ANTIPROLIFERATIVE AND CYTOTOXIC ACTIVITY OF *Morinda citrifolia* AGAINST HUMAN CANCER CELL LINES USING MTT ASSAY

JAHZIEL M. CULALA

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ABSTRACT

CULALA, JAHZIEL M., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **MAY 2019, ANTIPROLIFERATIVE AND CYTOTOXIC ACTIVITY OF *Morinda citrifolia* AGAINST HUMAN CANCER CELL LINES USING MTT ASSAY**

Adviser: KHRISTINA G. JUDAN CRUZ, Ph.D.

Morinda citrifolia, commonly called as Noni, has been reported to have a wide range of health benefits for cancer, infection, diabetes, asthma, hypertension and pain. Initial pharmacological studies on *M. citrifolia* have focused on cancer, inflammation and metabolic diseases, hence this study determined its potential anti-proliferative and cytotoxic activity against human cancer cell lines: breast adenocarcinoma cell line (MCF-7), colorectal carcinoma cell line (HCT-116) and lung adenocarcinoma cell line (A549) *in vitro*. The fruit and seeds of *M. citrifolia* were extracted with 95% ethanol and the filtrate was evaporated by a rotary evaporator. The excess moisture was evaporated and concentrated using vacuum concentrator. The ethanol extract underwent cytotoxicity testing through MTT cell viability assay. The cell viability assay revealed that all the three human cancer cell lines: lung adenocarcinoma (A549), colorectal carcinoma (HCT-116) and breast adenocarcinoma (MCF-7) exhibited resistance against *M. citrifolia* fruit ethanol extracts with IC_{50} values greater than 100 µg/mL. Hence, the ethanol extract of *M. citrifolia* fruits may not serve as anticancer agents using MTT assay. Nevertheless, the *M. citrifolia* fruits' compounds could be further studied in relation to other phytochemical constituents that could work with chemoprevention. Different cell viability assay could also be utilized in assessing *M. citrifolia* fruits' anti-proliferative activity and cytotoxicity.

LITERATURE CITED

Abdel-Hameed, E. S., Bazaid, S. A., Shohayeb, M. M., El-Sayed, M. M., & El-Wakil, E. A. (2012). Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of *Conocarpus erectus* L. growing in Taif, Saudi Arabia. *European Journal of Medicinal Plants, 2(2), 93-112*

Agarwal, E., Brattain, M., & Chowdhury, S. (2013). Cell survival and metastasis regulation by Akt signaling in colorectal cancer. *Cell Signalling, 25*(8), 1711-1719.

Akihisa, T., Matsumoto, K., Tokuda, H., Yasukawa, K., & Seino, K. (2007). Anti-inflammatory and potential cancer chemopreventive constituents of the fruits of *Morinda citrifolia* (Noni). *Journal of Natural Products, 70*, 754-757.

Akihisa, T., Tochizawa, S., Takahashi, N., Yamamoto, A., Zhang, J., Kikuchi, T., & Suzuki, N. (2012). Melanogenesis-inhibitory saccharide fatty acid esters and other constituents of the fruits of *Morinda citrifolia* (noni). *Chemistry & Biodiversity,* 9(6), 1172-1187.

Aldi, Y. H., Bakhtiar, A., Wardi, A., Yanwirasti, Nasrul, E., & Dillasamola, D. (2016). Effects of scopoletin from noni fruit (*Morinda citrifolia* L.) to IL-10 levels in white mice with hypertensitivity type I. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 7(4), 1404-1410.

Alshangiti, A., Chandhoke, G., & Ellis, P. M. (2018). Antiangiogenic therapies in non-small-cell lung cancer. *Current Oncology,* 1: S45-S58.

American Cancer Society. (2009). Cancer Facts & Figures. Retrieved from www.cancer.org: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2009.html

Archana, S., Paul, R., & Tiwari, A. (2011). Indian medicinal plants: A rich source of natural immuno-modulator. *International Journal of Pharmacology,* 7, 198-205.

Asian, C. (2016). *Special Section: Cancer in Asian Americans, Native Hawaiians, and Pacific Highlanders.*

Augestad, K.M.; Bakaki, PM; Rose, J; Crawshaw, BP; Lindsetmo, RO; Dorum, LM; Koroukian, SM; Delaney, CP. (2015). *The International Journal of Cancer Epidemiology, Detection and Prevention,* 39, 734-744.

