities were also investigated.

Methods

Preparation and extraction of mushrooms

Agaricus blazei Murill, *Cordyceps militaris* and *Ganoderma lucidum* were collected from Doi Pui Mushroom Research Center, Chiang Mai province, northeast Thailand. After dried at 50 °C by hot air oven for 48 h., the dehydrated mushrooms were cut into small pieces and then pulverized to powder using a grinder. The powder was then extracted with different solvents; 95% ethanol and water. For ethanol extraction, the powder was macerated with 95% ethanol (1:10) for 7 day each at room temperature. The supernatants were filtered using a Whatman No. 1 filter paper, combined, and then concentrated using a rotary evaporator (50 °C) under reduced pressure. For water extraction, the powder was boiled at 120 °C for 30 min. The supernatants were filtered using a Whatman No. 113 filter paper 2 time, then lyophilized using a vacuum freeze dryer.

Determination of total phenolic content

The total phenolic content of mushroom extracts was determined using Folin–Ciocalteu method described by Singleton and Rossi[7] with slight modifications. Briefly, the extract (1 mL) was diluted with distilled water (46 mL) in a volumetric flask. Folin-Ciocalteu reagent (1 mL) was added and mixed thoroughly for 5 min; Na2CO3 (2% (v/v), 3 mL) subsequently added to the mixture. After incubation at room temperature for 90 min, the absorbance of each mixture was measured at 765 nm. The total phenolic content was measured by plotting the calibration curve of a gallic acid standard, expressed as milligrams of gallic acid equivalents per gram of dried mushroom.

Determination of antioxidant activities

The DPPH radical-scavenging activity of mushroom extract was evaluated according to the methodology described by Kaewnarin et al.[8] with some modifications. Briefly, 3 mL of each mushroom extract with different concentrations were mixed with 1 mL of DPPH (0.1 mM) solution in methanol. Trolox was used as the positive controls. A mixture containing 3 mL of DPPH radical solution and 1 mL of 80% methanol was used as the negative control (blank). The mixture was shaken vigorously and left to incubate for 30 min in the dark at room temperature. After incubation, the absorbance of each mixture was measured spectrometrically at 517 nm. The ability to scavenge the DPPH radical scavenging was calculated using the following equation (1):

$$\% \text{ DPPH radical scavenging} = [(A_{blank} - A_{sample})/ A_{blank}] \times 100 \quad (1)$$

where A_{blank} and A_{sample} are the absorbance of the negative control reaction and the absorbance of the test extract. All samples were analyzed in triplicate. The activity of inhibition concentration at 50 percent (IC50) was also determined using an exponential regression of the plotted points for each extract, revealing the concentration required to reduce the initial concentration of DPPH radicals by 50%.

Determination of tyrosinase inhibitory activity

Mushroom tyrosinase inhibitory assay was performed using the DOPA-chrome method with some modifications. Briefly, the extracts were dissolved in distilled water at a concentration of 10 mg/mL. 100 µL of 2.5 mML-DOPA and 30 µL of 0.1 M phosphate buffer (pH6.8) were added to each well and subsequently incubated at room temperature for 10 minutes. After incubation, 50 µL of mushroom tyrosinase (200 U/mL) and 20 µL of the test sample solution were added. The final concentration of each extract in reaction tubes was 1 mg/mL. Blank solutions with and without enzyme were also prepared with no test sample solution added. The positive controls of 0.5 mg/mL kojic acid solutions was also prepared, with and without enzyme. Each test was prepared in 3 replications. After incubation at room temperature for 60 minutes, the absorbance of each well was measured at 490 nm using the microplate reader for monitoring the formation of the DOPA-chrome. Percentage of inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ Tyrosinase inhibition} = 100 \times [(A-B)-(C-D)]/ (A-B) \quad (2)$$

Where; A = absorbance of blank solution with enzyme

B = absorbance of blank solution without enzyme

C = absorbance of sample solution with enzyme

D = absorbance of sample solution without enzyme

Statistical analysis

All experiments were performed independently in triplicate, and the data were presented as the mean ± S.D. (standard deviation). The experimental data were subjected to one-way analysis of variance (ANOVA) to compare the antioxidant activity and phenolic acid content in each sample. Differences were considered to be statistically significant if the *p* value was less than 0.05.

