LARVICIDAL ACTIVITY OF *Vitex negundo Linn.* (LAGUNDI) AGAINST MOSQUITO LARVAE

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ABSTRACT

Mosquitoes have been a constant problem in the community as they continuously transmit serious diseases. Therefore, this study aimed to eliminate larvae of mosquitoes using *Vitex negundo Linn.* ethanol leaf extracts. This was done by comparing the span of time the larvae were exterminated upon exposure to treatments with three various ethanol leaf extract concentrations. The three concentrations of ethanol leaf extracts were 30%, 60%, and 90% with commercial insecticide as the positive control and the extracting solvent ethanol as the negative control. Three trials with three replicates with ten larvae each replicate were utilized in the study. The time (in minutes) of mortality was measured for each treatment application and the significance between the treatments was analyzed using the One-Way ANOVA and Tukey Test. Results showed a statistically significant difference between the 30% extract when compared to the 60% extract, 90% extract, positive control insecticide and control ethanol. The 60% extract, 90% extract and ethanol displayed a statistically insignificant difference when compared to the positive control. The *Vitex negundo Linn.* ethanol leaf extract therefore has larvicidal activity against mosquito larvae, though thorough processing is still necessary for assured efficiency.

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INTRODUCTION

1.1 The Problem and its Background

Mosquitoes are one of the most medically significant transmitters as they spread parasites and pathogens, which continue to have devastating effects on human beings (Maheswaran *et al.*, 2008). Mosquito-borne diseases such as malaria, filariasis, yellow fever and dengue cause extensive morbidity and mortality and are a major economic burden within disease-endemic countries (Sachs and Malaney, 2002; Boutayeb, 2006). Every year, about 300 million people are estimated to be affected by malaria, a major killer disease, which threatens 2,400 million (about 40%) of the world's population (Sharma, 1999; Snow *et al.*, 2005). Similarly, lymphatic filariasis caused by *Wuchereria bancrofti* affects about 106 million people worldwide. About 20 million people are infected every year by dengue viruses transmitted by *Aedes* mosquitoes with about 24,000 deaths (Poopathi *et al.*, 2010).

Current control is based on the use of commercial insecticides which have potential toxic effect on public health and the environment. Synthetic insecticides have been used during the past several decades to control varied dipteran pests (Poopathi *et al.*, 2010). Pesticides are indeed very effective in its use. But along with their useful effects, they also bring out serious harm to human health as well. Furthermore, these chemicals are expensive and are often toxic to both human and other animals and natural enemies (Cartilla and Dela Cruz, 2012). The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in a reduced control and often to a negative impact on various non-target organisms and on the environment in general (Charles and Nielsen-LeRoux, 2000).

However, the use of chemical insecticides has been greatly impeded due to development of physiological resistance in the insect intermediaries, environmental pollution resulting in bio-amplification of food chain contamination and harmful effects on beneficial non-target animals. In recent years, the increasing information on hazardous effect of synthetic insecticides on plant and animal health has alarmed scientists to seek some alternative ways such as insecticides which are eco-friendly (Montasser, 2011). Therefore, the need for alternate, more effective and environment-friendly control agents or insecticides became urgent (Poopathi *et al.*, 2010).

Botanical insecticides are one of the best alternatives for these hazardous chemicals. They are plant–drive insecticides which are either naturally occurring plant materials or the products simply derived from such plants such as *Vitex negundo Linn.* (Gupta *et al.*, 2005).

Vitex negundo Linn. or lagundi is credited with innumerable medicinal activities like analgesic, anti-inflammatory, anticonvulsant, antioxidant, bronchial relaxant and hepatoprotective. The plant products of *Vitex negundo* (lagundi) are variously reported to possess insecticidal ability against stored-product pests, i.e. pantry pests include several beetles, moths, and a mite that can infest whole grains or processed foods, pests, houseflies, and tobacco leaf eating larvae. Leaf oil of the plant is shown to have repellent action against stored product pests (Tandon, 2005). Therefore, it was settled to ascertain the larvicidal ability of *Vitex negundo Linn.* (lagundi) against adult mosquitoes.

1.2 Objectives of the Study

The main objective of the study was to test the larvicidal ability of *Vitex negundo Linn.* (lagundi) leaf extracts against mosquito larvae.

Specifically, the research aimed to:

a. determine the average time the mosquito larvae were exterminated when exposed to the following treatments: the positive control which is commercial insecticide, the control ethanol, and the leaf extracts with concentrations of 30%, 60%, and 90% *Vitex negundo Linn.*; and,
b. compare the span of time the mosquito larvae were exterminated when exposed to the abovementioned treatments using One-Way ANOVA and Tukey Test.

1.3 Significance of the Study

Vitex negundo Linn. (lagundi) is considered as a medicinally significant plant. The further results of this study could add up to lagundi's reputation and importance, which will therefore invoke people to preserve and further utilize the plant species. The results would also contribute to the insecticide industry as it helps find a cheaper, more acquirable, eco-friendly and effective alternative for their products. This would also benefit consumers and the populace to eliminate mosquitoes in their locality. Along with the control of mosquitoes, there is thus the decline in health issues and diseases which they transmit. This would also eradicate the mosquitoes before they could transmit the diseases that they carry. The study could also endorse and promote further queries and studies concerning the pest management of mosquitoes and other beneficial uses of *Vitex negundo Linn.*

1.4 Scope and Limitations of the Study

The study is limited on determining the larvicidal ability of *Vitex negundo Linn.* leaf extracts against mosquito larvae. The said extracts were collected from the leaves of the said plant species. The study only involved the mosquitoes at the larval stage, and does not include those of the egg, pupal and adult stage. The age or instar of the larvae will not be determined in the study. The species of the mosquito larvae will not be considered. The study is also limited to the procedure involving three treatments compared to a positive control which is a commercially available insecticide and control which is ethanol. The solvent used will only be limited to 95% ethanol. The concentration of said treatments will be 30%, 60% and 90% *Vitex negundo Linn.* leaf extracts. Bioactive compounds and components explaining the termination of adult mosquitoes will not be determined in the study.

