Phytochemical screening and Partial Characterization through GC-MS of Leaf Crude Extract of *Plectranthus scutellarioides* (Mayana), and *Plectranthus amboinicus* (Oregano) and their Larvicidal Activity Against 2nd – 3rd Instar Larvae of *Aedes aegypti* in Comparison to *Azadirachta indica*

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Abstract: Mosquitoes cause over one million death of people worldwide every year due to dengue fever. It is an endemic disease that cause great death percentage in the Philippines. Proliferation of this mosquito-borne disease can be prevented by using synthetic larvicide such as dichlorodiphenyltrichloroethane (DDT), but it has been found out that DDT can pose serious health effects on humans. In lieu of the synthetic one, biological control such as the use of *Azadirachta indica* was studied. Other plants like *Plectranthus scutellarioides* and *Plectranthus amboinicus* contain secondary metabolites which have potential larvicidal activity.

In this study, different concentrations of *P. scutellarioides* and *P. amboinicus* had been investigated for their larvicidal activity on 2nd-3rd instar larvae of *Aedes aegypti* and was compared to *A. indica.* Qualitative determination of the phytocomponents was done using phytochemical screening. The results revealed that the ethanolic leaf crude extract contains terpenoids, phenolic compounds, saponins and anthraquinones. Quantitative analysis was conducted through GC-MS and revealed the major components in each plant are terpenes and phenolic compounds that possessed insecticidal properties. After 24 hours, the lowest percentage mortality was observed in *P. amboinicus* leaf crude extract with 70.56 ± 6.371%. Highest mortality was observed in *P. scutellarioides* leaf crude extract with 96.11 ± 1.389%. Comparisons between the positive control, *A. indica and P. scutallarioides* showed no significant difference (α0.05 < 0. 398). On the other hand, comparison between *A. indica* and *P. amboinicus showed* significant difference (α0.05 > 0. 000). It can be concluded that *P. scutellarioides* has equal larvicidal activities with *A. indica* while *P. amboinicus* has lower larvicidal activity.

Key Words: *Azadirachta indica; Plectranthus scutellarioides; Plectranthus amboinicus;* Larvicidal activity; secondary metabolites

1. INTRODUCTION

Mosquitoes cause millions of death worldwide due to the disease it could transmit such as dengue fever, chikungunya, yellow fever and the worst, dengue hemorrhagic fever (Gutierrez, Jr. *et. al.,* 2014). Dengue fever is a common disease and is endemic to Philippines which is caused by *Aedes* mosquitoes (WHO,

2009). The peak incidence occurs during wet season since this is the time were they likely lay their eggs and reproduce. The risk is higher for those staying in places with nearby stagnant water reservoirs and no mosquito protection but transmission can occur in any urban areas including downtown business areas. The transmission of dengue is through the bite of mosquitoes specifically the female mosquitoes.

Proliferation of this mosquito-borne disease can be prevented with the use of major tool in mosquito control , the application of synthetic larvicide such as dichlorodiphenyltrichloroethane (DDT), but it has been found out that DDT can pose serious health effects causing genotoxicity, acute and chronic toxicity and other major complications on humans (Eskenazi et al., 2009). When it is also continually used to control the proliferation of these mosquitoes it would be absorbed and distributed in the environment thus causing damage to the biodiversity (Chowdhury *et al.,* 2008). Aside from these, synthetic larvicides have high cost that is why the researchers look for an alternative larvicide which is eco-friendly and a cheaper source in lieu of the synthetic one.

Biological control is an effective alternatives to synthetic and phytochemicals from plants are the ones commonly used. *Azadirachta indica* (Neem tree) from the family Meliaceae is a tree native in Asia. Meliaceae family is one of the sources of numerous bioactive secondary metabolites such as azadirachtin (Proksch *et al.,* 2001). This group of compounds has driven a lot of interest because of their high activity over the behavior and physiology of several phytophagous insect species (Castillo-Sánchez *et al.,* 2010). For this reason, *A. indica* is proven to be a good biological control of mosquitoes.

On the other hand, Lamiaceae family is a promising source of bioactive secondary metabolites. It contains essential oils such as monoterpenoids and phenolic compounds. Essential oils are known to act as antimicrobial, antispasmodic, carminative, and antiviral agents. In addition, essential oils of several mint species have been recently qualified as natural antioxidants (Mimica-Dukic, Bozin, 2008). Moreover, these plants could be seen in house backyards and these could be easily grown. Notable plants from this family which are usually seen in Filipino household are *Plectranthus scutellarioides* (Mayana) and *Plectranthus amboinicus* (Oregano).

