

**EVALUATION OF ANGIOSUPPRESSIVE ACTIVITY OF *Pleurotus florida*  
ON DEVELOPING CHICK EMBRYO USING CHORIOALLANTOIC  
MEMBRANE ASSAY**

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An Undergraduate Thesis Submitted to the Faculty of the Department of Biological  
Sciences, College of Arts and Sciences, Central Luzon State University,  
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for the Degree

**BACHELOR OF SCIENCE IN BIOLOGY  
(Major in Zoology)**

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## ACCEPTANCE SHEET

This undergraduate thesis entitled "EVALUATION OF ANGIOSUPPRESSIVE ACTIVITY OF *Pleurotus florida* ON DEVELOPING CHICK EMBRYO USING CHORIOALLANTOIC MEMBRANE ASSAY" prepared and submitted by **RODENE C. ZACARIAS**, in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOLOGY (ZOOLOGY)**, is hereby accepted.

  
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## BIOGRAPHICAL SKETCH

The author of this paper named Rodene Cabantac Zacarias, was a son of Mr. Rogelio Sison Zacarias Jr. and Mrs. Eden Cabantac Zacarias who hails at Purok 2, Brgy. Tomana East, Rosales, Pangasinan. He was 20-year-old student who celebrates his birthday every 1<sup>st</sup> day of September. His first educational milestone was at their barangay day care center where he spent his toddler years and began his journey as Kindergarten pupil at Rosales Christian Learning Center (RCLC), Rosales, Pangasinan wherein he graduated as an Outstanding Pupil. Eventually his parents sent him to IFI – Christian Home and Institute of Learning and Development (CHILD), Rosales Pangasinan for his elementary years and achieved the third highest honor during his graduation. He then continued his secondary years at Rosales National High School. By his time, he was honored to be one of the students belonging to the first batch of Special Science Class. During their commencement exercises, he was grateful because he reached his goal, to become one of those person standing and speaking in front of many people and proudly says he is their class Salutatorian. Before entering college, he was able to finish the TESDA Accredited Computer Literacy Program of AIE Colleges at Urdaneta City, Pangasinan. Now, he is currently finishing his baccalaureate degree in Biology major in Zoology at Central Luzon State University, Science City of Muñoz, Nueva Ecija.

During his college years he had been part of the following seminars and training: Symposiums of CLSU-Department of Biological Sciences with the themes “Current Trends in Food Safety and Quality Assurance”, HIV: AIDS “Survival of the Fittest. The Human Culture Media”, “Philippine Biodiversity and National Museum”, “Role of Biologists in the Environmental Impact Assessment and Management Projects”, “Bio-

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His main goal as of today is to finish his college degree and eventually further expand his knowledge in Medicine for him to become a successful medical doctor. He want to become a cardiologist because his family in both sides has a history of heart diseases. In order for this dream to be a successful one, he must work and study well. Because he know that education is the key to all success in life and believes that this is only the wealth that cannot be stolen by anyone. Lastly, he will be an obedient son of his parents because they are the one who sacrifices and who supports him all through his journey in schooling.

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## ABSTRACT

**ZACARIAS, RODENE C.**, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2018, EVALUATION OF ANGIOSUPPRESSIVE ACTIVITY OF *Pleurotus florida* ON DEVELOPING CHICK EMBRYO USING CHORIOALLANTOIC MEMBRANE ASSAY**

Adviser: ANGELES M. DE LON, Ph.D.

Angiogenesis is the proliferation of new blood vessels that promote normal embryonic development and numerous pathologies like tumor growth and cancer metastasis. The assays were used for both pro and anti-angiogenic agents. This study evaluated the angio-suppressive activity of *Pleurotus florida* on the developing chick embryo using chorioallantoic membrane (CAM) assay and used destructive sampling into four different treatment concentrations and a control. The result revealed that there is a decrease on the number of blood vessels formed after 24 hours, 48 hours, and 72 hours at a higher treatment concentrations. Statistical analysis showed that the 24 hours and 48 hours of exposure to various concentration have no significant difference. Nevertheless, after 72 hours of exposure, the mean blood vessels formed showed a significant difference among the treatment means between the highest concentration of *P. florida* (1%) and the control treatment (sterile water). In terms of the percentage angio-suppressive activity of *P. florida* with the greatest angiogenic effect which was the 1% *P. florida* treatment concentration possesses 35.63% and 38.65% angio-suppressive rate after 48 hours and 72 hours of exposure respectively. Thus, the study showed that *Pleurotus florida* lyophilized hot water extract exhibits angio-suppressive activity on the developing chick embryo using chorioallantoic membrane assay and is concentration and time exposure dependent.

