



Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Ligninolytic and leaf litter degrading mushrooms from the Philippines with antioxidant activities

Renato G. Reyes ^{*1} and Muraleedharan G. Nair ²

¹Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines,

²Bioactive Natural Products and Phytochemicals Laboratory, Plant & Soil Sciences, Michigan State University, East Lansing, Michigan, USA

*Email: renato.reyes@clsu.edu.ph

ABSTRACT

Methanol and hot water extracts of five ligninolytic mushrooms (*Ganoderma lucidum*, *Lentinus sajor-caju*, *Schizophyllum commune*, *Pleurotus florida* and *Pleurotus ostreatus*) and a leaf-litter-degrading mushroom (*Volvariella volvacea*) from the Philippines were evaluated *in vitro* for their antioxidant activities. MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to determine the antioxidant activities of the extracts of the different mushrooms. Both the methanol and hot water extracts of *G. lucidum* with 0.75 and 0.54 nm absorbances, respectively, showed better antioxidant activities than those of the other evaluated ligninolytic edible mushrooms and standard compounds (Vitamin C and TBHQ). Vitamin C and TBHQ had 0.49 and 0.39 nm absorbances, respectively. On the other hand, methanol extracts of *L. sajor-caju* had the highest bioactivity having an absorbance of 0.80 nm. Likewise, the different stages of the basidiocarps of *V. volvacea*, regardless of strain, exhibited antioxidant activities with partially and fully opened stages. Moreover, hot water extracts of *V. volvacea* demonstrated higher antioxidant activities than its methanol extracts. Results of the assay suggest that the five ligninolytic and leaf-litter-degrading mushrooms from the Philippines exhibited antioxidant activities.

Keywords: medicinal mushrooms, mushroom nutraceuticals, Philippine mushrooms

INTRODUCTION

Bioactive materials that prevent the proliferation of cancer cells and other physiogenic diseases, such as diabetes, arthritis and hypertension, are derived from natural sources that must be consumed on a regular basis either as functional foods/nutraceuticals or nutraceuticals. Nutraceuticals, which are commercially available in capsules or tablets, are extracts from food sources whose chemical characteristics have been refined or partially refined that enhance the immune response of the human body against diseases [1]. They are primarily for prophylactic purpose even though their therapeutic potential cannot be underestimated. On the other hand, nutraceuticals, which may include isolated nutrients, dietary supplements, modified/enriched foods, and herbal and processed products, such as cereals, soups and beverages, contain functional food ingredients, such as dietary fiber, polyunsaturated fatty acids, proteins, peptides, amino acids, minerals, vitamins and other antioxidants, for example, glutathione and selenium [2] [3] [4] [5]. These materials, which benefit the human body beyond basic nutrition, usually serve as antioxidants and anti-inflammatory, antimicrobial and immune boosters.

People regardless of geographical location are oftentimes confronted with both natural and manmade stresses owing to modern lifestyle coupled with the changing environment including climate changes. Oxidative stress due to the accumulation of reactive oxygen species has been linked to the above-mentioned diseases. Reactive oxygen species such as hydroxyl and superoxide radicals, which are produced by sunlight, ultraviolet radiation, chemical reactions

