

**NEEM LEAVES (*Azadirachta indica* A. Juss) CRUDE EXTRACT AGAINST
GUAVA FRUIT FLY (*Bactrocera dorsalis* Hendel)**

JOCELYN T. NEUDA

An Undergraduate Thesis Submitted to the Faculty of the Department of Environmental
Science, College of Arts and Sciences, Central Luzon State University,
Science City of Muñoz, Nueva Ecija, Philippines
in Partial Fulfilment of the requirements
for the Degree of

**BACHELOR OF SCIENCE IN ENVIRONMENTAL SCIENCE
(Environmental Chemistry)**

FEBRUARY 2020

ACCEPTANCE SHEET

This undergraduate thesis entitled “**NEEM LEAVES (*Azadirachta indica* A. Juss) CRUDE EXTRACT AGAINST GUAVA FRUIT FLY (*Bactrocera dorsalis* Hendel),**” prepared and submitted by **JOCELYN T. NEUDA**, in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN ENVIRONMENTAL SCIENCE (ENVIRONMENTAL CHEMISTRY)**, is hereby accepted:


ROSALIE R. RAFAEL, Ph.D.
Adviser

Jan. 21, 2020
Date Signed


DANILO S. PARAGAS, M.Sc.
Critic

1/21/2020
Date Signed


LUZVIMINDA S. QUITO, Ph.D.
Department Research Coordinator

1/21/2020
Date Signed


Accepted as partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN ENVIRONMENTAL SCIENCE (ENVIRONMENTAL CHEMISTRY)**:


SHARON E. LAZARO, Ph.D.
Chair, Department of Environmental Science

Jan. 21, 2020
Date Signed


KRISTINE GRACE D. WANG, M.Sc.
College Research Coordinator

1/22/2020
Date Signed


EVARISTO A. ABELLA, Ph.D.
Dean, College of Arts and Sciences

1/22/2020
Date Signed

BIOGRAPHICAL SKETCH

On the 9th day of September 1999 the author, JOCELYN T. NEUDA was born in Bibiclat, Aliaga, Nueva Ecija. She is the third eldest among five children of Mr. Orlando V. Neuda and Mrs. Josephine T. Neuda.

She finished her Elementary Education at Bibiclat Elementary School. She graduated her Secondary Education in Central Luzon State University Laboratory High School- Bibiclat with honor. Now, she is studying at Central Luzon State University taking Bachelor of Science in Environmental Science major in Environmental Chemistry.

She participated during the symposium entitled “Role of Biologists in the Environmental Impact Assessment and Management Projects held at the University Auditorium, Central Luzon State University on November 22, 2016. She attended a seminar on Environmental Awareness with the theme “Kilos Para sa Kalikasan: Building Resilience Philippines” on November 15, 2017 held at the College of Arts and Sciences Little Theater. These experiences provided her a valuable experience and insight in the awareness towards the environment, considering the concerns and issues. She was a recipient of the scholarship Iskolar ng Bayan during second semester of the school year 2016-2017 and whole semester of the school year 2017-2018.

ACKNOWLEDGMENT

The author would like to take this opportunity to express her deepest gratitude and appreciation to all the people who helped and contributed their efforts. The author would like to thank the following persons for the successful fulfillment of this study.

First and foremost, she would like to thank our Almighty God, for the good health, unconditional love, guidance and strength to overcome all the trials and achieve her dreams.

She would like to express a sincere gratitude to her thoughtful adviser and critic, Dr. Rosalie R. Rafael, and Prof. Danila S. Paragas for the continuous support, patience, guidance, life advices, motivation and great knowledge. Also, for cheering up that she can do it.

To Dr. Luzviminda S. Quitos, for all the knowledge that you clarify and lessons for the achievement and success of the study.

To Dr. Marilyn G. Patricio, for sharing her knowledge, guidance and motivation.

