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Toxicity activity of *Azadirachta indica* and *Murraya koenigii* against *Aedes albopictus* larvae in laboratory conditions

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Abstract

Dengue serves as major threat to human beings and has caused an increase in the rate of mortality every year without considering age, gender or ethnicity. Thus, an alternative by using botanical extracts against the larvae of this mosquito was carried out. *Azadirachta indica* and *Murraya koenigii* were used to evaluate the larvacidal activity against 3rd instar of *Aedes albopictus* larvae. The bioassay was carried out by using the methanolic extract of the *A. indica* and *M. koenigii* and the combined activity was done with the ratio 1:1 of the plant extracts. The result showed that at 3.75 mg/ml *A.indica* exhibits highest larvacidal activity by providing 96.30% of mortality. Furthermore, *M. koenigii* showed the percentage of mortality of 83.70% at 3.75mg/ml. In the basis of median lethal concentration (LC_{50}), it was found that the LC_{50} for *A. indica* was 1.45 mg/ml which is more susceptible than *M. koenigii* with 2.01 mg/ml as a LC_{50} value. The combination test showed that interaction between the 2 plants extract as an additivity effect. In nut shell, *A. indica* and *M. koenigii* was found to exhibit strong larvacidal activity. Thus, these plants have high potential to be developed as a biopesticide to control the emergence of *A. albopictus* larvae.

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Introduction

Aedes albopictus, has been described as a major human biting pest and commonly known as Asian tiger mosquito which belongs to the family of Culicidae. These species were found in tropical and subtropical areas of Southeast Asia and expands its territory to temperate regions of both urban and rural area around the globe. *Aedes albopictus* prefers rural, suburban and light or underdeveloped area as a breeding habitat and in host selection. Furthermore, the egg laid by *A. albopictus* able to withstand drought and hatch upon the climate is favorable (Kaplan *et al.*, 2010; Caminade *et al.*, 2012). Furthermore, *A. albopictus* has been described as a vector for a number of arboviruses that has an implication towards animals and human health which includes West nile fever, yellow fever, St. Louis encephalitis, Japanese encephalitis, Rift valley fever, chikungunya and dengue fever. In addition, the mosquito also transmits parasites such as *Dirofilaria immitis* which is commonly known as round worm that causes heartworm in dogs (Caminade *et al.*, 2012).

As an epidemiological concern, the annual dengue cases in Malaysia were found to be increase from 7103 in the year of 2000 to 46,171 in the year of 2010 and during the period of 2002-2010, the incidence rate of dengue cases were above 125 /100,000 population which were stated as high. Most of the dengue cases were reported as dengue fever which was found to be rise from 6692 in the year of 2000 to 42,140 in 2010. The ratio for dengue fever (DF) and dengue hemorrhagic fever (DHF) in the year of 2000 and 2010 was 16.3:1 and 10.5:1 respectively (Zaki *et al.*, 2014). In a study done at Negeri Sembilan, Malaysia in the year of 2010, it was found that the dengue cases for the time period of 12 months was 1,466 and reported that 8 month old child was the youngest patient and the oldest patient was 89 years old and in term of ethnicity, it was found that the Malays were found to be most affected, followed by the Chinese and Indian with the ratio of 4.1: 1.5: 1.0, and as per gender, males were found to be more dominant as compared to the female with the ratio of 1.4:1.0 (Cheah *et al.*, 2014).

The mortality rate from the dengue fever has increased from 45 in the year of 2000 to 134 in the year of 2010. The death rate from the DHF was 10.9% in the year of 2000 and considered as high in number and during the period of 2001- 2011 the mortality rate for DHF were found to be decrease with 2.5% and 6.9% respectively (Zaki *et al.*, 2014).The number of dengue cases that has been reported for the month of February 2015 in Malaysia was 23,966 which indicate an increase of 46% compared to February 2014 (WHO, 2015).

