

**OPTIMIZATION OF LIQUID CULTURE CONDITIONS OF
Pleurotus cystidiosus AS SOURCE OF MYCOCHEMICALS
AND ANTIOXIDANTS**

HZASELL S. ABALOS

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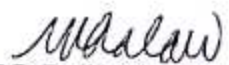
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ACCEPTANCE SHEET


This undergraduate thesis entitled "OPTIMIZATION OF LIQUID CULTURE CONDITIONS OF *Pleurotus cystidiosus* AS SOURCE OF MYCOCHEMICALS AND ANTIOXIDANTS" prepared and submitted by HZASELL S. ABALOS, in partial fulfillment of the requirements for the degree of BACHELOR OF SCIENCE IN BIOLOGY is hereby accepted.


RICH MILTON R. DULAY, M.Sc.
Adviser

6/13/18
Date Signed



SOFRONIO P. KALAW, Ph.D.
Critic

Date Signed



RICH MILTON R. DULAY, M.Sc.
Department Research Coordinator

6/13/18
Date Signed


Accepted as partial fulfillment of the requirements for the degree of
BACHELOR OF SCIENCE IN BIOLOGY


ANGELES M. DE LEON, Ph.D.
Chair, Department of Biological Sciences

Date Signed


RICH MILTON R. DULAY, M.Sc.
College Research Coordinator

6/13/18
Date Signed


EVARISTO A. ABELLA, Ph.D.
Dean, College of Arts and Sciences

Date Signed

BIOGRAPHICAL SKETCH

The author, Hzasell Santos Abalos, was born on November 25, 1996 at Bacag Villasis, Pangasinan and currently residing at Ladies Dorm 1 (Zinnia) CLSU, Science City of Muñoz, Nueva Ecija. She is the second eldest of Perfecto Ang Abalos and Erlinda Santos Abalos.

She took her primary education at Bacag Central School and her secondary education was at the Don Ramon E. Costales Memorial National High. She graduated with highest of honors in her section. Hzasell was not only academically inclined but also good in the other fields such as arts and dancing. In the four years she spent in high school, she was a performer and member of an Artist Club.

In 2013, she was admitted at the Central Luzon State University under the Bachelor of Science in Biology. She became a member and a scholar of Tanghalang Gagalawsa CLSU, a cultural dance group, and won the 4th place in DC-SUC III / CIRPS Socio-Cultural and Literary Festival 2014, and 2nd place in DC-SUC III / CIRPS Socio-Cultural and Literary Festival 2016. The author also joined an artist organization called GUHIT Pinas. The author completed her On-Job-Training under the Technology outreach Station- Freshwater, Looc, Castillejos, Zambales of the Bureau of Fisheries and Aquatic Resources Region III.

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ABSTRACT

ABALOS, HZASELL S., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **JUNE 2018, OPTIMIZATION OF LIQUID CULTURE CONDITIONS OF *Pleurotus cystidiosus* AS SOURCE OF MYCOCHEMICALS AND ANTIOXIANTS**

Adviser: RICH MILTON R. DULAY, M.Sc.

Pleurotus cystidiosus has a unique biology and one of the most interesting of all the oyster mushrooms because of its convenience and accessibility. However, it remains uncultivated for mycelial biomass production. Thus, the present work established the optimum liquid culture condition for the mycelial biomass production of *P. cystidiosus* for elucidation of functional activities. In order to provide information on the biomass production and biological activities of *P. cystidiosus*, optimization of liquid culture conditions, analysis of radical scavenging activity and total phenolics and mycochemical screening were analyzed. The coconut water with pH 6.5 incubated in room temperature (30°C), alternating light and dark, and static condition were the ideal cultural conditions for the mycelial growth of *P. cystidiosus*. Mycelia produced in agitated condition registered the highest radical scavenging activity of 35.55%, while the culture spent recorded 31.22%. However, mycelia produced in static condition exhibited 31.39% scavenging activity while its spent had 17.80%. Agitated spent recorded higher total phenolic content (112.13 mg GAE/g sample), while the mycelia produced gave 34.00 mg GAE/g sample when compared to static spent (71.71 mg GAE/ g sample) while its mycelia had 59.21 mg GAE/g sample. Mycochemical screening revealed that both mycelial mats contain varying amount of mycochemicals such as flavonoids, tannins,

alkaloids and steroids, essential oils, fatty acids, coumarines, phenols, and anthraquinones. Saponins, terpenoids, and cardiac glycosides were not detected in both samples.

