International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 6 (6): 950-953, 2010
ISSN 1811-7775

Antioxidant and Radical Scavenging Activity of Nine Edible Mushrooms Extract

L. Ramkumar, T. Ramanathan, P. Thirunavukkarasu and N. Arivuselvan
Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu, India

Abstract: This study revealed that antioxidant properties, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power were observed in different mushroom species namely *Agaricus bisporus*, *Pleurotus sajor-caju*, *Pleurotus euos*, *Hybsizus ulmarius*, *Pleurotus florida*, *Volveriela volvaciea*, *Pleurotus platypus*, *Pleurotus djamor* and *Calocybe indica*. Methonolic extract of *P. euos* was recorded maximum levels of DPPH free radical scavenging (6 mg mL^{-1}), reducing power (6 mg mL^{-1}) and enzymatic antioxidants viz., catalase, superoxide dismutase and peroxidise, 68.7±1.1, 0.39±0.01, 42.21±0.06, 37.12±0.04 and 7.21±0.05, respectively. The maximum amount of enzymatic antioxidants were recorded viz., CAT, SOD and POX; 42.21±0.06, 37.12±0.04 and 7.21±0.05 μmol, respectively.

Key words: Antioxidant, *Agaricus bisporus*, *Pleurotus florida,* 1, 1-diphenyl-2-picrylhydrazyl, reducing power, methanolic extract

INTRODUCTION

Free radicals are responsible for aging and causing various human diseases. A study shows that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. By donating hydrogen radicals, the primary radicals are reduced to non radical chemical compounds and are then converted to oxidize antioxidant radicals (Jadhav *et al*., 1995; El-Enshasy *et al*., 2010; Yamagushi *et al*., 1998). This action helps in protecting the body from degenerative diseases. Epidemiological studies have shown the beneficial effects of diets rich in vegetables, fruits and grain products in reducing the risk of cardiovascular disease and certain cancers (Beecher, 1999). The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts (Ames *et al*., 1993). Weisburger (1999) and Ames *et al*. (1993) reported that, the consumption of plant foods, such as fruits, vegetables, red wines and juices provides protection against various diseases, including cancer, cardio and cerebrovascular diseases. Exogenous chemical and endogenous metabolic processes in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of resulting in cell death and tissue damage. However, antioxidant supplements containing foods might be used to help human body cells to reduce oxidative scratch (Halliwell and Gutteridge, 1999). Mushrooms are considered to be a good source of antioxidants, such as variegatic acid and diboviquinone, which have been found in mushrooms (Kasuga *et al*., 1995). Cheung *et al*. (2003) reported that the methanol and water extract mushrooms were found to rich antioxidative activities. Mushroom species had been shown to possess antioxidant capacity in *in vitro* systems (Ribeiro *et al*., 2006). Seline and Johein (2007) reported that the l-carnitine concentration in mushroom ranged from 133 to 530 mg kg^{-1} DM (mean 320 mg kg^{-1} DM). The aim of this study was to determine the antioxidant property, scavenging activity and reducing power in the fruiting bodies of 9 edible mushroom species were collected from Namakkal district, Tamil Nadu and Southeast India.

MATERIALS AND METHODS

Mushroom collection: The species were collected from Namakkal district, Tamil Nadu and Southeast India, during April to June in 2009. The study was carried out of Centre of Advanced study in Marine Biology, Annamalai University, Parngipettai, Tamil Nadu, India.

Estimation of antioxidant activity: A fine dried mycelial mat (biomass filtrate paper) and harvested mushroom powder (20 meshes) samples (5 g) were continuously extracted with methanol in a Soxhlet apparatus for 24 h. The methanolic extract was evaporated to dryness at 40°C and redissolved in methanol at a concentration of 5 mg mL^{-1} and stored at 4°C prior to further use (Barros *et al*., 2007).

CAT: The mean CAT activity (μmol of H_2O_2 consumed/min/mg protein) was assayed following the method described by Sinha (1972).