The use of chemical insecticide such as organophosphates against the mosquitoes has led to toxicity to the vertebrates due its ability to interrupt with acetylcholine and cholinesterase synthesis. Temephos has been used as larvacidal agent and malathion is used against adult mosquitoes. Pyretherin has been used together with synergist such as piperonylbutoxide and shows less toxicity to mammals and birds but remain toxic to fish and tadpoles (Mazzacano and Black, 2013). Artificial Juvenile hormone (Jh), Methoprene shows decrease in the rate of pupa and adult emergence and also cause morphological alteration. (Mazzacano and Black, 2013). In addition, the species of *Bacillus* such as *Bacillus thuringiensis var. israelensis* and *Bacillus sphaericus* causes larvacidal activity by their production of protein toxin. Bait traps has been used against adult mosquitoes and since it is broad spectrum compared to larva control, it do affect the non-targeted organism. The trap is baited with pheromones, feeding stimulants, aggregation pheromone and oviposition attractants (Mazzacano and Black, 2013). The indigenous plant such as *Syzygiumcumini* L, *Jatrophacurcas* L, *Delonixregia* L, *Limoniaacidissima* L, *Millingtoniahortensis*L, *Capparisspinosa* L, *Piper cubeba* L, *Neriumindicum*has been reported for larvacidal activity against *Aedes*species (Murthy and Rani, 2009).

Azadirachta indica or commonly known as neem belongs to the family of Meliaceae which is believed as sacred tree by the hindu ancestors and has served human as medicinal agent during the ancient medical system. Neem has been scientifically proven for its medicinal activity such as a pain relieving agent (Kumar *et al.*, 2012), antipyretic effects (Parveen, 2013), antimicrobial activity (Aarati *et al.*, 2011), antiviral activity (Tiwari *et al.*, 2010), contraceptive (Bansal *et al.*, 2010), hepatoprotective (Gomase *et al.*, 2011), hypoglycemic agent (Patil *et al.*, 2013). Besides of medicinal activity, it also serves as larvacidal agent (Maragathavalli *et al.*, 2012), antihelminth in goats (Chandrawathani *et al.*, 2013), as a fertilizer in poultry (Lokanadhan *et al.*, 2012) and as an insecticide (Musabyimana *et al.*, 2001).

Murraya koenigii or commonly known as curry leaves belongs to the family of Rutaceae can been found mainly as a cookery item, by which it is widely used as aromatic agent in many food preparation and found commonly in Indian dishes. Curry leaves has been reported scientifically for medicinal activities such as antimicrobial activity (Malwal and Sarin, 2011), antipyretic activity (Rageeb *et al.*, 2012), hypoglycemic and hypolipedimic activity (Tembhurne and Sakarkar, 2012), hepatoprotective activity (Parimi *et al.*, 2014), anti-inflammatory effects (Darvekar *et al.*, 2011), cytotoxic activity (Mohan *et al.*, 2013), chemoprotective activity (Swati *et al.*, 2012), inotropic and nephroprotective activity (Shah and Juvekar, 2006; Bhandri, 2012). Furthermore, it also effective against the Indian earthworm (Sharma *et al.*, 2010). Moreover, the leaves have been reported to exhibit larvacidal activity against the mosquito species (Ajay *et al.*, 2011).

Thus, this study involves the use of the *Azadirachta indica* leaves and *Murraya koenigii* leaves against *Aedes albopictus* larvae and attempts to elucidate the type of combine activity of the extracts.

Material and methods

Plant Material

Azadirachta indica (neem) and *Murraya koenigii* (curry leaf) were collected from a vegetable farm at Ipoh, Perak. Both plants were sent for authentication at University Putra Malaysia (UPM). The plants were authenticated by Dr. Shamsul Khamis. The voucher number for *A. indica* is SK 2519/14 and *M. koenigii* is SK 2520/14.