LITERATURE CITED

- Abdullah, M. A., Ali, A. M., Marzian, M., Lajis, N. H., & Ariff, A. B. (1998). Establishment of cell suspension cultures of *Morinda elliptica* for the production of anthraquinones. *Plant cell, Tissue and Organ Culture*, 54, 173-182.
- Aberoumand, A. (2012). Screening of phytochemical compounds and toxic proteinaceous inhibitor in some lesser-known food based plants and their effects and potential applications in food. *International Journal of Food Science and Nutrition Engineering*, 2(3), 16-20.
- Adebayo, E.A., Oloke J.K., Ayandale, A.A. & Adegunlola C.O. (2012). Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius*. *Journal of Microbiology and Biotechnology*, 2 (2), 366-374.
- Agrawal, R.P, Chopra, A., Lavekar, G.S, Padhi, M.M., Srikanth N., Ota, S. & Jain, S. (2010). Effect of oyster mushroom on glycemia, lipid profile and quality of life in type 2 diabetic patients. *Australian Journal of Medical Herbalism*, 22(2), 50-54.
- Ahmed, S.A., Kadam, J.A., Mane, V. P., Patil, S.S., & Baig, M.M.V. (2009). Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) singer cultivated on different agro-wastes. *Nature and Science*, 7, 1545-0740.
- Alam, N., Yoon, K.N., Lee, J. S., Cho, H. J., & Lee, T. S. (2012). Consequence of the antioxidant activities and tyrosinase inhibitory effects of various extracts from the fruiting bodies of *Pleurotus ferulae*. *Saudi Journal of Biological Sciences*, 19, 111-118.
- Arbaayah, H. H. & Umi K. Y. (2013). Antioxidant properties in the oyster mushrooms (*Pleurotus* spp.) and split gill mushroom (*Schizophyllum commune*) ethanolic extracts. *Mycosphere*, 4(4), 661-673.
- Asatiani, M.D., Elisashvili, V.I., Wasser, S.P., Reznick, A, Z., & Nevo, E. (2007). Free-radical scavenging activity of submerged mycelium extracts from higher basidiomycetes mushrooms. *Bioscience, Biotechnology, and Biochemistry*, 71, 3090-3092.
- Badalyan, S. M. (2003). Edible and medicinal Higher Basidiomycetes mushrooms as a source of natural antioxidants. *International Journal of Medicinal Mushrooms*, 5, 153-162.
- Barros, L., Ferreira, M.J., Queiros, B., Ferreira, I.C.F.R., & Baptista, P. (2007). Total phenols, ascorbic acid, β -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry*, 103, 413-419.

