INHIBITORY ACTIVITY OF TANNIN FROM *Mimosa pudica* ON *Echinochloa crus-galli*

LORIE ANN R. ANDA

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ACCEPTANCE SHEET

This undergraduate thesis entitled "**INHIBITORY ACTIVITY OF TANNIN FROM *Mimosa pudica* ON *Echinochloa crus-galli***" prepared and submitted by **LORIE ANN R. ANDA** in partial fulfilment of the requirements for the degree of **BACHELOR OF SCIENCE IN CHEMISTRY,** is hereby accepted.

ROSALIE R. RAFAEL, Ph.D.
Adviser
6/15/18
Date Signed

MARY JANE T. DELA CRUZ, M.Sc.
Critic
06/14/18
Date Signed

REDEL L. GUTIERREZ, Ph.D.
Department Research Coordinator
6/15/18
Date Signed

Accepted as partial fulfilment of the requirements for the degree of **BACHELOR OF SCIENCE IN CHEMISTRY**

DANILA S. PARAGAS, M.Sc.
Chair, Department of Chemistry
6/18/2018
Date

RICH MILTON R. DULAY, M.Sc.
College Research Coordinator
6/18/18
Date

EVARISTO A. ABELLA, Ph.D.
Dean, College of Arts and Sciences
6/18/18
Date

BIOGRAPHICAL SKETCH

On August 14, 1996 at Gapan City District Hospital, a first born child named Lorie Ann Ramirez Anda was born. At a young age of 3, she was able to start her education as a Day Care student at Bagong Sikat, Gapan City, Nueva Ecija. She graduated 2nd Honorable Mention after finishing elementary at Divina Pastora College located at San Vicente, Gapan City, Nueva Ecija in the year 2009. By the year 2013, she completed her secondary education as a Special Science Class student at Juan R. Liwag Memorial High School in Bayanihan, Gapan City, Nueva Ecija and entered college at Central Luzon State University, Science City of Muñoz, Nueva Ecija taking up Bachelor of Science in Chemistry. With hardwork and perseverance, she was able to reach the Gross Percentage Average of a College Scholar in her first year followed by being a Dean's Lister in the succeeding year.

She was awarded scholarships from Department of Science and Technology Science Education Institute (DOST-SEI) for a three and a half year contract and a one year from Commission on Higher Education Tulong Dunong Scholarship. As a participant of DOST-SEI III Scholars' Summit held in Maimpis, City of San Fernando, Pampanga, gave her the opportunity to become the Vice Chairman of an organization of Department of Science and Technology Association of DOST Scholars (DOST ADS) Region III on the first year of its establishment. She had attended National DOST Scholar Summit held in Food Nutrition Research Institute, DOST Compound, Taguig City, Metro Manila. For the current year she was a member of the Central Luzon State University Association of DOST Scholar holding the position of Treasurer.

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ABSTRACT

ANDA, LORIE ANN R., Department of Chemistry, College of Arts and Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **JUNE 2018, INHIBITORY ACTIVITY OF TANNIN FROM *Mimosa pudica* ON *Echinochloa crus-galli.***

Adviser: ROSALIE R. RAFAEL, Ph. D.

Background: The use of allelopathy in controlling weeds has been a potential alternative as eco-friendly weed management. This study isolated tannin from *Mimosa pudica* and determined the inhibitory activity against *Echinochloa crus-galli.* **Methods:** Extraction of dried *Mimosa pudica* was done using 85% ethanol. Isolated tannin was obtained using Thin Layer Chromatography. Isolated compound was analysed through Ultraviolet-Visible and Fourier Transform Infrared (FTIR) Spectroscopy. Inhibitory activity of the tannin on the seed germination and seedling growth of *Echinochloa crus-galli* was evaluated. **Results:** The crude extract and tannin from *Mimosa pudica* inhibited the seed germination and seedling growth of *Echinochloa crus-galli* in all the concentrations used (1-10 ppm). All the tannin concentrations exhibited similar effects on the seed germination and seedling growth of *Echinochloa crus-galli.* The tannin isolated has absorption maximum at 250 nm in the UV-Vis spectroscopy and contain the functional groups, hydroxyl, substituted aromatic ring and an ester group through FTIR spectroscopy analysis. From the spectra gathered, the tannin could be a potential ellagitannin. **Conclusion:** The potential ellagitannin from *Mimosa pudica* had inhibitory activity on the seed germination and seedling growth of *Echinochloa crus-galli. Mimosa pudica* had weedicide property.