Aung, H., Mehendale, S., Wang, C., Xie, J., McEntee, E., & Yuan, C. (2007). Cisplatin's tumoricidal effect on human breast carcinoma MCF-7 cell was not attenuated by American ginseng. *Cancer Chemotherapy and Pharmacology,* 69, 369–374

Baek, S. J., Sukamporn, P., Rojanapanthu, P., & Chaichanasak, N. (2018). Anti-cancer activity of damnacanthal from noni. *Journal of Food, Nutrition and Population Health.*

Boncler, M., Różalski, M., Krajewska, U., Podsędek, A., & Watala, C. (2014). Comparison of PrestoBlue and MTT assays of cellular viability in the assessment of anti-proliferative effects of plant extracts on human endothelial cells. *Journal of Pharmacological and Toxicological Methods,* 69(1), 9–16.

Bussmann, R. W., Hennig, L., Giannis, A., Ortwein, J., Kutchan, T. M., & Feng, X. (2013). Anthraquinone content in noni (*Morinda citrifolia* L.). *Evidence-Based Complementary and Alternative Medicine,* 2013, 1-5.

Cai X, Yang J, Zhou J, Lu W, Hu C, Gu Z, Huo J, Wang X, Cao P. (2013). Synthesis and biological evaluation of scopoletin derivatives. *Bioorganic & Medicinal Chemistry,* 21: 84–92

Cassady, J., & Douros, J. (1980). *Anticancer agents based on natural product models.* New York: Academic Press.

CCP Cancer Coalition Philippines. (2017). Cancer. Retrieved from www.icanservefoundation.org:http://www.icanservefoundation.org/?attachment_id=4335

Chakravarti, B., Maurya, R., Siddiqui, J., Bid, H., Rajendran, S., Yadav, P., & Konwar, R. (2012). In vitro anti-breast cancer activity of ethanolic extract of *Wrightia tomentosa* role of pro-apoptotic effects of oleanolic acid and urosolic acid. *Journal of Ethnopharmacology,* 142, 72-79.

Cragg, G. M. & Newman, D. J. (2005). Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology,* 100, 72-79.

Cragg, G., Newman, D., & Snader, K. (1997). Natural products in drug discovery and development. *Journal of Natural Products,* 60(1), 52-60.

De Almeida, V. L., Leitão, A., Reina, L. C., Montanari, C. A., Donnici, C. L., & Lopes, M. T. (2005). Cancer and cell cicle-specific and cell cicle nonspecific anticancer. *Quimica Nova,* 28(1), 118-129.

De Los Reyes, M., Oyong, G., Ebajo, V. Jr., Ng, V. A., Shen, C., & Ragasa, C. (2015). Cytotoxic triterpenes and sterols from *Pipturus arborescens* (link) C.B. Rob. *Journal of Applied Pharmaceutical Science,* 5(11), 023-030.

De Souza Oliveira, R., Corrêa, R., Teixeira, R., Queiroz, D., Da Silva Souza, R., Garden, S., Ribeiro, E. (2016). Silica nanoparticles doped with anthraquinone for lung cancer phototherapy. *Journal of Photochemistry and Photobiology B,* 169, 1-9.

Dixon, A., McMillen, H., & Etkin, N. (1999). Ferment this: the transformation of noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae). *Ecological Botany,* 53, 51-68.

DOST. (2010). *First line of treatment for advanced lung cancer.* Retrieved from www.pchrd.dost.gov.ph: http://www.pchrd.dost.gov.ph/index.php/news/library-health-news/1952-first-line-of-treatment-for-advanced-lung-cancer

Dragsted, L., Strube, M., & Larsen, J. (1993). Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacology & Toxicology,* 72, 116-135.

Fearon, E., & Vogelstein, B. (1990). A genetic model for colorectal turmogenesis. *Cell,* 61(5), 859-767.

Fletcher S. W. (2008). Patient information: Risk factor for breast cancer. *Up-To-Date.* Retrieved from http://www.utdol.com/patients /content/topic.do?topicKey=~_rZvVFHbEjbw

Florento, L., Matias, R., Tuano, E., Santiago, K., Dela Cruz, F., Tuazon, A. (2012). Comparison of cytotoxic activity of anticancer drugs against various human tumor cell lines using *in vitro* cell-based approach. *International Journal of Biomedical Science,* 8(1), 76-80

Franek, G., Nowak-Kaputsa, Z., & Cabaj, M. (2004). Breast cancer. *Wiadomości Lekarskie,* 57, 82-84.

