

**TERATOGENIC AND CYTOTOXIC ACTIVITY OF *Fomitopsis feei*  
COLLECTED FROM PARACELIS, MOUNTAIN PROVINCE**

**AGANA REYN DULAY**

An Undergraduate Thesis Submitted to the Faculty of the Department of Biological  
Sciences, College of Arts and Sciences, Central Luzon State University,  
Science City of Munoz, Nueva Ecija, Philippines  
in Partial Fulfillment of the Requirements  
for the Degree of

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

**JUNE 2019**

## TABLE OF CONTENTS

	PAGE
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDICES	x
LIST OF APPENDIX TABLES	xi
LIST OF APPENDIX FIGURES	xii
ABSTRACT	xiii
INTRODUCTION	1
Background of the Study	1
Objectives of the Study	3
Significance of the Study	4
Scope and Limitation of the Study	4
Time and Place of the Study	5
REVIEW OF RELATED LITERATURE	6
Paracelis, Mountain Province	6
Mushroom	7
<i>Fomitopsis feei</i>	8
Medicinal Properties of Mushroom	9
Teratogenicity	10
Zebrafish as Model for Teratogenicity	11
Cytotoxicity	14
Brine Shrimp as Model for Cytotoxicity	14
MATERIALS AND METHODS	16
Source of Mushroom	16
Sub-study I. Teratogenicity	16
Preparation of Tank	16
Zebrafish Care, Culture, and Maintenance	16
Zebrafish Spawning and Egg Production	16
Harvesting of Fertilized Eggs	17
Preparation of Extract	17
Treatment Preparation	17

<i>Zebrafish (Danio rerio) Teratogenicity and Toxicity Assay</i>	18
Sub-study II. Cytotoxicity	19
Source of Brine Shrimp ( <i>Artemia salina</i> ) Eggs	19
Hatching Set-up	19
Hatching of Brine Shrimp Eggs	20
Dilution of Extract	20
Brine Shrimp Lethality Assay	21
Data Gathered	21
Statistical Analysis	22
RESULTS AND DISCUSSION	23
Sub-study I. Teratogenicity	23
Percentage Mortality	22
Percentage Hachability	25
Heartbeat Rate	26
Teratogenic Activity of <i>Fomitopsis feei</i>	27
Morphological Endpoints of Treated <i>D. rerio</i> Embryos	27
Growth Retardation	27
Other Morphological Abnormalities	28
Sub-study II. Cytotoxicity	31
SUMMARY, CONCLUSION, AND RECOMMENDATION	33
Summary	33
Conclusion	34
Recommendation	34
LITERATURE CITED	35
APPENDICES	42

## LIST OF TABLES

TABLE		PAGE
1	Stages of embryonic development of the <i>D. rerio</i> at 26±1°C (Kimmel <i>et al.</i> , 1995)	13
2	Treatment assignments of the different concentrations in assessment of teratogenicity of <i>F. feei</i> ethanol extract.	18
3	Different toxicological endpoints of zebrafish (Schulte & Nagel, 1994).	19
4	Treatment assignments of the different concentrations in assessment of cytotoxicity of <i>F. feei</i> ethanol extract.	20
5	Mean percentage mortality of <i>D. rerio</i> embryos after 36, and 48 hours of exposure to different concentrations of <i>F. feei</i> ethanol extract.	23
6	Hatchability of embryos treated with different concentrations of <i>F. feei</i> ethanol extract.	25
7	Heartbeat of <i>D. rerio</i> after 36 hours of exposure to various concentrations of <i>F. feei</i> ethanol extract.	26
8	Lethal and teratogenic effects of various concentrations of <i>F. feei</i> extract at 12, 24, 36, and 48 hours of exposure	30
9	Mean percentage mortality of <i>A. salina</i> nauplii after 24 hours of exposure to different concentrations of <i>F. feei</i> ethanol extract.	31

