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ANTIOXIDANT ACTIVITY OF FRUITING BODY EXTRACTS FROM *PYCNOPORUS SANGUINEUS* MUSHROOM

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Abstract. The objective of the this study is to provide an *in vitro* evidence for the potential antioxidant activity of the ethanolic and aqueous extracts from fruiting bodies of *Pycnoporus sanguineus* (L.: Fr.) Murrill mushroom via DPPH free radical scavenging and lipid peroxidation inhibition assays. DPPH free radical and lipid peroxidation inhibitory activities of ethanolic and aqueous extracts from fruiting bodies of *P. sanguineus* were examined in a dose-response manner. Ascorbic acid and trolox were used as a positive control for these assays. The results showed that the ethanolic extract of *P. sanguineus* possessed strong inhibitory activity on DPPH free radical and lipid peroxidation with IC_{50} values of 196.68 and 975.84 µg/mL, respectively. The aqueous extract also exhibited moderate activity against DPPH free radical and lipid peroxidation with IC_{50} values of 322.03 and 1311.24 µg/mL, respectively, which was significantly lower than that of ascorbic acid and trolox with IC_{50} values of 55.00 and 886.68 µg/mL, respectively. The IC_{50} values of *P. sanguineus* aqueous extract against DPPH and lipid peroxidation (322.03 and 1311.24 µg/mL, respectively) to be much higher than corresponding positive controls and therefore lower antioxidant activity inversely. The degree of DPPH free radical and lipid peroxidation inhibition correlated with the dose of samples (inhibitors). Based on the obtained results, the *P. sanguineus* mushroom possesses high potential in antioxidant activity and could be a potential source of natural antioxidants in food and pharmaceutical applications.

Keywords: antioxidant activity, DPPH free radical, lipid peroxidation, *Pycnoporus sanguineus*.

Classification numbers: 1.2.1; 1.3.1.

1. INTRODUCTION

Pycnoporus sanguineus (L.: Fr.) Murrill (Syn. *Trametes sanguinea* (L.) Lloyd) mushroom has been considered as one of the 25 major medicinal macrofungi worldwide [1]. This

mushroom is known to be rich in various bioactive substances with anti-bacterial, anti-fungal, anti-viral, antiparasitic, anti-oxidant, anti-inflammatory, anti-proliferative, anti-cancer, anti-tumour, cytotoxic, anti-HIV, hypocholesterolemic, anti-diabetic, anti-coagulant, hepatoprotective, and more other activities [2-5]. Qualitative phytochemical analysis of the extracts from fruiting bodies of the *P. sanguineus* revealed the presence of flavonoids, saponins, tannins, and terpenoids [5]. *P. sanguineus* mushroom has also been successfully cultivated on various agricultural by-products such as corn cobs, melaleuca bark, rice hulls [6-8].

Oxidative stress and cellular metabolism generate reactive oxygen species (ROS) that contribute to the etiology, pathogenesis and progression of several diseases including inflammations, cancer, and cardiovascular diseases [9]. Dietary antioxidants can inactivate ROS and provide protection from oxidative damage, and are therefore considered important therapeutic and prophylactic agents against disease development [10].

Lipid peroxidation is a free radical chain reaction, which causes oxidative damage to membrane lipids in biological systems. It impairs the biological functions of membrane, inactivates membrane bound enzymes and receptors and may change nonspecific calcium ion permeability [11, 12]. The basic pre-requisite for the occurrence of lipid peroxidation is inadequate free radical scavengers.

The interest in finding natural antioxidants for use in the food products and the pharmaceutical markets have grown notably since the early 1980s. The investigations aim at replacing the commonly used synthetic antioxidants such as butylhydroxytoluene and butylhydroxyanisole, which have been prohibited due to their carcinogenic potential as well as other health problems they may cause, including a gain in liver weight and the considerable proliferation of the endoplasmatic reticule [13, 14]. Many studies have highlighted the benefits of the consumption of antioxidant substances in daily diet. This can result in an effective action to protect the organism against the oxidative processes. It was discovered that several diseases, including cancer, arteriosclerosis, diabetes, arthritis, malaria, AIDS, and heart disease, may be linked to the damage caused by different forms of extremely ROS. These substances are also related to the human ageing process [15].

An endogenous antioxidant system of the body takes care of ROS that are generated under normal physiological conditions. However overproduction of ROS and inadequate presence of antioxidants have been related to pathogenesis of disease conditions like diabetes, cancer, atherosclerosis etc. [16, 17]. Our body's self antioxidant system comprises of enzymes like catalase, superoxide dismutase and glutathione which react to reactive species and neutralize them thereby protecting the body from damaging effect of free radical species and preventing oxidative stress [18].

