SCIENCE AND HUMANITIES JOURNAL
VOL. 11, 2017, pp. 91-101 **DOI:** https://doi.org/10.47773/shj.1998.110.5

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF VOLVARIELLA (*Volvariella volvacea*), WOOD EAR (*Auricularia auricula-judae*), AND SHIITAKE (*Lentinula edodes*) MUSHROOMS

JEBBEN PEREZ[1] and CANDELARIO L. CALIBO[2]

[1]*Intertek Testing Services Philippines Inc., NCR, Philippines,*
[2]*Department of Pure and Applied Chemistry,*
Visayas State University, Visca 6521-A, Baybay City, Leyte, Philippines

ABSTRACT

Edible mushrooms, whether fresh or processed, are widely consumed worldwide. In order to determine their nutritional and/or functional values, this study was conducted to evaluate the free radical scavenging activity of Volvariella (*Volvariella volvacea*), wood ear (*Auricularia auricula-judae*), and shiitake (*Lentinula edodes*) mushrooms. Trolox equivalent per 100g sample (TE/100g) was determined to describe the activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Results showed that wood ear exhibited the highest free radical scavenging activity (387.11±30.26 TE/100g) among the three species. Shiitake mushroom exhibited 368.13 ±27.03 TE/100g activity. Volvariella contained the lowest activity with 318.72 ±36.87 TE/100g. Based on the solvent system, ethanolic extracts showed the highest free radical scavenging activity (386.45±23.30 TE/100g) while hexane extract showed the least free radical scavenging activity (326.34 ±27.75 TE/100g). Water extract exhibited 361.17 ±49.11TE/100g activity. The results of this study showed that mushroom extracts could serve as natural antioxidants due to their significant antioxidative property.

Key words: free radical scavenging, mushrooms, DPPH assay, Trolox

Correspondence : J. Perez. Address: Intertek Testing Services Philippines Inc., Km 23. West Service Rd. Bo Cupang Muntinlupa City E-mail: jebbenperez@gmail.com

INTRODUCTION

Many diseases nowadays are often associated with free radical oxidation damage that results from an imbalance between the formation and neutralization of oxidants. Some of these diseases are heart diseases, macular degeneration, diabetes, hepatitis, and cancer. Free radical oxidation damage or oxidative damage is instigated by free radicals. These free radicals seek stability through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Wangensteenet al 2004). Free radical is a highly reactive atom or group of atoms with an unpaired electron which exists in different forms. These include superoxide, hydroxyl, hydroperoxyl, peroxyl, and alkoxyl radicals. Every day, our body is constantly attacked by these free radicals through our aerobic biochemical processes in the body which normally uses oxygen like respiration and cell-mediated immune functions. Environmental exposure of free radicals is also possible like from smoking and contact with radiation (Kamen 1987).

Antioxidants are substances that may protect cells from the damage caused by free radicals. Antioxidants interact with the free radicals to stabilize them; thereby preventing some of the damage free radicals might otherwise cause. Naturally occurring antioxidants, which can inhibit or delay the oxidation of oxidizable substrate in a chain reaction, have attracted interest because they can protect the human body from diseases caused by free radical damage.

Two of the most common mushroom species in the Philippines are Volvariella (*Volvariella volvacea*) and Wood Ear (*Auricularia auricula-judae*). Shiitake Mushroom from Japan is one of the most commonly commercialized mushroom species. The chemical composition of mushrooms is not well studied and the studies are greatly limited to European and American strains (Chang & Miles 2004). It was emphasized that the specimens with the same species grown under different conditions vary greatly in their chemical compositions. Furthermore, the flesh of the mushroom has been proven to be almost identical to meat and possesses the same nourishing properties. High-temperature mushrooms including Volvariella are inexpensive and can substitute meat products (Chang & Miles 2004).

Thus, evaluating the free radical scavenging of the three species of mushrooms could not only lead to potential therapeutic use of the said mushrooms, but also to popularizing the mushrooms as an economical substitute for meat products.

MATERIALS AND METHODS

Chemicals and Reagents

Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) were obtained from Merck & Co., Inc. Analytical grades of the following reagents were used: Ethanol and HCl.

Collection and Preparation of Mushroom Samples

The fresh mushroom samples were obtained from Ormoc City, Baybay City, and Davao City, Philippines. These samples were identified and authenticated by Prof. Yolanda Mangaoang of the Department of Pest Management. The test materials were washed with tap water followed by distilled water to remove dust particles and other contaminants. The cleaned sample was air-dried and stored in a clean beaker ready for extraction.