## LIST OF FIGURES

FIGURE		PAGE
1	<i>Fomitopsis feei</i>	8
2	Lethal and teratogenic effects of various concentrations of <i>F. feei</i> ethanol extract: (A) dead embryo (D) and curved body (cb) at 48hpta in 750 ppm; (B) yolk deformity (Yd), no gut (Ng), little pigmentation (Lp) and bent tail (bt) at 48 hpta in 750 ppm; (C) scoliosis (Sc) at 48 hpta in 750 ppm; (D) pericardial edema (Pe) and little pigmentation (Lp) at 48 hpta in 500 ppm.	28
3	Morphological development of embryos exposed to different concentrations of <i>F. feei</i> ethanol extract.	29
4	Point estimate of LC50 value of <i>F. feei</i> ethanol extract after 24 hours of exposure.	32

## LIST OF APPENDICES

APPENDIX		PAGE
A	Analysis of Variance and Probit Analysis	43
B	Treatment Preparation	45
C	Experimental Procedure	46

## LIST OF APPENDIX TABLES

APPENDIX TABLE		PAGE
1	Analysis of variance of percentage mortality of zebrafish embryo treated with <i>Fomitopsis feei</i> ethanol extract after 36 hours of exposure.	43
2	Analysis of variance of percentage mortality of zebrafish embryo treated with <i>Fomitopsis feei</i> ethanol extract after 48 hours of exposure.	43
3	Analysis of variance of percentage hatchability of zebrafish embryo treated with <i>Fomitopsis feei</i> ethanol extract after 48 hours of exposure.	43
4	Analysis of variance of percentage heartbeat of zebrafish embryo treated with <i>Fomitopsis feei</i> ethanol extract after 36 hours of exposure.	43
5	Analysis of variance of percentage mortality of brine shrimp larva treated with <i>F. feei</i> ethanol extract after 24 hours of exposure.	44
6	Probit analysis for the median lethal concentration (LC50).	44
7	Dilution of Ethanol Extract of <i>Fomitopsis feei</i>	45

## LIST OF APPENDIX FIGURES

APPENDIX FIGURE		PAGE
1	Zebrafish acclimatization	46
2	Zebrafish spawning	46
3	Transferring of zebrafish embryos into vials.	47
4	Microscopic examination of zebrafish embryos.	47
5	Hatching set-up for brine shrimp lethality assay	48
6	Observation of dead brine shrimp larvae using magnifying lens.	48

## ABSTRACT

**DULAY, AGANA REYN**, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija Philippines, **MAY 2019, TERATOGENIC AND CYTOTOXIC ACTIVITY OF *Fomitopsis feei* COLLECTED FROM PARACELIS, MOUNTAIN PROVINCE**

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

Mushrooms are being utilized by indigenous people and Paracelis, Mountain Province is one of the perfect place to search for mushrooms utilized by them. Several studies have been conducted on various macrofungi, however little is known about the *Fomitopsis feei* collected from Paracelis, Mountain Province. This study determined the teratogenic and cytotoxic activity of the ethanol extract of *F. feei* against zebrafish (*Danio rerio*) embryos and brine shrimp (*Artemia salina*), respectively. The mushroom exhibited teratogenic effects against the developing *D. rerio* embryos wherein growth retardation, malformation of tail, yolk deformity, pericardial edema, curved body, scoliosis and little pigmentation were the notable teratogenic effects of the ethanol extract to the developing embryos. Embryos treated with  $\geq 1000$  ppm recorded high mortality rate. Hatchability was most evident at lower concentrations  $\leq 750$  ppm. In terms of heartbeat, as the concentration of the extract increased, the heartbeat rate significantly decreased. For the cytotoxicity, 1250 ppm has the highest mortality rate with 73.33%. Using probit analysis, the  $LC_{50}$  of *F. feei* is 534.676 ppm which is considered as mildly toxic. Thus, *F. feei* in higher concentrations exhibit toxic effect.