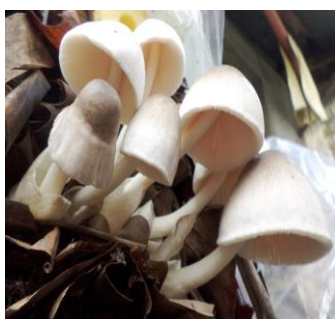
and metabolic processes, have a wide variety of pathological effects on cellular processes [6]. Although the cells in the human body are capable of producing oxidative enzymes, such as superoxide dismutase and catalase, as means of natural defense, there is still need to take in antioxidant supplements or food rich in antioxidants to prevent further cellular damage due to ROS. Thus, the consumption of functional foods containing alpha tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione from fruits, vegetables, pulses, cereals and their by-products, which possess antioxidant properties [7] [8] [9] [10] [11] is deemed imperative. This intake is necessary to balance ROS especially under physiological conditions where there are insufficient antioxidant defense and repair systems in the body. Edible mushrooms have also been evaluated for this purpose, some of which have shown promising results. For instance, mushrooms (*Pleurotus* sp., *Agaricus bisporus*, *Morchella esculenta*, and *Boletus edulis*) consumed in Europe exhibited an almost 90% 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity [9]. Similarly, *Lentinula edodes* also exhibited a potent radical scavenging activity [11]. *Grifola frondosa*, *Phellinus* spp., *Pleurotus porrigens*, *Hygrocybe conica*, *Volvariella volvacea*, *Coprinus comatus* and *Russula delicata* [12] [13] [14] [15] [16] [17] have also been found to have antioxidant activities. In this paper, the antioxidant activities of five species of ligninolytic mushrooms (*Ganoderma lucidum*, *Schizophyllum commune*, *Lentinus sajor-caju*, *Pleurotus florida* and *Pleurotus ostreatus*) and a leaf-litter-degrading (*V. volvacea*) mushroom from the Philippines are presented. *P. florida* and *P. ostreatus* are commercially grown in the country primarily for culinary purpose, while Philippine wild strains of *G. lucidum*, *S. commune* and *L. sajor-caju* have been successfully domesticated by the Center for Tropical Mushroom Research and Development at the Central Luzon State University and grown on a limited scale by a number of mushroom growers. On the other hand, *V. volvacea* is considered as the most popular and most highly preferred mushroom by Filipinos, being the first cultivated mushroom in the Philippines [18].

MATERIALS AND METHODS

Preparation of mushroom samples

Five strains of ligninolytic mushrooms (*G. lucidum*, *S. commune*, *L. sajor-caju*, *P. florida* strain 1 and *P. ostreatus*) from the Center for Tropical Mushroom Research and Development of the Central Luzon State University, Philippines were separately grown in 7 x 14² inch polypropylene bags. On the other hand, *P. florida* strain 2 was grown in a pulp and paper waste formulation (Fig. 1).

Spawn of strains of *V. volvacea* from Central Luzon State University and Butuan, Philippines were inoculated separately into beds of rice stubbles and banana leaves following the usual procedure of growing *V. volvacea* under outdoor condition [19]. Fruiting bodies were harvested and sorted into different growth stages such as button/egg stage, partially open and fully open. The harvested fruiting bodies were thinly shredded by hand and air-dried.

*Ganoderma lucidum**Lentinus sajor caju**Schizophyllum commune**Volvariella volvacea**Pleurotus ostreatus**Pleurotus florida***Fig. 1.** Fruiting bodies of test mushrooms

Preparation of mushroom extracts

Twenty five grams each of air-dried ligninolytic mushroom samples was pulverized separately in a waring blender and boiled in 600 ml of reversed osmosis water. The mushroom samples were vacuum-filtered using Whatman filter paper no. 1. Similarly, 25 g each of air-dried mushroom samples from each growth stage of the two strains of *V. volvacea* was separately soaked for 30 min in 600 ml of reversed osmosis water to rehydrate and subsequently boiled until the mushroom tissues became tender. The boiled samples were cooled for 12 h, homogenized and centrifuged at 10,000 rpm, 4°C for 10 min. Both the supernatant and residue of each mushroom sample were placed separately in a refrigerator for 12 h. The supernatants, which constituted the hot water extracts, were lyophilized for 48 h. On the other hand, the residues were soaked in methanol for 24 h. Soaking was repeated after the first methanol extract was collected. Methanol extracts from both collections were combined and evaporated in vacuum.

Both the lyophilized hot water and vacuum-dried methanol extracts were stored in a refrigerator in preparation for bioassay.

Preparation of mushroom extracts for thin layer chromatography

To screen for possible UV-active compounds from mushrooms, thin layer chromatography was performed using silica gel as the stationary phase and chloroform/methanol (4:1) and hexane/acetone (4:1) as the mobile phases.