GLOBOCAN. (2018). *Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018.* World Health Organization. International Agency for Research on Cancer . Retrieved from http://www.who.int/cancer/PRGlobocanFinal.pdf?ua=1

Greenwell, M., & Rahman, P. (2015). Medicinal plants: their use in anticancer treatment. *International Journal of Pharmaceutical Sciences and Research.,* 6(10), 4103-4112.

Gupta, R., Banerjee, A., Pathak, S., Sharma, C., & Singh, N. (2013). Induction of mitochondrial-mediated apoptosis by *Morinda citrifolia* (Noni) in human cervical cancer cells. *Asian Pacific Journal of Cancer Prevention,* 14(1), 237-42.

Handa, S., Khanuja, S., Longo, G., & Rakesh, D. (2008). Extraction technologies for medicinal plants. *International Centre for Science and High Technology.*

Hecht, S. (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Reviews Cancer,* 3(10), 733-44.

Heinicke, R. M. (1985). The pharmacologically active ingredient ofNoni. Bulletin of the national tropical botanical garden. *Bulletin of the National Tropical Botanical Garden, 165.*

Hirazumi, A., Furusawa, E., Chou, S., Okano, C., & Ching, C. (1992). Antitumor activity of *Morinda citrifolia* on intraperitoneally implanted Lewis lung carcinoma in mice. *Proceedings of the American Association for Cancer Research.*, 33, 515.

Huang, H., Liu, C., Chou, M., K. C., & Wang, C. (2015). Noni (*Morinda citrifolia* L.) fruit extracts improve colon microflora and exert anti-inflammatory activities in caco-2 cells. *Journal of Medicinal Food,* 18(6), 663-676.

Imaginis. (2008). *Breast Cancer - Symptoms and Treatment Resources.* Retrieved from Imaginis: http://www.imaginis.com/breast-cancer-resource-center

Istivan, T. S., Pirogova, E., Gan, E., Almansour, N. M., Coloe, P. J., & Cosic, I. (2011). Biological effects of a de novo designed myxoma virus peptide analogue: evaluation of cytotoxicity on tumor cells. *Public Library of Science One,* 6(9), e24809.

Jemal, A., Bray, F., & Center, M. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians,* 61, 69-90.

Kaur, H., Gurjar, N., & Gill, R. (2018). The noni fruit (*Morinda citrifolia* L.): A systematic review on anticancer potential and other health beneficial pharmacological activities. *Journal of Medicinal Plants,* 6(2), 86-93.

Khuwaja, G. A. & Abu-Rezq, A. N. (2004). Bimodal breast cancer classification system. *Pattern Analysis and Application,* 7, 235–242.

Lim, S.-L., Mustapha, N. M., Goh, Y.-M., Abu Bakar, N. A., & Mohamed, S. (2016). Metastasized lung cancer suppression by *Morinda citrifolia* (Noni) leaf compared to Erlotinib via anti-inflammatory, endogenous antioxidant responses and apoptotic gene activation. *Molecular and Cellular Biochemistry,* 416(1-2), 85-97.

Liu, G., Bode, A., Ma, W., Sang, S., Ho, C., & Dong, Z. (2001). Two novel glycosides from the fruits of *Morinda citrifolia* (Noni) inhibit AP-1 transactivation and cell transformation in the mouse epidermal JB6 cell line. *Cancer Research,* 61, 5749-5756.

Liu, R. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition,* 78, 517S–520S.

Liu, X., & Jiang, J. (2006). Molecular mechanism of matrine-induced apoptosis in leukemia K562 cells. *The American Journal of Chinese Medicine,* 34, 1095–1103.

Liu, X., Zhang, L., & Fu, X. (2001). Effect of scopoletin on PC3 cell proliferation and apoptosis. *Acta Pharmacologica Sinica*, 22, 929–933.

MacKinnon, A., Kopatz, J., & Sethi, T. (2010). The molecular and cellular biology of lung cancer: identifying novel therapeutic strategies. *British Medical Bulletin*, 95(1), 47-61.

Madhuri, S., & Pandey, G. (2009). Some anticancer medicinal plants of foreign origin. *Current Science, 96*(6).

Mahima, A., Rahal, R., Deb, S., & Latheef, S. H. (2012). Immuno modulatory and therapeutic potentials of herbal, traditional indigenous and ethno veterinary medicines. *Pakistan Journal of Biological Sciences*, 15, 754-774.

Manuele MG, Ferraro G, Arcos ML, López P, Cremaschi G, Anesini C. (2006). Comparative immunomodulatory effect of scopoletin on tumoral and normal lymphocytes. *Life Science*, 79: 2043–2048

McClatchey, W. (2002). From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae). *Integrative Cancer Therapies*, 1(2), 110-20.