***Keywords*:** allelopathy, *Echinochloa crus-galli, Mimosa pudica,* ellagitannin

LITERATURE CITED

Abdollahzadeh, S. H., Mashouf, R. Y., Mortazavi, H., Moghaddam, M. H., Roozbahani, & Vahedi, M. (2011). Antibacterial and antifungal activities of *Punica granatum* peel extracts against oral pathogens, *Journal of Dentistry, 8(1),* 1-6.

Abhilasha, D., Quitana, N. & Joshi J. (2008). Do allelopathic compounds in invasive *Solidago Canadensis* restrain the native European flora. *Journal of Ecology, 96,* 993-1001.

Abu-Romman S,, Shatnawi M., & Shibli R. (2010). Allelopathic effects of spurge (*Euphorbia hierosolyminata*) on wheat (*Triticum durum*). AEJAES 7(3), 298-302.

Ahmad, I. & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology, 74,* 113-123.

Angayarkanni, J., Ramkumar, K. M., Poormina, T. & Priyadarshini, U. (2007). Cytotoxic activity of *Amorphophallus paeoniifolius* tuber extracts *in vitro. American-Eurasian Journal of Agricultural & Environmental Sciences, 2,* 395-398.

Arafat, Y., Khalid, S., Lin, W., Fang, C., Sehrish, S., Ali, N. & Azeem, S. (2015). Allelopathic evaluation of selected plants extract against broad and narrow leaves weeds and their associated crops. *Academia Journal of Agricultural Research, 3(10),* 226-234.

Ashrafi, Z. Y., Sadeghi, S. & Mashhadi, H. R. (2007). Allelopathic Effects of barley (*Hordeum vulgare)* on germination and growth of Wild Barley (*H. spontaneum).* *Pakistan Journal of Weed Research, 13(1-2),* 99-112.

Bray E. A. (1993). Molecular Responses to Water Deficit. *Plant Physiology, 103(4),* 1035–1040.

Chaudhuri, A. & Ray, S. (2016). Allelopathic potential of tannic acid and its equivalent phenolics extracted from aerial parts of *Ampelocissus latifolia* (Roxb.) Planch. *IOSR Journal of Agriculture and Veterinary Science, 9(7),* 90-100.

Chauhan B. S. & Johnson D. E. (2011). Ecological studies on *Echinochloa crus-galli* and the implications for weed management in direct-seeded rice. *Crop Protection, 30,* 1385–1391.

Chou, C. H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture. *Critical Reviews in Plant Sciences, 18,* 609-636.

Chung, I. M. & Miller, D. A. (1995). Natural herbicide potential of alfalfa residues on selected weed species. *Agronomy Journal, 87,* 920-925.

Duke, S. O., Rimando, A. M., Baerson, S. R., Scheffler, E.O. & Belz R. G. (2002). Strategies for the Use of Natural Products for Weed Management. *Journal of Pesticide Science, 27*, 298-306.

El-Rokiek, Kowthar, G., Rafat, R. El-Masry, Messiha K. N. & Ahmed, S. A. (2010). The Allelopathic Effect of Mango Leaves on the Growth and Propagative Capacity of Purple Nutsedge (*Cyperus rotundus* L.). *Journal of American Science, 6(9),* 151-159.

Escudero, A., Albert, M. J., Pita, J. M, & Garcia, F. P. (2000). Inhibitory effects of *Artemisia herbaalba* on the germination of the gypsophyte *Helianthemumsquamatum. Plant Ecology, 148*, 71-80.

