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Original Article

ANTIOXIDANT ACTIVITY OF OYSTER MUSHROOM (*PLEUROTUS FLORIDA* [MONT.] SINGER) AND MILKY MUSHROOM (*CALOCYBE INDICA* P AND C)

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ABSTRACT

Objective: To evaluate the antioxidant activity of tropical edible mushrooms namely *Pleurotus florida* and *Calocybe indica*.

Methods: Antioxidant potential was evaluated by using various antioxidant assays such as DPPH free radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, and superoxide radical scavenging activities as well as lipid peroxidation inhibiting assay, reducing power assay, ferric reducing antioxidant power (FRAP), metal chelating activity, phospho-molybdenum reduction assay and anti-haemolytic activity.

Results: The results obtained from this antioxidant study strongly suggest that *Pleurotus florida* and *Calocybe indica* have significant antioxidant activity.

Conclusion: Edible mushrooms *Pleurotus florida* and *Calocybe indica* are having significant antioxidant activity, could serve as easily accessible natural food rich in antioxidant which may enhance the immune system against oxidative damage and may be utilized as the potential sources of therapeutic agents.

Keywords: Antioxidant, *Pleurotus florida*, *Calocybe indica*, Tropical edible mushrooms, Therapeutic agents

INTRODUCTION

Mushrooms are eukaryotic, non-photosynthetic organisms that form characteristic fruiting bodies. They have been used by human beings for thousands of years as food and medicine [1]. The increasing consumption of mushrooms can be attributed not only to the pleasant flavour and aroma of edible mushrooms but also to their vitamins and protein contents as well as the molecule that include antioxidants and natural products which are free from pesticides [2]. Mushrooms are now considered as potential therapeutics and preventive agents that may ensure the wellness of humans. As a result, mushroom cultivation has been increased tremendously throughout the world during the last few decades [3]. Mushrooms have been reported to have significant pharmacological effects such as regulation of biorhythm, maintenance of homeostasis, prevention and cure for various diseases such as cancer, cerebral stroke, heart diseases and improvement of life. They have also been demonstrated to contain effective biomolecules with hypolipidemic, antithrombotic, hypotensive, anti-inflammatory and other applications [4]. The present study aims to evaluate the antioxidant activities of the extract of edible mushrooms *Pleurotus florida* and *Calocybe indica*.

MATERIALS AND METHODS

The fruiting bodies of *Pleurotus florida* and *Calocybe indica* were obtained from Mushroom Unit, Department of Biology, The Gandhigram Rural Institute–Deemed University, Gandhigram (TN), India. Sample preparation [5] and antioxidant activities such as DPPH free radical scavenging [6], hydroxyl radical scavenging [7], nitric oxide radical scavenging [8], superoxide radical scavenging [9], lipid peroxidation inhibiting assay [10], reducing power assay [11], ferric reducing antioxidant power (FRAP) assay [12], metal chelating activity [13], phospho-molybdenum reduction assay [14] and anti-haemolytic activity [15] of methanol extract were carried out.

Statistical analysis

The results were expressed as mean values and standard deviation (SD). Linear regression analysis was used to calculate IC50 value. Data were analyzed using One-Way Analysis of Variance (ANOVA) followed by Turkey's multiple comparison post hoc tests using SPSS software 16.0 versions. Values of $p<0.05$ were considered as statistically significant.

RESULTS

The antioxidant capacity of *Pleurotus florida* and *Calocybe indica* was determined by the DPPH method and the results were presented in fig. 1. Different concentrations of *P. florida* and *C. indica* (200-1000 µg/ml) showed maximum DPPH radical scavenging activity of 37.04±0.15 and 28.04±0.41 % at 1000 µg/ml respectively. Results showed the percentage of inhibition in a dose-dependent manner. The IC_{50} value of *P. florida* and *C. indica* were found to be 413.28±5.87 µg/ml and 588.40±11.85 µg/ml respectively. The results of hydroxyl radical scavenging activity were recorded in fig. 2. The hydroxyl radical scavenging effects of extracts of *P. florida* and *C. indica* using deoxyribose assay in the different concentrations (200-1000 µg/ml) were investigated. The strongest hydroxyl radical scavenging activity was observed in *C. indica* (65.41±0.65 % at 1000 µg/ml) than *P. florida* (46.99±2.58 % at 1000 µg/ml). The IC_{50} value of *P. florida* and *C. indica* was found to be 220.70±6.0 µg/ml and 148.23±1.01 µg/ml respectively.