Results

Extractable phenolic

Phenolic compound has been considered the major contributors to antioxidant actions in mushrooms[9]. The effect between type of mushrooms and extractant on phenolic content were observed. The results are shown in **Figure 1A,** the type of mushrooms affected the extraction phenolic content which varied from 0.39-3.28 mg GAE/g. The highest of phenolic yield found in *Ganoderma lucidum* that extracted by 95% ethanol and water were 3.28 ± 0.18 and 1.89 ± 0.28 mg GAE/g, respectively. In *Cordyceps militaris,* this value was 0.39 ± 0.08 and 1.88 ± 0.10 mg GAE/g in water and ethanolic extracts, respectively. On the other hand, the phenolic content of water extract of *Agaricus blazei Murill* was 1.45 ± 0.43 mg GAE/g, and this value decreased to 1.08 ± 0.21 mg GAE/g in 95% ethanol extract.

Antioxidant activities

The DPPH• free radical scavenging ability is a widely used method to determine the antioxidant activities of extracts due to a short period of time. The DPPH free radical scavenging ability of the extracts was expressed as IC50, as shown in Table 1. There was statistically significant difference between type of mushroom and extraction method ($P< 0.05$). The IC50 values of all extracts ranged from 7.34 – 0.46 µg/ml. A lower IC50 meant better radical scavenging activity. The results indicated that the highest DPPH radical-scavenging activity was achieved with the 95% ethanol extract of *Ganoderma lucidum* (0.46±0.01 µg/mL). However, compared to the positive control, had lower activity than ascorbic acid (0.21±0.01mg/mL). Water extracts from all tested mushrooms showed lower DPPH radical scavenging activities than 95% ethanol.

Table 1 DPPH radical scavenging activity from various mushroom with different extraction method.

Type of mushrooms	DPPH radical scavenging activity (IC50, µg·mL^{-1})	
	Water	95 % (v/v) Ethanol
Cordyceps militaris	7.34±0.59	3.81±0.07
*Agaricus blaze*i Murill	4.20±0.14	2.39±0.05
Ganoderma lucidum	2.42±0.10	0.46±0.01
Ascorbic acid	0.21±0.01	

Tyrosinase inhibitory activity

The tyrosinase inhibitory activity was carried out by the DOPA-chrome method. The enzyme activity was investigated on the basis of color formation of DOPA-chrome by UV-Vis spectrophotometer. All the percentage inhibition values of the mushroom extracts and a standard Kojic acid were presented in **Figure 1B** The results showed that all mushroom extract presented slightly inhibition of tyrosinase activity (2.82-9.79%) comparable with kojic acid (98.35 ± 2.5%). The highest tyrosinase inhibitory activity was found in the ethanolic extract of *Ganoderma lucidum* extract (9.79±1.68 %), followed by the water extract (7.86±2.67%). When considering each type of solvent, the water extracts from *Cordyceps militaris* and *Agaricus blazei Murill* exhibited relatively higher tyrosinase inhibition levels than ethanolic extracts. The results were similar to those reported by Yoon et al [10] who indicated that the methanolic extract of *Lentinus lepideus* mushroom presented stronger tyrosinase inhibitory activity than its hot water extract.

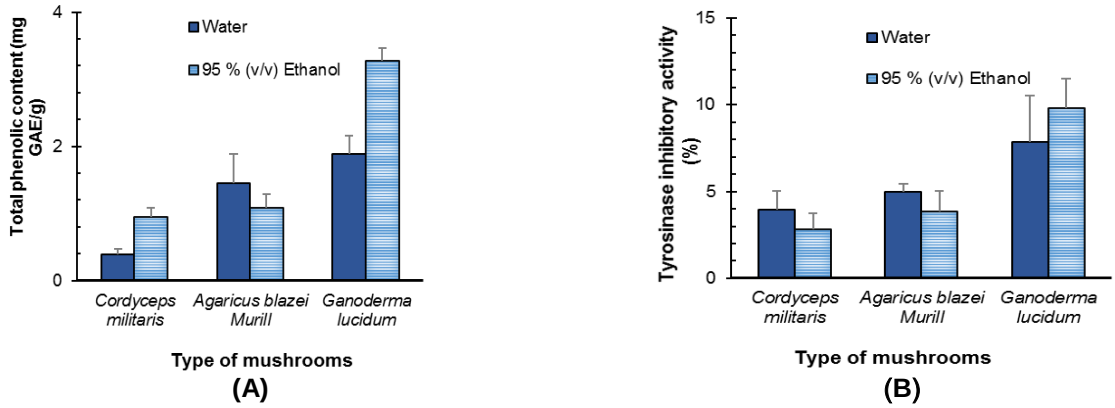

Figure 1 Total phenolic content (A) and tyrosinase inhibitory activity (B) from various mushroom with different extraction solvents.

