Available online at www.sciencedirect.com

ScienceDirect

Procedia - Social and Behavioral Sciences 153 (2014) 180 – 188

Procedia
Social and Behavioral Sciences

AicQoL2014Kota Kinabalu
AMER International Conference on Quality of Life
The Pacific Sutera Hotel, Sutera Harbour, Kota Kinabalu, Sabah, Malaysia
4-5 January 2014
"Quality of Life in the Built & Natural Environment"

Biolarvacidal Potential of Ipomoea Cairica Extracts Against Key Dengue Vectors

Ahmad Razali Ishak *, Nazri Che Dom, Hazilia Hussain, Nur Husna Sabri

Department of Environmental Health, Faculty of Health Sciences, Universiti Teknologi MARA

Abstract

Chemicals are used widely as insecticides causing adverse effects to human health and the environment. Natural insecticides derived from plants which is eco-friendly. This study is to assess the efficacy of Ipomoea cairica extract against two different mosquito larvae. Lethal Concentrations (LC_{50}) of petal, leaves and root extract against Ae. albopictus larvae were 20.5, 27.9 and 34.3 mg/L respectively. Lethal concentration (LC_{50}) were found to be lower in Ae. aegypti which were 12.7, 13.6 and 31.9 mg/L respectively. In conclusion, these extracts can be utilized as an option in vector control program.

Peer-review under responsibility of the Association of Malaysian Environment-Behavior Researchers, AMER (ABRA malaysia).

Keywords: Ae. Aegypti; ae. albopictus; dengue; ipomoea cairica

1. Introduction

Mosquito population is a well-known vector in transmitting many types of diseases causing serious public health problems (Gubler 1995; Lam, 1993; Nazri *et al.* 2013a). In Malaysia, there are about 434 species of mosquitoes representing 20 different genera (Zaridah *et al.* 2006). Many studies have indicated that *Ae. aegypti* and *Cx. quinquefasciatus* are the most abundant urban mosquitoes found in Malaysia (Raghavendran *et al.* 2011). The common method used currently in Malaysia to control mosquito's larvae is through the usage of chemicals such as abate. However, extensive use of chemical insecticides have

*Corresponding author. Tel.:+60-0332584509; fax: +60-0332584599
E-mail address: ahmadr2772@puncakalam.uitm.edu.my

Peer-review under responsibility of the Association of Malaysian Environment-Behavior Researchers, AMER (ABRA malaysia).
doi:10.1016/j.sbspro.2014.10.052

created problems in insecticide resistance indicates a reduction of sensitivity of a mosquito towards insecticides developed due to the widespread usage of chemicals in destroying larvae. Thus, it is considered to be a serious public health challenge that contributes to ineffective method for disease control (Nazri *et al*. 2013b). Moreover, in ecological perspective, the widely used pesticide also kills non-targeted predators for mosquitoes such as dragonfly and other insects. A study conducted by Hidayatulfathi et al. (2005) revealed that insecticide resistance has developed in three different species of mosquitoes (*Cx. quinquefasciatus, Ae. albopictus and Ae aegypti*) larvae against Malathion, Permethrin and Temephos. The study also reported that *Cx. quinquefasciatus* developed higher resistance to Malathion and Permethrin as compared to *Ae. Albopictus* and *Ae. aegypti*. There are known specific cure has been found for most vector borne diseases, reducing the larval population is the best in controlling the outbreak of dengue. Abu Bakar *et al.* (2009) tested on M. cajuputi plant extracts against dengue vector in the laboratory setting. The result showed high mortality of 60.2% – 61.4% and 60.8% - 64.0 % in *Ae. aegypti* and *Ae. albopictus* when sprayed with 5% and 10% M. cajaputi after exposure for 5 and 10 seconds respectively. Their study was extended using the same plant species in aerosol spray cans at low cost housing flat in Kuala Lumpur and discovered that there was a high knockdown and mortality rate in both *Aedes* species towards the MS aerosol (Abu Bakar *et al.* 2012).In addition, the repellent activities of several plants were also tested against *Aedes* species in Malaysia. Misni *et al.* (2008) investigated the repellency of Piper aduncum Linn essential oil against *Ae. aegypti* and their results indicated optimum repellency (ED_{50} value 1.236 $ugcm^{-2}$ and ED 900.8 $ugcm^{-2}$) occurred after 90 seconds exposure. For the effect against *Ae. albopictus*, the lowest median effective dose (ED_{50}) value was 2.1 ug/cm2 at 90 seconds of exposure (Misni *et al.* 2009). Furthermore, field evaluation by ultra-low-volume (ULV) spray against dengue vector using Litsea elliptica extracts and bifenthrin indicated low larvacidal effect but the extract caused 96.5 -97.6% mortality among *Ae. aegypti* adults (Sulaiman *et al*. 2006). Thus, these findings exhibit the potential of using natural products in combating vector borne diseases. Other studies have also shown the advantage of using plant extracts as larvacide because they do not induce pesticide resistance in the mosquitoes (Azmathullah, 2011; Borah *et al*. 2010; Choochote *et al*. 2004; Chowdry *et al*. 2006 and Katade *et al*. 2006). Recently, natural products derived from plants extracts are widely used as an alternative method against mosquitoes due to their excellent properties like low toxicity to human and a high degree of biodegradability thus reducing the risk of adverse ecological effects. In view of residue problems in the environment and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore the use of plants to obtain extracts that are safe for non-targets animals and do not pose any residual problem. The working concept of this research is to assess the ability of *Ipomea cairica* (morning glory) as a larvidcide and its efficacy to control vector mosquito population. This study addressed the two main objectives in achieving full understanding of this potential effect of natural product. (1) To screen the phytochemical properties of of Ipomea cairica. (2) To investigate the efficacy of different part of morning glory extract (*Ipomea cairica*) as larvacide to *Ae. aegypti* and *Ae. Albopictus* using larvacidal assay technique.