MTT antioxidant assay of mushroom extracts

The protocol of Liu and Nair [8] for MTT antioxidant assay was adopted with minor modifications. Extracts were prepared in DMSO (10 mg/ml) of ACS reagent grade (EMD Chemicals, Inc.). MTT (1 mg/ml) was dissolved in DMSO and temporarily stored in a refrigerator. An 10 μ L aliquot of each of the mushroom extracts in DMSO was mixed with 190 μ L of MTT solution and 200 μ L of DMSO (250 ppm) in a 2-ml glass vial with Titeseal closure (Fisher Scientific, Inc.) and vortexed. Duplicate glass vials containing the reaction mixture (250 ppm) were incubated at 37°C for 18 h. 200 μ L of the reaction mixture was pipetted out into each well of a 96-well cell culture plate. Vitamin C and TBHQ (both from Sigma Aldrich, Inc.) dissolved in DMSO at 25 ppm served as positive controls. The absorbance was read at 570 nm using an EL_x800 Universal Microplate Reader (BIO-TEK Instruments, Inc.).

RESULTS AND DISCUSSION

The ability of the five ligninolytic mushroom extracts to terminate the oxidation chain reactions via the removal of free-radical intermediates and the inhibition of other oxidation reactions was demonstrated in this experiment. Through the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay of Liu and Nair [8], the reduction of MTT to purple formazan in the presence of mushroom extracts was observed under in vitro condition. The thin layer chromatographic profile of the mushroom extracts showed no interesting UV-active spots. These spots usually indicate compounds containing chromophores. However, despite the absence of chromophores, both the methanol and hot water extracts of the five ligninolytic mushrooms exhibited varying antioxidant activities in the MTT assay (Fig. 2). This observation confirms previous reports that indeed mushrooms are antioxidants [20] [21] [22]. Mushrooms expressed varying antioxidant activities. For instance, among the five evaluated mushrooms, *G. lucidum* (both hot water and methanol extracts) had the highest bioactivity, which even surpassed the activities of the standard compounds (Vitamin C and TBHQ). The antioxidant activity of *G. lucidum* from the Philippines is congruent with the reports of previous researchers from other countries [23]. With regard to the type of solvent used in extraction, methanol extracts of *L. sajour-caju* showed the highest bioactivity. The hot water extract of *P. florida*, which was previously grown on rice straw and pulp and paper wastes, demonstrated comparable antioxidant activities to the standard compounds. This interesting observation suggested that the type of substrate used for growing the mushrooms did not affect the bioactivity. Although there was no marked significant difference, the variation in the bioactivity of the same strain of mushroom (*P. florida*) grown on different substrates was noted. Different forms (fruiting bodies, mycelia, stipe, and pileus) of mushroom from the same strain exhibited varying antioxidant activities [24] [25]. *P. florida*, being the most widely cultivated mushroom in the rural areas in the Philippines, is primarily consumed for culinary purpose owing to its favorable texture and flavor. Both hot water and methanol extracts of the other ligninolytic mushrooms, namely, *S. commune* and *P. ostreatus*, had relatively lower bioactivities than those of the other evaluated mushrooms and than standard compounds. Likewise, the most popular leaf-litter-degrading mushroom *V. volvacea* also exhibited antioxidant activities as manifested by the ability of its hot water and methanol extracts to transform yellow MTT into purple formazan after 18 h of incubation.