The present study was conducted in order to determine the partially characterize the phytochemical compounds present in the leaf crude extract of *Plectranthus scutellarioides* (Mayana) and *Plectranthus amboinicus* (Oregano) and to compare the larvicidal activity of the two leaf crude extract against *Aedes aegypti with Azadirachta indica* (Neem) as positive control.

2. METHODOLOGY

Collection and Identification of Plants and *Aedes aegypti*

Leaves of *Azadirachta indica* were obtained in from Gen. T. de Leon NHS, Valenzuela, while leaves of *Plectranthus scutellarioides* and *Plectranthus amboinicus* were gathered in Antipolo, Rizal. On the other hand, larvae of *Aedes aegypti* were obtained from the Entomology Dept. of Research Institute for Tropical Medicine (RITM) and the Dept. of Science and Technology (DOST). The collected plants were identified by the Botany Division of the National Museum (BDNM) while *Aedes aegypti* was identified by DOST.

Leaf Extraction and Filtration of Plants

The leaves of each plant were washed carefully and air dried for a week at room temperature. Dried leaves were subjected to grinding into pieces and weighed by 100g each species. The weighed plants were set in the bottle with 600ml of 95% EtOH for each setup and left for three days. Plant extract were then filtered through the use of cheese cloth and Whatman filter paper #40 and the filtrates were concentrated for 6 hours through the process of rotary evaporation as adopted by the method of Nathan *et al.* (2006).

Phytochemical Screening

Phytochemical screening was done by following Prashant Tiwari, *et al.* (2011) procedures in phytochemical analysis of crude extracts to test for the presence of terpenoids, phenolic compounds, saponins, anthraquinones and essential oils.

Dilution of Leaf Crude Extract in the Preparation of the Initial Concentration of the Stock Solutions

Three concentrations of the leaf crude extract was prepared, 500ppm, 100ppm, and 50ppm. For 500ppm concentration, 0.5g of each crude extract was dissolved in 1000ml distilled water. While for 100ppm concentration, 100ml of 500ppm conc. was diluted in 500ml distilled water. And finally for 50ppm concentration, 50ml of 500ppm concentration was diluted in 500ml distilled water.

Gas Chromatography-Mass Spectrometry

The remaining crude extract from each species that was rotary evaporated was suspended into dichloromethane (DCM) to purify the extract first before undergoing Gas Chromatography-Mass Spectrometry using the standard procedure of Adam (2007). This test was done at the Chemistry Laboratory of the De La Salle University.

Larvicidal Bioassay

Larvicidal suspension containing the food pellets were prepared 48 hours before the test of the efficacy of each extract with various concentrations.

Simple plastic cups with dimensions 5.7 cm in diameter and 4 cm in depth were. Using a graduated cylinder, 100 ml of distilled water was measured and transferred in each cup. An aliquot of twelve ml of 500ppm, 100ppm, and 50ppm concentration of crude extract was measured and placed on the same container with the distilled water to produce final concentrations: 60ppm, 12ppm, and 6ppm respectively. Twenty larvae of *Aedes aegypti larvae in 2nd - 3rd instar stage was transferred in the solution made.*

There were three trials with three replicates in each trial and was observed in the following hours (1st, 3rd, 6th, 9th and 24th hour) to test the larvicidal activity of each plant extract. Dead and moribund larvae were counted during the specified time of observation.

Analysis of Data

Analysis of data was done using SPSS version 20.0 and Graphpad Prism 6. LC50 was taken as well as minimum and maximum percentage mortality of each extract. The analysis of the effectiveness of each treatment was done using Independent Sample test.

3. RESULT AND DISCUSSION

Phytochemical Screening

A variety of bioactive compounds have been reported to be present in *A. indica and P. amboinicus* crude extract (Nostro et.al., 2007) and as one of the pioneer studies in *P. scutellarioides*, presence of the said bioactive compounds were also obtained from its leaf crude extract.

In this study, phytochemical screening revealed the chemical constituents of the crude leaf extract of *A. indica, P. scutellarioides and P. amboinicus*. The results showed the presence of terpenoids, phenols, saponins, and anthraquinones. (Table 1).