2. Material and Methods

2.1. Plant material extraction

The samples of *Ipomea cairica* plant were collected and washed using distilled water to avoid possible trace contamination. Then, the different parts of *Ipomoea cairica* such as petals, roots and leaves were dried at room temperature (25 - 28 °C) and grounded separately to produce fine particles. By using modern soxhlet extraction technique, Soxterm, 10 gram of each powdered material was inserted into soxterm extraction thimble. The samples were soaked in 500 ml of methanol in solvent vessel. The

soaked material were left for 3 hours in Soxterm extractor. The vessel containing the crude extract was dried in the dryer at 85 °C about 1 hour. The weight of crude extract was calculated according to the following formula:

$$\text{Crude extract (gram)} = (m2 - m1)/E \qquad (1)$$

The formula indicates m2 = weight of the dry empty vessel in grams, m1 = weight of the vessel in grams containing crude extract after evaporation of the solvent and E = the sample weight in grams (10 gram). The volume of stock solution should be 20 ml of 1%, obtained by weighing 200 mg of Ipomoea cairica crude extract and adding 20 ml methanol to it (WHO, 2005). The crude extract was kept in a screw-capvial, with aluminium foil over the mouth of the vial. These extractions were preserved in a refrigerator at 4°C temperatures with proper labeling until it was used in phytochemical screening and mosquito larvicidal bioassay tests.

2.2. Phytochemical screening

Qualitative determinations of phytochemical constituents were carried out on the methonolic extracts of Ipomoea cairica as described by Prashant *et al*, 2011. Nine phytochemical constituent were screened including saponins, alkaloid, phenol, tannin, glycoside, flavonoid, Amino acid, phytosterol and terpen.Ferric Chloride Test chloride solution. Formation of bluish black color indicates the presence of phenols. The extract was run through Gelatin's Test for tannin screening. In this method, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins. Detection of glycosides was carried out by modified Borntrager's Test. The plant extract was hydrolyzed with diluted hydrochloric acid. Then, the extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with an equal volume of benzene. The benzene layer was separated and then the solution was treated with ammonia solution. Rose-pink color was formed in ammonical layer indicates the presence of anthranol glycosides. For flavonoids screening, Alkaline Reagent Test was used. In this test, the extract was treated with few drops of sodium hydroxide solution. Intense yellow color was formed, which becomes colorless on addition of dilute acid indicates the presence of flavonoids. Froth test was used for saponins detection. Plant extract was diluted with distilled water to 200 ml. This solution was then shaken in a graduated cylinder for 15 minutes. 1 cm layer of foam formed which indicates the presence of saponins. Xanthoproteic Test was used to detect the presence of protein and amino acids. The extract was treated with few drops of concentration nitric acid. Yellow color formation indicates the presence of proteins. Salkowski's Test was used for phytosterols detection. The extract was treated with chloroform and filter. The filtrate was treated with few drops of concentrated sulphuric acid, mixes well and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.The extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Emerald green color formation indicates the presence of diterpenes.