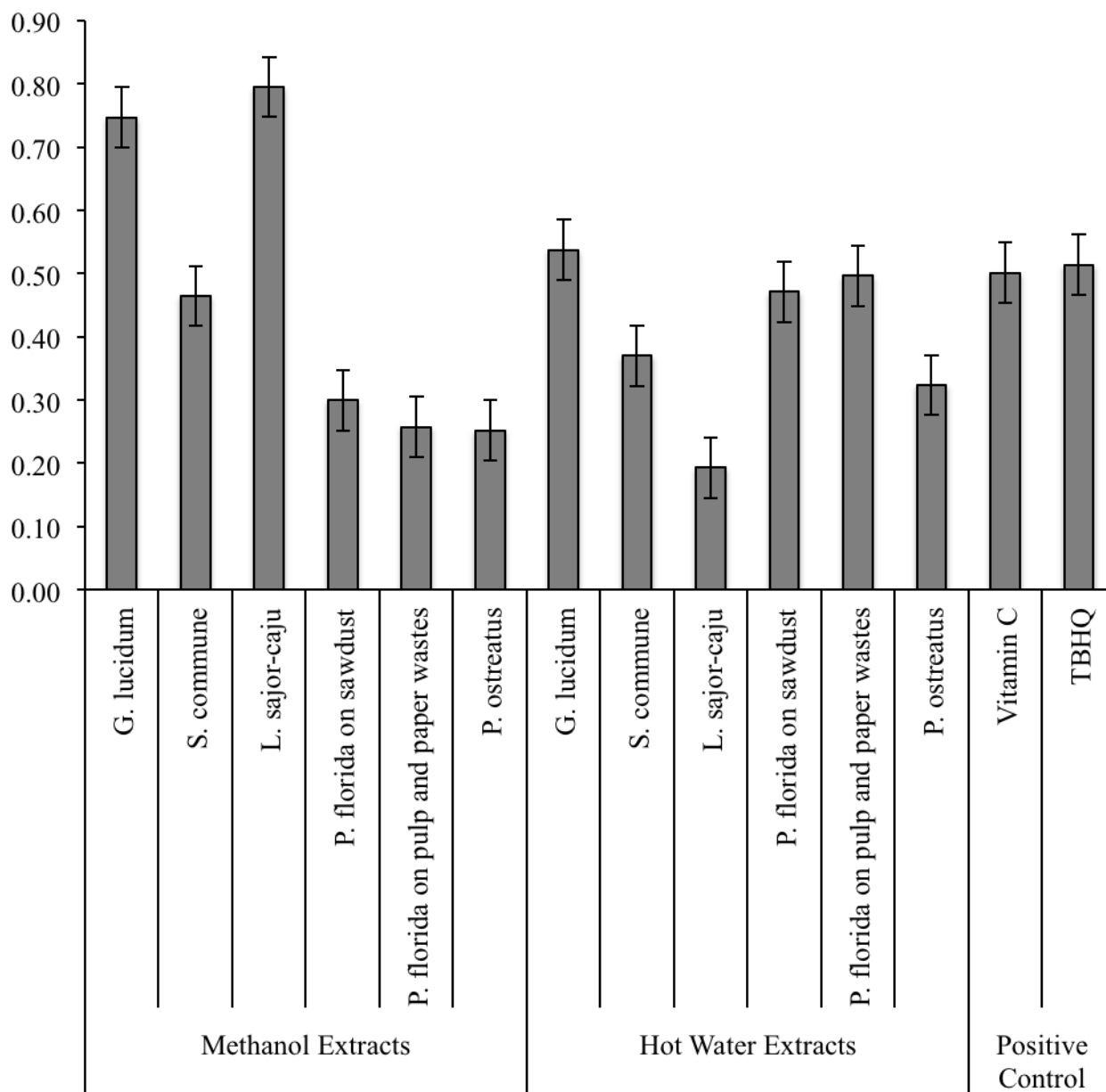


Fig. 2. Antioxidant activities of ligninolytic edible mushroom extracts at 250 ppm

The hot water extract of *V. volvacea* demonstrated a higher antioxidant activity than the methanol extract (Fig. 3). This interesting observation therefore supports the practice of cooking this culinary mushroom by boiling in order to maximize its nutraceutical potential. The different growth stages of the fruiting bodies of the two strains of *V. volvacea* also expressed varying antioxidant activities with the partially and fully opened basidiocarps as having the highest activities. The antioxidant activities of these stages are generally higher than those of the standard compounds. This finding is vital information for mushroom growers and even buyers as to what stage of the mushroom must be harvested in order to maximize its full nutraceutical potential. Usually, mushroom growers prefer not to harvest the partially or fully opened basidiocarps because of postharvest problems such as weight loss. Similarly, mushroom consumers prefer not to harvest the partially or fully opened stages owing to unfavorable texture compared with the button stage. Both the methanol and hot water extracts of the partially opened basidiocarp of the *V. volvacea* Butuan strain had higher bioactivities than those of the Central Luzon strain. However, the fully opened basidiocarps of the Central Luzon strain had higher bioactivities than those of the Butuan strain. This

interesting observation suggests that it is the stage of development of fruiting bodies rather than the strain that plays an important role in the expression of antioxidant activity.

