ZOOCHEMICALS AND FUNCTIONAL ACTIVITIES OF SEA CUCUMBER (*Holothuria leucospilota*) IN CASIGURAN, AURORA

JOB DANIEL NOAH G. CRUZ

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 Muñoz, 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

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURE

ABSTRACT

CRUZ, JOB DANIEL NOAH G., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **MAY 2019**, **FUNCTIONAL ACTIVITIES OF SEA CUCUMBER (*Holothuria leucospilota*) IN CASIGURAN, AURORA**

Adviser: MA. ELIZABETH DC. LEOVERAS, Ph. D.

The functional activities of *Holothuria leucospilota* was assessed using different assay. Collection Holothuria Casiguran, Aurora yielded two species, *Holothuria leucospilota* and *Holohuria hilla* .that belong to Order Aspidochirotida and Family Holothuriidae. *H. leucospilota* is the dominant species and was used in the study and in different functional activities. The body wall of *H. leucospilota* screened for different chemical components, and among the present compounds are Essential oil, Triterpene, Steroids, Phenols, Coumarines, Anthrones, Tannins and Flavonoids. It was also found out that *H. leucospilota* possess satisfactory level of antioxidant with 37.50% scavenging effect with phenolic content of 14.00 mg GAE/g of sample.

In addition, embryo toxicity and teratogenecity of *H. leucospilota* was observed in different concentration in Hot water and fresh body wall extract

In the toxicity to the embryo test, result shows that in hot water extract it showed non-toxic while fresh body wall extract in the highest concentration (10,000 ppm), it is toxic. Hot water extract do not have terratogenic effect but a delayed growth was monitored. In fresh body wall extract delayed growth was observed in all treatments and perverted tail in 1000 ppm concentration was seen.

Cam Irritation Inhibition Assay of *H. leucospilota* revealed that in 1000 ug/ml shows moderate inhibition. It concluded that *H. leucospilota* has potential functional activities.

LITERATURE CITED

Almeida, P. D., Boleti, A. P., Rüdiger, A. L., Lourenço, G. A., Júnior, V. F. & Lima, E. S. (2015). Anti-Inflammatory Activity of TriterpenesIsolated from *Protium paniculatum* Oil-Resins. *Evidence-based complementary and alternative medicine.*

Althunibat, O., Ridzwan, B., Taher, M., Daud, J., Ichwan, S. & Qaralleh, H. (2013). Antioxidant and cytotoxic properties of two sea cucumbers, *Holothuria edulis and Stichopushorrens. Acta biologica Hungarica*, 64, 10-20.

Althunibat, O., Hashim, R. B., Taher, M., Daud, J. M., Ikeda, M. & Zali, B. I. (2009). In vitro antioxidant and antiproliferative activities of three Malaysian sea cucumber species. *European Journal of Scientific Research,* 37, 376-387.

Beauregard K. A., Truong N. T., Zhang H., Lin W. & Beck G. (2001). The detection and isolation of a novel antimicrobial peptide from the echinoderm, *Cucumaria frondosa. Advances in Experimental Medicine and Biology*, 484, 55–62.

Bordbar, S., Anwar, F. & Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods - a review. *Marine Drugs*, 9(10), 1761-805.

Brannen, K. C., Panzica-Kelly, J., Danberry, T. & Augutine R. (2010). Development of zebra fish embryo teratogenicity assay and quantitative prediction model. *Birth Defects Research*, 89, 66-77.

Bruckner, A. W., Johnson, K. A. & Field, J. D. (2003). Conservation strategies for sea cucumbers: Can a CITES Appendix II listing promote sustainable international trade? *Secretariat of the Pacific Community Beche-de-Mer Information Bulletin*, 18, 24-33.

Cafirma, E. P., De Leon G. A., Garcia R. A. M. V., Miguel C. M., Milandres J. J. B., Waing, K. G. D, Dulay, R. M. R. (2018). Phytochemical compositions and biological activities of leaf extract of oregano (*Origanumvulgare*). *International Journal of Biology, Pharmacy and Allied Sciences*, 7(5), 792-801.

Ceesay, A., Shamsudin, M., Aliyu-Paiko, M., Ismail, I. S., Nazarudin, M. & Mohamed Alipiah, N. (2019). Extraction and Characterization of OrganComponents of the Malaysian Sea Cucumber *Holothurialeucospilota*Yielded BioactivesExhibiting Diverse Properties. *BioMed Research International*, 1-16.

