

Evaluation of the Antioxidant Properties of Cultivated Edible Mushroom: Agrocybe Aegerita

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Research article

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Abstract

The antioxidant activity of an edible mushroom *Agrocybe aegerita* was analysed by testing its methanolic extracts with that of cultivated mushrooms *Lentinus edodes* and *Calocybe* species. Results showed predominant activity with respect to all the assays with different chemical systems including reducing power, DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), superoxide scavenging, peroxide scavenging, ferrous ion chelating, total phenolic & flavonoid content. Scavenging effects on 2,2-diphenyl-1-picrylhydrazyl radicals were moderate to high (66.23-92.27%) at 1.5 mg/ml. Chelating effects on ferrous ions were moderate to excellent (78.41-96.65%) at 20 mg/ml. At 12 mg/ml, the reducing powers were excellent (84.04 – 92.82%). FRAP results were moderate in the range (18.60 – 72.35%) at 12 mg/ml. The ability to scavenge super oxide (% SOD scavenging) was moderate to excellent (26.21-92.32 %) at 20 mg/ml. The total phenols in the extracts ranged from 0.64 – 0.26 at 20 mg/ml. The total flavonoid content in the extracts ranged from 0.307 – 0.055 at 10 mg/ml.

Introduction

"An antioxidant is essentially a molecule that inhibits the oxidation of other molecules." Oxidation, a chemical reaction transfers electrons or hydrogen from substance to the oxidizing agent. It is an essential process during which energy is produced in biological systems. However, there are many reactive oxygen species and free radicals that are related or formed as a result of the oxidation process. These reactive species frequently cause cell death and are involved in other degenerative processes associated with ageing (WWW. en.wikipedia.org/wiki/Antioxidant). Reactive oxygen species (ROS) along with free radicals are also found to play an essential role in functional changes which are

associated with diseases like cancer, rheumatoid arthritis, cirrhosis etc. Cells are equipped with enzymes like superoxide dismutase, catalase and also chemicals like vitamin E, vitamin C, polyphenols, carotinoids and glutathione [1]. Antioxidants are also known as reducing agents such as ascorbic acid, thiols or polyphenols [2]. Antioxidant containing natural foods are used to reduce the oxidative damage. Mushrooms are extensively used as food because of their unique taste and fine flavour. Experimental evidence shows that mushrooms contain many biologically active components which offer health benefits and protection against degenerative diseases [3]. Mushrooms have been rich sources for compounds like lectins. terpenoids, beta-glucans, ascorbic acid.

tocopherols, carboxylic acids and various dietary fibres [4-7].

Agrocybe aegerita is the most widely cultivated edible mushroom in the regions of Chile, Japan, and the Far East, as well as southern Europe. They have also been developed as the primary valuable source possessing varieties of bioactive secondary metabolites such as indole derivatives with free radical scavenging activity, cylindan with anticancer activity, and also agrocybenine with antifungal activity [8]. Agrocybe aegerita has unique flavor, good nutritive and medicinal values. This mushroom is known to have an anti-tumour lectin (www.nrcmushroom.org). Wheat straw or sawdust supplemented with wheat bran is commonly used as substrates for its cultivation. Calocybe spp. (Milky mushroom) is a well-recognized tropical edible mushroom and promising for cultivation in India [9]. Lentinus edodes apart from being delicious, it has an added medicinal value with excellent nutritional profile and high levels of B vitamin and pro-vitamin D2 (ergosterol) [10]. Mushroom properties are known to change with the substrate. Antioxidant activity of Agrocybe aegerita has not been reported earlier. Invitro antioxidant assays have been used to investigate the antioxidant activity of mushroom. The mushroom Agrocybe aegerita was cultivated on paddy straw to assess the antioxidant activity by comparing it with two other cultivated edible mushrooms.

Materials and Methods

Mushroom samples:

All the mushroom samples namely, *Agrocybe aegerita*, *Lentinus edodes*, and milky mushroom (*Calocybe* spp) were collected from the Mushroom Lab, Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bangalore. All the mushrooms have been identified by Dr.Meera Pandey, Principal scientist, IIHR. *Agrocybe aegerita* and *Calocybe* spp were cultured under laboratory conditions using paddy straw as substrate. For *L.edodes*, saw dust was used as substrate. Voucher specimens of the three species were deposited at the herbarium of Department of Biosciences, Sri Sathya Sai Institute of Higher Learning.