Furthermore, the effect of said treatments will also be verified using the span of time the mosquito larvae were exterminated after the applications of these treatments only. Only three trials with three replicates each trial are utilized with ten larvae per replicate. The treatments which are able to eliminate mosquito larvae are the ones to be included in the statistical analysis.

1.5 Definition of Terms

ANOVA – Analysis of Variance; it is a statistical method for making simultaneous comparisons between two or more means and yields values that can be tested to determine whether a significant relation exists between variables.

Extermination - implies complete and immediate extinction by killing off all individuals.

Ethanol – a colorless volatile flammable liquid C_2H_5OH that is the intoxicating agent in liquors and is also used as a solvent and in fuel.

Insecticide – a substance used for killing insects; the chemical used for extermination.

Iodoform Test – a chemical color test for the presence of a $R(CO)CH_3$ functionality by treatment with aqueous base and iodine, evidenced by the formation of a yellow precipitate of CHI_3.

Lagundi – (scientific name: *Vitex negundo Linn*.) is a large native shrub that is indigenous in the Philippines and has been traditionally used as herbal medicine.

Larvicide – an agent that kills insect larvae.

Pesticide – a substance used for destroying insects or other organisms harmful to cultivated plants or to animals; the agents identified to exterminate pests.

Positive Control – an experimental sample whose result is already known to be positive; it is used to check for errors during the procedure. In this case, the positive control will be commercial insecticide.

Rotary evaporator – a piece of apparatus consisting of a motor unit that rotates the evaporation flask, a vacuum system, a heated water bath and a condenser, which is used to remove solvents from samples under reduced pressure.

Transfluthrin – is one of the best-tested insecticidal agents, and has been incorporated in products against flying insects. In regard to its structure, toxicology and principle of action on insects' nerves, Transfluthrin is regarded as one of the fast-acting pyrethroids with low persistency.

Tukey Test – a method that is used to determine which groups among the sample have significant differences. A post-hoc test is needed after we complete an ANOVA in order to determine which groups differ from each other.

CHAPTER II

REVIEW OF RELATED LITERATURE

2.1 Mosquitoes

Mosquitoes are flying, biting insects that develop in water during their immature stages. Mosquitoes have four life stages: the egg, larva, pupa, and adult. Eggs are laid on the surface of water (*Culex* and *Anopheles* types) or damp soil that is soon to flood (*Aedes* type). Most eggs hatch within 48 hours. The larvae live in water and breathe at the surface through tubes. Larvae, or wrigglers, feed on organic debris and microorganisms in the water, then molt into pupae, a resting stage that remains in the water. During this time the mosquito develops into an adult. After two days, the pupal skin splits and the adult emerges. The length of this life cycle varies by species from 4-30 days (Cornell Cooperative Extension, 2012).

Mosquitoes bites are itchy since when a certain mosquito bites, it injects chemicals to prevent the blood from clotting and reduce pain. These chemicals cause irritation. Mosquitoes are attracted by CO_2 (Carbon dioxide) in our breath. They can detect this from great distances. When the female mosquito gets close, she makes a final choice using skin temperature, odor and other chemical or visual factors. Mosquito larvae eat organic material, bacteria and microscopic plants and animals found in water. Pupae do not feed (Alameda County Mosquito Abatement District, 2000).

Mosquitoes spread disease to humans, domestic animals, and wildlife. Emergent mosquito-borne diseases, such as dengue fever and West Nile virus (WNV), are recognized as an imminent health risk for people (Hawaii Conservation Alliance, 2005).

2.1.1 Mosquitoes in the Larval Stage

The second life stage of a mosquito is the larva. Larvae always live in water and cannot survive long out of it. The larva is an active stage that feeds while floating at the water surface. They get air at the water surface through a snorkel-like device. When disturbed, larvae actively move in an s-shape motion which gives them their common name "wriggler." They can swim down from the surface but need to return to it shortly in order to breathe. Larvae are filter feeders, eating organic matter in the water, which they collect by using their bristle-like mouthparts.

Current control is based on removing the standing water in which the larvae have hatched. If removing the stagnant water is not possible, people may be able to treat it with an insecticide or with oil that covers the surface (Caron, 1996).

2.2 *Vitex negundo Linn.* (Lagundi)

Vitex negundo Linn. (lagundi) is a large aromatic shrub or sometimes a smaller slender tree with quadrangular, densely whitish tomentose branchlets up to 4.5-5.5 meters in height. The plant is used in a variety of means throughout its distribution points as an astringent, cephalic, stomachic, antiseptic, alterant, thermogenic, depurative, rejuvenating, ophthalmic, anti-gonnorhoeic, anti-inflammatory, antipyretic and useful in bronchitis, asthma, and the enlargement of the spleen. Its roots are primarily used as a tonic, febrifuge, antirheumatic, diuretic, expectorant, and are useful as a demulcent in dysentery, in cephalalgia, otalgia, colic, uropathy, wound and ulcers. The bark is also useful in odontalgia, verminosis and opthalmopathy. The flowers are mainly used as an astringent, carminative, hepatoprotective, digestive and are useful in hemorrhages and cardiac disorders. The leaves are chiefly utilized as astringents, anodyne, anti-inflammatory, antipyretic or febrifuge, tranquilizer, bronchial smooth muscle relaxant, anti-arthritic, antihelmintic and vermifuge. The

leaves (leaf oil) are also reported to have repellent action against stored product pests (Tandon, 2005).

