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Antioxidant Properties and Phytochemical Screening of Infusion and Decoction Obtained from Three Cultivated *Pleurotus* Species: A Comparative Study

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ABSTRACT

Natural products represent a rich source of biologically active compounds and an example of molecular diversity with recognized potential in drug discovery. Nowadays, medicinal mushrooms used in folk medicine are being increasingly studied for their successful application in pharmaceutical industries. In this context, *Pleurotus* species are one such potential mushroom with worldwide acceptance as a delicious food and famed for remarkable medicinal properties. However, till date no reports regarding the bioactivity of the most compatible herbal formulations of this mushroom (infusion and decoction) have been documented. Therefore, the present study has been undertaken to investigate the phytochemical composition and antioxidant properties of three cultivated species of this group namely, *P. eous*, *P. florida* and *P. ostreatus*. Phytochemical composition revealed that the infusion fraction of P. eous is the most enriched fraction having high amount of phenols (13.03 µggallic acid equivalent/mg of extract), flavonoids (3.57 µg quercetin equivalent/mg of extract) and ascorbic acid (16.66 µg/mg of extract). Moreover, free radical scavenging potentials of the corresponding fractions of these mushrooms were tested by different *in-vitro* systems. Results demonstrated infusion had noticeable scavenging activity in concentration dependent manner while decoction exhibited moderate activity for all three species. Decoction did not show any activity in hydroxyl radical scavenging assay. Together, our findings provide a detailed data explaining beneficial therapeutic properties of aqueous fraction of these *Pleurotus* species. Additionally, it also can be concluded that in comparison with other studied mushroom, *P. eous* infusion is superior in terms of antioxidant efficacy.

Keywords: Cultivated mushroom, Free radical scavenger, Nutraceuticals, Phytochemicals, Phenolic compounds.

INTRODUCTION

Since ancient past, mushrooms have been consumed as a low caloric diet food of unique aroma and taste. Besides its remarkable nutritional values, they have become an attractive source of nutraceuticals as a major attention has been paid on the discovery of its therapeutically active ingredients[1]. Nowadays, many Asian as well as Western countries devotes more consideration to cultivation of mushrooms as it not only improves livelihood through economical basis but also promotes bioconversion of waste products into a reliable source of nutritious food. The doctrine of 'white revolution' began with the view that mushrooms are now becoming a precious treasure with multidimensional application in human welfare[2]. As expected, in 21st century around 70% of agricultural waste products have been bioprocessed through mushroom cultivation for the need of mankind. A vast number of studies have been reported regarding optimal requirement for successful cultivation of mushrooms[3, 4]. Owing to the difference in solubility of bioactive compounds, suitable extraction

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procedure thus, plays a crucial role in therapeutic attributes of different mushroom species. Ethno-botanical inspection revealed that most of the herbal preparation of Ayurveda is relied on extraction with boiling water as they are easily digestible[5]. Traditionally, two methods; infusion and decoction are considered as time honoured methods, easy to prepare for delivering the goodness of herbs. It has been reported that these herbal preparations are mostly effective for the treatment of urinary tract ailments, kidney disorders and other health issues[6]. However, application of mushroom powder through infusion and decoction are often overlooked while some medicinal mushroom including Reishi mushroom, Chaga mushroom have been known to possess significant health promoting favour by virtue of these herbal preparations[7,8].

Pleurotus species are renowned as world's second most commercially cultivated mushroom and highly appreciated throughout the globe for their remarkable nutritional and medicinal benefits. They are widely cultivated in different regions of India, Europe, and Africa and commonly known as oyster mushroom[9]. Since ancient times, they have been valued as a rich source of carbohydrates, proteins, vitamins, minerals, dietary fibres and others. Several reports have been documented regarding their health benefits including antioxidant, antimicrobial, antitumor, immunomodulator etc[10]. However, there is no such report about its medicinal properties of traditionally used orally suitable formulation, infusion and decoction. In this present investigation, an attempt has been undertaken with the objective to evaluate and compare phytochemical profile and antioxidative properties of infusion and decoction of three commonly cultivated *Pleurotus* species, *P. eous*, *P. florida* and *P. ostreatus* using four different *in vitro* systems.

Table 1. Phytochemical profiling and comparison *between P. eous, P. florida* and *P. ostreatus*.