2.3. Preparation of mosquito's larvae

Ae. aegypti and *Ae. albopictus* were obtained as egg raft column on a filter paper from Vector Control Research Unit (VCRU) University Science of Malaysia (USM) Penang. Larval food was added to the distilled water 24 hours prior to the placement of the collected eggs to promote uniform hatching. The

hatched larvae were then transferred to shallow pans containing 2 liter of tap water. The containers were kept at 25 + 2 °C throughout the process.

2.4. Preparation of mosquito's larvae

Bioassays for the toxicity of the extracts were carried out against *Ae. aegypti* and *Ae. Albopictus* larvae using World Health Organization (WHO) Standard Method 2005. In the larvicidal assay, two types of mosquito larvae at the third instars stage were exposed to test concentration of 10, 25, 50, 75 and 100 pm of different part of Ipomoea cairica extract into the disposal test cups. Three replicates for eachconcentration together with one control with distilled water were set up simultaneously. The tests were conducted at room temperature at 25 to 27 oC. The knockdown rate was recorded every 5 minutes within 1 hour after 24 hours the test larvae exposed to the concentration. Mortality was recorded each 4 hours until 48 hours exposure. During this period of time, no food was offered to the test organisms (Zaridah *et al*. 2006). This same method will apply to the rest species of *Ae. albopictus* mosquito larvae.

$$Mortality\ (\%) = (X\text{-}Y)/Y \times 100 \qquad (2)$$

The formula above indicates X = percentage survival in the untreated control and Y = percentage survival in the treated sample. Any small, unhealthy or damage larvae were removed or replaced. The depth of the water in the cups or vessels was maintained between 5 cm to 10 cm because the deeper level may cause undue mortality.

2.5. Data analysis

Data collected were pooled for analysis. LC_{50} and LC_{90} were calculated from a log dosage probit mortality regression. The lethal concentration to 50% (LC_{50}) and lethal 90% (LC_{90}) of test organisms, 95% onfidence interval and their slopes of probit regression line will be determine using Microsoft excel 2010 (Zaridah *et al*. 2006).

3. Result

3.1. Phytochemical screening

Table 1. Result of phytochemical constituent

Plant species	Phytochemical	Petal	Leaf	Root
Ipomoea cairica	Alkaloids	+	+	-
	Glycosides	+	-	-
	Saponins	+	+	-
	Phytosterols	-	-	-
	Phenols	+	-	-
	Tannins	-	+	+
	Flavanoids	+	+	+
	Protein and acid amino	-	+	+
	Diterpens	-	+	+

Note: (+) Detected; (-) Not detected

The result of the phytochemical screening as tabulated in Table 1 reveals that flavonoids were present in the petals, leaves and roots extract of Ipomoea cairica. Alkaloids were detected both in the petals and leaves extract while glycosides were detected in the petals extract only. In addition, Saponins were

tected in both petals and leaves while phenols were detected only in the petals extract. For tannins, protein and amino acids and diterpens were detected in both leaves and roots extract but not present in the petals. Phytosterols test gave negative readings to all of the extracts.

3.2. Knockdown and cumulative mortality assessment

Figure 1 showed the comparison of cumulative knockdown rate between three different parts of Ipomoea cairica extracts. Based on the trend, the petal's extracts are more effective compared to the leaves and roots extract to both species of *Aedes* mosquito. Figure 1 also showed the percentage mortality of third instar larvae for *Ae. aegypti* and *Ae. albopictus* as larvacides. The results revealed that the methanol extractions of Ipomoea cairica under laboratories conditions have indicated the potential larvacidal activities. Furthermore, the treatment of increasing dosage of extracts (10, 25, 50, 75, 100 ppm) clearly indicates the effect of the mortality dependence on the concentration of the plant extracts.