Vitex negundo Linn. is a large aromatic shrub or small slender tree of about 3 meters in height with quadrangular branches. It is mostly found in moist areas, scattered all throughout Mediterranean countries and Central Asia. It is a plant of the Verbenaceae family and is commonly known as five leaved chaste tree. The plant is found throughout India, Ceylon- Afghanistan, tropical Africa, Madagascar, China and Philippines (Kirtikar and Basu, 2008). The plant occurs in Bengal, Southern India and Burma also (Nadkarni, 2002). It is common in waste places around villages, river banks, moist localities and in the deciduous forests (Sharma *et al.,* 2005).

2.2.1 Studies on *Vitex negundo Linn.* (Lagundi)

Vitex negundo Linn. has been reported to have various advantageous uses, and therefore, this has pushed scientists and researchers to test and further explore the capabilities of this plant.

Anti-cancer potential was tested by Diaz *et al.* (2003), on the cytotoxicity of flavones isolated from the chloroform extract of *Vitex negundo* leaves. Vitexicarpin, a flavone was investigated for its cytotoxic action in human cancer cell line.

Antimicrobial capabilities were also evaluated. Rideout *et al.* (1999) reported antibacterial & antifungal activity of Vitexilactone & Casticin from the chloroform extract of *Vitex negundo* leaves against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* using agar plate method. Alagarsamy *et al.* (1999) reported antibacterial & antifungal activity of *Vitex negundo* leaves. Antibacterial & antifungal activity of chloroform, methanol and water extracts were evaluated by Agar cup plate method at concentrations of 10 mg/ml, 20 mg/ml and 30 mg/ml against *E.coli, P. aeruginosa, S. aureus & Candida albicans*

employing co-trimoxazole & amphotericin as a reference standards for said screenings. Rana & Dayal (2003) and Kaushik *et al.* (2003) reported anti-microbial activity of hexane and methanolic extracts of *Vitex negundo* leaves. The extracts were active against *Mycogene perniciosa, Rhizoctonia solani, Bacillus megaterium, Pseudomonasa fluorescens, Staphylococcus* species and *Xanthomonas* species. Shin *et al.* (1997), Lee *et al.* (1998) & Iqbal *et al.* (2002) & Nyligira *et al.* (2004) reported anti-microbial activity from the chloroform extract of *Vitex negundo* leaves containing Vitexilactone and Casticin and were active against *Candida albicans, Aspergillus niger, Fusarium chlamdosporum, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Loganathan *et al.* (2004) investigated antibacterial activity from the methanolic and chloroform extracts of *Vitex negundo* leaves against *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus albus, Bacillus subtilis, Escherichia coli, Klabsiella aerogens, Proteus vulgaris and Pseudomonas aeruginosa* using Agar Plate Method. Singh *et al.* (2010) reported antibacterial and antifungal activity of volatile oil against *S. aureus, E. coli, K. pneumoniae, B. subtilis, M. luteus and Candida albicans* using ciprofloxacin and chloramphinicol as reference.

Chawla *et al.* (1992) investigated anti-inflammatory activity of chloroform extract of seeds of *Vitex negundo* in Sprague-Dawley male rats in carrageenan induced rat paw edema using Ibuprofen as standard drug. Rao *et al.* (1977), Ahmad *et al.* (1989), Chawla *et al.* (1991), & Nyligira *et al.* (2004) reported anti-inflammatory activity of bark, seeds, seed oil and essential oil of *Vitex negundo*. Jana *et al.* (1999) reported preliminary anti-inflammatory activity of *Vitex negundo* in albino rats along with *Zingiber officianale* and *Tinospora cordifolia*. Dharmasiri *et al.* (2003) investigated anti-inflammatory activity from the aqueous extract of *Vitex negundo*

leaves in Wistar rats (male) using carrageenan-induced & formaldehyde-induced rat paw oedema using indomethacin as standard. The early phase of carrageenan-induced rat paw oedema was significantly suppressed in an inversely dose-dependent manner.

Immuno-stimulant Activity was also assessed. Singh *et al.* (2005) reported immunostimulatory activity from the extracts of *Vitex negundo* in oxyburst phagocytic assay using human polymorph nuclear cells. Suri *et al.* reported immunostimulatory potential of two iridoid glucosoides from *Vitex negundo* leaves.

Zheng *et al.* (1999), Zheng and Luo (1999) and Onu *et al.* (2004) reported antioxidant potential of Vitedoin A, Vitedoin B and other lignans derivatives from the seeds of *Vitex negundo*. Tondon & Gupta (2005) reported anti-oxidant effect of Vitexin which is a new compound. Tiwari & Tripathi (2007) evaluated antioxidant property of different fractions of *Vitex negundo* by employing various invitro systems, such as 2, 2'-azino-bis-3-ethyl benzothiazioline-6-sulfuric acid (ABTS), Lipid peroxides (LPO), Superoxide, Hydroxyl radical scavenging and iron chelation. Total antioxidant capacity was determined by the assay based on the performed radical monocation ABTS. LPO was assessed in terms of thiobarbituric acid reactive substances by using egg yolk homogenates as lipid rich media.