## LITERATURE CITED

- Adams, R.H., & Alitalo, K. (2007). Molecular regulation of angiogenesis and lymphangiogenesis. *National Review Molecular Cell Biology*, 8, 464–478.
- Ahmed, S.A., Kadam, J.A., Mane, V.P., Patil, S.S., & Baig, M.M.V. (2008). Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) Singer cultivated on different agro-wastes. *Nature and Science*, 7(1), 1545-0740.
- Ajith, T.A. & Janardhanan, K.K. (2007). Indian medicinal mushroom as a source of anti-oxidant and antitumor agents. *Journal for Clinical Biochemistry Nutraceutical*, 40(3), 157-162.
- Alam, N., Amin, R., Khan, A., Aral, I., Shim, M.J., Lee, M.W., & Lee, T.S. (2008). Nutritional analysis of cultivated mushrooms in Bangladesh - *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. *Mycobiology*, 36(4), 228-232.
- Auerbach, R., Akhtar, N., Lewis, R.L., & Shinnars, B.L. (2000). Angiogenesis assays, problems and pitfalls. *Cancer Metastasis Review*, 19, 167–172.
- Auerbach, R., Auerbach, W., & Polakowski, I. (1991). Assays for angiogenesis, A review. *Pharmacological Therapy*, 51, 1–11.
- Auerbach, R., Lewis, R., Shinnars, B., Kubai, L., & Akhtar, N. (2003). Angiogenesis assays, A critical overview. *Clinical Chemistry*, 49(1), 32–40.
- Badalyan, S.M. (2014). Potential of mushroom bioactive molecules to develop healthcare biotech products. *International Conference on Mushroom Biology and Mushroom Products*, 373-738.
- Bankole, P. O. & Adekunle, A. A. (2012). Studies on biodiversity of some mushrooms collected in Lagos State, Nigeria using biotechnological methods. *Journal of Yeast and Fungal Research*, 3(4), 37 – 48.
- Bellairs, R. & Osmond, M. (2005). *The Atlas of chick development*, 2nd Edition, Elsevier Academic Press, Oxford.
- Bobek, P. (1989). Effect of mushroom *Pleurotus ostreatus* and isolated fungal polysaccharide on serum and liver lipids in Syrian hamsters with hyper lipidemia. *Nutrition*, 7, 105-108.
- Bobek, P., Nosálová, V., & Cerná, S. (2001). Effect of pleuran (beta-glucan from *Pleurotus ostreatus*) in diet or drinking fluid on colitis in rats. *Nahrung*, 45, 360-363.

- Brooks, P.C., Montgomery, A.M. & Cheresch, D.A. (1999). Use of the 10-day-old chick embryo model for studying angiogenesis. *Methods of Molecular Biology*, 129, 257–69.
- Carmeliet, P. (2003). Angiogenesis in health and disease. *Natural Medicine*, 9, 653–660.
- Carmeliet, P., & Jain, R.K. (2011). Molecular mechanisms and clinical applications of angiogenesis. *Nature*, 473, 298–307.
- Chandana, P., Tina, R., & Nirmalendu, D. (2017). Potentiality of Oyster Mushroom (*Pleurotus* Spp.) in Medicine- A Review. *Science Medicine Central*, 1-8.
- Chang, S.T. (1991). Recent trends in world production of cultivated edible mushrooms. *Mushroom Journal*, 503, 15-18.
- Chen, Z., Wen, Z. & Bai, X. (2013). *In vivo* chick chorioallantoic membrane (CAM) angiogenesis assays. *Bio-protocol*, 3(18), 1-5.
- Damjanović, A., Zdunić, G., Šavikin, K., Mandić, B., Jadranin, M., Matić, I.Z., & Stanojković, T.P. (2016). Evaluation of the anti-cancer potential of *Mahonia aquifolium* extracts via apoptosis and anti-angiogenesis. *Bangladesh Journal of Pharmacology*, 11, 741-749.
- Davey, M.G. and Tickle, C. (2007). The chicken as a model for embryonic development. *Cytogenetic and Genome Research*, 117, 231–239.
- De Castro, M.E.G. & Dulay, R.M.R. (2015). Toxic and teratogenic effects of *Lentinus sajor-caju* and *Pleurotus ostreatus* ethanolic extracts in *Danio rerio* embryo model. *International Journal of Biology, Pharmacy and Allied Sciences*, 4(4), 2261-2269.
- Deryugina, E.I. & Quigley, J.P. (2008). CHAPTER TWO, chick embryo chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. *Methods Enzymol*, 444, 21–41.
- Djajanegara, I. & Harsoyo. (2008). White oyster mushroom (*Pleurotus florida*) mutant With Altered Antioxidant Contents. *Biotropia*, 15(1), 65-73.
- Djarajah, N. M. & Djarajah, A. B. (2001). Budidaya jamur tiram. *Penerbit Kanisius, Yogyakarta*. 15(1), 77-82
- Dubost, N.J., Ou, B., & Beelman, R.B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry*, 105, 727–735.

