Anti-oxidant contents and activities from natural *Phellinus linteus* and *Phellinus igniarius* extracts

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ABSTRACT

The natural *Phellinus* mushroom has been widely used as a medicinal plant for the treatment of various diseases. There are many commercial products derived from the mushroom available on the market today; however, the biochemical substances involving antioxidants and the associated activities are still unknown or have yet to be reported. In this study, we investigated the antioxidant contents and activities from four commercial products containing natural *Phellinus linteus* (PL-65 and PL-38) and *Phellinus igniarius* (PI-65 and PI-16). The evaluation of anti-oxidant contents in this study included total phenolic compound, flavonoids, tocopherol, ascorbic acid and glutathione. The highest antioxidant contents were found in the *Phellinus igniarius* product, under the commercial name PI-65, followed by PL-65, PL-38 and PI-16 respectively. The total antioxidant activity was assessed by using DPPH and ABTS assays. The results were reported as trolox equivalent antioxidant concentrations (TEAC). The PI-65 and PL-65 showed the highest TEAC values in both DPPH (1.00 and 1.00 g/ml) and ABTS (1.88 and 1.79 g/ml) assays. Based upon these results, all these products may have potential anti-oxidants to prevent oxidative stress which can cause pathogenesis and ultimately induced cell death in the human body.

Keywords: Anti-oxidants, Natural mushroom, *Phellinus linteus*, *Phellinus igniarius*

1. INTRODUCTION

Free radicals are normally generated by being metabolized in cells. However, exogenous factors, such as sunlight, ultraviolet light, and chemicals can cause free radicals as well [1]. The overproduction of free radicals results in an imbalance between factors that generate it and factors that protect the cells, this can cause oxidative stress [2]. Oxidative stress is associated with biological molecules like proteins, lipids and DNA and creates lipid peroxidation, protein, and DNA damage. These damages lead to ageing, inflammatory disease, atherosclerosis, cardiovascular disease, diabetes, neurological disease and cancer [3-4]. Although the human body possesses an antioxidant system to protect cells from free radicals, these systems are insufficient to provide complete protection. Essential dietary foods are abundant sources of antioxidants, such as phenols, flavonoid, tocopherols, ascorbic acid, carotenoids and glutathione for supporting the antioxidant system and reducing oxidative damages [5]. *Phellinus linteus* and *Phellinus igniarius* have been popularly used as a medicinal mushroom since ancient times. Their medicinal properties have various positive effects, such as being antitumor, anticancer, immunomodulatory activities and can treat various diseases such as gastro-enteric disorder, lymphatic disorder and cancer [6]. The antioxidant contents and activities in these mushrooms have been reported in many research studies. Quantitation of properties was significantly found for being used as a source of anti-free radicals [6-8]. The present study aimed to evaluate the antioxidant contents and properties of hot water extracts from natural *Phellinus linteus* and *Phellinus igniarius* using *in vitro* assays. The scavenging abilities for antioxidant activity are on DPPH and ABTS. Potential antioxidant components in these extracts were determined as well.

2. METERIALS AND METHODS

Materials

Hot water extracts of natural *Phellinus linteus* and *Phellinus igniarius* were used from commercial products of Amazing Grace Health Products. The products of *Phellinus linteus* were PL-65 and PL-38 and the products of *Phellinus igniarius* were PI-65 and PI-16.

Determination of Antioxidant contents

Total phenols were measured by The Folin-Ciocalteau method [9] with some modification. 0 µl of sample was mixed with 50 µl of Folin-Ciocateau reagent, which made up a volume of 400 µl. With added water, it was incubated at room temperature for 5 minutes then 500 µl of 7% Na_2CO_3 was added into the solution, after which it was kept at room temperature for 90 minutes. The mixture measured the absorbance at 750 nm by a spectrophotometer; Gallic acid was used as a standard.

Total flavonoid was determined with a slightly modified method of Kurkina (2011) by using a complex-forming reaction with an aluminum chloride solution [10]. The reaction mixture contained 100 µl of sample mixed with 200 µl of 3% lCl_3ethanolic solution and made up a volume of 1 ml with 95% ethanol. The mixture was mixed and incubated for 30 minutes at room temperature. The absorbance was measured at 403 nm with a spectrophotometer; Rutin was used as a standard.

Tocopherol was estimated by using the method previously described [11]. 100 µl of sample was mixed with 350 µl of 0.07% (/v) 2,2´bipyridine, 50 µl of 0.2% (w/v) $FeCl_3$ then made up a volume of 1 ml with 95% ethanol. The mixture was incubated at room temperature for 1 minute and measured absorbance at 520 nm by using spectrophotometer; α-Tocopherol was used as a standard.

Ascorbic acid was then measured by following the method of Marschner and Cakmak (1992) [12]. The reaction mixture contained 10 µl of the sample, which was then added 250 µl; 0.15M sodium phosphate buffer (pH 7.4) and 100 µl of water. The color was developed by adding the followed reagents; 200 µl of 10% trichloroacetic acid (TCA), 200 µl of 42.5% ortho phosphoric acid, 200 µl of 4% 2,2´bipyridine and 100 µl 3% $FeCl_3$. The mixture was mixed and incubated at room temperature for 10 minutes. The absorbance was read at 525 nm with a spectrophotometer; L-ascorbic acid was used as a standard.