As for the study of Reddy *et.al* (2012); Terpenoids, saponins, phenols, and anthraquinones obtained from the leaf crude extracts *Sida cordifolia* are bioactive compounds which have strong anti-feedant activity that act on the chemoreceptors of insects and its ingestion leads to deleterious effects. When larval insects ingest these compounds, their growth and development are inhibited due to the blocking of biosynthesis of insect hormones such as ecdysteroids (Lee *et.al*., 2010). These bioactive compounds also cause degradation in the larval epidermis of insects preventing the larvae from molting (Aliero, 2003).

According to Coloma *et.al* (2005), terpenes are antifeedants that act on a broad spectrum of insects with divergent feeding adaptations. Sesquiterpenes have been reported to serve as toxic or feeding deterrents to herbivore insects (Fraga, 2004). Diterpenes on the other hand is a rich source of natural insect antifeedants and attractants (Nishida *et al.,* 2004). They are very lipophilic and tend to have strong flavors (Bernhoft, 2008). It has the ability to kill soft-bodied insects by penetrating their waxy cuticle, leading to desiccation. Once penetrated through the cuticle, certain components within essential oils may begin to block neurotransmitters, digestive enzymes or growth hormones (Gililan *et al*., 2009).

Table 1: Phytochemical screening of leaf crude extract of *A. indica, P. scutellarioides and P. amboinicus*.

Phytochemical Screening	*A. indica*	*P. scutellarioides*	*P. amboinicus*
Terpenoids	+	+	+
Phenols	+	-	+
Saponins	+	+	-
Anthraquinones	+	+	+

(-) indicates the absence of the phytochemical constituents
(+) indicates the presence of the phytochemical constituents

Saponins are a class of secondary plant metabolites which possesses clear insecticidal activities. According to Wiesman and Chapagain (2006) it exert a strong and rapid-working action against a broad range of pest insects that is different from neurotoxicity. The most observed effects are increased mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction. Main hypotheses in literature, saponins exert a repellent/deterrent activity, bear digestive problems, provoke insect molting defects or cause cellular toxicity effects.

Experiment done by Kekuda et.al (2011) revealed that phenolic content and other secondary metabolites in could be a suitable source for bioactive compounds against insect vectors such as *Aedes aegytpi* to control arboviral diseases such as chikungunya and dengue. Penetration of phenol to organisms is related with diffusion of the compound across a cell's membrane and enhances its toxicity. On the other hand, anthraquinones exhibit a wide spectrum of ecological impacts by mediating biotic and abiotic interactions of plants with their environment (Izhaki, 2002). It protect plants against herbivores, pathogens, competitors, and extrinsic abiotic factors. These compounds play an important role as anti-nutritional components of food and animal feed (Bilal *et.al.,* 2012). The activity of these metabolites is attributed to its ability to bind to the free protein available for larvae nutrition; reduced nutrient availability could have resulted in larvae starvation and death (Athanasiadou *et al*., 2001). Schultz (1989) reported that in insects and insect larvae that ingest the condensed tannins occurs connection of these compounds with intestinal mucosa causing autolysis, leading to an inability to use nutrients by the larva.

Composition identification analyzed by Gas Chromatography-Mass Spectrometry (GC- MS)

Based on the results of the peak areas of the screened phytocomponents, it was found that tetradecanoic acid, 10,13-dimethyl-, methyl ester and 3-methyl-2-(2-oxopropyl)furan were considered as the significantly major components present in *A. indica.* These components are essential oils (Sutha *et al.,* 2010), generally terpenes, that functions as an insecticide, repellent and insect feeding deterrent (Magnelli *et.al.*, 2010). Six minor ccomponents was also detected such as, 2- methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane,Z,Z-6,28-heptatriactontadien-2-one, tetradecanoic acid, 10,13-dimethyl-, methyl ester, 3-methyl-2-(2-oxopropyl)furan, 1,3- dioxolane, 4-ethyl-5-octyl-2,2-bis(triflouromethy)-, trans-, and 5-(2-isopropyl-5- methylcyclohexyloxy)-3-methyl-4-(phenylthio)- tetrahydrofuran-2-one.