- Eilken, H.M., & Adams, R.H. (2010). Dynamics of endothelial cell behavior in sprouting angiogenesis. *Curriculum Opinion Cell Biology*, 22, 617–625.
- Elaiya Raja. R., & Ganesh, P. (2013). Studies on cultivation of oyster mushroom (*Pleurotus florida*) on using different substrates. *Indian Streams Research Journal*, 3(3), 1-4.
- Elmastas, M., Isildak, O., Turkekul, I., & Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms, *Journal of Food Composition and Analysis*, 20, 337–345.
- Folkman, J. (2007). Angiogenesis, an organizing principle for drug discovery. *National Review Drug Discovery*, 6, 273–286.
- Fombang, E.N., Lobe, E.E. & Mbofung, C.M.F. (2016). *Pleurotus florida* aqueous extracts and powder influence lipid profile and suppress weight gain in rats fed high cholesterol diet. *Journal of Nutrition & Food Science*, 6, 473.
- Ganeshpurkar, A., Pardhi, P., & Bhadoriya, S.S. (2015). Antioxidant potential of white oysterculinary-medicinal mushroom, *Pleurotus florida* (higher basidiomycetes). *International Journal of Medical Mushrooms*, 17(5), 91–498.
- Hamburger, V. & Hamilton, H.L. (1992). A series of normal stages in the development of the chick embryo. *Journal of Experimental Zoology*, 108, 49-92.
- Im, K.H., Nguyen, T.K., Shin, D.O.B., Lee, K.R. & Lee, T.S. (2014). Appraisal of antioxidant and anti-inflammatory activities of various extracts from the fruiting bodies of *Pleurotus florida*. *Molecules*, 19, 3310-3326.
- Ivanova, T.S., Krupodorova, T.A., Barshteyn, V.Y., Artamonova, A.B., & Shlyakhovenko, V.A. (2014). Anticancer substance of mushroom origin. *Experimental Oncology*, 36(2), 58-66.
- Jayakumar, T., Ramesh, E., & Geraldine, P. (2007). Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats. *Experimental Gerontology*, 42, 183-191.
- Jayakumar, T., Ramesh, E., & Geraldine, P. (2006). Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl<sub>4</sub>-induced liver injury in rats. *Food and Chemical Toxicology*, 44, 1989-1996.
- Jose, N., & Janardhanan, K.K. (2000). Antioxidant and antitumor activity of *Pleurotus florida*. *Curriculum Science*, 79, 941-943.