To her beloved parents, Mr. Orlando V. Neuda and Mrs. Josephine T. Neuda for their endless love, sacrifices, financial and moral support, patience that made her to pursue her dreams and overcome the hardship of life.

To her sister Jemalyn, for her nonstop encouragements, attention, support and understanding. To the author's friends, Ate Mey, Ate V, Ate Cz, and Ate Nikki who showed concern, support and for the love and understanding given.

Finally, to the persons whose name were not mentioned but helped in special ways. Special thanks and gratitude to all of you, so much appreciation is extended to you.

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF APPENDICES	ix
LIST OF APPENDIX TABLES	x
LIST OF APPENDIX FIGURES	xii
ABSTRACT	xiii
INTRODUCTION	1
Background of the Study	1
Objectives of the Study	3
Significance of the Study	3
Scope and Limitation of the Study	4
Time and Place of the Study	4
REVIEW OF RELATED LITERATURE	5
Neem (<i>Azadirachta indica</i> A. Juss)	5
Botanical Description	5
Chemical Composition and Constituents	6
Biological Activity, Application and Uses	6
Chemistry of Natural Products	7
Phenolic Metabolites	7
Gallic Acid	8
Thin Layer Chromatography	8
Folin- Ciocalteu or Total Phenolic Assay	9
Guava Fruit fly (<i>Bactrocera dorsalis</i> Hendel)	9
METHODOLOGY	12
Chemicals and Reagents	12

Plant Material	12
Extract Preparation	12
Thin Layer Chromatography	13
Photochemical Screening	13
Determination of Total Phenolic Content	14
Adulticidal Assay	15
Larvicidal Assay	16
Collection of Ripe Guava Fruits	16
Preparation of Soil and Extract	17
Bioassay and Treatment	17
RESULTS AND DISCUSSION	18
Description of the Crude Extracts	18
Thin Layer Chromatographic Screening of the Crude Extracts	18
Total Phenolic Content (TPC) Assay	21
Adulticidal Assay	21
Larvicidal Assay	24
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS	26
Summary	26
Conclusions	27
Recommendations	27
LITERATURE CITED	28
APPENDICES	33

LIST OF TABLES

TABLE		PAGE
1	Solvent system for Thin Layer Chromatography	13
2	List of plant constituents, their visualizing agents and indication of a positive test	14
3	Percent Mortality of Trial 1 after 24 hours	22
4	Percent Mortality of Trial 2 after 24 hours	22
5	Percent Mortality of Trial 3 after 24 hours	23

LIST OF FIGURES

FIGURE		PAGE
1	Female fruit fly laying eggs to the ripe guava fruit	11
2	Trapped fruit flies to the alternative bait	15
3	Adulticidal assay set-up	16
4	<i>Azadirachta indica</i> A. Juss crude extract	18
5	<i>Azadirachta indica</i> A. Juss chromatogram	19
6	(a) larvae after 2-3 days and (b) emergence of pupa after 15 days	24
7	Total emergence of pupa after 15 days days and living fruit flies after 15 days	25

LIST OF APPENDICES

APPENDIX		PAGE
I	Schematic Diagram of the Methodology	34
II	Plant Sample	35
III	Regression Line of Gallic Acid with Folin-ciocalteu Reagent	36
IV	Statistical Analysis	37
V	Phytochemical Screening using Thin Layer Chromatography	44
VI	Larvicidal Assay	45
VII	Effect of Neem Extracts to Fruit fly	46
VIII	Effect of Different Concentrations of Neem to Fruit fly Pupa	47
IX	Certificate of Authentication of Plant Samples	49