- Bernas, E., Jaworska, G. & Lisiewska, Z. (2006). Edible mushrooms as a source of valuable nutritive constituents. *Acta Scientiarum Polonorum Technologia Alimentaria*, 5, 5-20.
- Bisko, N. A., & Bilay, V. T. (1996). Some physiological aspects of the cultivation of *Lentinula edodes* (Berk.) Singer In: DJ Royse (ed.). Proceed. II. *International Conference on Mushroom Biology and Mushroom Products*, 381-386.
- Bobek, P., Ginter, E., Jurcovicová, M., Kuniak, L. (1991). Cholesterol lowering effect of the mushroom *Pleurotus ostreatus* in hereditary hypercholesterolemic rats. *Annals of Nutrition and Metabolism*, 35, 191-5.
- Bustillos, R. G., Dulay, R. M. R., Bauto, J. J., Pascual, F., Baltazar, K., Bunag, H. W. & Reyes, R. G. (2014). Antioxidant activity and total phenolic content of *Volvariella volvacea* and *Schizophyllum commune* mycelia cultured in indigenous liquid media. *International journal of pure & applied bioscience*, 2(6), 175-181.
- Caulier, G. Van Dyck, S. Gerbaux, P. Eeckhaut, I. & Flammang, P. (2011). Review of saponin diversity in sea cucumbers belonging to the family Holothuriidae. *SPC Beche-de mer Information Bulletin*, 31, 48-54.
- Chang, S.T. & Buswell, J.A. (2003). Medicinal mushrooms as prominent source of nutraceuticals for the 21st century. *Current Topics in Nutraceutical Research*, 1, 257-280.
- Choi, I.Y., Joung, G. T., Ryu, J., Choi, J.S., & Choi, Y.G. (2003). Physiological characteristics of green mold (*Trichoderma* spp.) isolated from oyster mushroom (*Pleurotus* spp.). *Mycobiology*, 31, 139-44.
- De Silva, D.D., Rapior, S., Sudarman, E., Stadler, M., Xu, J., Alias, J.A. & Hyde, K.D. (2013). Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. *Fungal Diversity*, 62, 1-40.
- Dehariya, P., Vyas, D. & Kashaw, S.K. (2013). Mushroom nutraceuticals on different substrates. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5, 88-90.
- Deshmukh, A. J., Mehta, B. P., Sabalpara, A. N. & Patil, V. A. (2012). In vitro effect of various nitrogen, carbon sources and pH regimes on the growth and sporulation of *Colletotrichum gloeosporioides* Penz. and Sacc causing anthracnose of Indian bean. *Journal of Biopest*, 5, 46-49.
- Dulay, R.M.R., Cabalar A.C., De Roxas M.J.B., Concepcion J.M.P., Cruz N.E., Esmeralda, M., Jimenez, N., Aguila, Jc., De Guzman, Ej, Santiago, J.Q., Samoy, J.R., Bustillos, R.G., Kalaw, S.P. & Reyes, R.G. (2015). Proximate composition

- and antioxidant activity of *Panaeolus antillarum*, a wild coprophilous mushroom. *Current Research in Environmental & Applied Mycology*, 5(1), 52-59.
- Dulay, R. M. R., Flores, K. S., Tiniola, R. C., Marquez, D. H. H., Dela Cruz, A. G., Kalaw, S. P., & Reyes, R. G. (2015). Mycelial biomass production and antioxidant activity of *Lentinus tigrinus* and *Lentinus sajor-caju* in indigenous liquid culture. *Mycosphere*, 6(6), 634-642.
- Dulay, R. M. R., Ray, K., & Hou, C. T. (2015). Optimization of liquid culture conditions of Philippine wild edible mushroom as potential bioactive lipids. *Biocatalysis and Agricultural Biotechnology*, 4(3), 409-415.
- Dulay, R. M. R., Cardona, E. M., Kalaw, S. P., & Reyes, R. G. (2016). Optimization of liquid culture conditions of *Coprinus cinerea* for mycochemical composition and antioxidant properties. *Der Pharma Chemica*, 8(18), 313-319.
- Erbil, K. & Kalyoncu F. (2008). Mycelial Growth Rate of Some Morels (*Morchella spp.*) In Four Different Microbiological Media. *American Eurasian Journal Of Agricultural And Environmental Science*, 3(6), 861-864.
- Fernandes, A., Antonio, A. L., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, 135, 641-650.
- Ferreira, I.C.F.R., Baptista, P., Vilas-Boas, M., & Barros, L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. *Food Chemistry*, 100, 1511-1516.
- Food And Agricultural Organization (FAO) (2006). Corporate Document Repository. Wild Edible Fungi: A Global Overview of Their importance to people. www.fao.org/documents
- Friel, M. T., & McLoughlin, A. J. (2000). Production of a liquid inoculum spawn of *Agaricus bisporus*. *Biotechnology Letters*, 22, 351-354.
- Gapiński, M., Woźniak, W., Ziombra, M. & Murawska, J. (2007). Oyster mycelium on the liquid medium. *Acta Mycoogica*, 42(1), 125-128.
- García-Lafuente, A., Moro, C., Villares, A., Guillamón, E., Rostagno, M.A., D'arrigo, M. & Martínez, J.A. (2011). Mushrooms as a source of anti-inflammatory agents. *American Journal of Community Psychology*, 48(1-2), 125-141.
- Gregori, A., Svagelj, M. & Pohleven, J. (2007). Cultivation techniques and medicinal properties of *Pleurotus spp.* *Food Technology and Biotechnology*, 45, 238-249.