Although specific compounds have not been isolated, purified or identified in this investigation, it is presumed that polyphenols [11] [26] and polysaccharide-protein complexes [27] are responsible for the antioxidant activity, as previously reported for other edible mushrooms with bioactivities. Other bioactive compounds, such as fatty acid derivatives [20] and exopolysaccharides [28] [29] [30], may also be responsible for the antioxidant activity.

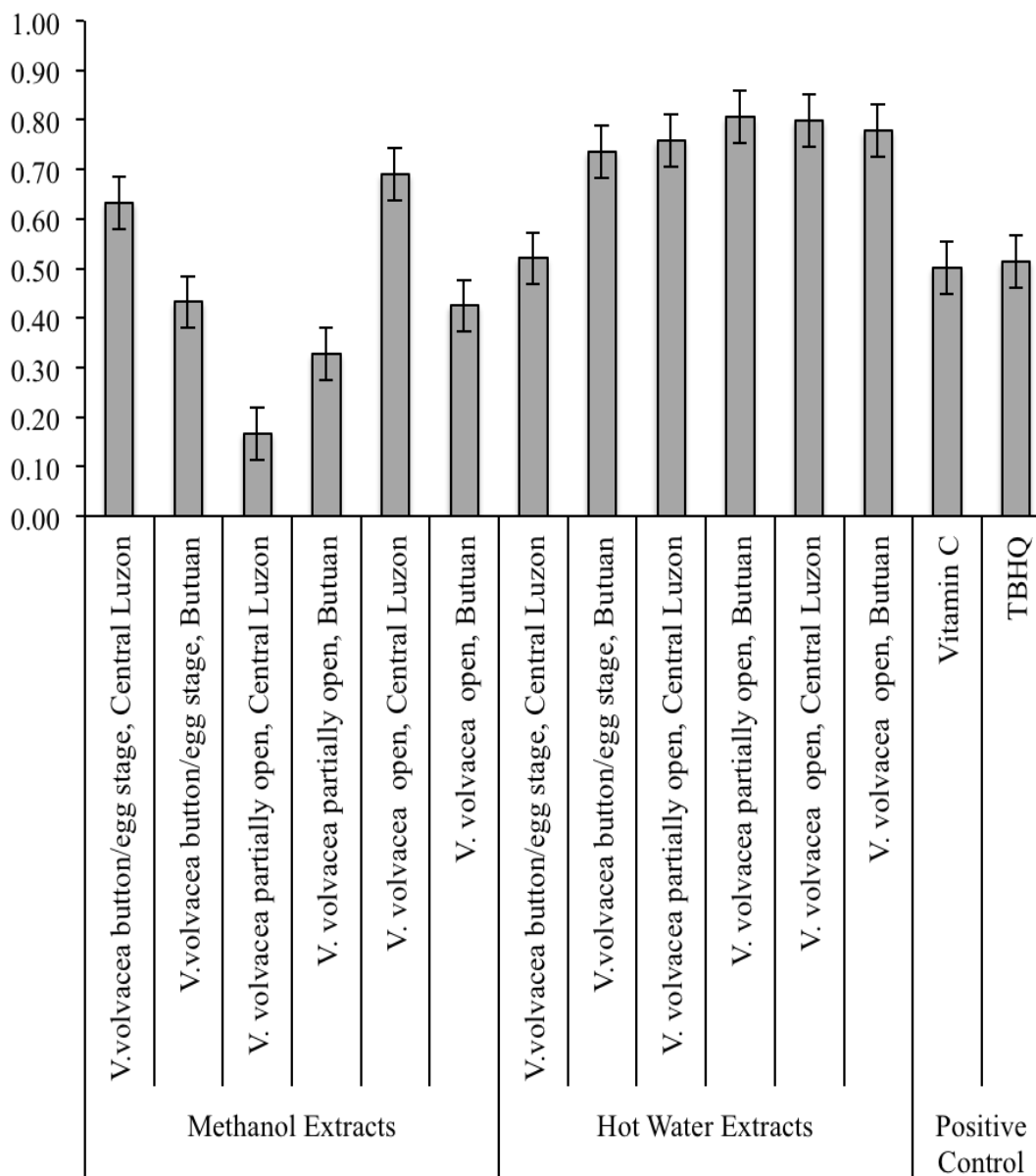


Fig. 3. Antioxidant activities of *V. volvacea* extracts at 250 ppm

Acknowledgment

Grateful acknowledgment is extended to the Philippine–American Educational Foundation, Fulbright Fellowship Program, the Bioactive Natural Products Laboratory at Michigan State University, USA and the Central Luzon State University, Philippines for making this research possible.

REFERENCES

- [1] Chang, S.T., Buswel, J.A., Mushroom nutraceuticals. *World J. Microbiol. Biotechnol.* **1996**; 12:473-476.
- [2] Andlauer, W., Fürst, P., Nutraceuticals: a piece of history, present status and outlook. *Food Res. Int.* **2002**; 35:171-176.
- [3] Barros, L., Cruz, T., Baptista, P., Estevinho, L.M., Ferreira, I., Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem. Toxicol.* **2008**; 46: 2742-2747.
- [4] Kruger, C.L., Mann, S.W., Safety evaluation of functional ingredients. *Food Chem. Toxicol.* **2003**; 41:793-805.
- [5] Babar, N., Oberoi, H.S., Uppal, D.S., Palil, R.T., Total phenolic and anti-oxidant capacity of extracts obtained from six important residues. *Food Res. Int.* **2011**; 44, 391-396.
- [6] Kim, S., Hwang, H.J., Lee, B.C., Yun, J.W., *Grifola frondosa* polysaccharides as cosmeceuticals. *Food Technol. Biotechnol.* **2007**; 45(3), 295-305.
- [7] Dimitrios, B., Sources of natural phenolics antioxidants. *Trends Food Sci Technol.* **2006**; 17, 505-512.