LIST OF APPENDIX TABLES

APPENDIX TABLE		PAGE
1	Statistical Analysis for Trial 1 (3 hours)	37
2	Statistical Analysis for Trial 1 (6 hours)	37
3	Statistical Analysis for Trial 1 (9 hours)	37
4	Statistical Analysis for Trial 1 (12 hours)	38
5	Statistical Analysis for Trial 1 (15 hours)	38
6	Statistical Analysis for Trial 1 (18 hours)	38
7	Statistical Analysis for Trial 1 (21 hours)	38
8	Statistical Analysis for Trial 1 (24 hours)	39
9	Statistical Analysis for Trial 2 (3 hours)	39
10	Statistical Analysis for Trial 2 (6 hours)	39
11	Statistical Analysis for Trial 2 (9 hours)	39
12	Statistical Analysis for Trial 2 (12 hours)	40
13	Statistical Analysis for Trial 2 (15 hours)	40
14	Statistical Analysis for Trial 2 (18 hours)	40
15	Statistical Analysis for Trial 2 (21 hours)	40
16	Statistical Analysis for Trial 2 (24 hours)	41
17	Statistical Analysis for Trial 3 (3 hours)	41
18	Statistical Analysis for Trial 3 (6 hours)	41
19	Statistical Analysis for Trial 3 (9 hours)	41
20	Statistical Analysis for Trial 3 (12 hours)	42

21	Statistical Analysis for Trial 3 (15 hours)	42
22	Statistical Analysis for Trial 3 (18 hours)	42
23	Statistical Analysis for Trial 3 (21 hours)	42
24	Statistical Analysis for Trial 3 (24 hours)	43

LIST OF APPENDIX FIGURES

APPENDIX FIGURE		PAGE
1	Calibration curve of gallic acid for total phenolic content (TPC) using folin-ciocalteu method	36
2	TLC plate sprayed with (a) SbCl_3 , (b) $\text{K}_3\text{Fe}(\text{CN})_6\text{-FeCl}_3$, (c) Vanillin- H_2SO_4 , (d) methanolic KOH	44
3	Total emergence of pupa in different concentrations	45
4	Empty stomach of fruit fly	46
5	Effect of neem extracts to fruit fly pupa in control, 1.25% and 2.5% concentration	47
6	Effect of neem extracts to fruit fly pupa in 5%, 7.5% and 10% concentration	48
7	Certificate of authentication of plant samples	49

ABSTRACT

NEUDA, JOCELYN T., Department of Environmental Science, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **February 2020**, **NEEM LEAVES (*Azadirachta indica* A. Juss) CRUDE EXTRACT AGAINST GUAVA FRUIT FLY (*Bactrocera dorsalis* Hendel)**

Adviser: ROSALIE R. RAFAEL, Ph.D.

Use of synthetic pesticides to control fruit flies in farming could be hazardous to human health and damage to the environment. An alternative to these synthetic agents are natural products that can be derived from various plant sources such as the neem tree leaves. Thus, this study was conducted to determine the total phenolic content of the leaves of neem (*Azadirachta indica* A. Juss) and test the adulticidal and larvicidal activity of the crude extract against guava fruit fly (*Bactrocera dorsalis* Hendel). Leaves of *Azadirachta indica* were extracted with 95% ethanol. Thin layer chromatography screening was conducted to identify the phytochemicals in the crude extract. Total phenolic content (TPC) was measured using the Folin-Ciocalteu method. Phenolic compound such as flavonoids, tannins and phenols, triterpenes and anthraquinones are found present in the neem leaf crude extract. The total phenolic content of *Azadirachta indica* leaf crude extract was 152.47 mg gallic acid equivalents (GAE)/g. Table 5 shows the percent mortality of Trial 3. Lowest percent mortality was recorded in the control after 24 hours with 53.3% in adulticidal assay. The treatments (1.25, 2.5, 5.0, 7.5, and 10%) neem crude extract seems to be more effective than the control after 24 hours. Emergence of pupa in 10% and 7.5% concentration was low compared to the control. Emergence of living fruit flies also affected by the concentration of neem. Higher concentrations (10%, 7.5% and 5%) has no

emergence of living fruit flies. The bioactivity can be attributed to the phytochemical components identified. *Azadirachta indica* exhibit adulticidal and larvicidal property against guava fruit flies and can be used as alternative plant-based pesticides.