Chen, S., Xue, C., Yin, L., Tang, Q., Yu, G. & Chai, W. (2011). Comparison of structures and anticoagulant activities of fucosylated chondroitin sulfates from different sea cucumbers. *Carbohydrate Polymers*, 83, 688-696.

Chin, K. Y., Chin, K. C., Kim, K. H. & Yaacob, H. B. (2011). Anti-anaphylactic shock properties of sea cucumber water extract ("Air Gamat"). *In Proceedings of the Malaysian. Science and Technology Congress; Melaka, Malaysia*, 8(10), 89.

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W. & Lin, Y. (1998). Tannins and human health. Critical Reviews in Food Science and Nutrition, 38(6), 421-464.

Conand, C. (2006). Sea cucumber biology, taxonomy, distribution: Conversation Status. In The Proceedings of the CITES workshop on the conservation of sea cucumbers in the family Holothuriidae and Stichopodidae. Bruckner, A. W. (Edition). *NOAA Technica Memorandum*, 244.

Dhinakaran, D. I. & Lipton, A. P. (2014). Pharmacological potentials of sea cucumber *Holothuriaatra*extracts from the Indian Ocean. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 4(35).

Demiray, S., Pintado, M. & Castro, P. (2009). Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plant: *Tiliaargentea, Crataegi folium* leaves and *Polygonumbistorta* roots. *World Academy of Science, Engineering and Technology*. 54.

Dulay, R. M., Kalaw, S., Reyes, R., Alfonso, N. & Eguchi, F. (2012). Teratogenic and toxic effects of Lingzhi or Reishi Medicinal mushroom *Ganodermalucidum* (W.Curt.:fr.) P. Karst. (Higher Basidiomycetes), on zebra fish embryo as model. *International Journal of Medicinal Mushrooms*, 14(5), 507-512.

Duthie, G. G., Duthie S. J. & Kyle, J. A. M. (2000). Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutritional. Research Review*, 13, 79-106.

Gates, K. W. (2010). Marine products for healthcare: Functional and bioactive nutraceutical compounds from the ocean, Vazhiyil Venugopal. *Journal of Aquatic Food Product Technology*, 19, 48-54.

Gowda, N. M., Goswami, U. & Islam, K. (2008). Purification and characterization of a T antigen specific lectin from the coelomic fluid of a marine invertebrate, sea cucumber (*Holothuria scabra). Fish & Shellfish Immunology*, 24(4), 450- 458.

Guevarra, B.(2005). A guidebook tophytochemical screening: phytochemical and biological.Manila: *UST Publishing House.*

Han, N. & Bakovic M (2015) Biologically Active Triterpenoids and Their Cardioprotective and Anti-Inflammatory Effects. *Journal of Bio analysis & Biomedicine*, 12.

Higgins, M. (2000). Sea cucumbers in a deep pickle. *Environmental News Network.*

Hu, X., Wang, Y., Wang, J., Xue, Y., Li, Z., Nagao, K., Yanagita, T. & Xue, C. (2009). Dietary saponins of sea cucumber alleviate orotic acid-induced fatty liver in rats via PPARα and SREBP-1c signaling. *Lipids in Health and Disease.*

Huizeng F. (2004). Sea cucumber: Ginseng of Sea Zhongguo. *Marine Medicine*, 82, 37-44.

Jain, P. K. & Himanshu, J. (2012). Coumarin: Chemical and pharmacological profile.

Jiang, J., Yang, B.L.&Tai, Y. (2004). Studies on resources and bioactive substances of sea cucumber. *Letters in Biotechnology*, 15, 537-540.

Khotimchenko, Y. (2018). Pharmacological potential of sea cucumbers. *International Journal of Molecular Sciences*, 19(5), 1342.

Kiew, P. L. & Don, M. M. (2012). Jewel of the seabed: sea cucumbers as nutritional and drug candidates. *International journal of food sciences and nutrition*, 63(5), 616-36.

Kimmel, C., Ballard, W., Kimmel, S., Ullmann, S. & Schilling, T. (1995). Stage of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), 253-310.

Kolak, U., Özturk, M., Özgokce, F. & Ulubelen, A. (2006). Norditerpene alkaloids from *Delphinium linearilobum* and antioxidant activity. *Phytochemistry*, 67(19), 2170-2175.