Mehendale, S., Aung, H., Wang, A., Yin, J., Wang, C., Xie, J., & Yuan, C. (2005). American ginseng berry extract and ginsenoside Reattenuate cisplatin-induced kaolin in take in rats. *Cancer Chemotherapy and Pharmacology*, 56, 63-69.

Mian-Ying W., Diane N., Gary A., Jarakae J. and Brett W. (2008). Liver protective effects of *Morinda citrifolia* (Noni). *Plant Foods for Human Nutrition*, 63(2), 59.

Mirzaei-Aghsaghali, A. (2012). Importance of medical herbs in animal feeding: A review. *Annals of Biological Research*, 3, 918-923.

Mohd, Z., Abdul, A., Osman, A., & Saari, N. (2006). Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). *Food Chemistry*, 94(2), 169.

Mossman, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65, 55-63.

Mueller, B., Scott, M., Sowinski, K., & Prag, K. (2000). Noni juice (*Morinda citrifolia*): hidden potential for hyperkalemia. *American Journal of Kidney Diseases.*, 35(2), 310-2.

NCCIH National Center for Complementary and Integrative Health. (2016). *Noni.* Retrieved from https://nccih.nih.gov/health/noni

Newman, D. J., Cragg, G. M., Holbeck, S., & Sausville, E. A. (2002). Natural products as leads to cell cycle pathway targets in cancer chemotherapy. *Current Cancer Drug Targets*, 2, 279-308.

Neubig, R.R., Spedding, M., Kenakin, T., Christopoulus, A., (2003). International union of pharmacology committee on receptor nomenclature and drug classification. Xxxviii. Update on terms and symbols in quantitative pharmacology. *Pharmacological Reviews*, 55, 597-606.

Ngelangel, C., & Wang, E. (2002). Cancer and the Philippine Cancer Control Program. *Japanese Journal of Clinical Oncology*, 32, 52-61.

NMCD Nutrition, Metabolism & Cardiovascular Diseases. (2017). *Traditional Medicine*.

Nualsanit, T., Rojanapanthu, P., Gritsanapan, W., Lee, S., Lawson, D., & Baek, S. (2012). Damnacanthal, a noni component, exhibits antitumorigenic activity in human colorectal cancer cells. *Journal of Nutritional Biochemistry*, 23, 915-923.

Parkin, M. B., Ferlay, J., & Pisani, P. (2005). Global Cancer Statistics. *CA: A Journal for Clinicians*.

Pawlus, A., Su, B., Keller, W., & Kinghorn, A. (2005). An anthraquinone with quinone reductase-inducing activity and other constituents of the fruits of *Morinda citrifolia* (noni). *Journal of Pharmacy and Pharmacology*, 68(12), 1720-2.

Pezzuto, J. (1997). Plant-derived anticancer agents. *Biochemical Pharmacology*, 53, 121-33.

Philippine Statistics Authority. (2016). Deaths in the Philippines. Retrieved from psa.gov.ph: https://psa.gov.ph/content/deaths-philippines-2016

Pilkington, K. (2017). *Noni (Morinda citrifolia)*. Retrieved from CAM Cancer and Alternative Medicine for Cancer: http://cam-cancer.com/Site-Summaries/Herbal-products/Noni/(merge)

Potterat, O. & Hamburger, M. (2007). *Morinda citrifolia* (Noni) fruit—phytochemistry, pharmacology, safety. *Planta Medica*, 73(3), 191-9.

Rang, H. P., Dale, M. M., & Ritter, J. M. (2001). *Pharmacology, 4th edition*. Churchill living stone, London.

Ravindranath, M.H., Saravanan, T.S., Monteclaro, C.C., Presser, N., Ye, X., Selvan, S.R., Brosman, S. (2006). Epicatechins purified from green tea (Camellia sinensis) differentially suppress growth of gender-dependent human cancer cell lines. *Evidence-Based Complementary and Alternative Medicine*, 3:237-24[illegible]

Saha, A., Kuzuhara, T., & Echigo, N. (2010). New role of (-)epicatechin in enhancing the induction of growth inhibition and apoptosis in human lung cancer cells by curcumin. *Cancer Prevention Research,* 3, 953–962.

Saif, M., & Chu, E. (2010). Biology of colorectal cancer. *The Cancer Journal,* 16(3), 196-201.

Santos, M. A., & Elisabetsky, E. (1999). Ethnopharmacology as a tool for the selection of medicinal plants for screening antitumour activity,. *Revista Brasileira de Plantas Medicinais,* 2(1), 7-17.