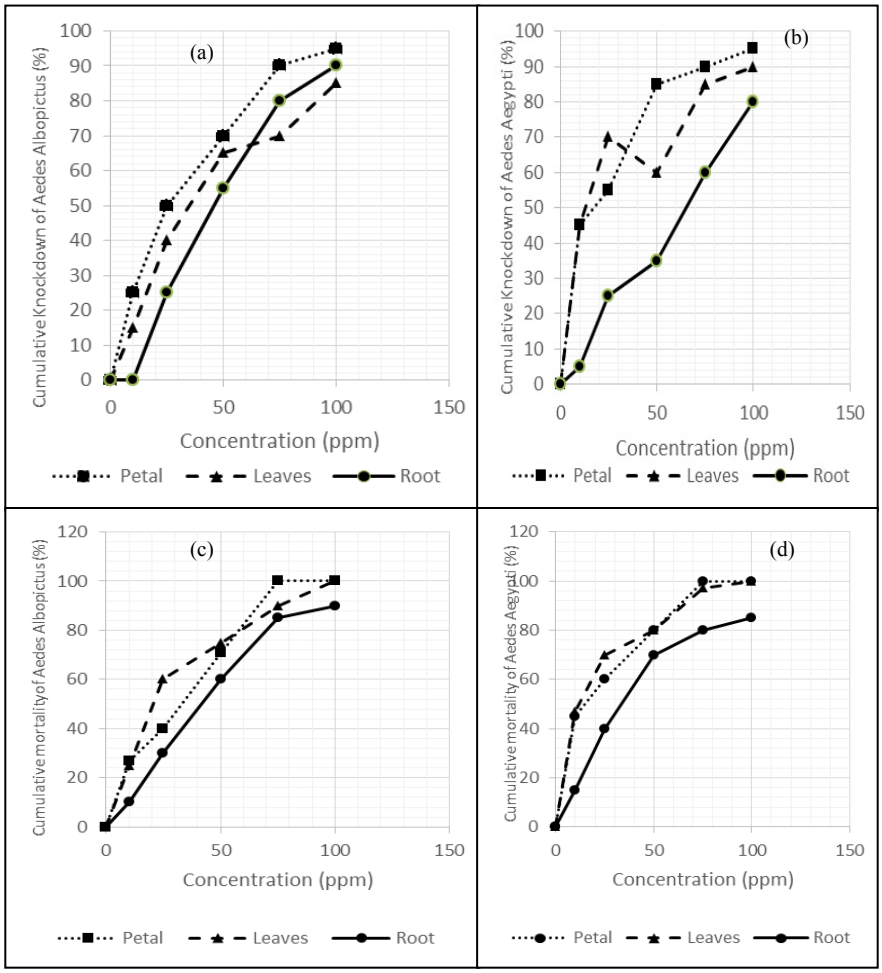

Fig. 1. Trend of knockdown rate and cumulative mortality rate induced by crude extract of three different part of *Ipomoea cairica* on the late third instar larvae. Noted (a&c) *Ae.albopictus* and (b&d) *Ae.aegypti* in different concentration exposure.

In addition, the comparison of cumulative larval mortalities between three different parts of *Ipomoea cairica* extracts indicates that the leaves and petal extracts provide more effective result compared to root extracts. The leaf extract was the most lethal although in a low concentration compared to other parts of plants for both *Ae. albopictus* and *Ae. aegypti* larvae. However, at higher concentration, the petal extracts were more lethal than leaves extracts.

3.3. Larvacidal efficacy

As shown in Table 2, all parts of Ipomoea cairica also exhibited strong larvacide activities against *Ae. aegypti* and *Ae.albopictus* larvae in 48 hour exposure. The LC_{50} values of the leaf, petal and root essential oils against *Ae.albopictus* were 20.5 mg/L (LC_{90}: 62.5 mg/L), 27.9 mg/L (LC_{90}:163.1mg/L) and 34.3 mg/L (LC_{90}:105.3mg/L), respectively. On the other hand, the essential oil of leaves and petals was also potent against *Ae. aegypti* larvae, showing LC_{50}:12.7 mg/L (LC_{90}:62.3 mg/L) and LC_{50}:13.6 LC_{90}:182.7 mg/L). The least larvacide activity was observed for the root extracts of Ipomoea cairica, with a LC50 = 31.9 mg/L (LC_{90}:157.7 mg/L).