Preparation of Mushroom Extracts

Using an osterizer, ten (10) grams of the air-dried mushroom were blended and homogenized with forty (40) milliliters of solvent. The homogenates were centrifuged at 2500 rpm for 30 minutes. The extract was then decanted and filtered using Whatman No.1 filter paper. The filtrate was transferred to a centrifuge tube wrapped with carbon paper ready for DPPH Assay.

Determination of Free Radical Scavenging Activity

The antioxidant activity of all extracts was determined according to the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay by Hatano et al (1988).

The stock of DPPH (2.3mg/100mL) was prepared using ethanol/water solvent. The Shimadzu UV-Vis spectrophotometer was scanned to determine the λ_{max} for DPPH which was found to be 517nm. The determined λ_{max} is the same value with the published one in literature. The standard for the assay, which is Trolox at varying concentrations, was reacted with DPPH solution for thirty minutes at room temperature. The change in the absorbance was measured at the established λ_{max}.

The different mushroom extracts (0.3 mL) were mixed with 2.7 mL of DPPH stock solution. The mixture was left to stand at ambient temperature for 30 minutes in the dark room (until stable absorption values were obtained), giving enough time for the reaction of the cellular antioxidants with the DPPH. The free radical scavenging activity was tested by reading its absorbance at the established λ_{max} using the Shimadzu UV-Vis spectrophotometer. The corresponding solvents used in the extracts were used as reference blank.

The free radical scavenging activity was evaluated using Trolox equivalent per 100 grams sample. Antioxidant activity of a sample was expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply Trolox units per 100 grams sample or TE/100g.

RESULTS AND DISCUSSION

The DPPH assay is the widely used model system for the evaluation and determination of free radical scavenging activities of several natural compounds such as crude extracts of different plant and food samples. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The antioxidants scavenge the DPPH radical by donating protons, thereby forming the reduced DPPH or the DPPH-H as shown in Figure 1.

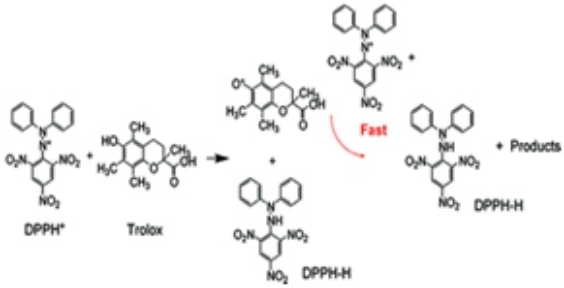

Figure 1. Reaction of DPPH with Trolox (Friaa&Brault, 2006)

The odd electron in the DPPH radical gives a strong absorption maximum at wavelength 517 nm. The reduction is noted through the color changes in the solution which is from purple to yellow as shown in Figure 2.

Figure 2. Color change of DPPH radical

The free radical scavenging activity of the three mushroom species is summarized in Table 1 and Figure 3.

Table 1. Free radical scavenging activity (TE/100g) of three mushroom species

MUSHROOM SPECIES	FREE RADICAL SCAVENGING ACTIVITY 1/ (TE/100grams)
Wood Ear	$387.11^{a} \pm 30.26$
Volvariella	$318.72^{c} \pm 36.87$
Shiitake Mushroom	$368.13^{b} \pm 27.03$

1/ Values followed by the same letter are not significantly different at $\alpha = 0.05$ %, based on Tukey's Honesty Significant Difference Test.

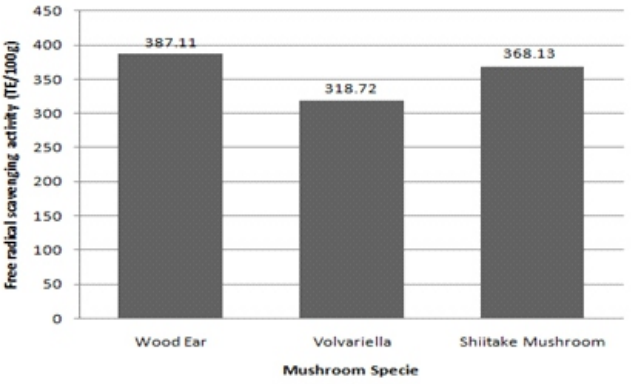

Figure 3. Free radical scavenging activity (TE/100g) of three mushroom species

Results showed that Wood Ear has the highest activity with $387.11^{a} \pm 30.26$ TE/100 grams followed by the commercialized Shiitake Mushroom with $368.13^{b} \pm 27.03$ TE/100 grams. Volvariella was found to have the least activity with $318.72^{c} \pm 36.87$ TE/100 grams. The free radical scavenging activity of these three mushroom species is higher compared with the activity of some common foods published in literature like tomato, green beans, and green cabbage (Figure 4). Furthermore, the activity of the green grapes as well as of spinach and broccoli flowers is comparable to that of the mushrooms.