- [8] Liu, Y.B., Nair, M.G., An efficient and economical MTT assay for determining the antioxidant activity of plant natural product extracts and pure compounds. *J. Nat. Prod.* **2010**; 73, 1193-1195.
- [9] Ramirez-Anguiano, A.C., Santoyo, S., Reglero, G., Soler-Rivas, C., Radical scavenging activities, endogenous oxidative enzymes and total phenols in edible mushrooms commonly consumed in Europe. *J. Sci. Food Agric.* **2007**; 87, 2272-2278.
- [10] Sowndhararajan, K., Siddhuraju, P., Manian, S., Antioxidant and free radical scavenging capacity of the underutilized legume, *Vigna vexillata* (L.) A. Rich. *J Food Compost Anal.* **2011**; 24,160-165.
- [11] Cheung, L.M., Peter, C.K., Cheung, V., Ooi, E.C., Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* **2003**; 81, 249-255.
- [12] Mathew, J., Sudheesh, N., Rony, K., Smina, T., Janardhanan, K., Antioxidant and antitumor activities of cultured mycelium of culinary-medicinal paddy straw mushroom *Volvariella volvacea* (Bull.: Fr.) Singer (Agaricomycetidae). *Int J Med Mushrooms.* **2008**; 10(2),139-147.
- [13] Türkoğlua, A., Durub, M.E., Mercanc, N., Antioxidant and antimicrobial activity of *Russula delica* Fr: an edible wild mushroom. *Eurasian Journal of Analytical Chemistry.* **2007**; 2(1),54-67.
- [14] Vaz, J.A., Barros, L., Martins, A., Santos-Buelga, C., Vasconcelos, M.H., Ferreira, I., Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. *Food Chem.* **2011**; 126, 610-616.
- [15] Wong, J.Y., Chye, F.Y., Antioxidant properties of selected tropical wild edible mushrooms. *J Food Compost Anal.* **2009**; 22, 269-277.
- [16] Yang, Y., Hu, J., Liu, Y., Feng, N., Chen, H., Tang, Q., Ye, L.B., Zhang, J., Antioxidant and cytotoxic activities of ethanolic extracts and isolated fractions of species of the genus *Phellinus* Quéél. (Aphyllophoromycetidae). *Int J Med Mushrooms.* **2011**; 13(2), 145-152.