- Jose, N., Ajith, T.A. & Janardhanan, K.K. (2004). Methanol extract of the oyster mushroom, *Pleurotus florida*, inhibits inflammation and platelet aggregation. *Phytotherapy Research*, 18, 43–46.
- Jung, H.J., Jeo, H.J., Lim, U.J., Ahn, E.K., Song, Y.S., Lee S., Shin, K.H., Lim, C.J., & Park, E.H. (2007). Anti-angiogenic activity of the methanol extract and its fractions of *Ulmus davidiana* var. *japonica*. *Journal of Ethnopharmacology*, 112(2), 406–409.
- Khan, A. & Tania, M. (2012). Nutritional and medicinal importance of *Pleurotus* mushrooms, An overview. *Food Reviews International*, 28, 313-329.
- Khan, M.A., Rahman, M., Mousumi, T., Uddin, M.N. & Ahmed, S. (2011). *Pleurotus sajor-caju* and *Pleurotus florida* mushrooms improve some extent of the antioxidant systems in the liver of hypercholesterolemic rats. *Journal of Nutrition*, 4, 20-24.
- Khatun, S., Islam, A., Cakilcioglu U., Guler P. & Chatterjee, N.C. (2015). Nutritional qualities and antioxidant activity of three edible oyster mushrooms (*Pleurotus* spp.). *Wageningen Journal of Life Sciences*, 72(73), 1–5.
- Knighton, D. R., Fiegel, V. D. & Phillips, G. D. (1991). The assay of angiogenesis, *Program for Clinical Biology Research*, 365, 291–299.
- Kim, M.Y., Seguin, P., Ahn, J.K., Kim, J.J., Chun, S.C., Kim, E.H., Seo, S.H., Kang, E.Y., Kim, S.L., Park, Y.J., Ro, H.M., & Chung, I.M. (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *Journal of Agriculture and Food Chemistry*, 56, 7265–7270.
- Kim, S.H., Song, Y.S., Kim, S.S., Kim, B.C., Lim, C.J., & Park, E.H. (2004). Anti-inflammatory and related pharmacological activities of the n-BuOH subfraction of mushroom *Phellinus linteus*. *Journal of Ethnopharmacology*, 93(1), 141-146.
- Kurz, H. (2000). Physiology of angiogenesis. *Journal of Neuro-Oncology*, 50, 17–35
- Laksmi, B., Tilak J.C., Adhikari, S., Devasagayam, T.P.A., & Janardhanan, K.K. (2004). Evaluation of antioxidant activity of selected indian mushroom. *Pharmaceutical Biology*, 42, 179-185.
- Larger, E., Marre, M., Corvol, P. & Gasc, J.M. (2004). Hyperglycemia-induced defects in angiogenesis in the chicken chorioallantoic membrane model. *American Diabetes Association*, 53, 752–761.
- Lee, Y.L., Huang, G.W., Liang, Z.C., & Mau, J.L. (2006). Antioxidant properties of three extract from *Pleurotus citrinopileatus*, *LWT-Food Science and Technology*, 40, 823–833.

- Lee, J.S., Park, B.C., Ko, Y.J., Choi, H.G., Yong, C.S., Lee, J.S., & Kim, J.A. (2008). *Grifola frondosa* (maitake mushroom) water extract inhibits vascular endothelial growth factor-induced angiogenesis through inhibition of reactive oxygen species and extracellular signal-regulated kinase phosphorylation. *Journal of Medical Food*, 11(4), 203-210.
- Lee, Y.S., Kim, Y.H., Shin, E.K., Kim, D.H., Lim, S.S., Lee, J.Y., & Kim, J.K. (2010). Anti-angiogenic activity of methanol extract of *Phellinus linteus* and its fractions. *Journal of Ethnopharmacology*, 112(2), 415-421.
- 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.
- 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., Lv, P.Y., Zhao, R., Shi, B., Wang, Y.P., Wang, X.Y., & Liu, F. (2012). Isolation and identification of a novel polysaccharide-peptide complex with antioxidant, anti-proliferative and hypoglycaemic activities from the abalone mushroom. *Bioscience Report*, 32, 221-28.
- Lin, S., Ching, L.T., Chen, J., & Cheung, P.C.K. (2015). Antioxidant and anti-angiogenic effects of mushroom phenolics-rich fractions. *Journal of Functional Foods*, 17, 802-815.
- Liu, Y.T., Sun, J., Luo, Z.Y., Rao, S.Q., Su, Y.J., Xu, R.R., & Yang, Y.J. (2012). Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity. *Food and Chemical Toxicology*, 50, 1238-1244.
- Lokman, N.A., Elder, A.S.F., Ricciardelli, C. & Oehler, M.K. (2012). Chick chorioallantoic membrane (CAM) assay as an *in vivo* model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. *International Journal of Molecular Science*, 13, 9959-9970.
- Madan, A. (2003). Angiogenesis and anti-angiogenesis in the neonate, relevance to retinopathy of prematurity. *NeoReviews*, 4(12), 1-10.
- Manimuthu, M. & Rajendran, S. (2015). Can oyster mushroom be cultivated on sea grass. *International Journal of Advanced Research*, 3(3), 962-966.
- Mansouri, K., Mahamed-Khosroushahi, L., & Rasouli, H. (2014). Antiangiogenic/inflammatory behavior of mushroom *Ganoderma lucidum* extract could be effective for treatment of corneal neovascularization: a hypothesis. *Journal of Reports in Pharmaceutical Sciences*, 3(1), 14-18.

