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Antioxidants Activities of *Pleurotus eous* (Berk.) Sacc.: (APK1) Mushroom

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Abstract

The objective of the study is to determine antioxidant activity of aqueous extract at three different harvesting stages of *Pleurotus eous* (Pink mushroom), an edible oyster mushroom. The PEAE1, PEAE2 and PEAE3 samples harvested at different stages and antioxidant properties were studied. At 0.5-2.5 mg/ml, PEAE exhibited DPPH scavenging activities in the range of 22.33%-84.76%. With regard to the effect on hydroxyl radicals, PEAE at 1-5 mg/ml exhibited antioxidant activities in the range of 16.22%-83.39%. The total antioxidant activity was also determined by FTC and TBA tests and results showed lipid peroxidation inhibition. Thus, all these suggest that aqueous extract of *Pleurotus eous* is rich in natural antioxidants and has many therapeutic properties.

Keywords: Antioxidants, Aqueous Extract, Free radicals, Mushroom, Oyster, *Pleurotus*

1. Introduction

In healthy individuals, the natural antioxidative defence systems continuously balance ROH production. Oxygen-centered free radicals and ROS are associated with many diseases [1].

Edible mushrooms have multiple functional properties [2]. Presently mushrooms available commercially have antitumour, antiviral, and immunomodulating effect, but little information is available on their antioxidant properties [3]. Commercial mushrooms are now probed for therapeutic value, but only very meagre information is available on its antioxidant properties. Commercial mushrooms are now probed for their therapeutic value.

Pleurotus belonged to edible basidiomycetes commonly called oyster mushroom: species are edible, and are cultivated commercially [4]. The genus *Pleurotus* has about 40 species [5] and is the second most important cultivated mushrooms in the world. It has non-starchy carbohydrates, dietary fiber, essential amino acids, minerals and vitamins [6].

Hence, the current study is aimed to evaluate the free radical scavenging properties by *in vitro* assay systems and the advantage in consuming *Pleurotus eous* as food.

2. Materials and Methods

2.1 Sample Extraction

APK1 is a pink edible oyster mushroom collected from Tamil Nadu Agricultural University, Coimbatore. The PE 1, PE 2 and PE 3 harvested for three consecutive days were dried and 10 g each were extracted by stirring with 100 ml of boiling water for 4 hrs, centrifuged at 5000 rpm for 15 min and filtered using Whatmann No. 1 filter paper. The residue was then extracted two times with 100 ml of boiling water. These extracts were freeze dried and labeled as PEAE 1, PEAE 2 and PEAE 3 respectively and stored at 4°C until analysis.

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2.2 *Invitro* Assays

Hydroxyl and 1,1-diphenyl-2-picrylhydrazyl (DPPH) quenching ability was measured using to a literature procedure [7], [8] with few modifications.

2.3 Total Antioxidant Activity

2.3.1 Ferric Thiocyanate (FTC) Test

FTC test was conducted according to the method described by Kikuzaki and Nakatani [9].

2.3.2 Thiobarbituric acid (TBA) Test

TBA test [10] was conducted to study lipid peroxidation by evaluating malonaldehyde formation.

3. Statistical Analysis

The data obtained in triplicate were expressed as mean ± S.D. Statistical differences at $p<0.05$ between the stages were analyzed by ANOVA using SPSS 15.0 software.

4. Results and Discussion

4.1 Scavenging Ability on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radicals

Table 1 depicts the radical scavenging activity of PEAE extracts.

Values are expressed as mean ± SD (n =3). Means within the same row with different letters (a-e) and means within the same column with different letters (x-z) not sharing common superscript letters differ significantly at 5% level by DMRT.

Table 1. Scavenging activity (%) of DPPH radical from *P. eous*

Extracts	Sample concentration (mg/ml)				
	0.5	1.0	1.5	2.0	2.5
PEAE1	22.93 ± 0.55^{ax}	43.74 ± 1.43^{bx}	64.33 ± 1.59^{cx}	78.24 ± 3.52^{dx}	83.76 ± 2.08^{ex}
PEAE2	24.09 ± 1.23^{ay}	35.77 ± 1.18^{by}	54.88 ± 1.35^{cy}	77.07 ± 3.86^{dx}	80.68 ± 1.52^{dy}
PEAE3	22.33 ± 1.10^{ax}	35.87 ± 0.88^{by}	55.03 ± 1.10^{cy}	77.25 ± 2.32^{dx}	84.76 ± 1.68^{ex}

At 2.5 mg/ml, scavenging activity of PEAE1 was 83.76 %, PEAE2 80.68 %, PEAE 3 84.76 %. PEAE3 showed higher radical scavenging activity followed by PEAE1 and PEAE2. EC_{50} values were 1.4, 1.24, 1.35 mg/ml (50% inhibition) respectively. In comparison, at 0.2 mg/ml, scavenging abilities of BHA, BHT and ascorbic acid were 86.88 %, 98.08 % and 98.09 % respectively.

PEAE3 showed higher radical scavenging activity against DPPH followed by PEAE1 and PEAE2 at 2.5 mg/ml. The scavenging ability was better and this might be due toextraction of hydrogen donating components from PEAE. Nonpolar phenolics such as carotenoids and tocopherols present in mushroom might be responsible for the high antioxidant activity [11]. The above results revealed the various extracts of PEAE had free radical scavengers, which act as primary antioxidants.

Inhibition of lipid oxidation by scavenging free radicals presents deleterious effects that can happen to cellular conponents and functions [12]. Synthetic antioxidants can be replaced by natural antioxidants present in mushrooms to reduce oxidative damage and can benefit degenerative illness [13], [14].