Corresponding Author: L. Ramkumar, Faculty of Marine Science, Annamalai University, Parangipettai-608 502, Tamil Nadu, India Tel: +91 04144 243223 (213), +91-9965553333 Fax: +91-04144 243555

SOD: The mean activity of SOD (units/min/mg protein) was determined by the method of Marklund and Marklund (1974), in which one unit was considered to be the amount of enzyme that inhibited pyrogallol autooxidation by 50%.

POX: Mean POX activity (μmol of pyrogallol oxidized/min/mg protein) of the mushroom extracts was measured according to the method described by Sadasivam and Manickam (2004).

DPPH radical scavenging activity: The scavenging activity of the free and bound extracts on 1, 1-diphenyl 2-picrylhydrazyl (DPPH) radical was measured according to the method of Cheung *et al.* (2003) with some modifications. Aliquots (various concentration of methanol extract) of 0.8 mL of 0.2 mM DPPH methanolic solution was mixed with 0.2 mL of the extracts. The mixture was vigorously shaken and left to stand for 10 min under subdued light. The absorbance was measured at 520 nm. The DPPH radical scavenging activity (%) was calculated by the following equation:

$$\text{Radical scavenging activity (\%)} = (1-A_{sample}/A_{control})\times 100$$

where, A_{sample} is the absorbance in the presence of sample and $A_{control}$ is the absorbance in the absence of sample, respectively. All extracts were analyzed in triplicate.

Reducing power: The reducing power was determined according to the method of Oyaizu (1986) with some modifications. Various concentrations of methanolic extracts from mushrooms (2.5 mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6, Wako Pure Chemical Co., Osaka, Japan) and 2.5 mL of 1% potassium ferricyanide (Sigma) and the mixture was incubated at 50°C for 20 min. After 2.5 mL of 10% trichloroacetic acid (w/v, Wako) were added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride (Wako) and the absorbance was measured at 700 nm in a 2020 double beem spectrophotometer. A higher absorbance indicates a higher reducing power.

Statistical analysis: All experiments were done in three replicates and mean values are presented. Statistical analysis was performed on the data by Duncan's multiple range test with means followed by a common letter are not significantly different at the 5% level by DMRT. Values are expressed as Mean±SD.

RESULTS AND DISCUSSION

Antioxidant, DPPH and reducing power results were clearly indicated that among sp., *P. eous* was significantly increased the enzymatic antioxidative substances, compared to other sp. The maximum amount of enzymatic antioxidants were recorded viz., CAT, SOD and POX; 42.21±0.06, 37.12±0.04 and 7.21±0.05 μmol, respectively (Table 1). Yang *et al.* (2002) was reported methanol extraction with; two mushrooms strains had the highest antioxidant activity. This report was similar values to compare our study. In the past few years, the suspected toxicity of some synthetic compounds used in food has raised interest in natural products (Stone *et al.*, 2003). Some food industries and pharmaceuticals have increased their efforts in preparing bioactive compounds from natural sources by extraction and purification. Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage (Halliwell and Gutteridge, 1999). So we need for identifying alternative natural and secure sources of food antioxidants has been created and the search for natural antioxidants, particularly of plant origin, has remarkably increased in recent years (Skerget *et al.*, 2005).

Jose and Janardhanan (2000) have reported that the methanol extracts of *Pleurotus* sp., exhibited effective radical scavenging. The concentrations of cysteine, methionine and aspartic acid are reported to be higher in *P. ostreatus* than those in other edible mushrooms, such as *Agaricus* sp. and *Lentinula* sp. (Mattila *et al.*, 2002). In addition, *P. ostreatus* has also been reported to possess excellent reducing power of ferric ions (Lin, 1999). In this study, methanolic extracts were prepared in different concentration; these are 2, 4 and 6 mg mL^{-1}. DPPH

Table 1: Antioxidant activities of catalase, superoxide dismutase and glutathione peroxidase in dried mushroom