Kozlowska, A. &Szostak-Wegierek, D. (2014). Flavonoids—food sources and health benefits.

Kress, J. P Hall, J. P. 2014 "ICU-acquired weakness and recovery from critical illness," *The New England Journal of Medicine*, 370, 1626–1635. 2014.

Lavitra T., Rachelle, D., Rasolofonirina, R., Jangoux, M. &Eeckhaut I. (2008). Processing and marketing of holothurians in the Toliara region, southwestern Madagascar. *Secretariat of the Pacific Community Beche-de-Mer. Information Bulletin*, 28, 24-33.

Laale, H. (1977). The biology and use of zebrafish, Brachydaniorerio in fisheries research. A literature review. *Journal of Fish Biology*. 10. 121 - 173.

Liu, H. H., Ko., W. C& Hu, M. L (2002). Hypolipidemic effect of glycosaminoglycans from the sea cucumber metriatylascabra in rats fed a cholesterol- supplemented diet. *Journal of Agricultural and Food Chemistry*, 50(12), 3602-6.

Luepke, N. (1985). Hen's egg chorioallantoic membrane for irritation potential. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*. 23. 287-91.

Macabante, P. B. (2016). Analgesic activity of *Holothuriaatra*on albino mice. Unpublished undergraduate thesis. Central Luzon State University, Science City of Munoz,NuevaEcija.

Mamelona, J., Pelletier, E. M., Lalancette, K. G., Legault, J., Karboune, S. &Kermasha, S. (2007). Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumariafrondosa*. *Food Chemistry*, 104, 1040-1047.

Mcgrath, P. & Li, C. (2008). Zebrafish: a predictive model for assessing drug-induced toxicity. *Drug Discovery Today*, 394-401.

Mehmet, A., Huseyin, S., Bekir, T., Yilmaz, E. &Sevim, K. (2011). Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science & Technology*, 46, 500-508.

Mena-bueno, S., Atanasova, M., Fernández-trasancos, Á.,Paradela-dobarro, B., Bravo, S. B., Álvarez, E. &Eiras, S. (2016). Sea cucumbers with an anti-inflammatory effect on endothelial cells and subcutaneous but not on epicardial adipose tissue. *Food & Function*, 7(2), 953-963.

Menaga D. &Ayyasamy P. (2012). Effect of horse gram on the cultivation of *Pleurotus florida* mushroom and their phytochemical analysis and antimicrobial activity. *International Journal of Reasearch in Pharmaceutical Sciences*, 3(1), 140-145.

Menaga, D., Mahalingam, P. U., Rajakumar, S. &Ayyasamy, P. M. (2012). Evaluation of phytochemical characteristics and antimicrobial activity of *Pleurotusflorida* mushroom. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4), 4-8.

Morgan, A. & Archer, J. (1999). Overview: Aspects of sea cucumber industry research and development in the south pacific. *Secretariat of the Pacific Community Beche-de-Mer Information Bulletin*, 1, 15-17.

Murniasih, T., Putra, M. Y.&Pangestuti R. (2015). Antioxidant capacities of Holothuria sea cucumbers. *Ann Bogorienses*, 19, 21-26

Naveed, R., Hussain, I., Tawab, A., Tariq, M., Rahman, M., Hameed, S. &Iqbal, M. (2013). Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against Salmonella and other multi-drug resistant bacteria. *BMC complementary and alternative medicine*, 13, 265.

Neves, A., Rosa, S., Gonçalves, J., Rufino, A., Judas, F., Salgueiro, L., Lopes, C. M., Cavaleiro, C. & Mendes, A. (2009). Screening of five essential oils for identification of potential inhibitors of IL-1-induced Nf- κ B activation and NO production in human chondrocytes: Characterization of the Inhibitory Activity of α -Pinene. *Planta Medica*, 76, 303-8.

Nobsathian, S., Tuchinda, P., Sobhon, P., Tinikul, Y., Poljaroen, J., Tinikul, R., Sroyraya, M., Poomton, T.,&Chaichotranunt, S. (2017). An antioxidant activity of the whole body of *Holothuriascabra*. *Chemical and Biological Technologies in Agriculture*, 4, 1-5.

Ouedraogo, M., Baudoux, T., Caroline, S., Nortier, J., Colet, J., Efferth, T., Qu, F., Zhou, J., Chan, K., Shaw, D., Pelkonen, O. &Duez, P. (2012). Review of current and "omics" methods for assessing the toxicity (genotoxicity, teratogenicity and nephrotoxicity)of herbal medicines and mushrooms. *Journal of ethnopharmacology*, 140, 492-512.