Amancharla *et al.* (1999) also tested mosquito repellent activity of aqueous extract of *Vitex negundo* leaves. A new chemical 'rotundial' was tested for the said activity. The chloroform fraction of the aqueous extract of the fresh leaves of *Vitex negundo* by bioactivity guided isolation yielded a pure compound rotundial which has shown mosquito repellent activity.

2.3 Pesticides and Insecticides

Pesticides have numerous beneficial effects. These include crop protection, preservation of food and materials and prevention of vector-borne diseases. For

example pesticides may be used in the prevention of malaria, which kills up to one million children per year, and for preventing other vector-borne diseases such as dengue, leishmaniasis and Japanese encephalitis (National Resource Council, 1993).

But, pesticides are toxic by design – they are designed to kill, reduce or repel insects, weeds, rodents, fungi or other organisms that can threaten public health and the economy. Their mode of action is by targeting systems or enzymes in the pests which may be identical or very similar to systems or enzymes in human beings and therefore, they pose risks to human health and the environment (National Resource Council, 1993).

Pesticides are ubiquitous in the environment and most are synthetic. There is growing concern about children's exposure to pesticides and their special susceptibility. Children are not little adults, and may have higher exposures and greater vulnerability at both high and low levels of exposure (National Resource Council, 1993).

An insecticide is simply a specialized type of pesticide used to kill insects. So all insecticides are pesticides and some pesticides are insecticides. Insecticides also range from industrial to ones designated for home use. There are even insecticides used to kill specific insects, like cockroaches or wasps (Organic and Chemical Pesticides, 2009).

The types of insecticides available in the market range from organochlorines organophosphates, carbamate esters, pyrethroids and botanical insecticides (Squibb, 2002).

2.4 Transfluthrin

Transfluthrin is the active ingredient in most of the commercial insecticides readily available in the market today (Baygon Agents, 2013). Transfluthrin is a fast

acting insecticide. It is used in household and hygiene products, mainly against flying insects, such as mosquitoes and flies, but also against material pests, such as moths. Transfluthrin is one of the best-tested insecticidal agents, and has been incorporated in commercial products against flying insects since 1996. In regard to its structure, toxicology and principle of action on insects' nerves, Transfluthrin is regarded as one of the fast-acting pyrethroids with low persistency.

Transfluthrin is highly selective. Low quantities are exceptionally powerful against hygiene, health and material pests in the indoor environment. The excellent knock-down action of Transfluthrin at an extremely low concentration permits its use especially in products to combat flies, mosquitoes and cockroaches. In products against crawling insects. Transfluthrin shows a flushing and knock-down effect. It is a relatively volatile substance and acts principally as a contact and inhalation agent (Baygon Agents, 2013).

The mechanism of action is through inhalation and contact; broad spectrum, effects insects presynaptic voltage gate sodium channels in nerve membranesrapid causing knockdown (AERU, 2012).

Unlike most of other synthetic pyrethroids, Transfluthrin is extraordinarily effective at very low application rates. Moreover, its evaporation rate proves to be particularly high, allowing the substance to evaporate even at room temperature, thus making it suitable to be applied both through heated and unheated systems. Owing to all of these features, Transfluthrin is the active choice and is applied in innovative aerosols, vaporizers (COILS, MATs, LEDs, unheated systems) and clothes-moth control products (Endura, 2000).

However, Transfluthrin has caused widespread worry due to its disadvantages. One major concern is the fetal exposure to environmental toxins & infant outcome,

and the effect of Transfluthrin to pregnant women is being studied. Studies using rat hepatocytes showed that Transfluthrin acts as a weak promotor of cell proliferation. Genotoxically, exposure to Transfluthrin has a genotoxic effect on the epithelial cells of human nasal mucosa (Fluoride Action Network Pesticide Project, 2012). Environmental toxicity tests showed that Transfluthrin is of low toxicity to algae, earthworms and birds but is highly toxic to fish and daphnia (UN, 2003). It has also been known to cause skin and eye irritation (AERU, 2012).

2.5 Ethanol as Extracting Solvent

Ethanol (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, $-OH^-$, bonded to a carbon atom.

Ethanol extraction is a type of solvent extraction used to extract fragrant compounds directly from organic solvent extraction or expression. Ethanol extracts from dry materials are called tinctures, while ethanol washes for purifying oils and concretes are called absolutes. The impure substances or oils are mixed with ethanol which is less hydrophobic than solvents used for organic extraction, dissolves more of the oxidized aromatic constituents, leaving behind the wax, fats and other generally hydrophobic substances. The alcohol is evaporated under low-pressure leaving behind absolute. The absolute may be further processed to remove any impurities that are still present from the solvent extraction. Ethanol extraction is not used to extract fragrance from fresh plant materials; these contain large quantities of water which would also be extracted into the ethanol (http://oilganic.com, 2010).

2.6 Basic Principles of the Iodoform Test for Ethanol

The Iodoform Test is a chemical reaction that involves the production of iodoform (CHI_3) by the multiple halogen of a methyl ketone in the presence of a base. In analytical chemistry, this reaction was traditionally used to determine the presence of a methyl ketone, or a secondary alcohol oxidizable to a methyl ketone through the iodoform test (Nuffield Foundation, 2011). This reaction is illustrated in Figure 1:

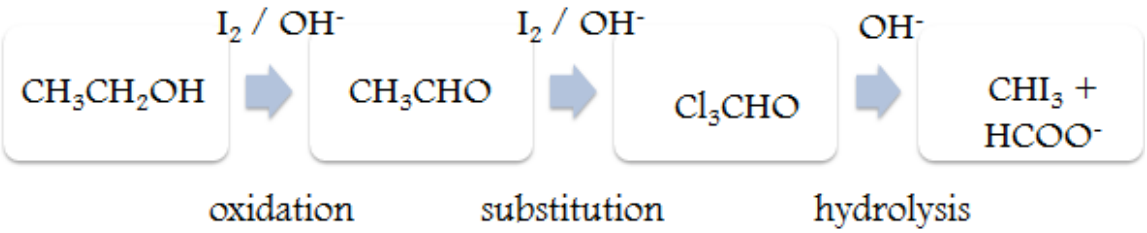

Figure 1. The chemical reaction involved in the Iodoform reaction when initiated from a primary alcohol (ethanol).