Mushroom sp.	CAT	SOD	POX
A. bisporus	36.15±0.06^{b}	30.31±0.06^{b}	4.04±0.03^{f}
P. sajor-caju	29.22±0.08^{e}	23.92±0.07^{f}	5.46±0.40^{c}
P. euos	42.21±0.06^{a}	37.12±0.04^{a}	7.21±0.05^{a}
H. ulmarius	31.89±0.05^{d}	26.01±0.08^{d}	5.11±0.04^{d}
P. florida	23.24±0.04^{g}	25.15±0.05^{e}	4.95±0.06^{e}
V. volvaciea	35.91±0.61^{b}	27.16±0.06^{c}	6.61±0.03^{b}
P. platypus	32.45±0.04^{c}	26.51±0.05^{d}	4.95±0.06^{e}
P. djamor	28.06±0.08^{f}	20.29±0.06^{g}	4.00±0.03^{f}
C. indica	32.93±0.05^{c}	21.46±0.07^{g}	5.01±0.04^{d}

Values are expressed as Mean±SD of nine mushroom sp. in each group. Mean of three replications. Means followed by a common letter are not significantly different at the 5% level by DMRT. Units: CAT: 1 μmole of H_2O_2 utilized /min /mg protein, SOD: μmole of Inhibition of 50% nitrite formation/min/mg protein, POX: 1 μmole of pyrogallol oxidized/min/mg protein

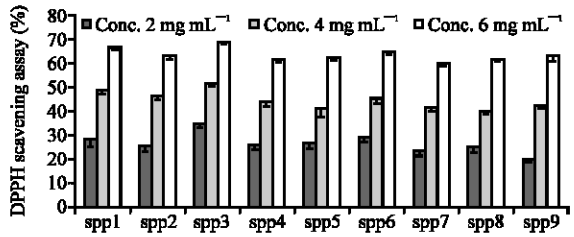

Fig. 1: Scavenging effect of methanolic extracts from 9 edible mushrooms on 1, 1-diphenyl-2-picrylhydrazyl radical. Each value is expressed as Mean±SD. spp1: *Agaricus bisporus,* spp2: *Pleurotus sajor-caju*, spp3: *Pleurotus euos*, spp4: *Hybsizus ulmarius*, spp5: *Pleurotus florida*, spp6: *Volveriela volvaciea*, spp7: *Pleurotus platypus*, spp8: *Pleurotus djamor* and spp9: *Calocybe indica*

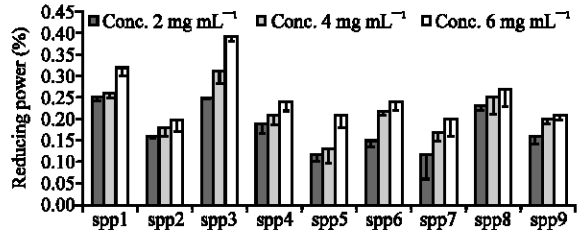

Fig. 2: Reducing power of different concentrations of methanol extract of 9 edible mushrooms. Values are expressed as Mean±SD. spp1: *Agaricus bisporus,* spp2: *Pleurotus sajor-caju*, spp3: *Pleurotus euos*, spp4: *Hybsizus ulmarius*, spp5: *Pleurotus florida*, spp6: *Volveriela volvaciea*, spp7: *Pleurotus platypus*, spp8: *Pleurotus djamor* and spp9: *Calocybe indica*

scavenging activities and reducing power in various concentrations of methanol extracts were also higher in *P. euos*; this was followed by *A. bisporus* (Fig. 1, 2). Different concentrations of DPPH scavenging activities of methanolic extracts from *P. eous* were 34.7 to 68.5%. The methanolic extract of mushroom species has presented in significant antioxidant activity. Antioxidant activity of the mushroom extracts include scavenging of free radicals also find good accountable. In addition, the mushroom is utilize, the source of natural antioxidants in food industry.

ACKNOWLEDGMENTS

We would like to thank Dean and the authorities of the Annamalai University, Parangipettai for the facilities provided. We also thank the anonymous reviewers and editor for helpful comments that improved the manuscript.