Mushrooms	Phenol (µg GAE/mg of extract)		Flavonoid (µg QE/mg of extract)		Ascorbic acid (µg/mg of extract)	
	Infusion	Decoction	Infusion	Decoction	Infusion	Decoction
P. eous	13.03±1.05^{a}	5.78±2.73^{b}	3.57±0^{a}	2.23±1.23^{b}	16.66±0.8^{a}	11.1±1.02^{b}
P. florida	8.2±1.87^{a}	2.17±1.02^{b}	2.57±1.03^{a}	1.19±1.24^{b}	16.66±0.77^{a}	12.5±0.25^{b}
P. ostreatus	9.4±1.34^{a}	5.2±1.23^{b}	3.71±0.73^{a}	0.73±1.71^{b}	10±1.05^{a}	6.66±0.23^{b}

Abbreviations: GAE as gallic acid equivalent; QE as quercetin equivalent. Results are presented in mean ± SD (n = 3). Different letters in column was showed statistically significant differences ($p<0.05$) according to ANOVA.

MATERIALS AND METHODS

Instrument

For microtiter based method, Bio-Rad iMarkTM Microplate Reader (USA) was used to measure absorbance of reaction mixtures in 96 well plate. The instrument is an eight-channeled vertical path length photometer which reads at 6 specific wavelengths including 415, 450, 490, 595, 655, and 750 nm.

Samples

The cultivated *Pleurotus* species viz., *P. ostreatus*, *P. florida* and *P. eous* were collected from cultivation center of Narendrapur, West Bengal. Each sample after collection were dried to make them crispy at 40°C for overnight and melded to fine powder using a mixer grinder.

Preparation of Extracts

Infusion was prepared by adding 0.1 g of dried mushroom powder to 20 ml of boiled distilled water and left the mixtures to stand for 5 min at room temperature and then filtered through Whatman filter paper.

For decoction, 0.1 g sample was mixed with 20 ml distilled water and boiled for 5 min on a heating plate.

Then after 5 min standing time, the fraction was filtered and stored. The obtained fractions should not be stored for more than one week.

Phytochemical Estimation

Mushroom bioactive phytochemicals including phenol, flavonoid, ascorbic acid, β carotene and lycopene content was estimated following our previously published article[11]. Total phenol and flavonoid content was calculated from calibration curve prepared using Gallic acid and Quercetin as a standard respectively. Total ascorbic acid was estimated by titration against 2, 6-dichlorophenol indophenol dye (DCPIP). β carotene and lycopene content was calculated from the following formula:

β carotene (mg/100 mg) – $0.0458\times A_{663}+0.373\times A_{505}-0.0806\times A_{453}$

Lycopene (mg/100 mg)– $0.216\times A_{663}-0.304\times A_{505}+0.452\times A_{453}$

Determination of Antioxidant Potential

DPPH radical scavenging activity:

DPPH radical scavenging activity of extracts was determined following the method of Pereira et al. with little modifications[12]. Reaction mixture (0.2 ml) comprising of different concentrations of each extracts and DPPH solution was incubated for 30 min in a 96 well microtiter plate. Following that radical scavenging activity was measured by taking absorbance at 575 nm in a microplate reader. Results were expressed with respect to ascorbic acid standard as percentages of DPPH radical quenching ability. EC_{50} value was noted that indicates the concentration of the extract at which 50% DPPH radicals were quenched.

ABTS radical scavenging potential:

For determining ABTS radical scavenging activity, a standard protocol by Khatua et al. was followed[13]. In brief, ABTS•+ reactive cation radical was prepared first by mixing 1:1 (v/v) 7 mM ABTS solution and 2.45 mM potassium persulfate and incubated 12-16 h in dark at room temperature. Before use in experimental sets, ABTS•+ solution was diluted with water to obtain an absorbance of 0.700 at 750 nm. 0.2 ml reaction sets were prepared by adding various concentrations of extract along with respective amounts of ABTS•+ solution in a 96 well microtiter plate and left for incubation of 10 min in dark. Trolox was used as standard and ABTS•+ radical scavenging activity (%) was measured spectroscopically at 750 nm wavelength in microplate reader. EC_{50} was calculated from calibration curve.