## LITERATURE CITED

- Aldahi, A., Khalid, H., Alhassan, M., Ali, A., Babiker, S., Albdeen, E., & Kabashi, A. (2015). Antioxidant and cytotoxic activity of *Cordia africana* Sudan. *Advancement in Medical Plant Research*, 3(2), 29-32.
- Asaduzzaman, Rana S., Hasan S. M. R., Hossain M., & Nittananda. (2015). Cytotoxic (brine shrimp lethality bioassay) and antioxidant investigation of *Barringtonia acutangula* (L.). *International Journal of Pharma Sciences and Research*, 6(8), 1179-1185.
- Badalyan, S. & Shahbazyan, T. (2015). Medicinal properties of two polypore species: *Fomes fomentarius* and *Fomitopsis pinicola*. Yerevan State University. Retrieved from [www.researchgate.net/publication/291148415](http://www.researchgate.net/publication/291148415).
- Badshah, H., Ullah, F., Khan, M.U., Mumtaz, A.S. & Malik, R.N. (2015). Pharmacological activities of selected wild mushrooms in South Waziristan, Pakistan. *South African Journal of Biology*, 97, 107-110.
- Bankole, P. O and 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.
- Blaise, C., & Féraud, J. F. (Eds.). (2005). Small-scale freshwater toxicity investigations. Retrieved from <https://onlinelibrary.wiley.com/doi/pdf/10.1002/tox.20402> on June 5, 2018.
- Bolhassan, M.H., Abdullah, N., Sabaratnam, V., Tsutomu, H., Abdullah, Sumaiyah, Mohd, N., Rashid, N. & Musa, Y. (2012). Diversity and Distribution of Polyporales in peninsular Malaysia. *Sains Malaysiana*, 4(2), 155-161
- Brannen, K. C., Panzica-Kelly, J. M., Danberry, T. L., & Augustine-Rauch, K. A. (2010). Development of a zebrafish embryo teratogenicity assay and quantitative prediction model. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, 89(1), 66-77.
- Braunbeck, T., & Lammer, E. (2006). Fish embryo toxicity assays. *German Federal Environment Agency*, 1-298
- Bustillos, R.G., Paguio, Z.K., Hermosa, D.P. & Dulay, R.M.R. (2016). Philippine coprophilus mushrooms (*Panaeolus antillarum* and *Panaeolus cyanescens*) exhibit toxic and teratogenic effects in zebrafish (*Danio rerio*) embryo model. *Advances in Environmental Biology*, 10(3), 75-80.

- Carranza, J. V. (1985). Cultural and other studies on the species of *Fomitopsis* with rose-colored context (fungi, decay, brown rots, polypores, sexuality). University of Arizona. Retrieved from [https://repository.arizona.edu/bitstream/handle/10150/187996/azu\\_td\\_8522806\\_sip1\\_m.pdf?sequence=1&isAllowed=y](https://repository.arizona.edu/bitstream/handle/10150/187996/azu_td_8522806_sip1_m.pdf?sequence=1&isAllowed=y).
- Chang, C. V., Felicio, A. C., de Paula Reis, J. E., de Oliveira Guerra, M., & Peters, V. M. (2002). Fetal toxicity of *Solanum lycocarpum* (Solanaceae) in rats. *Journal of Ethnopharmacology*, 81(2), 265-269.
- Chang, S.T. & Miles, P. (2011). Mushroom: Cultivation, nutritional value, medicinal effect and environmental impact. CRC Press, Second Edition, 1. Retrieved from <https://www.crcpress.com/Mushrooms-Cultivation-Nutritional-Value-Medicinal-Effect-and-Environmental/Miles-Chang/p/book/9780849310430>.
- Chelela, B. L., Chacha, M. & Matem, A. (2014). Cytotoxicity activity of some wild mushroom species from Southern Highlands of Tanzania. *American Journal of Research Communication*, 2(9), 192-201.
- Cheng, Jing-Jy, Lin, C., Lur, H., Chen, H. and Lu, M. (2008). Properties and biological functions of polysaccharides and ethanolic extracts isolated from medicinal fungus, *Fomitopsis pinicola*. *Process Biochemistry*, 43(8), 829-834.
- Chilkov, N. (2017). Six cancer-fighting medicinal mushrooms. *Huffpost: The Blog*. Retrieved from [https://www.huffingtonpost.com/nalini-chilkov/cancer-foods\\_b\\_1192207.html](https://www.huffingtonpost.com/nalini-chilkov/cancer-foods_b_1192207.html).
- Chongawen, G. (2013). Paracelis, Mt. Province history. Retrieved from <http://babyblueinmysoul.blogspot.com/2013/02/paracelis-mt-province-history.html?m=1>
- 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.
- De Castro, M.E. G., Dulay, R.M. R. & Enriquez, M.L. D. (2016). Toxic and teratogenic effects of medicinal and culinary mushroom, *Termitomyces clypeatus*, collected from the termite mound in Mt. Makiling forest reserve, Los Baños, Laguna, Philippines on developing embryos of zebrafish (*Danio rerio*). *Scholars Research Library*, 8(5), 237-242.
- Department of the Interior and Local Government-Cordillera Administrative Region (1999). *Local Governance Regional Resource Center*. Retrieved from <http://www.dilgcar.com/index.php/2015-07-10-09-39-31/municipality-of-paracelis#>.