REFERENCES

Ames, B.N., M.K. Shigenaga and T.M. Hagen, 1993. Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA., 90: 7915-7922.

Barros, L., P. Baptista and I.C.F.R. Ferreira, 2007. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food Chem. Toxicol., 45: 1731-1737.

Beecher, G.R., 1999. Phytonutrients' role in metabolism: Effects on resistance to degenerative processes. Nutr. Rev., 57: 3-6.

Cheung, L.M., P.C.K. Cheung and V.E.C. Ooi, 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem., 81: 249-255.

El-Enshasy, H., A. Daba, M. El-Demellawy, A. Ibrahim, S. El Sayed and I. El-Badry, 2010. Bioprocess development for large scale production of anticancer exo-polysaccharide by *Pleurotus ostreatus* in submerged culture. J. Applied Sci., 10: 2523-2529.

Halliwell, B. and J.M.C. Gutteridge, 1999. Free Radicals in Biology and Medicine. 1st Edn. Oxford University Press, New York.

Jadhav, S.J., S.S. Nimbalkar, A.D. Kulkarni and D.L. Madhavi, 1995. Lipid Oxidation in Biological and Food Systems. In: Food Antioxidants: Technological, Toxicological and Health Perspectives, Madhavi, D.L., S.S. Deshpande and D.K. Salunkhe (Eds.). Marcel Dekker, New York.

Jose, N. and K.K. Janardhanan, 2000. Antioxidant and antitumour activity of *Pleurotus folrida*. Curr. Sci., 79: 941-943.

Kasuga, A., Y. Aoyagi and T. Sugahara, 1995. Antioxidant activity offungus *Suillus bovines* (L:Fr.) O. Kuntze. J. Food Sci., 60: 1113-1115.

Lin, H.C., 1999. Evaluation of taste quality and antioxidant properties of edible and medicinal mushrooms. Masters Thesis, National Chung-Hsing University, Taichung, Taiwan.

Marklund, S. and G. Marklund, 1974. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

Mattila, P., P. Salo-Vaananen, K. Konko, H. Aro and T. Jalava, 2002. Basic composition and amino acids content of mushroom cultivated in Finland. J. Agric. Food Chem., 50: 6419-6422.

Oyaizu, M., 1986. Studies on products of browning reactions: Antioxidant activities of products of browning reaction prepared from glucose amine. Jpn. J. Nutr., 44: 307-315.

Ribeiro, B., J. Rangel, P. Valentáo, P. Baptista, R. Seabra and P. Andrade, 2006. Contents of carboxylic acids and two phenolics and antioxidant activity of dried portuguese wild edible mushrooms. J. Agric. Food Chem., 54: 8530-8537.

Sadasivam, S. and A. Manickam, 2004. Biochemical Methods. 2nd Edn. New Age International Publishers, New Delhi, India.

Seline, K.G. and H. Johein, 2007. The determination of L-carnitine in several food samples. Food Chem., 105: 793-804.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem., 47: 389-394.

Skerget, M., P. Kotnik, M. Hadolin, A.R. Hras, M. Simonic and Z. Knez, 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem., 89: 191-198.

Stone, W.L., I. Le Clair, T. Ponder, G. Baggs and B.B. Reis, 2003. Infants discriminate between natural and synthetic vitamin E. Am. J. Clin. Nutr., 77: 899-906.

Weisburger, J.H., 1999. Mechanisms of action of antioxidants as exemplified in vegetables tomatoes and tea. Food Chem. Toxicol., 37: 943-948.

Yamagushi, T., H. Takamura, T. Matoba and J. Terao, 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1,-diphenyl-2-picrylhydrazyl. Biosci. Biotechnol. Biochem., 62: 1201-1204.

Yang, J.H., H.C. Lin and J.L. Mau, 2002. Antioxidant properties of several commercial mushrooms. Food Chem., 77: 229-235.