Hydroxyl radical scavenging activity:

The antioxidant potential of the extracts can be measured in terms of hydroxyl radical scavenging activity using BHA as standard[14]. 1 ml reaction mixture consisted of KH_2PO_4 - KOH buffer (20 mM, pH 7.4), 2-deoxy-D-ribose (2.8 mM), variable concentration of extracts, $FeCl_3$ (100 mM), EDTA (104 µM), ascorbate (100 µM) and H_2O_2 (1 mM). Reaction mixture was incubated at 37˚C for 1 h. 2 ml thiobarbituric acid (TBA) and trichloroacetic acid (TCA) solution (100 ml contained 375 mg TBA, 15 mg TCA; 2 ml concentrated HCL added to 98 ml of TBA-TCA solution) was added and incubated at boiling water bath for 15 min. After cooling, absorbance was measured at 535 nm. EC_{50} value indicates the effective concentration at which radical scavenging activity was 50%. Degree of scavenging was calculated by the following equation:

Scavenging effect = {(A0-A1)/ A0} × 100

A0 was absorbance of the control and A1 was absorbance in the presence of sample.

Total Antioxidant Capacity:

The total antioxidant capacity of the extracts was measured according to the following method[15]. Briefly, 0.1 ml of each extracts was mixed with 3 ml freshly prepared reagent solution containing 0.6 M sulfuric acid (H_2SO_4), 28 mM sodium phosphate (Na_3PO_4) and 4 mM ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) and incubated for 90 min at 95°C. After cooling down following incubation, absorbance was measured at 695 nm against blank. The antioxidant capacity was expressed as the number of equivalents of ascorbic acid.

Statistical Analysis

The assays were carried out in triplicate and results are expressed as mean ± standard deviation (SD). Data analysis is carried out using Microsoft®Office Excel (Microsoft®, USA).). Results were compared by *ANOVA* to determine variances among samples and values of $p<0.05$ were considered as statistically significant

Table 2. Antioxidant activities of infusion and decoction obtained from three *Pleurotus* species

Antioxidant parameters		*P. eous*		*P. florida*		*P. ostreatus*	
		Infusion	**Decoction**	**Infusion**	**Decoction**	**Infusion**	**Decoction**
EC_{50} values (µg/ml)	DPPH radical scavenging activity	97±1.23[a]	81±0.89 [b]	108±2.04[a]	71±1.5[b]	105±0.78[a]	83±1.49[b]
	ABTS radical scavenging activity	35±0.23[a]	30±1.75[b]	74±1.52[a]	56±0.66[a]	59±2.23[a]	59±1.05[a]
	Hydroxyl radical scavenging potentiality	640±0.55	Nil	526±1.46	Nil	554±0.71	Nil
Total antioxidant capacity (µg Ascorbic acid equivalent/mg of extract)		21.78±1.39[a]	14.65±0.76[b]	13.0±0.76[a]	11.38±0.56[b]	14.08±0.2[a]	13.46±0.23[a]

Results are presented as mean ± SD; n = 5. Different letters in column was showed statistically significant differences ($p<0.001$) according to ANOVA.

RESULTS AND DISCUSSION

Phytochemical Composition

In the present study, we have evaluated the phytochemical composition specially the bioactive metabolites content and their concurrent biological activity in terms of antioxidative potentiality of two extracts, infusion and decoction. Mushrooms are an excellent resource of bioactive phytochemicals that are responsible for their significant medicinal benefits as an antioxidative, antimicrobial, anticancer, immunomodulatory agent[16]. Mushrooms gather a huge number of secondary metabolites that includes phenols (phenolic acid and flavonoid), ascorbic acid, carotenoids and others. Although extraction procedure greatly affects the relevant quantity of these phytochemicals that demands an easy approach to obtain maximum bioavailability. In this finding, we have prepared two orally compatible fractions; infusion and decoction of investigated mushroom species. These are traditionally used oldest mode of herbal drug preparation, while some bioactive compounds are more effectively extracted in boiling condition[17].