Ozcan, T., Akpinar-Bayizit, A., Yilmaz-Ersan, L. &Delikanli, B. (2014). Phenolics in human health. *International Journal of Chemical Engineering*, 5(5), 393-396.

Pacheco, R. G., Vicente, C. P., Zancan, P. &Mourao, P. A. S. (2000). Different antithrombotic mechanisms among glycosaminoglycans revealed with a new fucosylated chondroitin sulfate from an echinoderm. Blood coagulation & fibrinolysis: *An International Journal in Haemostasis and Thrombosis*, 11, 563-73.

Pangestuti, R. & Arifin, Z. (2018). Medicinal and health benefit effects of functional sea cucumbers. *Journal of Traditional and Complementary Medicine*, 8.

Raquibul, S. M., Hossain, M. M., Aktar, R., Jamila, M., Mazumder, M. E. H. &Alam, M. A. (2010). Analgesic activity of the different fractions of the aerial parts of *Commelina benghalensis*Linnaeus. *International Journal of Pharmacology*, 6, 63-67.

Rascón-Valenzuela, L., Torres Moreno, H., Velazquez, C., Garibay-Escobar, A. & Robles-Zepeda, R. (2016). Triterpenoids: Synthesis, Uses in Cancer Treatment and other Biological Activities. *Advances in Medicine and Biology*, 106, 166

Ribatti, Domenico. (2016). The chick embryo chorioallantoic membrane (CAM) assay, *Reproductive Toxicology*, 70.

Ridzwan, B. H. (2007). Sea cucumbers, a Malaysian heritage, 1st edition. *Research centre of international islamic university Malaysia (IIUM): Kuala Lumpur Wilayah Persekutuan, Malaysia*, 1–15, 89–128.

Ridzwan, B. H., Kaswandi, M. A., Dayang, K. M., Fredalina, B. &Haniza, H. (1996). Crude extracts of three local species of sea-cucumbers: their inhibitory effects on histamine induced anaphylactic shock in guinea-pigs. *In Proceedings of the European Histamine Research Society, Antwerp, Belgium*, 2, 22.

Romero-Estrada, A., Maldonado-Magaña, A., González-Christen, J., Bahena, S. M., Garduño-Ramírez, M. L., Rodríguez-López, V. & Alvarez, L. (2016). Anti-inflammatory and antioxidative effects of six pentacyclictriterpenes isolated from the Mexican copal resin of *Burseracopallifera*. *BMC complementary and alternative medicine*.

Schulte, C.,Nagel, R. (1994).Testing acute toxicity in the embryo of zebrafish *Brachydanio rerio*as an alternative to the acute fish test: preliminary results. *Atla*, 22(1),12-19.

Selderslaghs, I. W. T., Van Rompay, A., De Coen, W. &Witters, H. (2009). Development of a screening assay to identify teratogenic and embryotoxic chemicals using the zebrafish embryo. *Reproductive Toxicology*, 28, 308-320.

Serra, A. T., Rocha, J., Sepodes, B., Matias A. A, Feliciano R. P. (2012). Evaluation of cardiovascular protective effect of different apple varieties-Correlation of response with composition. *Food chemistry*, 135, 2378-2386.

Shahram, K., Alborz, E., Jasem, S.,&Masoud F. (2016). "Cytotoxic activity of *Holothuria leucospilota* extract against Leishmania infantum In Vitro," *Advances in Pharmacological Sciences*, 6.

Shi, X., Du, Y., Lam, P. K. S., Wu, R. S. S. & Zhou, B. (2008). Developmental toxicology and alteration of gene expression in zebra fish embryos exposed to PFOS. *Toxicology and Applied Pharmacology*, 230, 23-32.

Silchenko, A. S., Avilov, S. A., Kalinin, V. I., Kalinovsky, A. I., Dmitrenok, P. S., Fedorov, S. N., Stepanov, V. G., Dong, Z. &Stonik, V.A. (2008). Constituents of the sea cucumber *Cucumariaokhotensis*. Structures of okhotosides B1-B3 and cytotoxic activities of some glycosides from this species. *Journal of Natural Products*, 71(3), 351-6.

Smith, A. C. (1978). A proposed phylogenetic relationship between sea cucumber Polian vesicles and the vertebrate lymphoreticular system. *Journal of Invertebrate Pathology*, 31, 53-357.