Table 2. Lethal concentration of larvacidal activity from different part of *Ipomoea cairica* against *Ae aegypti* and *Ae. albopictus*

Mosquito species	Treatment	24 hours			48 hours		
		LC_{50} (mg/L)	LC_{90} (mg/L)	Regression	LC_{50} (mg/L)	LC_{90} (mg/L)	Regression
Aedes albopictus	Petal	31.8	174.4	Y= 1.7317x + 2.3985	27.9	163.1	Y= 1.6687x + 2.5879
	Leaf	21.7	118.2	Y= 1.7378x + 2.6779	20.5	62.5	Y= 2.1195x + 2.2181
	Root	37.6	`114.8	Y= 2.6418x + 0.8379	34.3	105.3	Y= 2.6314x + 0.9586
Aedes aegypti	Petal	18.0	180.4	Y= 1.2802x + 3.3916	13.6	182.7	Y= 1.1346x + 3.7138
	Leaf	12.2	82.8	Y= 1.5363x + 3.3337	12.7	62.30	Y= 1.8573x + 2.9471
	Root	37.5	121.1	Y= 2.5163x + 1.0381	31.9	157.7	Y= 1.8331x + 2.2513

LC_{50}: Lethal concentration of required to kill 50 per cent the population exposed
LC_{90}: Lethal concentration required to kill 90 per cent of the population exposed.

4. Discussion

The control of mosquito larvae using larvicidal agents is a major component in the control of vector borne diseases. Thus, investigation into plants as potential larvicides is considered as viable and preferred alternative in the control of the community level. Moreover, the plant should be locally available or easily found at the local level. Phytochemicals compound act as general toxicants both against adult as well as against larval stages of mosquitoes, while others interfere with growth and development or reproduction or produce olfactory stimuli acting as repellent or attractant (Mathew *et al*., 2009). In this study, the phytochemical screening test reveals the present of phytochemical constituents in all part of Ipomoea cairica extracts. These phytochemicals found in this study was responsible as a larvacide agent to kill the mosquito's larvae (Kabaru & Gichia, 2009). The finding of the current study is consistent with Chapagain *et al.* (2008) who discovered that the increasing concentration of root derived from callus saponins of B. aegyptiaca increased the mortality of the larvae. Furthermore, it supported the idea of the saponin compound in the plant can help to destroy the mosquito larvae. In addition, the finding of Mathivanan et *al*. (2010) also suggested that the presence of alkaloid from Piper longum fruit was found to be active against mosquito larvae of *Cx. Pipiens*. Many studies have been conducted on the knockdown effect of adult mosquitoes (Xue *et al*. 2004), but few study had done of knockdown rate for larvae mosquitoes. In this study, the knockdown effect occurred for all test concentration in all different parts of Ipomoea cairica extraction. Knockdown is defined as the initial effect such as morbid or unusual behaviour due to

the alteration of a specific physiological process or processes that taken placed upon contact with the toxicant (Hidayatulfathi *et al*, 2004). Before exposure, all the third instar larvae of both *Aedes* sp. exhibited a natural behaviour with the siphon had pointed up through the water surface and heavy hung down. However, after 5 minutes of exposure, abnormal evidence of excitation, restlessness, and sluggishness was initially observed. Excitation and restlessness persisted for between 10-30 minutes, and other abnormal motions were seen such as coiling movement. The treated larvae frequently sank down and floated up again quickly. During the period of 30-60 minutes, some larvae showed more toxic symptoms including vibration and convulsion at the bottom of the plastic cup. Afterwards, more larvae exhibited the toxic symptoms. In the larvicidal bioassays, there were no pupation and mortalities that exceeded 20% in the negative control solutions thus, discarding the bioassay experiments or correcting the mortality data was not necessary. The result of LC_{50} and LC_{90} of all extracts, showed that the leaf of Ipomoea cairica essential oil were more toxic to *Ae.aegypti* compared to *Ae. albopictus*. The least toxic to both these species is the root extracts which probably contain slow-acting insecticidal ingredients. Jantan *et al*. (2003) evaluated 17 methanol extracts and nine essential oils of Malaysian plants for their larvicidal activity against Ae. aegypti. The essential oils of *Cinnamomum impressicostatum, Cinnamomum microphyllum* and *Curcuma domestica* showed significant effects, reaching LC50 values of 13.7, 20.6 and 20.9 mg/ml, respectively. Except for the oil of *Zingiber cassumunar*, the essential oils of the other species were effective against larvae, with LC_{50} values less than 200 mg/ml. *Garcinia praniana*, *Garcinia griffithii*, *Labisia pumila var. alata*, *L. pumila var. pumila* and *Mitragyna speciosa* showed relatively high activity with LC_{50} values ranging from 103–271 mg/ml. Although, *Ipomoea cairica* plant extracts have been studied for various medicinal properties, there is no study conducted on the mosquito larvicidal properties of this plant reported to our knowledge. From the results of the present investigation, the larvicidal activities observed for all extraction (leaf, petal and root) are of special interest. The low LC_{50} and LC_{90} indicating their high potential to serve as a larvicidal agent against key dengue vectors. Further study on the isolation of the active principles responsible for the mosquito larvicidal activity may pave the way for the development of a botanical insecticide for the control of *Ae.aegypti* and *Ae.albopictus*, the dengue vector. At present, mosquito control is mostly directed against larvae because the fight against adult is temporary, unsatisfactory and polluting the environment, but larvae treatment is more localized in time and space resulting in less dangerous outcomes (Chowdhury *et al*., 2008). Larvae control can be an effective control tool due to the low mobility of larvae mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified (Howard *et al*. 2007). These studies have proven the principle in controlling larvae breeding using natural products is feasible and cost effective. Eco-friendly chemicals are recommended in larviciding mosquito breeding sites. In this case, a plant species that is used in managing of mosquitoes was analyzed for its larvicidal properties. Plants being a natural source of compounds are known to contain larvicidal agents, which may act independently or in combination, even in their semi purified form. The plant extract can be used together with chemical larvicide to produce a synergistic effect which is more effective and reduce negative physiological impact to the environment.