Phytochemical screening revealed that among the two extracts, infusion was found to be phytochemically more enriched in comparison with decoction. Quantitatively, investigated phytochemicals followed the similar pattern in both of these extracts; ascorbic acid>phenol> flavonoids>lycopene>β carotene (Table 1). Although with respect to mushroom species, *P. eous*showed highest quantity of phytochemicals. These results are in resemblance with our previous study of infusion and decoction obtained from *Lepistasordida* that presented infusion was comparatively superior to decoction[18]. Although the phenol content of infusion obtained from *P. eous* was higher than wild species of *P. djamor*[19]. Another study of cultivated species of *P. eous*showed that phenolic compounds were better extracted by hot water than other nonpolar solvents such as methanol and ethyl acetate[20]. Likewise, our results showed much higher content of phenolics compared with that of ethanolic extract of *Pleurotus* sp. studied by Loganathanet al[21]. In addition, flavonoids are one of the members of naturally

occurring phenolic compounds and known as a potent free radical scavenger. Herein, the flavonoid content was expressed in µg quercetin equivalent/ mg of extract which exhibited variation in the ranges of 2.57-3.71 µg quercetin equivalent/ mg of extract and 0.73-1.19 µg quercetin equivalent/ mg of extract for infusion and decoction, respectively. In drug development domain, preliminary screening of metabolites promotes the discovery of several unique compounds that might be beneficial for mankind.

Infusion was the fraction with highest quantity of ascorbic acid ranging between 10 to 16.66 µg/mg of extract, while the decoction exhibited moderate amount. However, with respect to infusion, *P. eous* and *P. florida* showed similarity in their ascorbic acid content. Ascorbic acid is one of the highly water soluble antioxidant which cannot be synthesized by human body itself, thus a healthy supplement is required to fulfill the need of this antioxidative agent[22]. With this respect, infusion as well decoction of these investigated *Pleurotus* sp. could be a good dietary food choice due to its remarkable metabolites content.

Antioxidant Activity

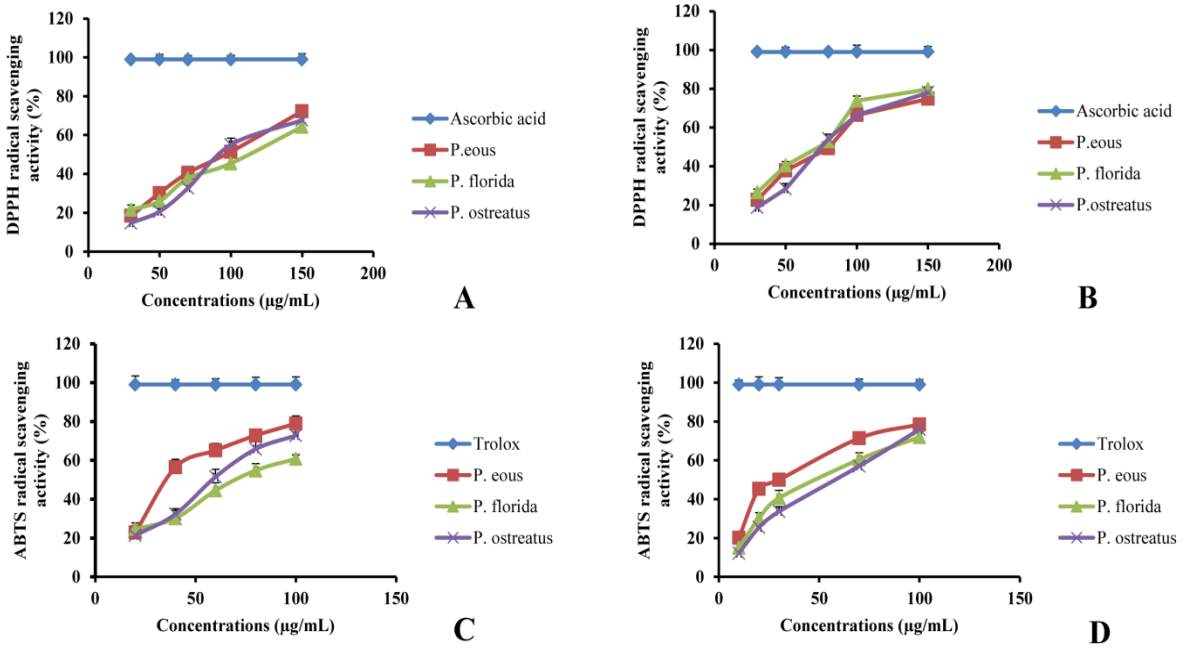
Abovementioned bioactive compounds are well related with the mushroom antioxidant properties. Antioxidants are compounds that help our body to combat oxidative stress related tissue damage and other highly reactive free radical activities. Thereafter exogenous supply of dietary antioxidants can effectively boost our in-built antioxidant system by increasing the production of antioxidative enzymes as well as play a protective role against accumulation of excessive free radicals[23]. In this present investigation, freshly prepared infusion and decoction of all three cultivated *Pleurotus* sp. were subjected for determining free radical quenching ability in four experimental *in-vitro* systems.