The high free radical scavenging activity of the mushrooms might be attributed to its high Ergothioneine content. Ergothioneine (ERT; 2-mercaptohistidine trimethylbetaine) is a fungal metabolite that may have antioxidant functions in mammalian cells. Several studies showed that mushrooms provide more ergothioneine than any other food (Ey et al 2007). Its structure is shown in Figure 5. Its role as an antioxidant is well documented (Melville 1958). The antioxidant properties of mushrooms appear to be related to at least four activities which include the molecules ability to: scavenge directly reactive oxygen species; chelate various divalent metallic cations; activate antioxidant

enzymes such as glutathione peroxidase (Se-GPx) and MnSOD and to inhibit superoxide-generating enzymes such as NADPH-Cytochrome c reductase; and affect the oxidation of various hemoproteins such as hemoglobin and myoglobin. Aside from being a potent antioxidant, ergothioneine also helps maintain and conserve levels of other antioxidants such as glutathione, Vitamin C, and Vitamin E.

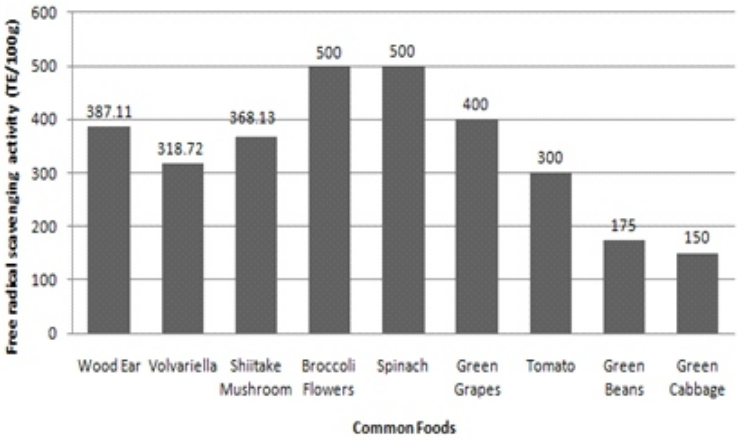

Figure 4. Free radical scavenging activity (TE/100g) of mushroom vs. common foods

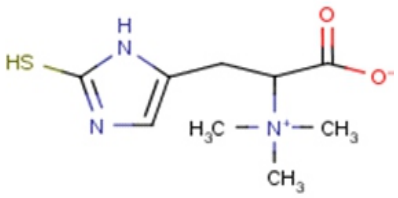

Figure 5.Structural formula of Ergothioneine

The difference in the free radical scavenging activity of the mushroom extracts is ascribed to the different availability of extractable components resulting in varied chemical composition of the mushroom. The difference could also be attributed to the individual compounds in the mixture and the structural factors such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, and other structural features.

Free radical scavenging activities of a crude extract strongly depend on the nature of the extracting solvent, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. The different solvent systems used in the experiment are 95% ethanol, water, and hexane. The free radical scavenging activity of the mushroom extracts based on the solvent system is shown in Table 2 and Figure 6.

Table 2. Free radical scavenging activity (TE/100g) of mushroom extracts based on the solvent system

SOLVENT SYSTEM	FREE RADICAL SCAVENGING ACTIVITY[1/] (TE/100grams)
95% Ethanol	$386.45^{a} \pm 23.30$
Water	$361.17^{b} \pm 49.11$
Hexane	$326.34^{c} \pm 27.75$

1/ Values followed by the same letter are not significantly different at $\alpha = 0.05$ %, based on Tukey's Honesty Significant Difference Test.