5. Conclusion

This study proof that the extracts of *Ipomoea cairica* has remarkable larvicidal properties and should be further explore in the future to reduce the dependency on chemical larvicide such as Abate, Temephos for mosquito control program. Further study is needed to extract Ipomoea cairica using different types of solvents and identifying the active component of the plant that causes larvae mortality. Field trials are also required to determine their potential as an alternative larvicide. Plant extracts could be an alternative method for mosquito larvicide because they contain bioactive chemicals which are generally free from

harmful effects. This would reduce the use of chemicals which is hazardous to the living environment and promote sustainable utilization for available bio-resources. Tropical country like Malaysia has advantage because indigenous plants such as Ipomoea cairica are available in large quantities. Moreover, it is cost effective to produce their extracts and for field use in vector control program.

Acknowledgement

The authors sincerely thank the Research Management Institute (RMI) for awarding research funding from Universiti Teknologi MARA (UiTM) and Department of Higher Learning, Ministry of Education, Malaysia.

References

Abu Bakar, A., Sulaiman, S., Omar, B., Mat Ali, R. 2012. Evaluation of *Melaleuca cajuputi* (Family; Myrtaceae) essential oil in spray cans against dengue vectors in low cost housing flats. *Journal Arthropod-Borne Dieases* 6(1):28-35

Ananth, S., Thangamathi, P., Pazhanisamy, S., Meena, S. 2009. Larvicidal Activities of three medicinal Plants against the Filarial Mosquito *Culex Quinquefasciatus*. *Journal of Basic and Applied Biology* 3(1 & 2):53-5

Azmathullah, N.M. 2011.Phytochemical screening of calotropis procera flower extracts and their Bio-control potential on *Culex* sp mosquito larvae and pupae. *International Journal of Pharmaceutical & Biologycal Archive* 2(6):1718-1721

Borah,R., Kalita, M.C., Kar, A. & Talukdar, A.K. 2010. Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vector *Aedes Aegypti* and *Culex quinquefasciatus*. *African Journal of Biotechnology* 9(16):2527-2530.

Chapagain, Bishnu P, Saharan, Vinod, & Wiesman, Zeev. (2008). Larvicidal activity of saponins from Balanites aegyptiaca callus against Aedes aegypti mosquito. *Bioresource technology, 99*(5), 1165-1168.

Choochote,W., Tuetub, B., Kanjanapothi, D., Rattanachanpichai, E., Chaithong, U., Chaiwong, P., Jitpakdi, A., Tippawangkosol, P., Rivong, D., Pitasawat, B. 2004. Potential of crude seed extracts of celery, Apium graveolens L., against the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *Journal of Vector Ecology* 29(2):340-6

Chowdhury, N., Ghosh, A., Chandra, G. 2006. Mosquito larvicidal activities of solanum villosum berry extraction against the dengue vector *stegomyia aegypti*. *BMC Complementary and Alternative Medicine* 8:10

Gubler, D.J. & Clark, G.G. 1995. Dengue/dengue hemorrhagic fever: the emergence of a global health. *Emerging Infectious Disease* 1:55-57

Hidayatulfathi, O.,Sallehuddin, S., Shafariatul, A.I., Zaridah, M.Z. 2005. Studies on the Larvacidal , Adulticidal and repellent activities of *Acorus calamus* Linn. Against *Aedes Aegypti* Linn. (Diptera: Culicidae) *Anns. Medical Entomology* 14(1):6-13.