- Herraiz, T. & Galisteo, J. (2002). Tetrahydro- β -carboline alkaloids that occur in foods and biological systems act as radical scavengers and antioxidants in the ABTS assay. *Free Radical Research*, 36, 923-928.
- Huang, H.C. & Liu, Y.C. (2008). Enhancement of polysaccharide production by optimization of culture conditions in shake flask submerged cultivation of *Grifola umbellata*. *Journal of the Chinese Institute of Chemical Engineers*, 39, 307-311.
- Imaga, N.O.A. Adenekan, S.O. Fakanye, T.J. Olaniyan F.K. & Alakaloko, A.A. (2012). Saponin, preponderant phytochemical in *Carica papaya* leaf extract, possibly responsible for its reported antisickling and antioxidant activity. <https://papayaleaves.wordpress.com>.
- Imtiaj A, Jayasinghe C, Lee GW, & Lee TS. (2008). Comparative study of environmental and nutritional factors on mycelial growth of edible mushrooms. *Journal of Culture Collections*, 97-105.
- Jagadish, L.K., Krishnan, V.V., Shenbhagaraman, R. & Kaviyaran, V. (2009). Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) Imbach before and after boiling. *African Journal of Biotechnology*, 8(4), 654-661.
- Jedinak, A. & Sliva, D. (2008). *Pleurotus ostreatus* inhibits proliferation of human breast and colon cancer cells through p53-dependent as well as p53-independent pathway. *International Journal of Oncology*, 33, 1307-1313.
- Jiang, T., Luo, Z. & Ying, T. (2015). Fumigation with essential oils improves sensory quality and enhanced antioxidant ability of shiitake mushroom (*Lentinus edodes*). *Food Chemistry*, 172, 692-698.
- Jacob, J. K. S., Kalaw, S. P., Reyes, R.G. (2015). Mycelial growth performance of three species of *Pleurotus* on coconut water gelatin. *Current Research in Environmental & Applied Mycology*, 5(3), 263-268.
- Jonathan, S. G., Fasidi, I. O. & Ajayi E. J. (2004). Physico-Chemical studies on *Volvarella esculenta* (Mass) Singer, a Nigerian edible fungus. *Food chemistry*, 85(3), 339-342.
- Kalaw, S. P. & Albinto, R. F. (2014). Functional activities of Philippine wild strain of *Coprinus comatus* (O. F. Müll.: Fr.) Pers and *Pleurotus cystidiosus* O. K. Miller grown on rice straw based substrate formulation. *Mycosphere*, 5(5), 646-655.
- Karthika, K., & Murugesan, S. (2015). Cultivation and determination of nutritional value on edible mushroom *Pleurotus Ulmarius*. *International Journal of Emerging Research in Management & Technology*, 4(11), 29-30