Keywords: *Azadirachta indica* A. Juss leaves; Total Phenolic Content; Thin Layer Chromatography; Larvicidal and adulticidal activity

LITERATURE CITED

- Abarca, N. A., Alvarado, E. D., Reyes, J. A., Soto, J. U., & Valdez, L. G. (2013). The phenols of the genus *Agave* (Agavaceae). *Journal of Biomaterials and Nanobiotechnology*, 4, 9-16.
- Abubakar, N., Shehu, K., Yahaya, M. M., Tafinta, I. Y., & Imonikhe, M. A. (2016). Phytochemical screening and thin layer chromatographic studies of *Guiera senegalensis* G. F Gmel (Egyptian mimosa). *Annals of Biological Sciences*, 4(1), 26-30.
- Al-jadidi, H. S. K., & Hossain, M. A. (2015). Studies on total phenolics, total flavonoids and antimicrobial activity from the leaves crude extracts of neem traditionally used for the treatment of cough and nausea. *Beni-Suef University Journal of Basic and Applied Sciences*, 4, 93-98.
- Alvarenga, C. D., França, W. M., Giustolin, T. A., Paranhos, B. A. J., Lopes, G. N., Cruz, P.L., & Barbosa, P. R. R. (2012). Toxicity of neem (*Azadirachta indica*) seed cake to larvae of the mediterranean fruit fly, *Ceratitidis capitata* (Diptera: Tephritidae), and its parasitoid, *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *Florida Entomologist*, 95(1), 57-62. doi.org/10.1653/024.095.0110
- Amur, A., Memon, N., Shah, M. A., Ansari, A., Solangi, D. A., & Mal, B. (2017). Biology and morphometric of different life stages of the oriental fruit fly (*Bactrocera dorsalis* Hendel) (Diptera: Tephritidae) on three varieties of mango of Sindh, Pakistan. *The Journal of Animal & Plant Sciences*, 27(5), 1711-1718.
- Awad, A. A., Ali, N. A., & Mohamed, H. O. (2014). Ultrastructure of the antennal sensillae of male and female peach fruit fly *Bactrocera zonata*. *Journal of Insect Science*, 14(45), 1-15. doi.org/10.1673/031.014.45
- Chauhan, P., Shivakumar, M. S., Muthusamy, R., & Kumar, D. (2011). Larvicidal activity of solvent leaf extracts of *Cassia fistula* (Linn.) and *Clerodendron inerme* (Gaertn) on the *Spodoptera litura* (Insecta: Noctuidae): a potential alternative. *Journal of Ecobiotechnology*, 3(7), 1-4.
- Chewe, L. A. (2015). *Effect of aqueous neem (Azadirachta indica A. Juss) leaf extract on selected soil chemical and biological properties*, Unpublished master's thesis, University of Zambia, Lusaka, Zambia.
- Dash, S. P., Dixit, S., & Sahoo, S. (2017). Phytochemical and biochemical characterizations from leaf extracts from *Azadirachta indica*: an important medicinal plant. *Biochemistry & Analytical Biochemistry*, 6(2), 1-4. doi.org/10.4172/2161-1009.1000323