In the first assay, antioxidant potential of the extracts was determined by an easy and most popular method, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay. The method is based on reduction of purple chromogen radical solution to pale yellow hydrazine in the presence of any antioxidant compounds which can be monitored by changes in the absorbance at 595 nm[24]. Here the results revealed that scavenging activity was gradually increased with the increasing concentrations of the extracts (Figure 1A & 1B). *P. eous*infusion showed highest scavenging effect of 72.3% at a concentration of 150 µg/ml while *P. florida*and *P. ostreatus*exhibited 64.33% and 67.72% scavenging activity, respectively. Though the EC_{50} values are very close, still from the data, lower EC_{50} value was obtained for *P. eous* infusion in comparison to other *Pleurotus* sp. (Table 2). The finding showed a close similarity with the phenol and ascorbic acid content of each investigated species indicating a strong correlation with extraction procedure[25]. Moreover, our results presented comparatively better activity than *Lentinusedodes* and *Volvariellavolvacea* for all tested extractants[26]. In addition González-Palma et al. also reported boiling of extract selectively enhanced the DPPH radical scavenging potential compared to the fraction prepared at room temperature[27].

Freshly prepared extracts were further subjected to check ABTS radical scavenging ability depending on the visible discoloration of green coloured ABTS radical solution by hydrogen donating activity of antioxidant compounds. As shown in figure 1C & 1D, the quenching ability showed a dose dependent increment in both extracts. Herein, infusion and decoction of *P. eous* were seen to scavenge significantly from 20% to 78% ABTS•+ over the tested concentration range (20-100 µg/ml) representing lower EC_{50} values than other *Pleurotus* sp. Among the three species, *P. ostreatus* showed no such differences in ABTS radical scavenging activity of infusion and decoction. Besides the EC_{50} values obtained in this assay indicates potent scavenging ability of *Pleurotus* sp. infusion and decoction due to presence of more hydrogen donating constituents extracted from the mushrooms.

An important marker of oxidative stress related disease is the excessive generation of harmful highly reactive free radicals such as hydroxyl radical (•OH), nitric oxide (NO) radical, singlet oxygen etc. Hydroxyl radical has the ability to cause DNA strand breakage that leads to several deadly ailments[28]. On account of this, it is necessary to ascertain the hydroxyl radical scavenging potentiality of any investigated antioxidant compounds. In this method, the scavenging activity of •OH was measured using Fenton's reaction where hydroxyl radicals were generated in an *in-vitro* environment that degraded 2-deoxy-2-ribose into orange colouredmalondialdehyde–TBA complex[29]. When respective mushroom extracts were added, hydroxyl radicals are removed thus preventing sugar degradation that displayed a characteristic absorbance at 535 nm. The figure 1E denoted that infusion of all three *Pleurotus* sp. was an excellent scavenger of hydroxyl radical, since decoction presented no such effect. However, comparing the EC_{50} values, *P. florida*infusion was more effective than crude polysaccharide isolated from *P. ostreatus*according to the findings of Mitra et al[28].Another study by Sudha et al reported significantly higher EC_{50} values than our findings for hydroxyl scavenging activities of *P. eous*[20].

Furthermore, total antioxidant capacity of each extracts was determined by phosphomolybdenum method. Different mushroom fractions showed significant variations in their total antioxidant capacity. Although the infusion displayed highest activity than decoction, while mushroom species followed the trend; *P. eous*>*P. ostreatus*>*P. florida*. Corresponding values has been presented in Table 2. Decoction seems to affect quantity of phytochemicals and antioxidant activity which might be related to longer boiling process than infusion preparation. Due to longer boiling conditions, active constituents of the decoction might be destroyed that can affect their concurrent bioactivity. The present findings are in similar line with the study carried out with water and methanol extracts of *P. djamor* as total antioxidant activity was found to be highest in water extract than methanol extract[30].