- Karwa, A. & Rai, M. (2009). Mycoenhancing the potential of synthetic antibiotics using wild mushrooms: First report. Proc. 5th *International Medicinal Mushroom Conference*.
- Kashangura C. (2008). Optimisation of the growth conditions and genetic characterisation of *Pleurotus* species. Dissertation. Harare: Department of Biological Sciences, Faculty of Science, University of Zimbabwe.
- Khan, M.A., & Tania, M. (2012). Nutritional and medicinal importance of *Pleurotus* mushroom: An overview. *Food Reviews International*, 28(3), 313-329.
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008). Dictionary of the Fungi (10th ed.), CABI, Wallingford, UK.
- Kittakoop, P., Mihidol, C., & Ruchirawat, S. (2014). Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis and smoking cessation. *Current Topics in Medicinal Chemistry*, 14(2), 239-255.
- Klomklung, N., Karunarathna S.C, & Hyde, K. D. (2014) Optimal conditions of mycelial growth of three wild edible mushrooms from northern Thailand. *Acta Biologica Szegediensis*, 58, 39-43.
- Komaraiah P., Kishor P. B. K., Carlsson M., Magusson KE & Mandenius, C. F. (2005). Enhancement of anthraquinone accumulation in *Morinda citrifolia* suspension cultures. *Plant Science*, 168, 1334-1337.
- Kuforiji, O.O., & Fasidi, I.O. (2005). Factors affecting the yield of *Volvariella volvacea* in various agro-wastes. *Nigerian Journal for Microbiology*, 19, 550-555.
- Kurtzman, R.H., & Martinez-Carrera, D. (2003). Light, what it is and what it does for mycology. *Micol. Aplic. Internac.*, 25, 23-33.
- Larraya, L.M., Alfonso, M., Pisabarro, A.G. & Ramirez, L. (2003). Mapping of genomic regions (quantitative trait loci) controlling production and quality in industrial cultures of the edible basidiomycete *Pleurotus ostreatus*. *Applied and Environmental Microbiology*, 69(6), 3617-3625.
- Lee, U.Y., Jayasinghe, C., Intiaj, A., Hur, H., Lee, G.W., & Lee, T.S. (2008). Favorable culture conditions for mycelial growth of Korean wild strains in *Ganoderma lucidum*. *Mycobiology*, 36, 28-33.
- Li, L., Ng, T.B., Song, M., Yuan, F., Liu, Z.K., Wang, C.L., Jiang, Y., Fu M. & Liu F. (2007). A polysaccharide-peptide complex from abalone mushroom (*Pleurotus abalonus*) fruiting bodies increases activities and gene expression of antioxidant enzymes and reduces lipid peroxidation in senescence-accelerated mice. *Applied Microbiology and Biotechnology*, 75, 863-869.

- Lv, Y.L., Sun, L.H., Zhang, F.S., Zhao, Y. & Guo, S.X. 2010. The effect of cultivation conditions on the mycelia growth of a dark-septate endophytic isolate. *African Journal of Microbiology Research*, 4, 602-607.
- Mane, V.P., Patil, S.S., Syed, A.A., and Baig, M.M. (2007). Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. *Journal of Zhejiang University Science B*, 8, 745-51.
- Mao, L-C., Pan, X., Que, F., & Fang, X-H. (2006). Antioxidant properties of water and ethanol extracts from hot air-dried and freeze-dried daylily flowers. *European Food Research and Technology*, 222, 236-41.
- Mau, J. L., Lin, H. C. & Chen, C. C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agricultural and Food Chemistry*, 50(21), 6072-6077.
- Menaga, D. Mahalingam, P.U. Rajakumar, S. & Ayyasamy, P.M. (2012). Evaluation of phytochemical characteristics and antimicrobial activity of *Pleurotus florida* mushroom. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4), 4-8.
- Mohamed, M. E., & Farghaly, A. F. (2014). Bioactive compounds of fresh and dried *Pleurotus ostreatus* mushroom. *International Journal of Biotechnology For Wellness Industries*, 3, 4-14.
- Mugdha, H., Gauri, S.B., Kiran, R.R., Jitendra, G.V. & Subhash, S.D. (2010). In-vitro susceptibility testing of some *Phellinus* species against *Acinetobacter baumannii* from Maharashtra India. *Journal of Medicinal Plant Research*, 4, 1335-1338.
- Muruke, M.H. (2014). Evaluation of Antioxidant and Iron Chelating Activities of A Wild Edible Oyster Mushroom *Pleurotus cystidiosus* from Tanzania. *Food Science and Quality Management*, 29, 18-28.
- Myers, N., Mittermeier, R., Mittermeier, C., Da Fonseca, G. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858.
- Nakano, Y., Fuji, H., & Kojima, M. (2010). Identification of blue-light photoresponse genes in oyster mushroom mycelia. *Bioscience, Biotechnology and Biochemistry*, 74, 2160-2165.
- Nuran, C. Y., Semra, T., Numan, Y., & Olcay Kaplan, I. (2012). Antioxidant properties of wild edible mushroom *Pleurotus eryngii* collected from Tunceli province of Turkey. *Digest Journal Nanomaterials and Biostructures*, 7, 1647-1654.
- Ntougias, S., Baldrian, P., Ehaliotis, C., Nerud, F., Antoniou, T. & Merhautova, V. (2012). Biodegradation and detoxification of olive mill wastewater by selected