Fig. 3 depicts the nitric oxide radical scavenging activity of the extracts. Different concentrations of *Pleurotus florida* and *Calocybe indica* (200-1000 µg/ml) showed 21.90±0.88 % and 23.13±1.32 % inhibition at the concentration of 1000 µg/ml. Concentrations required for 50% inhibition (IC_{50}) of nitric oxide radical scavenging activity in *P. florida* and *C. indica* were 893.44±28.14 and 781.63±20.83 µg/ml respectively. *P. florida* and *C. indica* were found to scavenge superoxide generated by photoreduction of riboflavin (fig. 4). The IC_{50} value of *P. florida* and *C. indica* were found to be 357.29±8.93 µg/ml and 441.92±7.81 µg/ml respectively. The inhibitory effects of *P. florida* and *C. indica* on lipid peroxidation inhibition were increased with increasing concentration. The inhibitory effect of the different concentrations of extract such as 200-1000 µg/ml of *P. florida* and *C. indica* were determined using the liver homogenate model and the results were recorded in fig. 5. The results of lipid peroxidation inhibition were found to be 80.0±4.01 % at 1000 µg/ml in *P. florida* and 87.59±0.32 % at 1000

µg/ml in *C. indica*. The IC_{50} value of *P. florida* and *C. indica* was found to be 17.44±0.64 µg/ml and 16.06±0.25 µg/ml respectively.

Fig. 6 summarizes that the reducing power of the extracts of *Pleurotus florida* and *Calocybe indica* was found to be excellent which steadily increased in direct proportion to the increasing concentrations of the extract. The reducing power inhibition percentages were found to be 0.43±0.007 % in *P. florida* and 0.43±0.005 % in *C. indica*.