Sample Preparation

Fresh mushrooms were harvested, separated and dried in tray drier at 38°C for 48 h. The dried material was ground into a coarse powder using mortar and pestle. Dried powders, 50 grams each, were defatted by refluxing with petroleum ether (60–80°C) for 6 h. The defatted

material was then dried and the extracts were prepared by taking each of the powdered mushrooms in to a 1 lit round bottom flask with 500ml of methanol and refluxed at 25°C for 3 hours. The extract was then filtered through Watman paper. The remaining residue was put for reextraction with methanol. The procedure was repeated thrice after which the combined extracts were concentrated to 10ml at 40°C by using Rota evaporator and kept for complete dryness in a china dish and stored at 4°C till further analysis. The dried extracts were dissolved in methanol to a concentration of 20 mg/ml and used for analysis.

Reagents and Chemicals

Homovanillic acid (HVA), L-ascorbic acid, nitro blue tetrazolium salt (NBT), butylatedhydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid, Horseradish peroxidase (HRP) type II, methionine, ferrozine, riboflavin, ethylenediaminetetraacetic acid (EDTA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid (TROLOX) were all purchased from Sigma-Aldrich Chemicals (India) Ltd. All other chemicals used are of analytical grade.

Chemical Assays

DPPH radical scavenging activity: Various concentrations of the methanolic extracts of mushrooms (0.25-1.5 mg/ml, 2.5 ml) were mixed with methanolic solution containing DPPH radicals (1 mM, 0.5 ml). The mixtures were shaken vigorously and left to stand in dark for 30 min. The reduction in the DPPH radical concentration was then determined by measuring the absorbance at 517 nm (Shimadzu UV-spectrophotometer, UV-2450). Methanol was taken as blank and DPPH solution without the extracts was taken as control. The percentage of DPPH scavenged was calculated using the equation: % Scavenged = $[(A_c-A_s)/A_c] \times 100$, where A_c is the absorbance of control, and A_s is the absorbance of solution containing sample extracts. Trolox and ascorbic acid were used as standard.

Ferrous ion chelating activity: The chelating of ferrous ions by the mushroom extracts was estimated by the method of Dinis, et al. [12,13]. The Fe²⁺ chelating ability was monitored by measuring the absorbance of the ferrous iron-ferrozine complex at 562 nm. Briefly, methanolic mushroom extracts (2–20 mg/ml, 0.4 ml) were added to a solution of 2mM FeCl₂ (0.2 ml). The reaction was initiated by adding 5 mM ferrozine (0.4 ml). The total volume was adjusted to 4 ml with methanol. The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance's of the solutions

were measured spectrophotometrically at 562 nm (Shimadzu UV-spectrophotometer, UV-2450). The percentage of chelation was calculated by using the equation: % Chelation = $[(A_C-A_S)/A_C] \times 100$, where A_C is the absorbance of control, and A_S is the absorbance of solution containing sample extracts. Control contains only FeCl₂ and ferrozine. Ascorbic acid was used as standard.

Reducing power: The reducing power was measured by the method of Oyaizu [14]. Methanolic extracts of mushroom (2– 12 mg/ml, 2.0 ml) were mixed with sodium phosphate buffer (0.2 M, pH 6.5, 2.0 ml) and potassium ferricyanide [K₃Fe(CN)₆] (1%, 2.0 ml). The mixture was then incubated at 50°C for 20 min. Tricloroacetic acid (10% w/v, 2.0 ml) was then added and the mixture was centrifuged at 3000 rpm for 10 min (REMI R23). To the supernatant layer (1.5 ml), 1.5 ml of deionised water and ferric chloride (0.1%, 0.25 ml) were added and the absorbance was measured at 700 nm (Shimadzu UV-spectrophotometer, UV-2450). Higher absorbance indicates better reducing power. Trolox and Ascorbic acid were used as standard.

Ferric reducing antioxidant power (FRAP): In FRAP method, the complex formed when ferric tripyridyltriazene (Fe³⁺ TPTZ) complex was reduced to the ferrous (Fe²⁺) ion was determined using UV-Vis Spectrophotometer. The oxidant in the FRAP assay was prepared by mixing TPTZ (10 mM in 40 mM HCl, 2.5 ml), acetate buffer (0.3 M pH 3.6, 25 ml), and 2.5 ml of FeCl₃.6H₂O (20 mM). To 1800 µl of freshly prepared FRAP reagent, 180 µl of water and 60 µl of mushroom extracts (2-12 mg/ml) were added. The mixture was then incubated at 37°C for 30 min. The absorbance was measured spectrophotometrically at 595 nm (Shimadzu UV-spectrophotometer, UV-2450). Higher absorbance indicates better ferric reducing ability of the extracts. Trolox and ascorbic acid were used as standard [15,16].