- Dulay R. M. R., Kalaw S. P., Reyes R. G., Alfonso N. F., & Eguchi F. (2012). Teratogenic and toxic effects of lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.Fr.) P. Karst. (Higher Basidiomycetes), on zebrafish embryo as model. *International Journal of Medicinal Mushrooms*, 14(5), 507–512.
- Dulay, R. M. R., Kalaw S. P., Reyes R. G., & Cabrera, E. C. (2014). Embryo-toxic and teratogenic effects of Philippine strain of *Lentinus tigrinus* (tiger sawgill basidiomycetes) extract on zebrafis (*Danio rerio*) embryos. *Annals of Biological Research*, 5(6), 9-14.
- Dulay, R. M. R., Rivera, A. C., & De Castro, M.E.G. (2017). Wild paddy straw mushroom (*Volvariella volvacea*) exhibit toxic and teratogenic effects in zebrafis (*Danio rerio*) embryo. *International Journal of Biology, Pharmacy and Allied Sciences*, 6(5), 910-917.
- Dumitrascu, M. (2011). *Artemia salina*. *Balneo-Research Journal*, 2(4), 119-122.
- Eguchi, F., Watanabe, Y., Zhang, J., Miyamoto, K., Yoshimoto, H., Fukuhara, T., & Higaki, M. (1999). Inhibitory effect of hot water extract from *Agaricus blazei* fruiting bodies (Cj-01) on hypertension development in spontaneously hypertensive rats. *Traditional Medicines*, 16, 201-207.
- Faridur, R.M., Rezaul, K.M., Farhadul, I.M., Rowshanul, H.M. & Tofazzal, H.M. (2010). Phytochemical and cytotoxic investigation on oyster mushroom (*Pleurotus ostreatus*). *International Research Journal of Pharmacy*, 1(1), 342-345.
- Finn, R. N. (2007). The physiology and toxicology of salmonid eggs and larvae in relation to water quality criteria. *Aquatic Toxicology*, 81(4), 337-354.
- Golla, U. R., Gajam, P. K., Mohammad, A. R., Kumar, K. A., & Raj, B. S. S. (2011). Assessment of bioactivity of *Desmostachya bipinnata* (L.) Stap using brine shrimp (*Artemia salina*) lethality assay. *Pharmacologyonline*, 3, 982-990.
- Gort, E. H., Groot, A. J., van de Ven, T. D., Van Der Groep, P., Verlaan, I., Van Laar, T., & Shvarts, A. (2006). Hypoxia-inducible factor-1 $\alpha$  expression requires PI 3-kinase activity and correlates with Akt1 phosphorylation in invasive breast carcinomas. *Oncogene*, 25(45), 6123.
- Gupta, S., Summuna, B., Gupta, M. & Anneper, S.K. (2018). Edible mushrooms cultivation. Bioactive, molecular and health benefits. *Bioactive Molecules in Food*, 1-33.
- Haque, A., Rana, M., Aktar, K., Haqu, U., Chouduri, A. U. & Islam, A.U. (2016). In-vitro antioxidant and cytotoxic activity of crude extracts of *Pleurotus highking*, a potential oyster mushroom. *British Journal of Pharmaceutical Research*, 14(2), 1-7.