Essential oils are the fragrant, highly concentrated natural constituents. It gives the plants their characteristic odor and contain the healing power of the plant from which it was extracted. They are primarily lipophilic in nature, which act as toxins, feeding and oviposition deterrents to many insect pests (Koul *et al*., 2008). They are presumed to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects (Mann and Kaufman, 2012). A gradual disappearance of cellular organelles and significant vacuolation will occur. Cytoplasm was less homogeneous. Ruiz *et al*., (2004) found a significant vacuolation, swollen nuclei and elongated epithelial cells of the mosquito larvae treated with essential oil. These epithelial cells were disrupted at the apical region with vesicle formation, lysis and leakage of cytoplasm material into the gut lumen. In addition, it is also known that the mosquito larvae and pupae breathe through spiracles. The oils block the spiracles, resulting in lack a respiratory siphon (asphyxiation) and death (Rotimi *et al*., 2011). Lipophilic plant oils are known to kill soft-bodied insects by penetrating their waxy cuticle, leading to desiccation. There is also evidence that once penetrated through the cuticle, certain components within essential oils may begin to block neurotransmitters, digestive enzymes or growth hormones (Gililan *et al*., 2009). In addition to the physical effects of disrupting cellular tissue, the rapid toxic effects of essential oils indicate that there is a neurotoxic effect on pests (Gillilan, 2012).

There was no study done yet with regards to the larvicidal ability of *P. scutellarioides.* As a pioneer study, it was found out that *P. scutellarioides* have phytol. This bioactive compound had the highest peak area and considered as the significantly major component within the ethanolic leaf crude extract of *P. scutellarioides*. According to Santos et.al (2013), phytol (3,7,11,15 tetramethylhexadec-2-en-1-ol) is a diterpene, a member of the group of branched-chain unsaturated alcohols. They are very lipophilic and tend to have strong flavours (Bernhoft, 2008). This only shows that *P. scutellarioides* has the potential as an insecticide against *A. aegypti* possessing not only phytol but also other essential oils such as sesquiterpenes and diterpenes. Other phytocomponents in *P. scutellarioides* were detected such as, Z,Z-6,28- heptatriactontadien-2-one, Tetradecanoic acid, 10, 13-dimethyl-, methyl ester, phytol, 1, 6, 10, 14, 18, 22-tetracosahexaen-3-ol-2, 6, 10, 15, 19, 23-hexamethyl-, (ALL-E)-, cholestan-3-ol,2- methylene, (3.BETA.,5.ALPHA.)-, and 4, 4, 6A, 6B, 8A, 11, 11, 14B-octamethyl-1, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-octadecahydro-2 respectively.

Based on the given results of GC-MS constituents of *P. amboinicus,* (Z)-9-hydroxy-2,4-dimethyl-non-7-enoic acid lactone had the highest peak area. This constituent is a phenolic compound which functions as a defense against insects (Magnelli *et,al.*, 2010). It plays an important role as anti-nutritional components of food and animal feed (Bilal *et.al.*, 2012). These include cell wall phenolic components lignification of cells and the presence of polyphenols such as condensed tannins. Molan *et al.*, 2003 observed that monomers of condensed tannins were responsible for decrease of the egg hatching, larval development and viability of infective larvae. The activity of these metabolites is attributed to its ability to bind to the free protein available for larvae nutrition; reduced nutrient availability could have resulted in larvae starvation and death (Athanasiadou *et al.*, 2001). Schultz (1989) reported that in insects and insect larvae that ingest the condensed tannins occurs connection of these compounds with intestinal mucosa causing autolysis, leading to an inability to use nutrients by the larva.

In addition, eight phytocomponents were determined such as, 1-methylene-2B-hydroxymethyl-3, 3-dimethyl-4B-(3-methylbut-2-enyl)-cyclohexane, 1, 6-cyclodecadiene 1- methyl 5methylene-8-(1-methylethyl)-, [S-(E,E)]-, 2R-acetoxymethyl-1, 3, 3-trimethyl-4T-(3- methyl-2-buten-1-yl)-1T-cyclohexanol, (Z)-9- hydroxy-2,4-dimethyl-non-7-enoic acid lactone, 1-formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2- enyl)-5-cyclohexane, 3-methyl-2-(2- oxopropyl)furan, Tritetracontane, and 1- (hexahydropyrrolizin-3-ylidene)-3,3-dimethyl-butan-2-one.

Larvicidal Activity

The graph shows percentage mortality of *Ae. aegypti larvae in P. scutellarioides*, and *P. amboinicus* leaf crude extract as well as the positive control with various concentrations.