The iodoform test or iodoform reaction is a qualitative chemical test for the detection of ketones carrying an alpha methyl group. The reagents are iodine and sodium hydroxide. Iodoform or triiodomethane is a pale yellow substance with a relatively high molar mass due to the iodine atoms. It is therefore solid at room temperature. It is insoluble in water and has an antiseptic smell. A visible precipitate of this compound will form upon reaction with ethanol (Iodoform Reaction, 2012).

CHAPTER III

METHODOLOGY

3.1 Research Design

The research study was experimental and conducted so as to determine the larvicidal potential of *Vitex negundo Linn*. (lagundi). Three various ethanol leaf extracts were used, classified according to their concentrations: 30%, 60%, and 90% *Vitex negundo Linn.* leaf extract solutions. These solutions were extracted using ethanol, an organic solvent. The assigned positive control was the commercial insecticide as well as ethanol for the control. Three trials with three replicates with ten mosquito larvae placed in each replicate were exposed to the said treatments.

3.2 Mosquito Larvae Collection

Mosquito larvae were acquired from containers with stagnant water. The larvae acquired were carefully transferred to small plastic cups and allowed to acclimatize for two minutes to prevent stressing the larvae. The larvae were then exposed to the treatments and transferred to a petri dish and held against the light for accurate viewing of larvicidal activity.

3.3 Collection of Leaves

Fresh, unsullied leaves of *Vitex negundo Linn.* (lagundi) were collected and used for the tests. The leaves which have withered, have dust and dirt particles, and have insect bites were discarded. The accumulated leaves were rinsed and washed by running water.

3.4 Ethanol Leaf Extract Preparation

The collected leaf samples were oven-dried for four days until brittle and fully dried. Thereafter, the leaves were sliced and cut into small portions and homogenized

in a blender. Four hundred seventy-six grams of the homogenized leaves were then soaked in approximately 1,200 mL 95% ethanol for 48 hours, accompanied with occasional shaking. The shaking causes the ethanol to cover the leaf samples inside the container. The ratio between the *Vitex negundo Linn.* leaves and the ethanol solvent did not matter as long as the ethanol solvent would overlap and fully obscure and cover the lagundi leaves. Filter paper was used to separate and filter the slurry from the solution. The ethanol left in the solution was then evaporated by means of the Rotary Evaporation Method.

3.5 Iodoform Test for Ethanol

The Iodoform Test for Ethanol was conducted to test the presence of ethanol in the extract, and was done by adding ten drops of 1 M NaOH and 25 drops of 0.5 M iodine solution to ten drops of the ethanol extract.

A visible yellow precipitate would indicate presence of ethanol in the sample, while no precipitation formation would confirm its absence.

3.6 Treatment Application

The concentrations were then constituted: 3 mL of the ethanol extract was added to 7 mL of the larvae water to obtain the 30% concentration, 6 mL of the ethanol extract was added to 4 mL of the larvae water to obtain 60% concentration, and 9 mL ethanol extract was added to 1 mL of larvae water to obtain the 90% concentration. The span of time (in minutes) at which the mosquitoes were exterminated became the basis for the larvicidal potential of *Vitex negundo Linn.* All glasswares used in the experiment were properly washed and rinsed with distilled water.

3.7 Analysis of Acquired Data

The One-Way ANOVA was used for the interpretation of the data to ascertain if prominent differences occur in the span of time the mosquito larva were exterminated between the five applied treatments: the positive control commercial insecticide, the control ethanol, the 30%, 60% and 90% ethanol leaf extract concentrations. The Tukey Test was also used to compare the extermination time among the treatments which displayed mortality in the subject organisms (mosquito larvae) and which among the treatments possessed the highest insecticidal activity against mosquito larvae. Statistical Package for the Social Sciences (SPSS) was used to calculate the statistical data.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Iodoform Test

A yellow precipitate of the compound iodoform will form from a sample upon reaction with ethanol. Upon performing the Iodoform Test, no yellow precipitate formed implying an absence of ethanol from the *Vitex negundo Linn.* leaf extract.

4.2 Extent of Extermination Time

Following the application of the five treatments, the length of time in which the adult mosquitoes were exterminated was recorded. The various corresponding time interval for each trial per treatments after application were averaged and are presented in Table 1.

Table 1. Average Extermination Time of Adult Mosquitoes in Minutes

Treatment	Positive Control, P (in minutes)	30% ethanol extract, E_{30} (in minutes)	60 % ethanol extract, E_{60} (in minutes)	90% ethanol extract, E_{90} (in minutes)	Control, C (in minutes)
Trial 1	1.65	25.18	7.56	3.92	3.71
Trial 2	1.27	12.31	7.81	4.37	4.30
Trial 3	0.99	15.62	7.13	3.75	3.00
Average	**1.30**	**17.70**	**7.50**	**4.02**	**3.67**

**depicts the results taken after 24-hour observation after application*

Average extermination time results showed that commercial insecticide, the positive control, yielded the lowest time extent with a total average of 1.30 minutes in comparison to the ethanol leaf extracts. The least concentrated ethanol extract, the 30% ethanol leaf extract, reflected the highest time interval with an average of 17.70 minutes. The 60% ethanol leaf extract procured an average of 7.50 minutes, and the

90% ethanol leaf extract obtained an average of 4.02 minutes. The 90% ethanol leaf extract acquired the lowest average time length among the three ethanol leaf extracts, and therefore the second lowest time interval for all the five treatments. Ethanol, the control, obtained a total average time of 3.67 minutes.