- Hobbs, C. (2014). Medicinal mushroom. Retrieved from <https://www.christopherhobbs.com/webdocs/presentations/Medicinal-Mushrooms-2014.pdf>.
- Islam S. (2013). "Cultivation techniques of edible mushrooms: *Agaricus bisporus*, *Pleurotus* spp., *Lentinula edodes* and *Volvariella volvacea*." *The magic of mushroom and mould biology*, 1-34.
- Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA Cancer J Clin*, 6(12), 69-90.
- Jo, W.S., Hossain, M. & Park, S. (2014). Toxicological profiles of poisonous, edible and medicinal mushrooms. *Mycobiology*, 42(3), 215-220.
- Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), 253-310.
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008). Dictionary of the Fungi. *Mycobank*. Retrieved from <http://www.mycobank.org> BioloMICS.
- Krisnaraju, A.V., Rao, T.V.N., Sundararaju, D., Vanisree, M., Tsay, H.S. & Subbaraju, G.V. (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science and Engineering*, 3(2), 125-134.
- Kumar, K. (2015). Role of edible mushrooms as functional foods- a review. *South Asian Journal Food Technology Environment*, 1, 211-218.
- Mabley, J. D. & Childs, S. J. (2010). "Zebrafish Cardiovascular System". *Fish Physiology*. New York City: Academic Press, 29, 141-159.
- Mau, J., Lin, H. & Chen, C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agriculture and Food Chemistry*, 50(21), 6072-6077.
- McLaughlin, J. L., Rogers, L. L., & Anderson, J. E. (1998). The use of biological assays to evaluate botanicals. *Drug information journal*, 32(2), 513-524.
- Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Plantae Md*, 45, 31-34.
- Mizuno, T. (2002). Medicinal properties and clinical effects of culinary-medicinal mushroom *Agaricus blazei* Murrill (Agaricomycetidae) (Review). *International Journal of Medicinal Mushrooms*, 4(4), 1-14.

- Morel, H. (2017). What is a mushroom? *Gourmet Mushroom Home Page*. Retrieved from [www.gmushrooms.com/info.htm](http://www.gmushrooms.com/info.htm).
- Mueller, G.M., Schmit, J.P., Leacock, P.R., Buyck, B., Cifuentes, J., Desjardin, D.E., & Ledge, D.J. (2007). Global diversity of distribution of macrofungi. *Biodiversity and Conservation*, 16(1), 37-48.
- Musngi R. B., Abella E. A., Lalap A. L., & Reyes R. G. (2005). Four species of wild *Auricularia* in Central Luzon, Philippines as sources of cell lines for researchers and mushroom growers. *Journal of Agricultural Technology*, 1(2), 279-299.
- Njiwa, J. R. K., Müller, P., & Klein, R. (2004). Life cycle stages and length of zebrafish (*Danio rerio*) exposed to DDT. *Journal of Health Science*, 50(3), 220-225.
- Oberst, L. (2016). Mushrooms have stunning powers to heal people and the planet. *Food Revolution Network*. Retrieved from <https://foodrevolution.org/blog/food-and-health/health-benefits-of-mushrooms/>.
- Oyetayo, O.V. (2011). Medicinal uses of mushrooms in Nigeria: Towards full and sustainable exploitation. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(3), 267-274.
- Parrng, C. (2005). In vivo zebrafish assays for toxicity testing. *Current Opinion in Drug Discovery and Development*, 8(1), 100-106.
- Peteros, N. P., & Uy, M. M. (2010). Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. *Journal of Medicinal Plants Research*, 4(5), 407-414.
- Quazi, S., Fatema, C.A. & Mishbahuddin, M. (2017). Brine shrimp lethality assay. *Bangladesh J Pharmacol*, 12, 186-189.
- Raghunath, A & Perumal, E. (2016). Analysis of lethality and malformations during zebrafish (*Danio rerio*) development teratogenicity testing. *Methods in Molecular Biology*, 337-363.
- Rahi, D. & Malik, D. (2016). Diversity of mushrooms and their metabolites of nutraceuticals and therapeutic significance. *Journal of Mycology*, 1-18.
- Reed, B. & Jennings, M. (2011). Guidance on the housing and care of zebrafish. Retrieved from <https://www.aaalac.org/accreditation/rcfresources/zebrafishreference.pdf>.
- Reneses, M.A. M., Dulay, R.M. R. & De Leon, A. M. (2016). Proximate nutritive composition and teratogenic effect of *Lentinus sajor-caju* collected from Banaue, Ifugao Province, Philippines. *International Journal of Biology, Pharmacy and Allied Sciences*, 5(7), 1771-1786.