The trend shows that percent mortality increases upon exposure to higher concentration. After 24 hours of exposure, the lowest mortality was observed at 6 ppm under *P. amboinicus* leaf crude extract having 70.56 ± 6.371%. Highest mortality was observed at 60 ppm of *P. scutellarioides* leaf crude extract which obtained a value of 96.11 ± 1.389%. However the positive control with a concentration of 60 ppm exhibited a higher mortality rate of 99.44 ± 0.556 than that of the *P. scutellarioides* leaf crude extract which has the highest mortality among the three concentrations of the two leaf crude extract. The exhibited trend implies that the effect of the leaf crude extracts of *P. scutellarioides* and *P. amboinicus* as well as the positive control (*A. indica*) to the mortality of larvae of *Ae. aegypti* at a specific time was directly related on the concentrations of each leaf crude extract. An independent-samples t-test was conducted to compare larvicidal activity of leaf crude extract of *A. indica and P. scutellarioides* against *Ae. aegypti*. There was no significant difference in the mortality of *Ae. aegypti* in *A. indica* (M=14.6667, SD= 5.88953) and *P. scutellarioides* (M=14.0741, SD=5.61399) leaf crude extracts; t (268)= .846, p = .398. These results suggest that the larvicidal activity of *P. scutellarioides* is comparable with that of *A. indica.* Specifically, this suggest that *A. indica* and *P. scutellarioides* have equal larvicidal activity. For the comparison of *A. indica* and *P. amboinicus* leaf crude extract larvicidal activity against *Ae. aegypti*, the results showed that there was a significant difference in the mortality of *Ae. aegypti* in *A. indica* (M=14.6667, SD= 5.88953) and *P. amboinicus* (M=10.0593, SD=5.61815) leaf crude extracts; t (268)= .846, p = .000. These results suggest that the larvicidal activity of *P. scutellarioides* is far comparable with that of *A. indica* and the larvicidal activity of *P. amboinicus* is lower than of *A. indica.*

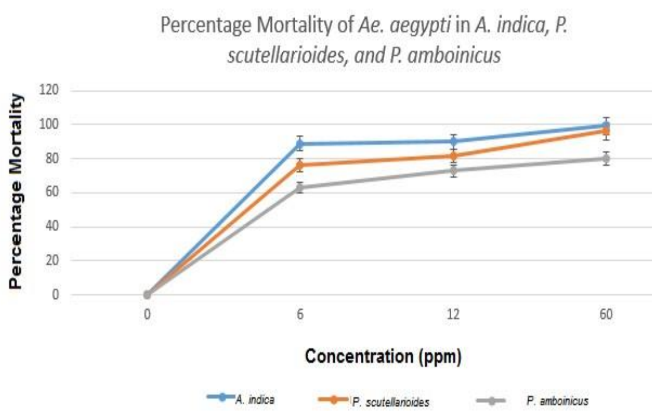

Figure 1. Percentage mortality of *Ae. aegypti* in *A. indica* (positive control), *P. scutellaroides* and *P. amboinicus*

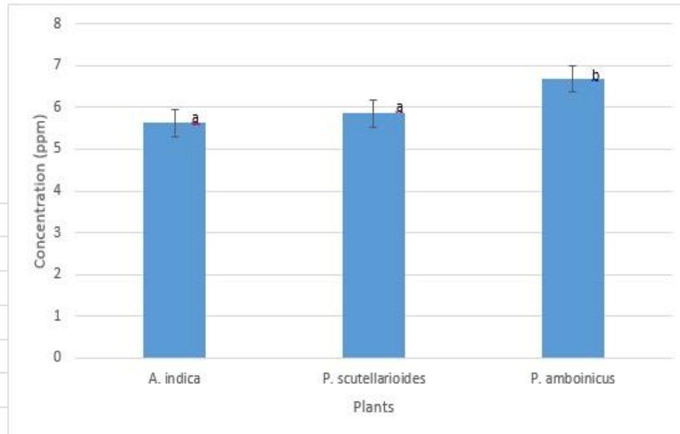

Figure 2. The lethal concentration 50 of *Ae. aegypti* in *A. indica* (positive control), *P. scutellarioides,* and *P. amboinicus*

Based on Figure 2, the calculated LC50 values of *P. scutellarioides* and *P. amboinicus* leaf crude extract were 5.85 ± 0.4719ppm and 6.68 ± 0.6017 ppm respectively. For the calculated value of LC50, *P. scutellarioides* leaf crude extract obtained lower value than *P. amboinicus*. This indicates that at a lower value of 5.85 ppm, *P. scutellarioides* can already be lethal to 50% of the population of *Ae. Aegypti* while *P. amboinicus* leaf crude extract needs to have a higher concentration of 6.68 ppm to kill 50% of the test population.