Superoxide scavenging activity: This assay is based on the capacity of the extracts to inhibit the superoxide radical formed by photochemical reduction of nitro bluetetrazolium (NBT) in the riboflavin–light–NBT system. The method followed was used by Rajesh Babu [17]. Each 3 ml reaction mixture contained sodium phosphate buffer (200 mM, pH 7.8, and 0.5 ml), methionine (104 mM, 0.25 ml), riboflavin (8 μ M, 0.5 ml), EDTA (100 μ m, 0.5 ml), NBT (600 μ M, 0.25 ml) and 1 ml test sample solution. The production of blue formation was followed by monitoring the increase in absorbance at 560 nm after 40 min. illumination from a fluorescent lamp. The percentage of superoxide scavenged is calculated using the equation: % Scavenged = [(A_C-A_S)/ A_C] × 100, where A_C is the absorbance of control, and A_S is the absorbance of solution containing sample extracts. Trolox was used as standard.

Determination of total phenolic content and total flavonoid content: Total phenolic content was measured at 20 mg/ml concentration of the extracts. To 0.1 ml of mushroom extracts in 13% HCl / MeOH (60:40, v/v), 2 ml of 2% sodium carbonate was added. The mixture was incubated at room temperature for 3 min. Folinciocalteu reagent (0.1 ml) was added to the mixture. After 30 min. absorbance was measured at 750 nm [18]. Gallic acid was used as standard. The results were expressed as mg of gallic acid equivalents (GAEs) per gram of mushroom extract.

Total flavonoid content was measured at 20 mg/ml concentration of the extracts. To 1 ml of mushroom extracts, 1 ml of 10% AlCl₃, potassium acetate (1 M, 0.1 ml) and 3.8 ml of MeOH were added and the mixture was incubated for 40 min at room temperature. Then absorbance was measured at 415 nm [19]. The results were expressed as μ g of quercetin equivalents (CEs) per gram of mushroom extract.

Statistical Analysis

All the analyses were carried out on triplicate samples and the mean values of the results were reported. The experimental results were analyzed using one-way ANOVA (analysis of variance) followed by student's t-test to determine the least significant difference at α =0.05 using Microcal Origin ver. 6.0 software.

Results and Discussions

DPPH radical scavenging activity

The methanolic extracts of the three cultivated mushrooms exhibited increasing scavenging effect with increased concentration (Figure 1). The activity of *Agrocybe aegerita* was found to be excellent even at 0.75mg/ml concentration (90.70%) when compared to that of *Calocybe sp.* and *Lentinus edodes* whose activity, even at 1.5mg/ml concentration, was observed to be 66.23% and 70.28% respectively. The radical scavenging activity of *Agrocybe aegerita* exhibited excellent scavenging activity of nearly 81% at 0.02 mg/ml concentration when compared with other edible mushrooms reported by Mujić I [20]. The scavenging effect of Trolox at 10 μ g/ml was 89.79% and the scavenging effect of Ascorbic acid at 100 μ g/ml was 65.96%.



Ferrous Ions Chelating Activity

The study of the chelating effects on the ferrous ions is advantageous since they are the most active pro-oxidants in the food system [21]. The methanolic extracts from the three cultivated mushrooms exhibited increasing chelation effect on ferrous ions with the increased concentrations and were high to excellent (78.41-96.65%) at 20 mg/ml (Figure 2). Among the three mushrooms methanolic extracts, *Lentinus edodes* exhibited excellent chelating ability than *Agrocybe aegerita* and *Calocybe sps.* However the chelating ability of *Agrocybe aegerita* was found to be (79.81%) at 10 mg/ml concentration, whereas *Lentinus edodes* and *Calocybe sps.* exhibited chelating ability of (90.27% and 60.21%) respectively. The chelating effect of L-ascorbic acid was high 93.53% at 0.5 μ g/ml concentration.



Reducing power

The reducing power ability of the methanolic extracts were excellent and increased steadily with increase in

concentration (Figure 3). At 2 mg/ml concentration, the reducing powers were (83.33 – 56.16%). The values were found to be much better than the reducing powers of the extracts reported by Yang JH [22]. The reducing power of

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Agrocybe aegerita is excellent when compared to the value reported by Barros L [3]. The reducing power of *Agrocybe aegerita* according to Mujić I [20] showed good activity of 55% at 1 mg/ml concentration. The high reducing power presented by the extracts might be suggestive of the hydrogen donating ability of active

species present in the extracts [23]. Results show that *Agrocybe aegerita* presented excellent reducing power (83.33%) followed by *Lentinus edodes* (65.32%) and *Calocybe sps* (56.16%). However the reducing power of TROLOX and Ascorbic acid at 60 μ g/ml and 6 μ g/ml was 82.27% and 71.05% respectively.