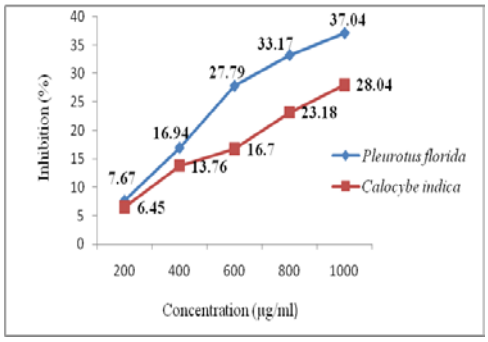

Fig. 1: DPPH radical scavenging activity

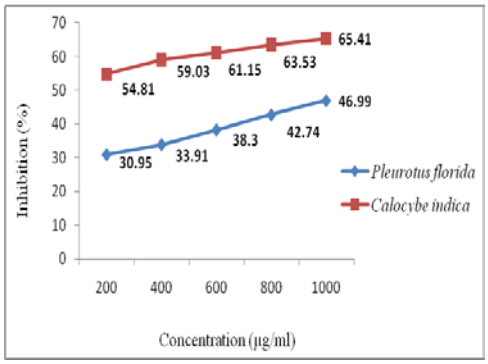

Fig. 2: Hydroxyl radical scavenging activity

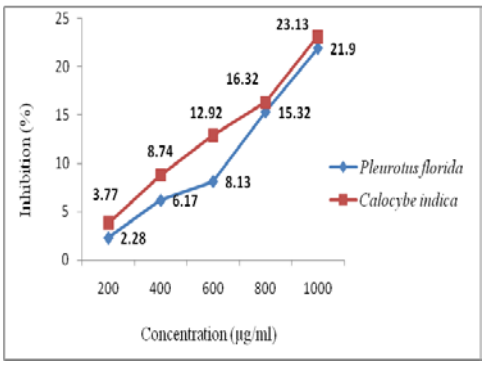

Fig. 3: Nitric oxide radical scavenging activity

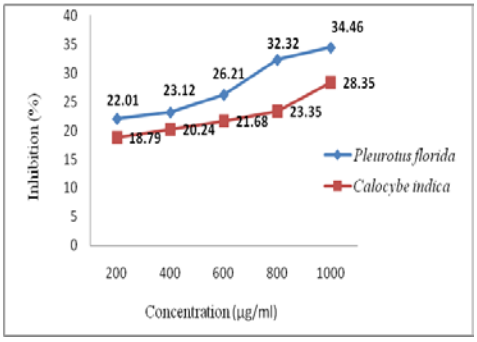

Fig. 4: Superoxide radical scavenging activity

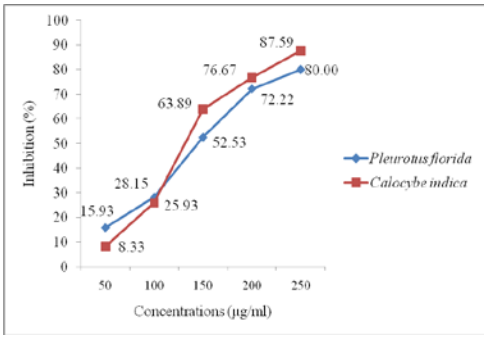

Fig. 5: Lipid peroxidation assay

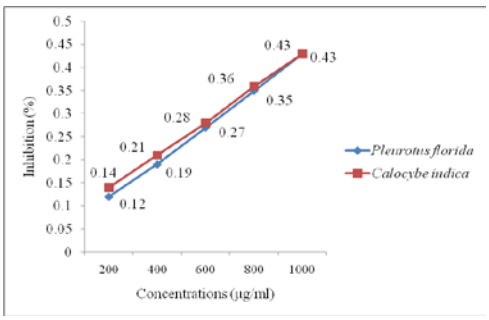

Fig. 6: Reducing power assay
***Values are mean±SD (*n = 3*) (*p*<0.05)**

In order to examine the reducing power, the reduction of $Fe^{3+}$to $Fe^{2+}$was investigated in the *Pleurotus florida* and *Calocybe indica* mushroom extract and the result is shown in table 1. The absorbance of the reaction mixtures at 593 nm was found as 59.65±0.46 m. mol [Fe (II)]/g and 57.38±0.37 m. mol [Fe (II)]/g in *P. florida* and *C. indica* respectively. Metal chelating activity was found to be higher in *C. indica* (0.97±0.05 mg EDTA E/g extract) than *P. florida* (0.77±0.08 mg EDTA E/g extract) (table 1). The antioxidant capacity (phospho-molybdenum reduction assay) of the extract of *P. florida* (197.26±1.19 %) was recorded to be higher than *C. indica* (190.64±0.40 %) (table 1).

Table 1: Ferric reducing antioxidant power (FRAP) assay, Metal chelating activity and phospho-molybdenum assay

Sample	FRAP mmol [Fe (II)]/g extract	Metal chelating activity (mg EDTA Eq/g extract)	Phosphomolybdenum (mg ascorbic acid Eq/g extract)
Pleurotus florida	59.65±0.46 %	0.77±0.08 %	197.26±1.19 %
Calocybe indica	57.38±0.37 %	0.97±0.05 %	190.64±0.40 %

Values are mean±SD (*n = 3*) (*p*<0.05), In the present study, extract of *Pleurotus florida* (20.23±3.33 %) and *Calocybe indica* (10.21±3.66 %) exhibited potent anti-haemolytic activity (table 2).

Table 2: Anti-haemolytic activity

Sample	Concentration(µg/ml)	Percentage activity (%)
Pleurotus florida	250	10.21±3.66 %
Calocybe indica	250	20.23±3.33 %

Values are mean±SD ($n = 3$) ($p<0.05$).

DISCUSSION

The Oyster and milky mushrooms produce a very impressive array of antioxidant compounds and have the potential to lower the risk of diseases [16, 17]. The free radical scavenging ability of these extracts of *Pleurotus florida* and *Calocybe indica* were found to be on the high side. This result is well supported by Blois [6] and Gezer *et al.* [18]. Their findings revealed that cysteine, glutathione, ascorbic acid, α-tocopherol, polyhydroxy aromatic compounds and aromatic amines reduce and decolorize α, α-diphenyl-β-picrylhydrazyl by their hydrogen donating ability. The antioxidative activities dose dependency and associated it the presence of reductones are reported to be the terminators of free radical chain reactions [19].

The hydroxyl radical is an enormously reactive free radical created in biological systems and has been concerned with an extremely harmful species in free radical pathology able to damage nearly each molecule found in living cells. This radical has the power to bond nucleotides in DNA and cause strand rupture, which leads to cause cytotoxicity carcinogenesis and mutagenesis. The ability of extracts to quench hydroxyl radicals seems to be the good scavenger of active oxygen species, thus reducing the rate of the chain reaction [20-24].