- Rogers, R. (2011). Three under-utilized medicinal polypores. *Journal of the American Herbalist Guild*, 12(2), 15-21.
- Santos, I.V.F., Duarte, J.L., Fernandes, C.P., Keita, H., Amado, J.R., Moyado, J.A., Navarrete, A., & Carvalho, J.C. (2016). Use of zebrafish (*Danio rerio*) in experimental models for biological assay with natural products. *Academic Journals*, 10(42), 883-891.
- Selderslaghs, I. W., Van Rompay, A. R., De Coen, W., & Witters, H. E. (2009). Development of a screening assay to identify teratogenic and embryotoxic chemicals using the zebrafish embryo. *Reproductive toxicology*, 28(3), 308-320.
- Shnyreva, A. V., Shnyreva, A. A., Espinoza, C., Padron, J. M. & Trigos, A. (2018). Antiproliferative activity and cytotoxicity of some medicinal wood-destroying mushroom from Russia. *International Journal of Medicinal Mushrooms*, 20(1), 1-11.
- Silva, A.G., Rapior, S., Sudarman, E., Stadler, M., Xu, J., Alias, S. & Hyde, K.D. (2013). Bioactive metabolites from macrofungi: Ethnopharmacology, biological activities, chemistry. *Fungal Diversity*, 62(1), 1-40.
- Sipes, N., Padilla, S., & Knudsen, T. (2011). Zebrafish-As an integrative model for twenty-first century toxicity testing. *Birth Defects Research Part C: Embryo Today: Reviews*, 93(3).
- Spence, R., Fatema, M. K., Ellis, S., Ahmed, Z. F., & Smith, C. (2007). Diet, growth and recruitment of wild zebrafish in Bangladesh. *Journal of Fish Biology*, 71, 304-309.
- Syahmi, A.R.M.S., Vijayaratha, S., Sasidharan, L.Y., Latha, Y.P.J., Kwan, Y.L, Lau, L.N., & Chen, Y. (2010). Acute oral toxicity of brine shrimp lethality of *Elaeis guineensis* method extract. *Molecules*, 15, 8111-8112.
- Thapa, C.D. (2012). Mushroom culture. *Department of Plant Pathology. Dr. Yashwant Singh Parmar University of Horticulture and Forestry*. Retrieved from <http://agropedia.iitk.ac.in/content/dr-yashwant-singh-parmar-university-horticulture-and-forestry>.
- Wasser, S. (2010). Medicinal mushroom science: history, current status, future trends and unsolved problems. *International Journal of Medicinal Mushrooms*, 12(1), 1-16.
- Ware, M. (2017). What is the nutritional value of mushroom? *Medical News Today*. Retrieved from <https://www.medicalnewstoday.com/articles/278858.php>.
- Wong, F., Chai, T., Tan, S. & Yong, A. (2013). Evaluation of bioactivities and phenolic content of selected edible mushrooms in Malaysia. *Tropical Journal of Pharmaceutical Research*, 12(6), 1011-1016.

- Weigt, S., Huebler, N., Strecker, R., Braunbeck, T. & Broschard, T. (2011). Zebrafish (*Danio rerio*) embryos as a model for testing proteratogens. *Toxicology*, 281(1-3), 25-36.
- Wu, H.T., Lu, F., Su, Y., Ou, H., Hung, H.C., Wu, J.S., Yan, Y.C & Chang, C.J. (2014). In vivo and in vitro anti-tumor effects of fungal extracts. *Molecules*, 19, 2546-2556.
- Younis, A., Stewart, J., Wu, F.S., Shikh, H.E., Hassan, F., & Elaasser, M. (2014). Cytotoxic activity of edible mushrooms extracts against tumor cell lines. *International Journal of Science and Technology*, 3(11), 736-749.