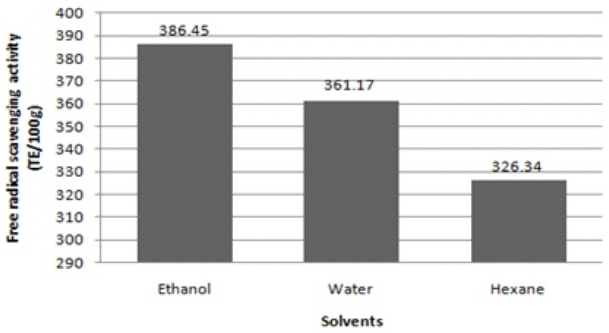

Figure 6. Free radical scavenging activity (TE/100g) of mushroom based on the solvent system

Results showed that 95% ethanol has the highest activity with 386.45a± 23.30 TE/100 grams, followed by water with 361.17b± 49.11 TE/100 grams, and lastly, hexane with 326.34c ± 27.75 TE/100 grams. The efficiency of the extracting solvent to dissolve endogenous compounds is vital in explaining the differences in the activity between solvent systems. The difference in the polarity between the three extracts correlates to their differences in activity. It was found out that polar extracts (water and ethanol) were more effective in scavenging the DPPH radical compared to the nonpolar extract (hexane). Furthermore, researchers proved that the extracts using moderately polar solvents have more potent antioxidative activity (Wangensteen et al 2004). This is why ethanol extracts have higher free radical scavenging activity compared to water extracts.

Hydrophilic and hydrophobic antioxidants are present in the mushroom extracts. Hydrophilic antioxidants are polar and water soluble antioxidants. The reactions between these antioxidants and free radicals take place within the cell cytosol, or the fluid inside the cells themselves.

The hydrophilic antioxidants include ascorbic acid (Vitamin C) which is found in some species of mushroom, glutathione which is an altruistic metabolite in fungi, lipoic acid, and uric acid. These antioxidants work within the cells themselves to fight against free radical damage. On the other hand, hydrophobic antioxidants are nonpolar and fat or lipid soluble antioxidants.

Unlike the hydrophilic antioxidants which fight against free radicals, hydrophobic antioxidants work within the membrane of the cell to help protect against free radical damage. Hydrophobic antioxidants include carotenes, alpha tocopherol (Vitamin E), ubiquinol, and lipoic acid. In recent studies, there is a high correlation between levels of antioxidant and its antioxidative activities (Chen & Yen 2006). Therefore, it can be deduced that more hydrophilic antioxidants are present in the three mushroom species.

CONCLUSION AND RECOMMENDATIONS

Based on the results, the free radical scavenging activity of the three mushrooms by the DPPH assay was comparable with common fruits and vegetables with Wood Ear exhibiting the highest activity and Volvariella the lowest antioxidative property. The 95% ethanol as extractant showed the greatest activity (386.45±23.30 TE/100 g), then water at 361.17±49.11 TE/100 g, and hexane the least (326.34±27.75 TE/100 g). Other tests may be used to evaluate the antioxidant activity of the mushroom extracts employing the ferric thiocyanate method and reducing power assay and the scavenging ability on different radicals such as hydroxyl and superoxide radicals (Boonsong et al 2016).

REFERENCES

Boonsong S, Klaypradit W & Wilaipun P. 2016. Antioxidant activities of extract from five edible mushrooms using different extractants. *Agriculture and Natural Resources.* 50(2):89-97. DOI: http//dx.doi.org/10.1016/j.anres.2015.07.002

Chang S and Miles PG. 2004. Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact. CRC Press. 2nd Ed

Chen H and Yen G. 2006. Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidiumguajava L.) leaves. National Science Council, Republic of China.

Ey J, Schömig E & Taubert D. 2007. Dietary sources and antioxidant effects of ergothioneine. J. Agric. Food Chem. 55 (16): 6466–74.

Friaa O and Brault D. 2006. Kinetics of the reaction between the antioxidant Trolox® and the free radical DPPH in semi-aqueous solution. *Org. Biomol. Chem.* 4(12):2417-23

Hatano T, Kagawa H, Yasuhara T & Okuda T. 1988. Two new flavonoids and their constituents in licorice root: their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin 36: 2090–2097.

Kamen B. 1987. Germanium: A New Approach to Immunity, Nutrition Encounter, Inc. Box 689, Larkspur, CA, 94939. http://www.egermaniumcom/book_view.php.

Melville DB. 1958. L-Ergothioneine. Vitam&Horm, 17:155-204.

Prakash A, Rigelhof F & Miller E. 2000. Antioxidant Activity. Medallion Laboratories.

Wangensteen H, Samuelsen AB & Maltrud KE. 2004. Antioxidant activity in extracts from coriander. *FoodChemistry Journal* 88: 293–297 Retrieved from http://www.healthcastle.com/antioxidant.shtml.