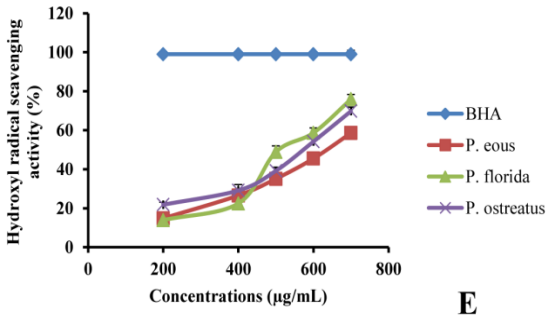


Figure 1: Antioxidant activity of infusions and decoctions (A) DPPH radical scavenging activity of infusions (B) DPPH radical scavenging activity of decoctions (C) ABTS radical scavenging activity of infusions (D) ABTS radical scavenging activity of decoctions (E) Hydroxyl radical scavenging activity of infusions. Data are shown mean and S.D. of 4 experiments.

CONCLUSION

Overall, the present investigation mainly features on the bio-medicinal aspects of an effortless and lucid means of herbal preparation of three most commonly cultivated *Pleurotus* sp. such as *P. eous*, *P. florida* and *P. ostreatus*. Both phytochemically enriched fractions; infusion and decoction showed strong correlation with their relevant free radical scavenging activity. Therefore, our finding adds another mode of consumption of these oyster mushrooms that can be used as a dietary supplement with remarkable antioxidative efficacy.

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CONFLICT OF INTERESTS

The Authors declare that they have no conflict of interest.

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خواص مضادات الأكسدة والفحص الكيميائي النباتي للتسريب وديكوتيون تم الحصول عليها من ثلاثة أنواع Pleurotus المزروعة(دراسة مقارنة)

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ملخص

تمثل المنتجات الطبيعية مصدرا غنيا للمركبات النشطة بيولوجيا ومثالا على التنوع الجزيئي مع معترف بها في اكتشاف الدواء. في هذه الأيام، يتم بشكل متزايد دراسة الفطر الطبي المستخدم في الطب الشعبي لتطبيقه بنجاح في الصناعات الدوائية. في هذا السياق،تعد أنواع الPleurotus واحدة من عيش الغراب المحتمل القبول في جميع أنحاء العالم كطعام لذيذ وتشتهر بخصائص طبية رائعة. ومع ذلك، حتى الآن لم يتم توثيق أي تقارير بشأن النشاط الحيوي للتركيبات العشبية الأكثر توافقا في هذا الفطر (التسريب وديكوتيون).لذلك أجريت هذه الدراسة لاستقصاء التركيب الكيميائي النباتي وخصائص مضادات الأكسدة لثلاثة أنواع من هذه المجموعة وهي P. eous و P. florida و P. ostreatus. وقد كشف التركيب الكيميائي النباتي أن جزء التسريب من P. eous هو الجزء الأكثر إثراء الذي يحتوي على كمية عالية من الفينولات (13.03 ميكروغرام من حمض الغاليك / ملغ من المستخلص)، والفلافونيدات (3.57 ميكروغرام من مكافئ كيرسيتين / ملغ من المستخلص) وحمض الأسكوربيك (16.66 ميكروغرام / ملغ من استخراج). علاوة على ذلك تم اختبار إمكانات تفكيك الجذور الحرة للكسور المقابلة لهذه الفطر بواسطة أنظمة مختلفة داخل المختبر. وأظهرت النتائج أن التسريب كان له نشاط ملحوظ في إزالة الزلازل بطريقة تعتمد على التركيز بينما أظهرت مغلي النشاط المعتدل للأنواع الثلاثة. لم ديكوتيون لا تظهر أي نشاط في مقايسة جذرية هيدروكسيل المسح. معًا تقدم نتائجنا بيانات مفصلة توضح الخواص العلاجية المفيدة للجزء المائي من هذه الأنواع Pleurotus. بالإضافة إلى ذلك، يمكن أيضًا استنتاج أنه بالمقارنة مع الفطر الذي تم دراسته، يعتبر P. eousinfusion الأفضل من حيث فعالية مضادات الأكسدة.

الكلمات الدالة: المغذيات، المواد الكيميائية النباتية، مركبات فينوليه.

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