- [17] Zhang, Y., Mills, G.L., Nair, M.G., Cyclooxygenase inhibitory and antioxidant compounds from the mycelia of the edible mushroom *Grifola frondosa*. *J. Agric. Food Chem.* **2009**; 50, 7581–7585.
- [18] Reyes, R.G., Abella, E.A., Eguchi, F., Higaki, M., State of the art of mushroom production activities in the province of Nueva Ecija, Philippines. *Mushroom Science and Biotechnology.* **1997**; 4 (1), 3-8.
- [19] Reyes, R.G., Eguchi, F., Kalaw, S.P., Kikukawa, T., Mushroom Growing in the Tropics: A Practical Guide. Science City of Munoz, Central Luzon State University Press. **2009**, 89.
- [20] Makropoulou, M., Aligiannis, N., Gonou-Zagou, Z., Pratsinis, H., Skaltsounis, A.L., Fokialakis, N., Antioxidant and cytotoxic activity of the wild edible mushroom *Gomphus clavatus*. *J Med Food*, **2012**; 15 (2), 216–221.
- [21] Puttaraju, N., Venkateshaiah, S., Dharmesh, S., Mysore, S., Urs, N., Somasundaram, R., Antioxidant activity of indigenous edible mushrooms. *J. Agric. Food Chem.* 2006; 54, 9764–9772.
- [22] Seephonkai, P., Samchai, S., Thongsom, A., Sunaart, S., Kiemsanmuang, B., Chakuton, K., DPPH radical scavenging activity and total phenolics of *Phellinus* mushroom extracts collected from northeast of Thailand. *Chin J Nat Med.* **2011**; 9 (6), 0441–0445.
- [23] Mohsin, M., Negi, P.S., Ahmed, Z., Determination of the antioxidant activity and polyphenol contents of wild lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt. Fr.) P. Karst. (Higher Basidiomycetes) from Central Himalayan Hills of India. *Int J Med Mushrooms.* **2011**; 13(6), 535–544.
- [24] Ferreira, I.C.F.R., Baptista, P., Vilas-Boas, M., Barros, L., Free radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. *Food Chem.* **2007**; 100, 1511-1516.
- [25] Tsai, S.Y., Huang, S.J., Mau, J.L., Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. *Food Chem.* **2006**; 98, 670–677.
- [26] Lee, E.J., Jang, H.D., Antioxidant activity and protective effect of five edible mushrooms on oxidative DNA damage. *Food Sci. Biotechnol.* **2004**; 13, 443–449.
- [27] Li, N., Li, L., Fang, J.C., Wong, J.H., Ng, T.B., Jiang, Y., Wang, C.R., Zhang, N.Y., Wen, T.Y., Qu, L.Y., Lu, P.Y., Zhao, R., Shi, B., Wang, Y.P., Wang, X.Y., Liu, F., Isolation and identification of a novel polysaccharide-peptide complex with antioxidant, anti-proliferative and hypoglycemic activities from the abalone mushroom. *Biosci. Rep.* **2012**; 32, 221-228.
- [28] Chen, Y., Xie, M.Y., Nie, S.P., Li, C., Wang, Y.X., Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. *Food Chem.* **2008**; 107, 231–241.
- [29] Lin, E.S., Production of exopolysaccharides by submerged mycelial culture of *Grifola frondosa* TFRI1073 and their antioxidant and antiproliferative activities. *World J. Microbiol. Biotechnol.* **2011**; 27, 555–561.
- [30] Mantovani, M.S., Marilanda, F., Jose, B., Angeli, P., Oliveira, R., Silva, A., Ribeiro, L., Beta glucans in promoting health: prevention against mutation and cancer. *Mutat. Res.* **2008**; 658, 154–161.