4.3 Data Analysis

Established by the data acquired in the study, the 30% ethanol leaf extract displayed the longest extermination time. The positive control displayed the shortest extermination time, followed by the control ethanol, the 90% ethanol leaf extract and the 60% ethanol leaf extract (Table 1) respectively. Although the extermination time differed, there was a statistically significant difference between the commercial insecticide and the 30% ethanol leaf extract. The mean difference is significant at 0.05 level. Any Sig. value below the 0.05 level is considered as a statistically significant difference. The One-Way ANOVA resulted in a Sig. value of 0.000 implying that there was a generally significant difference in comparison to each other (Table 2 in Appendix A). The Tukey Test for comparison of Sig. values showed that the 30% extract had a statistically significant difference when compared to the commercial insecticide while the 60% extract, 90% extract, and the ethanol displayed a statistically insignificant difference in comparison with the commercial insecticide (Table 3 in Appendix A).

The average extermination time for the mosquito larvae to be eliminated upon exposure to each treatment was shown graphically (see Figure 2). The graph showed that as the concentration of the extract increases, the span of time for extermination decreases.

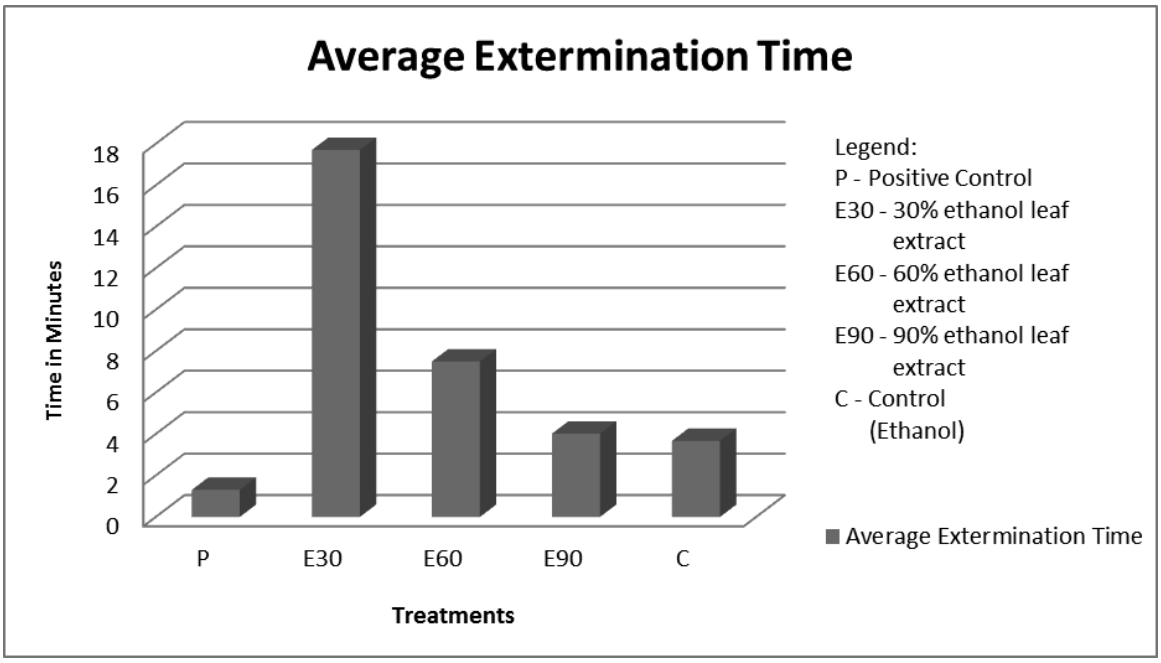

Figure 2. Average Time of Extermination vs. Applied Treatments graph

Factors considered as the cause of the potential of *Vitex negundo Linn.* ethanolic extracts are the phytochemicals and bioactive compounds or components present in the leaf extracts. A study on the preliminary phytochemical screening of both petroleum ether and methanolic extract of *Vitex negundo Linn.* done by Murthy *et al.* (2010), revealed the presence of tannins, flavonoids, flavones and coumarins. Tannins are proved to have feed-constraining assets against larvae of insects by the study of Cardinal-Aucoin *et al.* (2009). A study by Morimoto *et al.* (2000), showed that flavonoids have antifeedant properties on exposure to particular insects. Various studies on coumarin have reflected that coumarin exhibited strong insecticidal ability, and caused high percentage of mortality on eggs and larva of insects (Reda and El-Banhewy, 1986, Sharma *et al*., 2006). Furthermore, a bioassay done by Romanelli *et al*. (2010) proved that flavones have moderate insecticidal activity.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

To compare the span of time the mosquito larvae were exterminated upon exposure to the treatments, the average time of extermination of each treatment was determined. The average extermination time for the ethanol leaf extracts with different concentrations 30%, 60% and 90% were 17.70, 7.50 and 4.02 minutes, respectively. For the positive control, the average time interval recorded was 1.30 minutes. The control ethanol obtained an average time of 3.67 minutes. As shown in the Tukey Test results, only the Significant value of the insecticide and 30% extract showed a significant difference between each other. The 60% extract, 90% extract and the control ethanol displayed a statistically insignificant difference compared to the commercial insecticide.