Ferric Reducing Antioxidant Power (FRAP)

The mushroom extracts showed increased FRAP with increased concentration (Fig.4). At 12 mg/ml concentration the FRAP values of measured absorbance at 595nm were 13.25 – 72.35%. The FRAP values presented by these extracts were good when compared to the values

reported by Rajesh Babu [24]. Results showed that the maximum FRAP activity was exhibited by *Agrocybe aegerita* 72.35%. Least was exhibited by *Lentinus edodes* 13.25%. However the FRAP activity of Trolox at 125 mg/ml was found to be 67.70% and Ascorbic acid was found to be 65.70% at 1000 μ M/ml.



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Superoxide scavenging activity

The SOD activity of methanolic extracts of edible mushrooms which were assayed by the non-enzymatic riboflavin-light-NBT system, showed increased activity with increased concentrations (Figure 5) and were 92.32 – 39.81% at the concentration of 20 mg/ml. Results showed that the % Superoxide scavenging activity was more exhibited by *Agrocybe aegerita* 92.32%. This was followed by *Lentinus edodes* 41.73% and *Calocybe* spp. 39.81%. The SOD values exhibited by these extracts were excellent when compared to the values reported by Babu and Rao [24]. However the scavenged activity of Trolox was found to be 77.75% at 25 mg/ml of concentration.



Antioxidant Components

The major antioxidant components found in the methanolic extracts of the mushrooms are total phenols. Phenols such as tocopherols, BHT and gallate are found and are known to be effective antioxidants [22]. The total phenol and flavonoid content of all the extracts are summarized in Table 1. The total phenolic content of mushroom extracts recorded at concentration of 20 mg/ml was ranging from 0.64 – 0.26. The total phenolic content was observed to be more in the extracts of *Agrocybe aegerita 0.64*, which was followed by, *Lentinus edodes 0.33* and the least was shown by *Calocybe* spp 0.26. However, the absorbance of gallic acid at 0.6 mg/ml concentration was found to be 0.693. The highest total phenol content in *Agrocybe aegerita* might account for the

better results found in DPPH scavenging ability, FRAP, reducing power.

The total flavonoid content of mushroom extracts recorded at concentration of 10 mg/ml was ranging from 0.307 – 0.055. The total flavonoid content was observed to be more in the extracts of *Agrocybe aegerita* 0.307, which was followed by *Lentinus edodes* 0.072 and the least was shown by *Calocybe* spp 0.055. However, the absorbance of quercetin at 100 µg/ml concentration was found to be 0.457. The highest total flavonoid content in *Agrocybe aegerita* might account for enhanced results found in ferrous ion chelation and superoxide scavenging activities. Total flavonoid content of *Agrocybe aegerita* is good when compared to the value reported by Barros [3]. The total phenol and flavonoid content of the mushroom extracts were shown in Figure 6.

Compound concentration (mg/ml)	Agrocybe aegerita	Calocybe sps.	Lentinus edodes
Total Phenolic Content	26.91±0.03(a)	10.41±0.01(b)	13.32±0.02(c)
Total Flavonoid Content	3.328±0.011(a)	0.594±0.002(b)	0.773±0.014(c)

Table 1: Content of total flavonoids and total phenols of methanolic extracts of edible mushrooms (a) Each value is expressed as mean \pm standard deviation (n=3). Means with different letters within a row are significantly different (P<0.05).

Mushroom	DPPH Scavenging	FRAP	Ferrous Ion Chelation	Reducing Power	Super Oxide Scavenging
Agrocybe aegerita	0.128	7.116	3.501	1.114	11.443
Calocybe sps.	0.576	10.770	2.989	1.496	10.360
Lentinus edodes	0.687	6.395	2.401	1.341	9.073

Figure 6: Total phenol and flavonoid content of three edible mushrooms.

Table 2: The IC50 values of the methanolic extracts from edible mushrooms for various antioxidant assays

Conclusion

In the present study, we have compared the antioxidant activity of Agrocybe aegerita with that of *Calocybe* spp and *Lentinus edodes*. The activities increased steadily with increase in the concentration. Agrocybe aegerita has excellent DPPH radical scavenging, peroxide scavenging, FRAP and reducing power abilities. This may be attributed to its highest total phenol content. The excellent ferrous ion chelation and superoxide scavenging abilities exhibited by Agrocybe aegerita may be attributed to its highest flavonoid content. Of all the assays studied, total phenol content of the extracts showed better relationship with chelation with ferrous ions and peroxide scavenging abilities and the total flavonoid content of the extracts showed excellent correlation with DPPH scavenging, FRAP and reducing power abilities. In Table 2, the IC50 values were calculated form the plots for each assay are summarized. The results indicate that *Agrocybe aegerita* is a promising mushroom with excellent antioxidant potential than that of Lentinus edodes and Calocybe spp. The active components of the mushroom Agrocybe aegerita responsible for these activities needs to be further investigated.

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