- Eid, A., Jaradat, N., & Elmarzugi, N. (2017). A review of chemical constituents and traditional usage of neem plant (*Azadirachta indica*). *Palestinian Medical and Pharmaceutical Journal*, 2(2), 75-81.
- El-Aw, M. A. M., Draz, K. A. A., Hashem, A. G., & El-Grendy, I. R. (2008). Mortality comparison among spinosad-, actara-, malathion-, and methomyl-containing baits against peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) under laboratory conditions. *Journal of Applied Science and Research*, 4(2), 216-223.
- Giongo, A. M. M., Vendramim, J. D., & Forim, M. R. (2016). Evaluation of neem-based nanoformulations as alternative to control fall armyworm. *Ciência e Agrotecnologia*, 40(1), 26-36. doi.org/10.1590/S1413-70542016000100002
- Guevara, B. Q. (Ed.). (2005). *A guidebook to plant screening: phytochemical and biological*. España, Manila: University of Santo Tomas Publishing House.
- Gujjeti, R. P., & Mamidala, E. (2013). Phytochemical screening and thin layer chromatographic studies of *Aerva lanata* root extract. *International Journal of Innovative Research in Science, Engineering and Technology*, 2(10), 5725-5730.
- Hashmat, I., Azad, H., & Ahmed, A. (2012). Neem (*Azadirachta indica* A. Juss)- a nature's drugstore: an overview. *International Research Journal of Biological Sciences*, 1(6), 76-79.
- Hossain, D., Sarwar, S., Dewan, S. M. R., Hossain, S., Shadid-Ud-Daula, A. F. M., & Islam, M. S. (2014). Investigation of total phenolic content and antioxidant activities of *Azadirachta indica* roots. *Avicenna Journal of Phytomedicine*, 4(2), 97-102.
- Heve, W. K., El-Borai, F. E., Carrillo, D., & Duncan, L. W. (2016). Biological control potential of entomopathogenic nematodes for management of caribbean fruit fly, *Anastrepha suspensa* Loew (Tephritidae). *Pest Management Science*, 73, 1220-1228. doi.org/10.1002/ps.4447
- Hussein, A. S. M. (1984). Effect of the galloyl group on the molluscicidal activity of tannins. *Fitoterapia*, 5, 343-345.
- John, B., Sulaiman, C. T., George, S., & Reddy, V. R. K. (2014). Total phenolics and flavonoids in selected medicinal plants from Kerala. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(1), 406-408.
- Johnson, R. (2007). Identification of leaf pigments. *Colby Journal of Research Methods*, 9, 8-10.

- Karamaæ, M., Kosiñska, A., & Pegg, R. B. (2006). Content of gallic acid in selected plant extracts. *Polish Journal of Food and Nutrition Sciences*, 15(1), 55-58.
- Khan, M., Hossain, M. A., & Islam, M. S. (2007). Effect of neem leaf dust and commercial formulation of a neem compound on the longevity, fecundity and ovarian development of melon fly, *Bactrocera cucurbitae* (Coquillett) an oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Pakistan Journal of Biological Sciences*, 10(20), 3656-3661. doi.org/10-3923/pjbs.2007.3656.3661
- Khan, S., Ahmad, R., Shah, M. M., & Haq, I. U. (2016). The insecticidal potential of botanical extracts for management of peach fruit fly *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae). *Turkish Journal of Entomology*, 40(4), 445-453. doi.org/10.16970/ted.47318
- Mahmoud, D. A., Hassanein, N. M., Youssef, K. A., & Abou Zeid, M. A. (2011). Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. *Brazilian Journal of Microbiology*, 42, 1007-1016.
- Mard, S. A., Mojadami, S., Farbood, Y., & Naseri, M. K. G. (2015). The anti-inflammatory and anti-apoptotic effects of gallic acid against mucosal inflammation and erosions-induced by gastric ischemia-reperfusion in rats. *Veterinary Research Forum*, 6(4), 305-311.
- Murugesan, A. (2001). Neem tree- a review. *The Indian Forester*, 127(12), 1336-1342.
- Naik, H. S., Jagadeesh, K. S., & Basavaraju, B. S. (2017). Biology and biometrics of oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on custard apple, *Annona squamosa* L. *International Journal of Current Microbiology and Applied Sciences*, 6(12), 3859-3864. doi.org/10.20546/ijcmas.2017.612.445
- Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Anthony, S. (2009). Agroforestry database: a tree reference and selection guide version 4.0. Retrieved from <http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>
- Pandey, G., Verma, K. K., & Singh, M. (2014). Evaluation of phytochemical, antibacterial and free radical scavenging properties of *Azadirachta indica* (neem) leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(2), 444-447.
- Pankaj, S., Lokeshwar, T., Mukesh, B., & Vishnu, B. (2011). Review on neem (*Azadirachta indica*): thousand problems one solution. *International Research Journal of Pharmacy*, 2(12), 97-102.