The objective of this study is to provide an *in vitro* evidence for the potential antioxidant activity of the ethanolic and aqueous extracts from fruiting bodies of *Pycnoporus sanguineus* mushroom via DPPH free radical scavenging and lipid peroxidation inhibition assays.

2. MATERIALS AND METHODS

2.1. Materials

The fruiting bodies of *Pycnoporus sanguineus* MH225776.1 were collected in Viet Nam. This mushroom was identified by Tran Duc Tuong of Dong Thap University, a corresponding author of the paper, based on morphological and molecular characterisation of mycelia and the

fruiting bodies. The *P. sanguineus* MH225776.1 was planted on the formula of compost consisting of 50 % corn cobs and 50 % rubber sawdust at the Biotechnology Research and Development Institute, Can Tho University, Viet Nam.

2.2. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (BDH, England); Cyclophosphamide (Baxter Oncology GmbH, Germany); Methanol, phosphate buffer pH 7.4, DMSO buffer, and ascorbic acid (Prolabo, France); Trolox, thiobarbituric acid, and tricloacetic acid (Sigma-Adrich Co., USA).

2.3. Methods

2.3.1. Extraction

After being crushed, the fruiting bodies of *P. sanguineus* were soaked in solvents (ethanol 96 % v/v at room temperature during 72 hours and water at 80 ºC during 7 hours) at the ratio of 1:15. Next, soaking solution was filtered through filter paper. Finally, the filtrate was concentrated to remove solvents with a vacuum rotary evaporator (IKA RV 05 Basic - Germany), yielding total ethanolic and aqueous extracts.

2.3.2. Determination of DPPH free radical scavenging activity

This assay was carried out using a modified procedure of Blois [19]; Chi and Huong [20]; Phuong *et al*. [21]; National Institute of Medicinal Materials [22]. DPPH solution was prepared in methanol (MeOH) and stored in the dark at room temperature for about 30 minutes. The samples were prepared in DMSO into solution with a range of concentrations of 50, 100, 200, 300 and 400 (µg/mL). 1 mL each of the samples with various concentrations were placed in tubes containing 1 mL DPPH solution (0.2 mM). The reaction mixture was shaken for 15 seconds and stabilized in the dark at room temperature for 30 minutes. A control was prepared using the same procedure replacing the extracts with 1 mL of DMSO. Color reaction absorption was measured at 517 nm using a microplate reader (Thermo Spectronic Genesys 10 UV Vis Spectrophotometer). Ascorbic acid used as a positive control for this assay was investigated with ranges of concentrations of 5, 10, 20, 40 and 80 (µg/mL). The experiment was arranged with three replications. Percentage of DPPH free radical scavenging activity is calculated as:

$$\text{DPPH free radical scavenging activity (\%)} = [(Abs_{control} - Abs_{extracts/ascorbic\ acid})/Abs_{control}] \times 100$$

Notes: $Abs_{control}$: The optical density values of negative control.

$Abs_{extracts/ascorbic\ acid}$: The optical density values of the extracts/ascorbic acid samples.

Concentrations of the extracts resulting in 50 % inhibition of DPPH free radical (IC_{50}) were determined graphically.

2.3.3. Determination of lipid peroxidation inhibitory activity

Lipid peroxidation inhibitory activity assay was performed as previously described by National Institute of Medicinal Materials [22], Robak *et al*. [23]; Stroev and Makarova [24]; and Huong and Nghia [25] with modification as follows. Mice (40 - 50 g) were induced to increase MDA by cyclophosphamide (single dose 400 mg/kg body weight) for 7 days before they were used for the preparation of brain homogenate. Brain homogenate was prepared with homogenizer at 0 - 5ºC with phosphate buffer pH 7.4 (50 mM) (1:10) for 30 min. The homogenate was centrifuged for 15 min, and clear cell free supernatant was used for the study of *in vitro* lipid peroxidation. 0.2 mL each of the samples with various concentrations (10, 50, 100,