The data gathered in the study helped establish the larvicidal potential of *Vitex Negundo Linn.* (lagundi) against mosquito larvae. Average Extermination Time results indicate that among the ethanol leaf extracts, the 90% ethanol leaf extract held the lowest and therefore fastest time extent of death and mortality in direct comparison to the 30% and 60% ethanol leaf extracts. It is also concluded that the time span of extermination is inversely proportional to the concentration of the ethanol treatments. The 90% extract, however, does not surpass the capacity of extermination by the positive control as well as the control ethanol, both with the lowest extermination time among all the five applied treatments, although their time extent of mortality was close.

In totality, the data collected show that *Vitex negundo Linn.* indeed has larvicidal potential when treated to larvae in high concentrations, and can be used as a

substitute for commercial insecticides, though proper processing is still needed to ensure its appropriateness as substitute.

5.2 Recommendations

For future studies on the larvicidal potential of *Vitex negundo Linn.* (lagundi), it is therefore recommended to use other commercial insecticides as positive control to further ascertain if *Vitex negundo Linn.* can indeed be used as alternative for these pesticides. The application of other types of solvents is also recommended in leaf extraction to contrast its efficacy with the ethanol leaf extracts, such as methanol, is also highly endorsed. The determination of the age or instars of the larvae are also recommended. The detection of the specific bioactive compounds found in the plants which are responsible for the mortality of the adult mosquitoes is also recommended. The testing and application of the ethanol leaf extracts to other pests, such as ants or termites, so as to ascertain if these treatments have noteworthy effects on said pests would also be of help to gather more information pertaining to the study and further studies to be conducted.

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APPENDIX A

EXPERIMENTAL RAW DATA

Table 2. One-Way ANOVA Results

	Sum of Squares	df	Mean Square	F	Significant value
Between Groups	501.249	4	125.312	13.802	.000
Within Groups	90.791	10	9.079		
Total	592.040	14			

Table 3. Tukey Test Results, Multiple Comparison

(I) Substance	(J) Substance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Insecticide	30% extract	-16.3984333*	2.4602263	.000	-24.495240	-8.301626
	60% extract	-6.1964667	2.4602263	.162	-14.293274	1.900340
	90% extract	-2.7132333	2.4602263	.802	-10.810040	5.383574
	Ethanol	-2.3675333	2.4602263	.866	-10.464340	5.729274
30% extract	Insecticide	16.3984333*	2.4602263	.000	8.301626	24.495240
	60% extract	10.2019667*	2.4602263	.013	2.105160	18.298774
	90% extract	13.6852000*	2.4602263	.002	5.588393	21.782007
	Ethanol	14.0309000*	2.4602263	.001	5.934093	22.127707
60% extract	Insecticide	6.1964667	2.4602263	.162	-1.900340	14.293274
	30% extract	-10.2019667*	2.4602263	.013	-18.298774	-2.105160
	90% extract	3.4832333	2.4602263	.632	-4.613574	11.580040
	Ethanol	3.8289333	2.4602263	.553	-4.267874	11.925740
90% extract	Insecticide	2.7132333	2.4602263	.802	-5.383574	10.810040
	30% extract	-13.6852000*	2.4602263	.002	-21.782007	-5.588393
	60% extract	-3.4832333	2.4602263	.632	-11.580040	4.613574
	Ethanol	.3457000	2.4602263	1.000	-7.751107	8.442507
Ethanol	Insecticide	2.3675333	2.4602263	.866	-5.729274	10.464340
	30% extract	-14.0309000*	2.4602263	.001	-22.127707	-5.934093
	60% extract	-3.8289333	2.4602263	.553	-11.925740	4.267874
	90% extract	-.3457000	2.4602263	1.000	-8.442507	7.751107

*. The mean difference is significant at the 0.05 level.

Table 4. Extermination Time by Positive Control commercial insecticide

Replicate	Trial 1 (in minutes)	Trial 2 (in minutes)	Trial 3 (in minutes)
P_1R_1	1.5260	1.3550	1.2444
P_1R_2	1.1296	1.1437	0.5716
P_1R_3	2.2966	1.3044	1.1484
Average	1.6507	1.2677	0.9881

Table 5. Extermination Time by 30% Ethanol Leaf Extract

Replicate	Trial 1 (in minutes)	Trial 2 (in minutes)	Trial 3 (in minutes)
$E_{30}R_1$	28.1150	13.5075	17.4787
$E_{30}R_2$	23.3506	11.2272	15.2509
$E_{30}R_3$	24.0622	12.1906	14.1228
Average	25.1759	12.3084	15.6175

Table 6. Extermination Time by 60% Ethanol Leaf Extract

Replicate	Trial 1 (in minutes)	Trial 2 (in minutes)	Trial 3 (in minutes)
$E_{60}R_1$	10.2300	9.0381	8.1431
$E_{60}R_2$	5.4257	6.1085	7.1700
$E_{60}R_3$	7.0125	8.2690	6.0907
Average	7.5561	7.8052	7.1346

Table 7. Extermination Time by 90% Ethanol Leaf Extract

Replicate	Trial 1 (in minutes)	Trial 2 (in minutes)	Trial 3 (in minutes)
$E_{90}R_1$	5.1925	4.4965	6.0147
$E_{90}R2$	4.5212	6.3838	3.1687
$E_{90}R_3$	2.0535	2.2356	2.0719
Average	3.9224	4.3700	3.7518

Table 8. Extermination Time by Control Ethanol

Replicate	Trial 1 (in minutes)	Trial 2 (in minutes)	Trial 3 (in minutes)
N_1R1	5.0597	5.5172	4.1072
N_1R_2	3.4969	4.2250	2.4215
N_1R_3	2.5779	3.1444	2.4775
Average	3.7115	4.2955	3.0021

APPENDIX B

PHOTOS

Figure 3. *Vitex negundo Linn.* plant.