Discussion

From the results, the phenolic compound of *Ganoderma lucidum* and *Cordyceps militaris* mushrooms are well extracted by using 95% ethanol. In contrast, the phenolic compound of *Agaricus blazei Murill* well extracted by using water. It is probably due to the polarity of phenolic compounds contained in these mushrooms[9] Moreover, a significant correlation between extractable phenolic content and DPPH free radical scavenging activity and tyrosinase inhibitory activity of all mushroom samples were observed. Strong DPPH radical-scavenging and tyrosinase inhibitory activity were exhibited in the mushroom extracts which contained high total phenolic and flavonoid contents. This result was supported by the finding of previous studies by Kaewnarin et al[8], which revealed that the extracts with higher contents of phenolic showed higher DPPH free radical scavenging activity and also higher tyrosinase inhibitory activity. This effect is most likely due to a high content of phenolic lead to enhance the ability to donate electrons to scavenge free radicals[9]. Furthermore, the number of hydroxyl groups of the phenolic compounds could influence the tyrosinase inhibitory activity by the result of the forming a hydrogen bond to the active site of enzyme that reducing the enzyme activity[11]. It shall be noted that, the presence of compounds with antioxidant activity may have synergistic effects involved in tyrosinase inhibition. However, the chemical constituents of these extracts could be further studied.

Conclusion

In this work, three popular edible mushroom species were selected to investigate their amounts of phenolic compounds, antioxidant and anti-tyrosinase inhibitory activities. The results showed that the ethanolic extract of *Ganoderma lucidum* contained high amounts of phenolic compounds and also exhibited great antioxidant and anti-tyrosinase inhibitory activities. These findings suggest that, these extracts could be further studied and developed as a natural antioxidants ingredient for the cosmetic and dietary supplement industries.

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References

1. Harman D. Aging. A Theory Based on Free Radical and Radiation Chemistry. Journal of Gerontology. 1956;11(3):298-300.
2. Isabel CFRF, Lillian B, Rui MVA. Antioxidants in Wild Mushrooms. Current Medicinal Chemistry. 2009;16(12):1543-60.
3. Perluigi M, De Marco F, Foppoli C, Coccia R, Blarzino C, Luisa Marcante M, et al. Tyrosinase protects human melanocytes from ROS-generating compounds. Biochemical and Biophysical Research Communications. 2003;305(2):250-6.
4. Taofiq O, González-Paramás AM, Martins A, Barreiro MF, Ferreira ICFR. Mushrooms extracts and compounds in cosmetics, cosmeceuticals and nutricosmetics—A review. Industrial Crops and Products. 2016;90:38-48.
5. Kues U, Liu Y. 2000. Fruiting body production in basidiomycetes. Applied Microbiology and Biotechnology. 2000; 54:141-152.
6. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha J-P, Pihlaja K, Kujala TS, et al. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agricultural and Food Chemistry. 1999;47(10):3954-62.
7. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture. 1965;16(3):144-58.
8. Kaewnarin K, Suwannarach N, Kumla J, Lumyong S. Phenolic profile of various wild edible mushroom extracts from Thailand and their antioxidant properties, anti-tyrosinase and hyperglycaemic inhibitory activities. Journal of Functional Foods. 2016; 27:352-64.
9. Boonsong S, Klaypradit W, Wilaipun P. Antioxidant activities of extracts from five edible mushrooms using different extractants. Agriculture and Natural Resources. 2016;50(2):89-97.
10. Yoon KN, Alam N, Lee KR, Shin PG, Cheong JC, Yoo YB, et al. Antioxidant and Antityrosinase Activities of Various Extracts from the Fruiting Bodies of Lentinus lepideus. Molecules. 2011;16(3):2334.
11. Alam N., Yoon K.N., Lee T.S. Evaluation of the antioxidant and antityrosinase activities of three extracts from *Pleurotus nebrodensis* fruiting bodies. African Journal of Biotechnology. 2011; 10:2978–2986.