500, 1,000, 1,500 and 2,000 µg/mL) were placed in tubes containing 1 mL of added brain homogenate. Continue adding phosphate buffer to a sufficient 2 mL mixture. After being incubated at 37 ºC for 15 min, the reaction was stopped by addition of 1 mL tricloacetic acid (10 %). The reaction mixture was centrifuged at a rate of 10,000 rpm at 5 ºC for 10 min. Take 1 mL of clear solution after centrifugation to react with 1 mL of thiobarbituric acid reagent (0.8 %). The mixture was then heated at 100 ºC for 15 min. The samples were cooled and the absorbance was measured at 532 nm using a microplate reader (Thermo Spectronic Genesys 10 UV Vis Spectrophotometer). A control was prepared using the same procedure replacing the extracts with 0.2 mL of DMSO. Trolox was used as a positive control for this assay. The experiment was arranged with three replications. Percentage of lipid peroxidation inhibition is calculated as:

$$\text{Lipid peroxidation inhibition (\%)} = [(Abs_{control} - Abs_{extracts/trolox})/Abs_{control}] \times 100$$

Notes: $Abs_{control}$: The optical density values of negative control.

$Abs_{extracts/trolox}$: The optical density values of the extracts/trolox samples.

Concentrations of the extracts resulting in 50% inhibition of lipid peroxidation (IC_{50}) were determined graphically.

2.3.4. Statistical analysis

The data were statistically analyzed by One-Way analysis of variance (ANOVA) and Independent-Samples T Test of the SPSS Statistics 22.0 software. Statistical differences at p-values under 0.05 were considered significant and subsequently compared using the Tukey's test with 95 % confidence intervals.

3. RESULTS AND DISCUSSION

3.1. DPPH free radical scavenging activity

As shown in Figure 1 and Figure 2, ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus* and ascorbic acid showed DPPH free radical scavenging effects in a dose-dependent manner at the examined concentrations. Inhibition at various concentrations is significantly different ($p < 0.05$).

The highest DPPH free radical scavenging activity (at the concentration of ascorbic acid 80 µg/mL and extracts 400 µg/mL) was observed from the ascorbic acid (72.40 %), ethanolic extract (89.25 %) and aqueous extract (59.12 %) from fruiting bodies of the *P. sanguineus* with IC_{50} values being 55.00 µg/mL, 196.68 µg/mL and 322.03 µg/mL, respectively (Tab. 1). The results showed that ethanolic extract of mushroom had good DPPH free radical scavenging activity at all the examined concentrations. However, this inhibitory activity of ethanolic and aqueous extracts was significantly lower than that of ascorbic acid (positive control) (Fig. 1 & Tab. 1). DPPH free radical scavenging activity of the ethanolic extract was significantly higher than aqueous extract in the concentrations range of 50 - 400 µg/mL ($p < 0.05$) (Fig. 2 & Tab. 1). DPPH free radical scavenging activity of extracts from fruiting bodies of the *P. sanguineus* in this study is higher than that of the ethanolic extract (IC_{50} value at 1,326 µg/mL) and aqueous extract (IC_{50} value at 1,708 µg/mL) from fruiting bodies of *Ganoderma lucidum* reported by Huong and Hang [26] and methanolic extract with IC_{50} value at 9,000 µg/mL [27].

The previous study of Borderes *et al*. [28] showed the methanolic extract from *P. sanguineus* mycelium against DPPH free radical with IC_{50} values being 1,620 µg/mL [28]. This

inhibition of extract from *P. sanguineus* mycelium was lower than that of extracts from fruiting bodies of the *P. sanguineus* mushroom.