Glutathione (reduced form) was determined by using Ellman's reagent according to method of Greppin and Castillo (1988) [13]. The mixture solution contained with 50 µl of sample mixed with 950 µl of 60 mM potassium phosphate 2.5 mM EDTA buffer (pH 7.5), 2 mM 5,5´-dithiobis-2-nitrobenzoic acid (DTNB). The mixture was incubated at room temperature for 10 min. The absorbance was measured at 412 nm by using spectrophotometer. L-glutathione (reduced form) was used as a standard.

Antioxidant scavenging capacity

DPPH scavenging activity was determined by standard method [2] with modifications. 50 µl of sample was mixed with 350 µl of absolute methanol and then combined with 400 µl of 0.15 mM methanolic DPPH. The mixture

was incubated at room temperature for 30 minutes in the dark. The absorbance was read at 517 nm with a spectrophotometer. The results were expressed in grams; trolox equivalent antioxidant capacity (TEAC)/ml.

ATBS scavenging activity was estimated by following Jung et al. (2008) [8] with some modifications. The stock solution was prepared by mixing 7 mM ATBS in water and 2.45 mM potassium persulfate in equal volume then left in the dark for 12 hours. The working solution was diluted by mixing stock solution with absolute methanol to obtain an absorbance 1.0 ± 0.1 at 734 nm. $ATBS^{+}$ solution was freshly prepared for each assay. Twenty µl of sample was added into 980 µl of $ATBS^{\cdot+}$ solution for 5 minutes in the dark. The absorbance was measured at 734 nm by using spectrophotometer. The scavenging activity was expressed in gram TEAC/ml.

Statistical analysis

Data was shown as mean ± standard error (SE). All analyses were replicated three times. The results were analyzed by using one-way ANOVA and Duncan's multiple range test (DMRT) to test any significant differences.

3. RESULTS

The five antioxidants from four hot water extracts of *Phellinus linteus* and *Phellinus igniarius* in table 1 included total phenols, flavonoid, tocopherol, ascorbic acid and glutathione. The highest contents of all antioxidants was found in *Phellinus igniarius* product, PI-65. There were 16.50 ± 0.84, 0.71 ± 0.04, 37.76 ± 0.75, 1.21 ± 0.01 and 1.57 ± 0.07 mg/ml in total phenols, flavonoid, tocopherol, ascorbic acid and glutathione respectively. The lowest contents were found in *Phellinus igniarius* product, PI-16 as well. Its total phenols, flavonoid, tocopherol, ascorbic acid and glutathione contents were 6.01 ± 0.64, 0.26 ± 0.03, 14.67 ± 1.66, 0.36 ± 0.03 and 0.72 ± 0.01 mg/ml, respectively. This difference should be due to the concentration in both formulas being different as well as from the *Phellinus linteus* products. The PL-65 product was found to have lower antioxidant than PI-65 and higher than PL-38. However, there is no significant difference between PL-65 and PL-38 in flavonoid content.

Antioxidant activities were evaluated in hot water extracts using ABTS and DPPH assays. The DPPH and ABTS activity results were expressed in the term trolox equivalent antioxidant capacity (TEAC, g/ml). For DPPH assay, the synthetic DPPH radical can be reduced by an electron or hydrogen donation from extracts. The results showed that there were no significant differences of DPPH scavenging activity in both PL-65 and PI-65. The ability of extracts capable of scavenging DPPH free radicals follows this order: PI-65 = PL-68 > PL-38 > PI-16 (table 1). The ABTS assay was based on generating free radicals from the ABTS salt in the dark. As the results in table 1 revealed, all the hot water extract products were able to neutralize the ABTS radicals. The maximum capacity was found in PI-65 and PL-65 products. While activity in PL-38 was higher than PI-16, it was lower than PI-65 and PL-65.

Table1. Contents and activities of antioxidant components from hot water extract of natural *Phellinus linteus* and *Phellinus igniarius*.

Products	Antioxidant content (mg/ml)					Antioxidant scavenging activity (TEAC, g/ml)	
	Total phenols	Flavonoids	Total tocopherols	Ascorbic acid	Glutathione	DPPH	ABTS
PL-65	14.17±0.84 b	0.57±0.01 b	29.78±1.92 b	0.85±0.02 b	0.90±0.03 b	1.00±0.01 a	1.79±0.05 a
PL-38	9.03±0.84 c	0.48±0.03 b	18.78±1.61 c	0.37±0.03 c	0.78±0.00 c	0.87±0.02 b	1.23±0.06 b
PI-65	16.50±0.32 a	0.71±0.04 a	37.76±0.75 a	1.21±0.01 a	1.57±0.07 a	1.00±0.01 a	1.88±0.06 a
PI-16	6.01±0.64 d	0.26±0.03 c	14.67±1.66 c	0.36±0.03 c	0.72±0.01 c	0.72±0.03 c	0.88±0.02 c

Each value is expressed as mean ± standard error (n = 3)
Values indicated by different letters in a column are significantly different ($p < 0.05$)

4. CONCLUSION

Hot water extracts from *Phellinus linteus* and *Phellinus igniarius* in commercial products were evaluated in antioxidant contents and activities. According to the results, PI-65 was found the highest in antioxidant contents and PI-16 was found the lowest in antioxidant contents. In antioxidant activities, all the products had ability to reduced free radicals in DPPH and ABTS assays. The highest activities of DPPH and ABTS scavenging capacities were found in PI-65 and PL-65 products. These results showed that hot water extract products by *Phellinus linteus* and

Phellinus igniarius may contain a good source of antioxidants, which prevent oxidative damages from free radicals that cause human diseases.

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