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc., and is involved in the regulation of various physiological processes. Excess concentration of nitric oxide is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions which act as free radicals [25]. Similar antioxidant activity for nitric oxide in *Pleurotus florida* and *Calocybe indica* were reported by different research groups [26-31].

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using 4-electron chain reactions reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anion. It plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen in living systems [24, 29, 32, 33].

The lipid peroxidation has been broadly defined as the oxidative deterioration of polysaturated lipids. Peroxyl and hydroxyl radicals are important agents that mediate lipid peroxidation, thereby damaging cell membranes. A number of toxic compounds are generated during this process of lipid peroxidation. Thiobarbituric acid reactive substances (TBARS) are produced as by-products of lipid peroxide that occurs in the hydrophobic core of biomembranes. A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom [22, 24, 34-36]. The effect of mushroom extracts on lipid peroxide showed significant inhibition of TBARS formation. The present finding strongly suggests that the use of the mushroom extracts prevent lipid peroxide and this arrests membrane damage.

The antioxidant activities of certain mushroom extracts have been related to their reducing potential. The reducing potential of the extract of *Pleurotus florida* and *Calocybe indica* was evaluated using ferric reducing assay. The reducing potency is generally associated with the presence of substances called reductones, which exert antioxidant action by breaking the free radical chains *via* hydrogen atom donation. Reductones are reported to prevent peroxide formation by reacting with certain precursors of peroxides. In this assay, the presence of reductants in the samples would result in the reducing of $Fe^{3+}$to $Fe^{2+}$by donating the electron. The amount of Fe^{2+}complex can be measured by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance indicates an increase in reductive ability [37-43].

Further, the reducing capacity was investigated by measuring $Fe^{3+}$to Fe^{2+} conversion and serve as a significant indicator of its potential antioxidant activity. The antioxidant activities of putative antioxidants have been attributed to various mechanisms such as prevention of chain reaction, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued proton obstruction and radical scavenging [25].

Antioxidants inhibit the interaction between metal and lipid through the formation of insoluble metal complexes with a ferrous ion. The iron chelating capacity test measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion [17]. Transition metals have been proposed as the catalysts for the initial formation of radicals. Chelating agents may stabilize transition metals in living systems and inhibit generation of radicals, consequently reducing free radical induced damage. To estimate the antioxidant potential of *Pleurotus florida* and *Calocybe indica* mushroom extracts, their chelating activity was evaluated against Fe^{2+}. Ferrozine quantitatively forms complexes with Fe^{2+}. The chelating effects of the *P. florida* and *C. indica* mushroom extracts were found to be excellent.

Total antioxidant capacity by phospho-molybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyze and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phospho-molybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [14]. Phosphomolybdenum assay with the methanolic extracts of *Pleurotus florida* and *Calocybe indica* was determined. Comparatively, *P. florida* shows a better reduction of Mo than *C. indica*. The phospho-molybdenum assay results indicate that the methanolic extracts of *Grewia hirsuta* Vahl (Kalunnu) are more powerful antioxidant in the reduction of phospho-molybdenum complex [44].

Erythrocytes are considered as the major target for the free radicals owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the oxygen transport associated with redox active haemoglobin molecules, which are potent promoters of activated oxygen species. The extent of haemolysis was found to be much greater when red blood cells were treated with hydrogen peroxide (toxicant). This could be attributed to the oxidizing nature of hydrogen peroxide with respect to the destruction of a cell membrane and subsequent liberation of haemoglobin from the cells. Mobilization of $Fe^{2+}$by Ca^{2+}*via* Fenton reaction is also caused due to hydrogen peroxide which further leads to the production of OH radicals. All these factors, in unison, cause deterioration of cell membrane, which may, perhaps, be the key episode of the lyses of the cell. Nevertheless, the antihaemolytic activity is the expression of collaborative action of the various antioxidant mechanisms which function in nature [45-47].

CONCLUSION

The results obtained from this antioxidant study strongly suggest that the extract of *Pleurotus florida* and *Calocybe indica* has significant antioxidant activity, could serve as an easily accessible item of natural rich antioxidant food which may enhance the immune system against oxidative damage or it may be utilized as a potential source of therapeutic agent.

CONFLICT OF INTERESTS

We declare that we have no conflict of interests

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