- Manzi PA, Agguzzi A, & Pizzoferrato, L. (1999). Nutritional mushrooms widely consumed in Italy. *Food Chemistry*, 73, 321-325.
- Mccain, E.R., & Mclaughlin, J.S. (1999). Chapter 4, developmental and physiological aspects of the chicken embryonic heart. Association for biology laboratory education. Retrieved from <http://www.zoo.utoronto.ca/able>.
- 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), 102-106.
- Narayanasamy, P., Suganthavel, P., Sabari, P., Divya, D., Vanchinathan, J. & Kumar, M. (2008). Cultivation of mushroom (*Pleurotus florida*) by using two different agricultural wastes in laboratory condition. *The Internet Journal of Microbiology*, 7(2), 1-4.
- Nkembo, A.T., Ntantie, E., Salako, O.O., Amissah, F., Poku, A.F., Latinwo, L.M., & Lamango, N.S. (2016). The antiangiogenic effects of polyisoprenylated cysteinyl amide inhibitors in HUVEC, chick embryo and zebrafish is dependent on the polyisoprenyl moiety. *Oncotarget Journal*, 7(42), 68194-68205
- Nowak-Sliwinska, P., Segura, T., & Iruela-Arispe, M.L. (2014). The chicken chorioallantoic membrane model in biology. *Medicine and Bioengineering, Angiogenesis*, 17(4), 779–804.
- Odendo, M., Kirigua, V., Kimenju, J. W., Wasilwa, L., Musieba, F. & Orina, M. (2010). Analysis of mushroom value chain in Kenya. Retrieved from <http://www.primopdf.com>.
- Oklu, R., Walker, T.G., Wicky, S. Hesketh, R. (2010). Angiogenesis and current antiangiogenic strategies for the treatment of cancer. *Journal of Vascular Intervention Radiology*. 21(12), 1791–1805.
- Onuoha, C. I., Oyibo, G. & Judith, E. (2009). Cultivation of straw mushroom (*Volvariella volvacea*) using some agro-waste material. *Journal of American Science*, 5(5), 135 - 138.
- Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M.A., Martinez, J.A., Garcia-Lafuente, A., Guillamon, E., & Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry*, 128, 674–678.
- Panjwani, R., Saraf, A., & Pandey, A.K. (2015). Antioxidative property of pleurotus florida: an edible oyster mushroom in m.p. *World Journal Of Pharmacy And Pharmaceutical Sciences*, 5(1), 1383-1392.

- Parsons-Wingerter, P., Lwai, B., Yang, M.C., Elliott, K.E., Milaninia, A., Redlitz, A., Clark J.I. & Sage, E.H. (1998). A novel assay of angiogenesis in the quail chorioallantoic membrane, stimulation by bFGF and inhibition by angiostatin according to fractal dimension and grid intersection. *Microvascular Research*, 55, 201–214.
- Phang, L.K., & Gerhardt, H. (2009). Angiogenesis, a team effort coordinated by notch. *Developmental Cell*. 16, 196–208.
- Potente, M., Gerhardt, H., & Carmeliet, P. (2011). Basic and therapeutic aspects of angiogenesis. *Cell*. 146, 873-887.
- Qi-zhen, C.A.O. & Zhi-bin, L.I.N. (2004). Antitumor and anti-angiogenic activity of *Ganoderma lucidum* polysaccharides peptide. *Acta Pharmacologica Sinica*, 25(6), 833-838.
- Ramkumar, L., Ramanathan, T., & Thirunavukkarasu, P. (2010). Antioxidant and radical scavenging activity of nine edible mushrooms extract. *International Journal of Pharmacy*. 6, 950-953.
- Ramos, I.R. (2015). Health and nutritional properties of mushrooms. *Technological Centre for Research on Mushroom*. 1-63.
- Reis, F.S., Martins, A., Barros, L., & Ferreira, I.C.F.R. (2012). Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: a comparative study between in vivo and in vitro samples. *Food and Chemical Toxicology*, 50, 1201–1207.
- Reyes, R.G., Kalaw, S.P., Dulay, R.M.R., Yoshimoto, H, Miyazawa, N., Seyama, T. & Eguchi, F. (2013). Philippine native and exotic species of edible mushrooms grown on rice-straw-based formulation exhibit nutraceutical properties. *Philippine Agricultural Scientist*, 96(2), 198-204.
- Ribatti, D. (2012). Chicken chorioallantoic membrane angiogenesis model. *Methods in Molecular Biology*, 843, 47-57.
- Ribatti, D., Vacca, A., Roncali, L., & Dammacco, F. (1996). The chick embryo chorioallantoic membrane as a model for *in vivo* research on angiogenesis. *International Journal of Developmental Biology*, 40, 1189-1197.
- Satiroglu-Tufan, N. L. (2005). Amelioration of ethanol induced growth retardation by all-trans-retinoic acid and alphatocopherol in shell-less culture of the chick embryo. *Reproductive Toxicology*. 18, 407-412.