Figure 4. Rotary Evaporator.

Figure 5. Mosquito larvae in stagnant water.

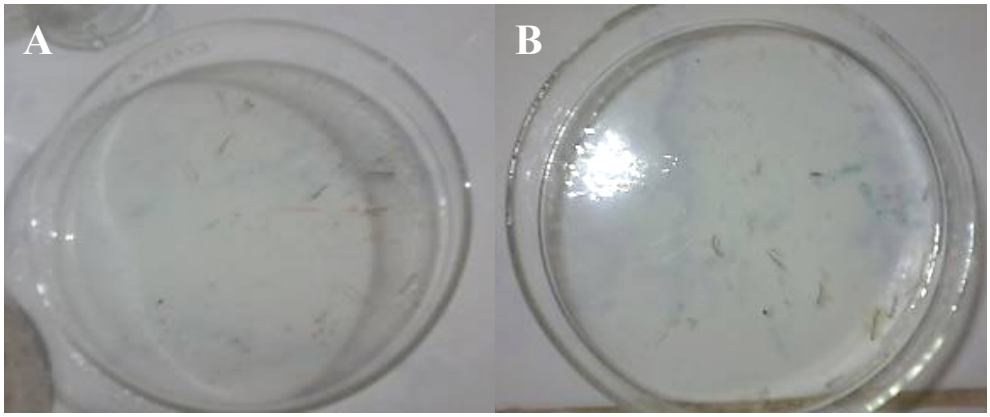

Figure 6. (A) The larvae after treatment of Positive Control Commercial Insecticide, P. (B) The larvae after treatment of Control Ethanol, C.

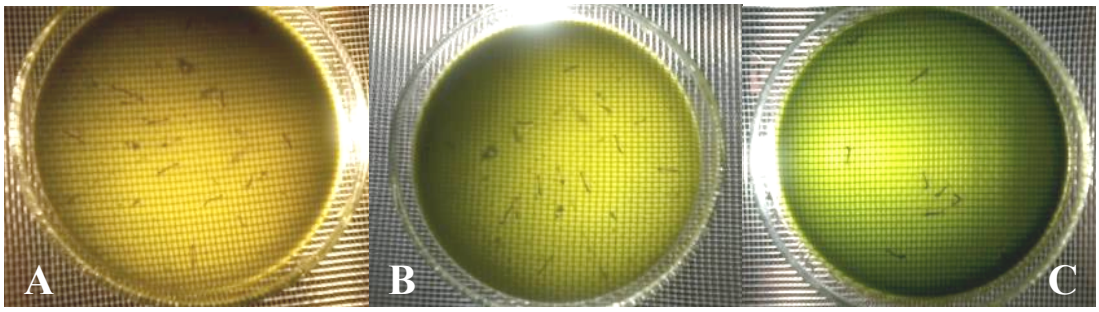

Figure 7. (A) The larvae after treatment of 30% Ethanol Leaf Extract, E_{30}. (B) The larvae after treatment of 60% Ethanol Leaf Extract, E_{60}. (C) The larvae after treatment of 90% Ethanol Leaf Extract, E_{90}.

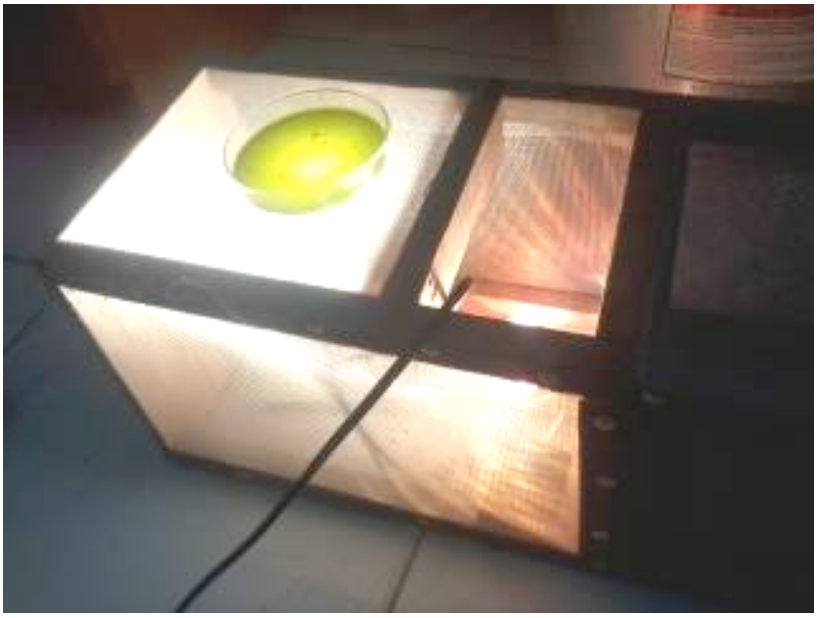

Figure 8. Observation of Subject Organisms. This was done using a box with wood as support and screen as the walls. A light source was placed inside the box and petri plates were placed on top of the screen.

Figure 9. The ethanolic leaf extracts after performing the Iodoform Test for Ethanol. The absence of the yellow precipitate indicated the absence of ethanol.