Fachinetto J.M., Bagatini M.D., Durigon J., Silva A.C.F., & Tedesco S.B. (2007). Efeito anti-proliferativo das infusões de *Achyrocline satureioides* DC (Asteraceae) sobre o ciclo celular de *Allium cepa. Revista Brasileira de Farmacognosia (Brazilian Journal of Pharmacognosy) 17*: 49-54.

Fang, B. Z., Yu, S. X., Wang, Y. F., Qiu X., Cai, C. X., & Liu, S. P. (2009). Allelopathic effects of *Eucalyptus urophylla* on ten tree species in South China. *Agroforestry Systems, 76*, 401-408.

Ferguson, J. J., Rathinasabapathi, B. & Chase A. C. (2003). Allelopathy: How Plants Suppress Other Plants. *Horticulural Science Department, UF/IFAS Extension,* Document: HS944, para. 4, p. 1.

Gibson, L. R. & Liebman, M. (2003). A laboratory exercise for teaching plant interference and relative growth rate concepts. *Weed Techonology, 17*, 394-402.

Gunawardhana, C. B., Ranasinghe S. J., & Waisundara V. Y. (2015) Review: *Mimosa pudica* Linn.: the garden weed with therapeutic properties. *Israel Journal of Plant Sciences,* 1-8.

Hussein, H. F. (2001). Estimation of critical period of crop-weed competition and nutrient removal by weeds in onion (*Allium cepa* L.) in sandy soil. *Egypt Journal of Agronomy, 24,* 43-62.

Jafari, L., Ghadiri, H., & Moradshahi, A. (2011). Allelopathic Potential of Rice (*Oryza sativa* L.) Cultivars on Barnyard Grass (*Echinochloa crus-galli). Journal of Agricultural Science and Technology 1*, 853-864.

Kaur, P., Kumar, N., Shivananda, T. N. & Kaur, G. (2011). Phytochemical screening and antimicrobial activity of the plant extract of *Mimosa pudica* L. against selected microbes. *Journal of Medical Plants Research. 5,* 5356-5359.

Kiemnec, G. L. & McInnis, M. L. (2002). Hoary cress (*Cardaria draba)* root extract germination and root growth of five plant species. *Weed Technology, 16*, 231-234.

Klewicka, E., Sojka, M., Klewicki, R., Kolodziejczyk, K. Lipinska, L. & Nowak, A. (2016). Ellagitannins from Raspberry (*Rubus idaeus* L.) Fruit as Natural Inhibitors of *Geotrichum candidum. Molecules, 21,* 908.

Krishnaraju, A. V., Rao, T. V. N., Sundararaju, S., Vanisree, M., Tsay, H. S., Gottumukkala, V. & Subbraju, G. (2006). Use of Medical plants in Aurveda. *International Journal of Applied Science and Engineering Research, 4,* 115-125

Meksawat, S. & Pornprom T. (2010). Allelopathic effect of itchgrass (*Rottboellia cochinchinesis*) on seed germination and plant growth. *Weed Biology and Management, 10,* 16-24.

Miolanen, J., Sinkkonen, J., & Sakminen J.P. (2013). Characterization of Bioactive Plant Ellagitannins by Chromatographic. *Chemoecology, 23(3)*, 165-179.

Mole, S. & P.G. Waterman. 1987. Tannins as Antifeedants to Mammalian Herbivores: Still An Open Question? In: *Allelochemicals: Role In Agriculture And Forestry* (Eds.): G.R. Waller, 572-587.

Muscolo A., Pauccio M. R., & Sidari M. (2001). The effects of phenolic acids extracted from two different solis in Aspromonte on germination of Pinus laricio seeds. *FEBS Journal, 10,* 659-663.

Muthukumar, G. (1980). Effect of Tannins on Soil Microorganisms and Crop; Ph. D. Thesis, University of Madras, Madras, p. 178.

Nam, S., Smith, D. M. & Dou, Q. P. (2001). Tannin acid potently inhibits tumor cell proteasome activity, increases p27 and Bax expression, and induces G1 arrest and apoptosis, *Cancer Epidemiology, Biomarkers and Prevention, 10,* 1083-1088.