Jantan, I., Wong, O.P., Sheila, D.V., Nazni, W.S., (2003), Larvicidal Activity of the Essential Oils and Methanol Extracts of Malaysian Plants on Aedes aegypti, Pharmaceutical Biology, 2003, Vol. 41, No. 4 : Pages 234-236

Kabaru, JM, & Gichia, L. (2009). Insecticidal activity of extracts derived from different parts of the mangrove tree Rhizophora mucronata (Rhizophoraceae) Lam. against three arthropods. *African Journal of Science and Technology, 2*(2).

Katade, S.R., Pawar, P.V., Wakharkar, R.D., Deshpande, N.R. 2006. Sterculia guttata seeds extractives – An effective mosquito larvicide. *Indian Journal of Experimental Biology* 44:662-665.

Lam, S.K. 1993. Two decade of Dengue in Malaysia. *Tropical Medicine*, 35(4):195-200.

Mathew, Nisha, Anitha, MG, Bala, TSL, Sivakumar, SM, Narmadha, R, & Kalyanasundaram, M. (2009). Larvicidal activity of Saraca indica, Nyctanthes arbor-tristis, and Clitoria ternatea extracts against three mosquito vector species. *Parasitology research, 104*(5), 1017-1025.

Mathivanan, T, Govindarajan, M, Elumalai, K, Krishnappa, K, & Ananthan, A. (2010). Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria Stapf.*(Family: Apocynaceae). *Journal Vector Borne Disease, 47*, 178-180.

Misni, N., Sulaiman, S., Othman, H. 2008. The repellent Activity of *Piper aduncum* Linn (Family : Piperaceae) essential oil against Aedes aegypti using human volunteers. *Journal of Tropical Medicine & Parasitology* 31:63-9

Misni, N., Sulaiman, S., Othman, H., Omar, B. 2009. Repellency of essential oil of *Piper aduncum* against *Aedes albopictus* in the laboratory. *Journal of American Mosquito Control Association* 25 (4): 442:447

Nazri Che Dom., Ahmad, A. H., & Ismail, R. (2013a). Habitat Characterization of Aedes Sp. Breeding in Urban Hotspot Area. *Procedia-Social and Behavioral Sciences*, *85*, 100-109.

Nazri Che Dom., Ahmad, A. H., Ishak, A. R., & Ismail, R. (2013). Assessing the Risk of Dengue Fever Based On the Epidemiological, Environmental and Entomological Variables. *Procedia-Social and Behavioral Sciences*, *105*, 183-194.

Prashant, T., Bimlesh K., Mandeep K., Gurpreet K., Harleen K., (2011) Phytochemical screening and Extraction: A Review, Journal of Internationale Pharmaceutica Sciencia, Vol. 1 Issue 1

Raghavendra, B.S., Prathibha, K.P., & Vijayan, V.A. 2011. Larvicidal efficacy of Eugenia Jambolana Linn. Extracts in three mosquito species at Rahman, W.A., Che'Rus, A. & Ahmad, A.H. (1997) Malaria and *Anopheles* mosquitoes in Malaysia Southeast Asian .*Journal of Tropical Medicine and Public Health* 28:599-605.

Sulaiman, S., Abang Kamaruddin, D.S.F., Othman, H. 2008. Evaluation of Bifenthrin and Acorus calamus Linn. Extract against *Aedes aegypti* L. and *Aedes albopictus* (Skuse). *Iranian Journal Arthropod-Borne Disease* 2(2): 7-11

Sulaiman, S., Othman, H., Shaari, N., Pawanchee, Z.A., Wahab, A., Abdullah, A.R.L. 2006. Field evaluation of Litsea elliptica extract and bifenthrin by ULV spraying against dengue vectors. *Journal of Tropical Medicine Parasitology* 29:51-5

Xue, Rui-De, Ali, Arshad, & Barnard, Donald R. (2003). Laboratory evaluation of toxicity of 16 insect repellents in aerosol sprays to adult mosquitoes. *Journal-American Mosquito Control Association, 19*(3), 271-274.

Zaridah, M.Z., Nor Azah M.A., Rohani A. 2006. Mosquitocidal activities of Malaysian plants. *Journal of Tropical Forest Science*, 18(1):74-80