- Sanchez, C. (2017). Chapter 2: Bioactives from mushroom and their application. *Food Bioactives*, 1-36.
- Song, Y.S., Kim, S.H., Sa, J.H., Jin, C., Lim, C.J., & Park, E.H. (2009). Anti-angiogenic, antioxidant and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus*. *Journal of Ethnopharmacology*, 112(2), 410-414.
- Soucy, N.V., Ihnat, M.A., Kamat, C.D., Hess, L., Post, M.J., Klei, L.R., Clark, C., & Barchowsky, A. (2003). Arsenic stimulates angiogenesis and tumorigenesis *in vivo*. *Toxicological Sciences*, 76, 271-279.
- Spanel-Borowski, K., Schnapper, U. & Heymer, B. (1988). The chick chorioallantoic membrane assay in the assessment of angiogenic factors. *Biomedicine Resources*, 9, 253-260.
- Swift, M.R., & Weinstein, B.M (2009). Arterial-venous specification during development. *Circulatory Research*, 104, 576-588.
- Thillaimaharani, K.A., Sharmila, K., Thangaraju, P., Karthick, M. & Kalaiselvam, M. (2013). Studies on antimicrobial and antioxidant properties of oyster mushroom *Pleurotus florida*. *International Journal of Pharmacological Science Research*, 4, 1540-1545.
- Tosoc, J.S.P., Frediles, V.C.P., Canda, C., & Demayo, C.G. (2016). Antiangiogenic, antitoxic and antioxidant properties of methanolic extracts of *Caladium bicolor* (Aiton) Venten. *International Journal of the Bioflux Society*, 8(1), 10-16.
- Tufan, A.D. & Satiroglu-Tufan, N.L. (2005). The chick embryo chorioallantoic membrane as a model system for the study of tumor angiogenesis, invasion and development of anti-angiogenic agents. *Current Cancer Drug Targets*, 5(4), 249-267.
- Van, D.H., Onguene, N.A. & Kuyper, T.W. (2003). Knowledge and utilization of edible mushrooms by local populations of the rain forest of south Cameroon. *American Biology*, 32, 19-23.
- Wang, J.C., Hu, S.H., Liang, Z.C., & Yeh, C.J. (2005). Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect. *Application of Microbiology and Biotechnology*, 67, 759-66.
- Wasser, S.P. (2002), Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, 60, 258-274.

- Wasser, S.P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Applied Microbiology and Biotechnology*, 89: 1323–1332.
- Wong, J.Y., & Chye, F.Y. (2009). Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis*, 22, 269-277.
- Yang, C.M., Zhou, Y.J., Wang, R.J., & Hu, M.L. (2009). Anti-angiogenic effects and mechanisms of polysaccharides from *Antrodia cinnamomea* with different molecular weights. *Journal of Ethnopharmacology*, 123, 407–412.
- Yao, L.H., Jiang, Y.M., Shi, J., Tomas-baeberan, F.A., Datta, N., & Singanusong, R. (2004). Flavonoids in food and their health benefits. *Plant Food Human Nutrition*, 59, 113–122.
- Yongabi, K., Agho M. & Carrera, D. (2004). Ethnomycological study of wild mushrooms in Cameroon, Central Africa. *Journal title*, 16, 34-36.
- Yoo, S.Y. & Kwon, S. (2013). Angiogenesis and its therapeutic opportunities. *Hindawi Publishing Corporation Mediators of Inflammation*, 127170, 1-12.
- Yu, S., Weaver, V., Martin, K. & Cantorna, M.T. (2009). The effects of whole mushrooms during inflammation. *Immunology*, 10, 12.
- Zetter, B.R. (1998). Angiogenesis and tumor matastasis. *Annual Review of Medicine*, 49, 407-420.
- Zhuang, C., Mizuno, T., Shimada, A., Ito, H., Suzuki, C., Mayuzumi, Y., Okamoto, H., Ma, Y., & Li, J. (1993). Antitumor protein-containing polysaccharides from a Chinese mushroom Fengweigu or Houbitake, *Pleurotus sajor-caju* (Fr.) sings. *Biological Science Biotechnology Biochemistry*, 57, 901-06.