A larvicide with lower LC50 value has higher toxicity effect compared to a larvicide with a higher LC50 value. This means that the larvicide only requires low concentration to have a toxic effect on the 50 % of the population.

4. CONCLUSION

The study revealed that *P. scutellarioides* containing terpenoids, saponins and anthraquinones could kill 50 % of the larval population with lower concentration compared to *P. amboinicus.* Lowest percentage mortality was observed in *P. amboinicus* while the highest was in *P. scutellarioides.* It also showed no significant difference in terms of larvicidal activity with that of *A. indica.* Thus, it can be established that *P. scutellarioides* could be an effective biological control in lieu of the synthetic one. The present study explored the possibilities of using a biological control through the use of plants to prevent the proliferation of mosquito which in turn will lessen the incidence of dengue and other mosquito-borne diseases.

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6. REFERENCES

Bilal, H., Akram, W., Ali-hasan, S. 2012. Larvicidal Activity of Citrus Limonoids against *Aedes albopictus* Larvae. J Arthropod-Borne Dis, 2012, 6(2): 104–11

Castillo - Sanchez, L. E., Jimenez - Osornio, J. J., Delgado - Herrera, M.A., 2010. Secondary Metabolites of the Annonaceae, Solanaceae and Meliaceae families Used as Biological Control of Insects. Tropical and Subtropical Agroecosystems, 12 :445 -462

Eskenazi, B., J., Chevrier, L. G., Rosas, H. A. Anderson, M.S. Bornman, H. Bouwman. A. Chen, B. A. Cohn, C., de Jager, D. S., Henshel, F., Leipzig, J. S., Leipzig, S. M., Snedeker, D., Stapleton. 2009. The Pine River Statement:Health Consequences of DDT Use. Environ Health Perspect; 117(9): 1359–1367

Fraga B. M. (2004), Natural sesquiterpenoids. Nat. Prod. Rep. 21, 669D693.

Gillilan, J. 2012. Improvement of U.S. EPA Minimum Risk Essential Oils' Pesticide Activity Through Surfactant Enhancement and Synergy.

Gutierrez Jr, P. M., Antepuesto, A. N., Eugenio, B. A. L. and Santos, M. F. L. 2014. Larvicidal Activity of Selected Plant Extracts against the Dengue vector *Aedes aegypti* Mosquito. International Research Journal of Biological Sciences. 3(4): 23-32.

Isman, M.B. (2000). Plant essential oils for pest and disease management. University of British Columbia, Suite 248, 2357 Main Mall, Vancouver, BC, Canada V6T 1Z4.

Kishore, N. *et al.*, (2011). A Review on Natural Products with Mosquitocidal Potentials. Research Signpost 37/661 (2), Fort P.O. Trivandrum-695 023 Kerala, India.p.335-365

Lee, K., Lynn, O.M., Shim, J., and Kim, J. (2010). Effects of Azadirachtin and Neem-based Formulations for the Control of Sweetpotato Whitefly and Root-knot Nematode.J. Korean Soc. Appl. Biol. Chem. 53(5), 598-604.

Molan, A.L.; Meagher, L.P.; Spencer, P.A.; Sivakumaran, S. Effect of flavan-3- ols on in vitro egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. *International Journal of Parasitology*, v. 33, p. 1691-1698, 2003.

Nishida R., Kawai K., Amano T. and Kuwahara Y. (2004), Pharmacophagous feeding stimulant activity of neo-clerodane diterpenoids for the turnip sawfly, Athalia rosae ruficornis. Biochem. Syst. Ecol. 32, 15-25.

Pierre, D. S., Nchiwan, N. E., Okechukwu, Lame, Y. 2014. Larvicidal and Pupicidal Toxicities of *Plectranthus glandulosus* and *Callistemon rigidus* Leaf Essential Oils against Three mosquito Species. Journal of Mosquito Research, 2014, Vol. 4, No. 2 doi: 10.5376/jmr.2014.04.0002

Senthilnathan S, Choi MY, Seo HY, Paik CH, Kalaivani K, Kim JD. Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata* lugens (Stål)Ecotox Environ Safety. 2008;70:244–50.

The Norwegian Academy of Science and Letters Ed. by Aksel Bernhoft (2008). Bioactive compounds in plants – benefits and risks for man and animals.

Athanasiadou, S.; Kyriazakis, I.; Jackson, F.; Coop, R.L. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology*, v. 99, p.205-219