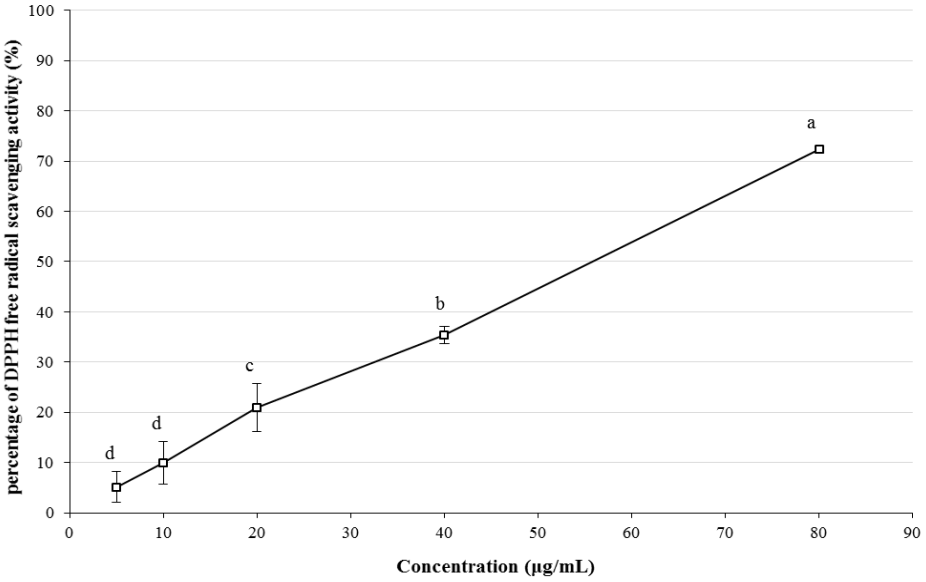

Figure 1. DPPH free radical scavenging activity of ascorbic acid (positive control) at various concentrations. The values are expressed as the mean ± standard deviation of three replicates (One-Way ANOVA followed by Tukey's test). Means not sharing a common letter were significantly different ($p < 0.05$).

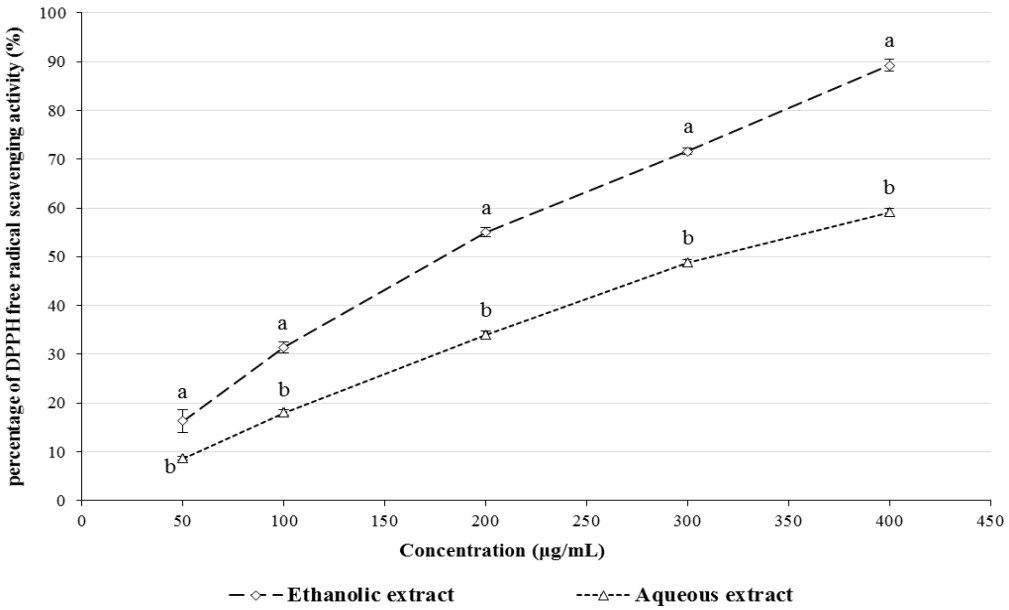

Figure 2. DPPH free radical scavenging activity of ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus* at various concentrations. The values are expressed as the mean ± standard deviation of three replicates (Independent-Samples T Test). Means not sharing a common letter at the same concentration were significantly different ($p < 0.05$).

Table 1. IC_{50} values of samples (ascorbic acid, ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus*) against DPPH free radical with correlation coefficient.

Samples	IC_{50} values (µg/mL)*	Correlation coefficient
Ascorbic acid	55.00	$R^2 = 0.9979$
Ethanolic extract	196.68	$R^2 = 0.9888$
Aqueous extract	322.03	$R^2 = 0.9914$

Note: *Concentrations of the samples resulting in 50 % inhibition of DPPH free radical.

3.2. Lipid peroxidation inhibitory activity

Similar to the results of DPPH free radical scavenging activity, ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus* and trolox showed lipid peroxidation inhibitory effects in a dose-dependent manner at the examined concentrations. Inhibition at various concentrations range of 100 - 2000 µg/mL is significantly different ($p < 0.05$) (Fig. 3).