- Prasad, S. B., Kaur, N., & Chauhan, S. K. (2014). Fruit flies: biodiversity, species complex and economic importance. *Popular Kheti*, 2(2), 137-140.
- Prashanth, G. K., & Krishnaiah, G. M. (2014). Chemical composition of the leaves of *Azadirachta indica* Linn (Neem). *International Journal of Advancement in Engineering Technology, Management and Applied Science*, 1(5), 21-31.
- Rashmi. M. A., Abraham, V., Shivashankar, S., Chakravarthy, A. K., Sumathi, M., & Subhash, K. (2017). Does change in tannin content in mango (*Mangifera indica*) fruits influence the extent of fruit fly (*Bactrocera dorsalis* Hendel) herbivory?. *Journal of Entomology and Zoology Studies*, 5(4), 381-385.
- Rispail, N., Morris, P., & Webb, K. J. (2005). Phenolic compounds: extraction and analysis. In Marquez A. J. (eds.) *Lotus Japonicus Handbook*. Springer, Dordrecht. doi.org/10.1007/1-4020-3735-X_34
- Sharma, R., & Sohal, S. K. (2013). Bioefficacy of quercetin against melon fruit fly. *Bulletin of Insectology*, 66(1), 79-83.
- Sharma, V. K., Tiwari, M., Chauhan, N. S., & Nema, R. K., (2012). Phytochemical investigation on the ethanolic extract on the leaves of *Zizyphus xylopyrus* (Retz.) Willd. *International Journal of Agronomy and Plant Production*, 3(1), 26-37.
- Stanković, M. S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac Journal of Science*, 33, 63-72.
- Tan, K. H., & Nishida, R. (2012). Methyl eugenol: its occurrence, distribution, and role in nature, especially in relation to insect behaviour and pollination. *Journal of Insect Science*, 12, 1-74.
- Tiwari, P., Joshi, A., & Dubey, B. K. (2017). Phytochemical screening and thin layer chromatographic studies of *Annona squamosa* (seeds), *Azadirachta indica* (leaves) and *Lavandula angustifolia* (flower) aqueous extract. *Asian Journal of Pharmaceutical Education and Research*, 1(6), 45-53.
- Vargas, R. I., Piñero, J. C., & Leblanc, L. (2015). An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the pacific region. *Insects*, 6, 297-318. doi.org/10.3390/insects6020297

- Verma, O. P., Poonam, S., & Kamin, A. (2019). Phytochemical screening of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) and *Phyllanthus emblica* (Amla). *International Journal of Current Microbiology and Applied Sciences*, 8(4), 682-686. doi.org/10.20546/ijcmas.2019.804.073
- Wani, M., Sharma, A., Deshpande, J., Mathew, S., & Khetmalas, M. B. (2013). Qualitative phytochemical screening of three indigenous medicinal plants. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 4(1), 287-291.
- Wei, D., Dou, W., Jiang, M., & Wang, J. (2017). Oriental fruit fly *Bactrocera dorsalis* (Hendel). *Biological Invasions and Its Management in China*, 267-283. doi.org/10.1007/978-94-024-0948-2_15
- Zivanov-curils, J., Tomin, J., Bojanic, V., Bojanic, Z., Najman, S., Katic, K., ... E., Dindic, B. (2004). Effect of chronic phenol intoxication on fertility and life span of *Drosophila melanogaster*. *Archive of Oncology*, 12, 17-18.