Oerke, E. C. (2006). Crop losses to pest. *Journal of Agricultural Sciences, 144,* 31-43.

Ohno, T. 2001. Oxidation of phenolic acid derivatives by soil and its relevance to allelopathic activity. *J. Environ. Qual.,* 30: 1631-1635.

Peres, R. S., Cassel, E. & Azambuja, D. S. (2012). Black Wattle Tannin As Steel Corrosion Inhibitor. *International Scholarly Research Network: ISRN Corrosion.* Article ID 937920.

Racadio, S. P., Molina, G. V. & Tacla, R. (2008). Phytochemical and Microbiological Testing of Makahiya (*Mimosa pudica* Linn.) Leaf Extract. *University of Northern Philippines Research Journal, 17,* 11-18.

Rafael, R. R., Espiritu A. P. N., & dela Cruz, M. J. T. (2017). Allelopathic Ellagitannin from *Annona muricata L.* (Guyabano) Leaf Extract against Rice Weed *Echinochloa crus-galli. International Journal of Agricultural Technology, 13(7.3),* 2597-2607.

Raja, P.B., Rahim, A.A., Qureshi, A.K. & Awang, K. (2014). Green Synthesis of silver nanoparticles using tannins. *Material Science-Poland, 32(3),* 409.

Ranjan, R. K., Kumar, M., Sathish, S. I. & Rao, M. R. K. (2013). Phytochemical analysis of leaves and roots of *Mimosa Pudica* collected from Kalingayaram, Tamil Nadu. *Journal of Chemical and Pharmaceutical Research, 5,* 53-55.

Reignose, M.J., Sanchez-Moreiras A., & Gonzalez, L. (1999). Ecophysiological approach in allelopathy. *Critical Reviews in Plant Sciences, 18,* 577-608.

Rice, E. L. (1984). Allelopathy, 2nd Edition, Academic Publishers, New York, 424.

Riedl, M. K., Carando, S., Alessio, H. M., McCarthy, M. & Hagerman A. E. (2002). Antioxidant Activity of Tannins and Tannin Protein Complexes: Assessment In Vitro and In Vivo. In Free radical in Food; Morello, M., et al.; ACS Symposium Series; American Chemical Society: Washington, DC. *14,* 188-200.

Sia, F. Y., Vejayan, J., Jamuna A., & Ambu S. (2011). Efficacy of tannins from *Mimosa pudica* and tannic acid in neutralizing cobra (*Naja kaouthia)* venom. *The Journal of Venomous Animals and Toxins including Tropical Diseases, 17(1),* 42-48.

Socrates, G. (2004) Infrared and Raman Characteristics Group Frequencies, Tables and Chart. 3a. ed., John Wiley & Sons, Inc., USA pp: 125-142.

Soni, A. & Sosa S. (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicainal plant extracts. *Journal of Pharmacognosy and Phytochemistry, 2(4),* 22-29.

Tabaglio, V., Gavazzi, C., Schulz, M. & Marocco A. (2008). Alternative weed control using the allelopathic effect of natural benzoxanoids from rye mulch. *Agronomy for Sustainable Development, Springer Verlag/ EDP Sciences/ INRA, 28(3),* 397-401.

Tsai F.Y., Lin C.C., & Kao C.H. (1997). A comparative study of the effects of abscisic acid and methyl jasmonate on seedling growth of rice. *Plant Growth Regulation, 21 (1),* 37–42.

Zeng, R. S., Mallik A. U. & Luo S. M. (2008). Allelopathy in Sustainable Agriculture and Forestry; Springer Science + Business media, LLC: New York, NY, USA.

Zhang, J., Yuan, K. Zhou, W., Zhou, J., & Yang, P. (2010). Studies on the active components and antioxidant activities of the extracts of *Mimosa pudica* Linn. from southern China. *Pharmacognosy Magazine, 7 (25),* 35-39.