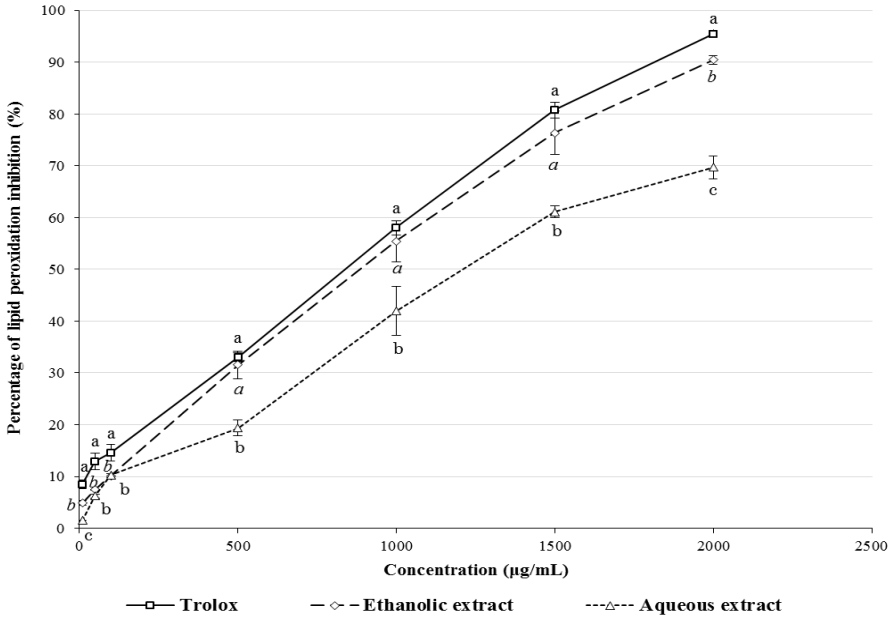

Figure 3. Lipid peroxidation inhibition of trolox (positive control), ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus* at various concentrations. The values are expressed as the mean ± standard deviation of three replicates (One-Way ANOVA followed by Tukey's test). Means not sharing a common letter at the same concentration were significantly different ($p < 0.05$).

Table 2. IC_{50} values of samples (trolox, ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus*) against lipid peroxidation with correlation coefficient.

Samples	IC_{50} values (µg/mL)*	Correlation coefficient
Trolox	886.68	$R^2 = 0.9941$
Ethanolic extract	975.84	$R^2 = 0.9906$
Aqueous extract	1311.24	$R^2 = 0.985$

Note: *Concentrations of the samples resulting in 50% inhibition of lipid peroxidation.

The highest lipid peroxidation inhibitory activity at the concentration of 2000 µg/mL of each samples was observed from trolox (95.53 %), ethanolic extract (90.45 %) and aqueous extract (69.71 %) from fruiting bodies of the *P. sanguineus* with IC_{50} values being 886.68 µg/mL, 975.84 µg/mL and 1311.24 µg/mL, respectively (Tab. 2). The results showed that ethanolic extract of mushroom had good lipid peroxidation inhibitory activity equivalent to trolox in the concentrations range of 500 - 1500 µg/mL ($p < 0.05$). However, this inhibitory activity of aqueous extracts was significantly lower than that of trolox (positive control). Lipid peroxidation inhibitory activity of the ethanolic extract was significantly higher than aqueous extract in the concentrations range of 500 - 2000 µg/mL ($p < 0.05$) (Fig. 3 & Tab. 2). Lipid peroxidation inhibition of extracts from fruiting bodies of the *P. sanguineus* in this study is higher than that of the ethanolic extract (IC_{50} value at 1,368 µg/mL) and aqueous extract (IC_{50} value at 2,818 µg/mL) from fruiting bodies of *Ganoderma lucidum* reported by Huong and Hang [26].

The result showed potent free radical scavenging activity of extracts from fruiting bodies of the *P. sanguineus* with the superior inhibitory activity in ethanolic extract. These inhibitory actions might be due to both ethanolic and aqueous extracts from fruiting bodies of this mushroom containing several phytochemicals such as flavonoids, saponins, and terpenoids reported by Tuong *et al*. [5]. The high antioxidant activity of the ethanolic extract from fruiting bodies of the *P. sanguineus* may be attributed to the high content of flavonoids. The earlier studies proved that, the secondary metabolites such as flavonoids, terpenoids, saponins had strong free radical scavenging capacities [29-32].

4. CONCLUSION

Pycnoporus sanguineus (*Trametes sanguinea*) presented a satisfactory antioxidant potential when compared to other mushrooms. The best results indicated that this was proved by DPPH free radical scavenging and lipid peroxidation inhibitory activities of ethanolic and aqueous extracts from fruiting bodies of this mushroom with the predominant inhibitory activity in ethanolic extract. *P. sanguineus* showed potential to activity as a natural source of antioxidant compounds. Hence, it could be of great importance to develop further studies addressing the issues such as the purification and identification of these compounds which are responsible for antioxidant activity of *P. sanguineus* mushroom.

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