- strains of the mushroom genera *Ganoderma* and *Pleurotus*. *Chemosphere*, 88, 620-626.
- Orsine, V. C. J., Novaes, M.R., Cavalho, G., & Ramirez, A. E. (2012). Nutritional value of *Agaricus sylvaticus*: mushroom grown in Brazil. *Nutricion Hospitalaria*, 27, 449-455.
- Palacios, I., Lozano, M., Moro, C., Arrigo, D., Rostagno, M.A., Martinez, J.A., Garcia-Lafuente, A., Quillamon, E. & Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry*, 128, 674-678.
- Patel, Y., Naraian, R. & Singh, V. K. 2012. Medicinal properties of *Pleurotus* species (oyster mushroom): a review. *World Journal of Fungal and Plant Biology*, 3(1), 1-12.
- Pietta, P.G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*, 63, 1036-1042.
- Pokhrel, C.P. & Ohga, S. (2007). Submerged culture conditions for mycelial yield and polysaccharides production by *Lyophyllum decastes*. *Food Chemistry*, 105, 641-646.
- Puttaraju, N. G., Venkateshaiah, S. U., Dharmesh Urs, S. M. N., & Somasundaram, R. (2006). Antioxidant activity of indigenous edible mushrooms. *Journal of Agricultural Food Chemistry*, 54, 9764-9772.
- Qing, H.F. & Jian, J.Z. (2002). Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. *Process Biochemistry*, 37, 769-774.
- Reyes R, Grassel W, & Rau U. (2009). Coconut water as a novel culture medium for the biotechnological production of schizophyllan. *Journal of Nature Studies*, 7, 43-48.
- Royse, D. I., & Zaki, S. A. (1991). Yield stimulation of *Pleurotus* sp. by dual nutrient supplementation of pasteurized wheat straw. In: *Science and cultivation of edible fungi* (ed.) M.J. Maher. Balkema, Rotterdam, 545-547.
- Saha, A., Mandal, P., Dasgupta, S. & Saha, D. (2008). Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplotia theobromae* (Pat.) Griffon and Maubl. *Journal of Environmental Biology*, 29(3), 407-410.
- Soytong, K., & Asue, T. (2012) Study on physiological and cultural requirements of *Pleurotus giganteus*. *Journal of Agricultural Technology*, 10(4), 923-930.