Sausville, E. (1997). *Targeted toxins. In: Bertinoo, J. (Ed.), Encyclopedia* (3). Academic Press, San Diego.

Senawong, T., Somprasong, K., Misuna, S., Komaikul, J., Senawong, G., Wongphakham, P., & Yunchalard, S. (2014). Phenolic acid composition and anticancer activity against human cancer cell lines of the comercially available fermentation products of Houttuynia cordata. *Science Asia,* 40, 420-427.

Sharma, A., Chakravarti, B., Gupt, M., Siddiqui, J., Konwar, R., & Tripathi, R. (2010). Synthesis and anti-breast cancer activity of biphenyl based chalcones. *Bioorganic and Medicinal Chemistry,* 17, 4711-4720.

Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010). Various types and management of breast cancer: An overview. *Journal of Advanced Pharmaceutical Technology & Research,* 1(2), 109-126.

Sharma, K., Pachauri, S. D., Khandelwal, K., Ahmad, H., Arya, A., Biala, P., Dwivedi, A. K. (2015). Anticancer effects of extracts from the fruit of *Morinda citrifolia* (noni) in breast cancer cell lines. *Drug research,* 66.

Soladoye, M., Amusa, N., Roji-Esan, N., & Taiwo, A. (2010). Ethnobotanical survey of anti-cancer plants in Ogun State, Nigeria. *Annals of Biological Research,* 1(4), 261-273.

Suffness, M. & Pezzuto, J.M. (1990). Assays related to cancer drug discovery. *Methods in Plant Biochemistry: Assays for Bioactivity,* 6, 71–133.

Sunder, J., Kundu, A., & Sujatha, T. (2016). Effect of *Morinda citrifolia* in growth, production and immunomodulatory properties in livestock and poultry: a review. *Journal of Experimental Biology and Agricultural Sciences,* 3.

Tan, M., Tan, C., & H.C.W. (2013). Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems. *International Food Research Journal,* 20, 3117-3123.;

Thani, W., Vallisuta, O., Siripong, P., & Ruangwises, N. (2010). Anti-proliferative and anti-oxidative activities of Thai noni/Yor (*Morinda citrifolia* Linn.) leaf extract. *Southeast Asian Journal Trophical Medicine Public Health,* 41, 482-489.

Verdadero, N. (2018). National Consciousness Week: A snapshot of cases in the Philippines. Health, Health Issues. *Modern Filipina.*

Wang, C., Ni, M., Wu, R., Aung, H., Xie, J., Tong, R., Yuan, C. (2007). Red American ginseng: ginsenoside constituents and antiproliferative activities of heat-processed Panax quinquefolius roots. *Planta Medica,* 73, 669-674.

Wang, C., Zhang, B., Song, W., Wang, A., Ni, M., Luo, X., Yuan, C. (2006). Steamed American ginseng berry: ginsenoside analyses and anticancer activities. *Journal of Agricultural and Food Chemistry,* 54, 9936-9942.

Wang, M., & Su, C. (2001). Cancer preventive effect of *Morinda citrifolia* (Noni). *Annals of the New York Academy of Sciences,* 952, 161-168.

Wang, M., West, B., Jensen, C., Nowicki, D., Su, C., Palu, A., & Anderson, G. (2002). *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. *Acta Pharmacologica Sinica,* 23(12), 1127-1141.

Westendorf, J., Effenberger, K., Iznaguen, H., & Basa, S. (2007). 2007. Toxicological and analytical investigations of noni (*Morinda citrifolia*) fruit juice. *Journal of Agricultural Food Chemistry,* 55, 529–537.

World Cancer Research Fund. (2017). The latest evidence on lifestyle and breast cancer. Retrieved from www.wcrf.org: https://www.wcrf.org/informed/articles/latest-evidence-lifestyle-and-breast-cancer

You, L., An, R., Liang, K., & Wang, X. (2013). Anti-Breast cancer agents from Chinese herbal medicines. *Mini-Reviews in Medicinal Chemistry,* 13, 101-105.

Young, M., Wakefield, M., Smyth, G., & Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biology.*

Zenk, M., El-Shagi, H., & Schulte, U. (1975). Anthraquinone production by cell suspension cultures of *Morinda citrifolia. Planta Medica,* 79-101.

Zin, M., Zamzahaila, Hamid, A., Azizah, & Osman, A. (2002). Antioxidative activity of extracts from mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chemistry,* 78, 227-231.