Strahle, U., Scholz, S., Geisler, R., Greiner, P., Hollert, H., Rastegar, S., Schumacher, A., Selderslaghs, I., Weiss, C., Witters, H.,&Braunbeck, T. (2012). Zebrafish embryo as an alternative to animal experiments-a commentary on the deffinition of the onset of protected life stages in animal welfare regulation. *Reproductive Toxicology*, 33(2), 128-132.

Soltani, M., Parivar, K., Baharara, J., Kerachian, A. A. &Asili, J. (2014). Hemolytic and cytotoxic properties of saponin purified from *Holothurialeucospilota* sea cucumber. *Reports of Biochemistry and Molecular Biology*, 3(1), 1-8.

Sunita, M. & Dhananjay, S. (2010). Quantitative analysis of total phenolic content in *Adhatoda vasica* nees extracts. 2(4), 2403-2406.

Taiyeb-ali, T., Zainuddin, S. L., Swaminathan, D. & Yaacob, H. B. (2003). Efficacy of ' Garnadent' toothpaste on the healing of gingival tissues: A preliminary report. *Journal of Oral Science*, 45(3), 153-9.

Tian, F., Zhang, X., Tong, Y., Yi, Y., Zhang, S., Li, L., Sun, P., Lin, L. & Ding, J. (2005). PE, a new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activities in vitro and in vivo. *Cancer biology & therapy*, 4(8), 874-82.

Venugopal, V. (2008). Marine products for healthcare: Functional and bioactive nutraceutical compounds from the ocean. *Boca Raton: CRC Press.*

Weight, S., Huebler, N., Strecker, R., Briunbeck, T. & Broschard, T. (2011). Zebra fish (*Daniorerio*) embryos as a model for testing proteratogens. *Toxicology*, 28, 25-36.

Wen, J., Hu, C. & Fan, S. (2010). Chemical composition and nutritional quality of sea cucumbers. *Journal of the Science of Food and Agriculture*, 90(14), 2469-74.

Werner, E., Muller, G., Heinz, C., Wiens, M., Ottstadt, S. P. &Batel, R. (2004) Traditional and modern biomedical prospecting: Part II-the benefits approaches for a sustainable exploitation of biodiversity (Secondary metabolites and biomaterials from sponges). *Evidence-based complementary and alternative medicine*, 1, 133-144.

Wu, M., Xu, S., Zhao, J., Kang, H. & Ding, H. (2010). Free-radical depolymerization of glycosaminoglycan from sea cucumber *Thelenota ananas* by hydrogen peroxide and copper ions. *Carbohydrate Polymers*, 93, 1116-1124.

Yaacob, H., Kim, K. H., Shamimi, M., Aziz, N. S. & Sahil, S. M. (1997). Malaysian sea cucumber (Gamat): A prospect in health food and therapeutic. *Perubatan University Kebangsaan Malaysia*, 19, 24-39.

Yen, G. C., Duh, P. D. & Chuang D. Y. (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chem.*70, 437-441

Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. *Journal of clinical pathology*, 54(3).

Yu R. F. B. (2016). Anti-inflammatory potential of *Ficus pseudopalma* Blanco (Niyog-niyogan) leaf ethanolic fraction phytochemicals on the chorioallantoic membrane of 10-Day Old Anas Luzonica (Duck) Eggs. *Philippine Journal of Health Research and Development*, (3):50-55.

Yuan, W. P., Liu, C. H., Wang, X. J., Meng, X. M., Xia, X. K., Zhang, M. S. & Hu, W. (2010). Evaluation and analysis of nutritional composition of different parts of sea cucumber *Apostichopus japonicas*. *Science and Technology of Food Industry*, 920, 187-202.

Yumnamcha, T., Roy D., Damanyati, D. M. &Nongthomba, U. (2015). Evaluation of developmental toxicity and apoptotic induction of the aqueous extract of *Milettia pachycarpa* using zebrafish as model organism. *Toxicological & Environmental Chemistry*, 97(10), 1363-1381.

Zou, Z., Yi, Y., Wu, H., Wu, J., Liaw, C. & Lee, K. (2003). Intercedensides A-C, new cytotoxic triterpene glycosides from the sea cucumber *Mensamaria intercedens*Lampert. *Journal of Natural Products*, 66(8), 1055-60.