4.2 Hydroxyl Radical Scavenging Activity

Oxidative damage to DNA, lipids and proteins was caused by highly reactive OH ions [15]. Hydrogen atom can neutralize OH, as in the case of many free radicals.

The PEAE1, PEAE2 and PEAE3 were shown to scavenge the hydroxyl radical directly to an extent of 2-10 mg/ml with an inhibition percentage of 16.22-78.04 %, 30.74-82.09% and 37.64-83.39% respectively (Table 2). The EC_{50} values were found to be 2.8, 2.7, 2.2mg/ml (50% inhibition) respectively.

Table 2. Hydroxyl radical scavenging activity (%) of various extracts from *P. eous*

Extracts	Sample concentration (mg/ml)				
	2	4	6	8	10
PEAE1	16.22 ± 0.39^{ax}	34.12 ± 1.66^{bx}	53.38 ± 2.15^{cx}	70.27 ± 1.79^{dx}	78.04 ± 2.93^{ex}
PEAE2	30.74 ± 1.35^{ay}	41.55 ± 1.05^{by}	57.43 ± 1.76^{cy}	67.91 ± 3.40^{dy}	82.09 ± 2.09^{ey}
PEAE3	37.64 ± 0.03^{az}	49.45 ± 1.40^{bz}	54.61 ± 1.32^{cx}	68.63 ± 1.80^{dy}	83.39 ± 2.10^{ey}

Values are expressed as mean ± SD (n =3). Means within the same row with different letters (a-e) and means within the same column with different letters (x-z) not sharing common superscript letters differ significantly at 5% level by DMRT.

PEAE3 showed higher radical scavenging activity against hydroxyl radicals followed by PEAE2 and PEAE1 at 10 mg/ml. By abstracting hydrogen atoms, lipid

peroxidation are brought about by hydroxy radicals [16]. Mushrooms have show to protect biomembranes from lipid peroxidation and scavenge the hydroxy radicals and superoxide anions at initiation stage and terminates peroxy radicals. Overall the scavenging might be due to the active hydrogen donating ability of hydroxy substitutions.

4.3 Total Antioxidant Activity (FTC and TBA Tests)

Figure 1 shows the hydroperoxides inhibitory activity of PEAE through FTC test. According to Steinbrecher [17], one of the reasons for atherosclerotic development is peroxidation of LDL fractions. Antioxidants curtail the concentration of peroxides in the initial stages of peroxidation of lipids.

When compared to control, the PEAE1, PEAE2 and PEAE3 extracts diminish hydroperoxidation of linoleic acid during the entire incubation period. The PEAE showed less increase in absorbance values from 12 hours to 72 hours, indicating high antioxidant potential.

This result suggests that the PEAE are found to terminate the radical chain reaction and reduce hydroperoxide generation [18], [19]. After the controls reached its maximum absorbance, FTC and TBA tests (Figure 2) were conducted on the samples. The extent of lipid peroxidation is evaluated based on the formation of malonaldehyde. At low pH and high temperature (100°C) malonaldehyde binds TBA to form a red complex which correlates with the oxidative rancidity of the lipid that can be measured at 532 nm.

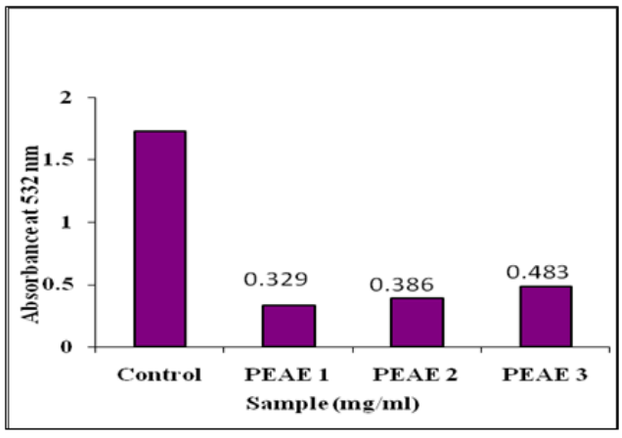

Figure 1. FTC assay.

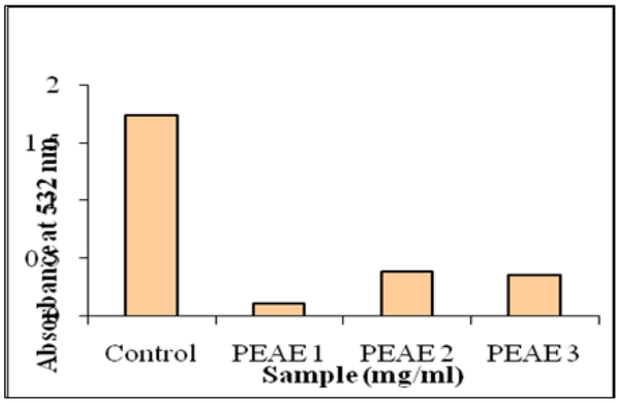

Figure 2. TBA inhibitory activity.

The trends of thiobarbituric acid reactive substances inhibitory activity of PEAE1, PEAE2 and PEAE3 and FTC are similar which might be due to the lower hydroperoxides accumulation in the extracts. Besides, secondary antioxidant compounds that might be present in PEAE may also cause inhibition of hydroperoxides decomposition.

5. Conclusion

Our results concluded that PEAE possesses high antioxidant activity. Current research confirms the antioxidant activity of Pleurotus eous pink oyster mushroom (APk1). From this it is confirmed that the use of this mushroom in food brings a significant increase in the component of antioxidants.

6. References

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