- Selvakumar, P., Rajasekar, S., Periasamy K. & Raaman N. (2008). Isolation and characterization of melanin pigment from *Pleurotus cystidiosus* (telomorph of *Antromycopsis macrocarpa*). *World Journal of Microbiology and Biotechnolo*, 24, 2125-2131.
- Sharma, B. B. (2004). Effect of duration of light on radial growth of pink oyster mushroom. *Indian Phytopathology*, 57(2), 234.
- Shih, I.L., Chou, B.W., Chen, C.C., Wu, J.Y. & Hsieh, C. (2008). Study of mycelial growth and bioactive polysaccharide production in batch and fed-batch culture of *Grifola frondosa*. *Bioresource Technology*, 99, 785-793.
- Sribusarakum, A., Bunyapraphatsara, N., Vajragupta, O. & Watanabe, H. 2004. Antioxidant activity of *Linnophila aromatica* Merr. *Thai Journal of Phytopharmacy*, 11(2), 11-17.
- Stamets, P. (2000) *Growing Gourmet and Medicinal Mushrooms*. Ten Speed Press, Berkeley.
- Szabová, E., Rohařová, L. & Hedvigy, M. (2013). Semi-solid fermentation of *Pleurotus ostreatus*. *Journal of Microbiology and Biotechnology Food Sciences*, 2, 1950-8.
- Tang, Y.Z., Zhu, L.W., Li, H.M. & Li, D.S. (2007). Submerged culture of mushrooms in bioreactors challenges, current-state-of-the-art, and future. *Food Technology and Biotechnology*, 45, 221-229.
- Turkoglu, A., Duru, M.E, Mercan, N., Kivrak, I. & Gezer, K. (2007a). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chemistry*, 101, 267-273.
- Turkoglu, A., Duru, M.E. & Mercan, N. (2007b). Antioxidant and antimicrobial activity of *Russula delica* Fr: an edible mushroom. *Eurasian Journal of Analytical Chemistry*, 2(1), 54-67.
- Valdez, A. S. (2014). Optimization of culture conditions for mycelial growth of *Pleurotus djamour* (rumph. Ex fr.) Boedjin. Undergraduate thesis. Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.
- Vaz, A.J., Barros, L., Martins, A., Santos-Buelga, C., Vasconcelos, M. H. & Ferreira, I. C. F. R. (2011). Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. *Journal of Food Composition and Analysis*, 126, 610-616.
- Wandati, T. W., Kenj, G.M. & Onguso, J. M. (2013). Phytochemicals in edible wild mushrooms from selected areas in Kenya. *Journal of Food Research*, 2(3), 137.

- Wang, Q., Li, H., Chen, T. T., & Han, J. R. (2012). Yield, polysaccharides content and antioxidant properties of *Pleurotus abalonus* and *Pleurotus geesteranus* produced on asparagus straw as substrates. *Scientia Horticulturae*, 134, 222-226.
- Wei, Y. & Lee, H. (2013). Oxidative Stress, Mitochondrial DNA Mutation, and Impairment of Antioxidant Enzymes in Aging. *Experimental Biology and Medicine*, 227, 671-682.
- Wu, G. H., Hu, T., Li, Z.Y., Huang, Z. L., & Jiang J. G. (2014). In vitro antioxidant activities of the polysaccharides from *Pleurotus tuber-regium* (Fr.) Sing. *Food Chemistry*, 148, 351-356.
- Wu, S. & Ng, L. 2008. Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) in Taiwan. *Food Science Technology*, 41, 323-330.
- Yang, F. C. & C. B. Liau. (1998). The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. *Process Biochemistry*, 33, 547-553.
- Yang, F.C., Huang, H.C. & Yang, M.J. (2003). The influence of environmental conditions on the mycelial growth of *Antrodia cinnamomea* in submerged cultures. *Enzyme and Microbial Technology*, 33, 395-402.
- Yildirim, A., Mavi, A., Oktay, M., Kara, A.A., Algur, O., & Bilaloglu, V. (2000). Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argen tea* Desf ex DC), sage (*Salvia triloba* l.), and black tea (*Camellia sinensis*) extracts. *Journal and Agricultural Food and Chemistry*, 48, 5030-5034.
- Yim, H. S., Chye, F. Y., Koo, S.M., Matanjun, P., How, S. E., & Ho, C. W. (2012). Optimization of extraction time and temperature for antioxidant activity of edible wild mushroom, *Pleurotus porrigens*. *Food Bioprocesses*, 90, 235-242.
- Zervakis, G., Dimou, D. & Balis, C. (2004). First record of the natural occurrence in Europe of the basidiomycete *Pleurotus cystidiosus* on a new host. *Mycological Research*, 96, 874-876.
- Zhang, J., Ma, Z., Zheng, L., Zhai, G., Wang, L., Jia, M., & Jia, L. (2014). Purification and antioxidant activities of intracellular zinc polysaccharides from *Pleurotus cornucopiae* SS-03. *Carbohydrate Polymers*, 111, 947-954.
- Zhao, H., Huang, L., Xiao, C. L., Liu, J., Wei, J. & Gao, X. (2010). Influence of culture media and environmental factors on mycelial growth and conidial production of *Diplocarpon mali*. *The Society for Applied Microbiology, Letters in Applied Microbiology*, 50, 639-644.