Collection of larvae

Third instar of *Aedes albopictus* larvae were collected from the Unit of Entomology, Institute of Medical Research (IMR), Malaysia and the larvae were maintained until the bioassay.

Extraction

The maceration technique that was used in this study was the modification technique used by Arivoli and Tennyson, (2012). *A. indica* leave was washed thoroughly in running tap water and was shade dry under the room temperature. The dried leaves were reduced into size by using hand and powdered by using an electric blender. The powdered leaves were allowed to soak in methanol for 72 hours. After 72 hours of soaking, the *A. indica* leaves extract were filtered and the filtrate was dried in a hot air oven at 50˚C ± 2˚C for 3 days. Similar method was used for the extraction of *M. koenigii* leaves.

Toxicity bioassay

The method used to evaluate the toxicity of the leave extract was modified method from Subramaniam *et al.* (2012). A plastic cup was filled with 190ml of distilled water and added with 10ml of *A. indica* leaf extract. Final desired concentration used was 1.25mg/ml, 2.50mg/ml and 3.75mg/ml. Ten larvae of 3rd instar of *Aedes albopictus* were introduced into the plastic cup. The test was replicated 3 times. The same method was used for other concentration, whereas Abate (temephos) and 1,8-cineole was used as positive control and methanol were used as negative control. Mortality was recorded for 24, 48 and 72 hours. The corrected percentage of mortality was calculated by using Abbott's formula (1925) as shown below. Similar method was used for *M.*

koenigii leaves extracts.

$$\text{Percentage of corrected mortality} = \frac{\% \text{ of mortality in treated} - \% \text{ of mortality in control}}{100 - \% \text{ of mortality in control}} \times 100$$

Combine activity

These studies were followed by minor modification from the method used by Mansour *et al.* (2010). A combination of 3.75mg/ml of *A.indica* leaves extract with 3.75mg/ml of *M.koenigii* leaves extract with the ratio 1:1 was prepared. Three replicates were carried out and mortality was recorded for 24, 48 and 72 hours and the percentage of corrected mortality were calculated by using Abbott's formula (1925). The controls remain same as for toxicity bioassay. The combined action of the different mixtures was expressed by using Co-toxicity Factor formula which is developed by Sun and Johnson (1960) to differentiate between potentiation, antagonism and additivity.

$$Co - toxicity\ Factor = (O - E) \times \frac{100}{E}$$

From the formula, O indicates observed mortality (%) and E indicates expected mortality (%). The Co-toxicity factor consists range of value in which a positive factor of ≥ 20 denotes potentiation, a negative factor of ≤ -20 denotes antagonism and the intermediate value of > -20 to < 20 denotes additive effect (Mansour *et al.*, 2010).

Statistical Analysis

Paired T test was done via SPSS version 16.0 to obtain the *P* value. Probit analysis was done to calculate LC_{50} for the first 24 hours by using the method used by Randhawa (2009).

Results

The concentration of 1.25mg/ml of *Azadirachta indica* extract commences the larvacidal activity by providing the mean mortality of 4.33 ± 1.15 with the percentage of mortality of 46.30% at the 24 hours of observation. These concentration shows significant difference ($P<0.05$) compared to other concentrations that was included in this studies. The concentration of 2.50mg/ml of *A. indica* extract exhibit 8.00 ± 0.00 as the mean mortality of the *Aedes albopictus* and the percentage of mortality was 74.82% which shows an increase of 28.52% compared to 1.25mg/ml.

The maximum concentration that was used in these studies was 3.75mg/ml and expresses the larvacidal activity with the mean mortality of 9.67 ± 0.58 and the percentage of mortality was 96.30%. The increment of percentage of mortality in 3.75mg/ml was 21.48% compared to 2.50mg/ml. Similarly, Abate and 1,8-cineole remain potent by causing 100% of mortality to the entire test larvae . Similar effects were observed at 48 and 72 hours (Table 1).

Table 1. Mean mortality and percentage of mortality of *Aedes albopictus* larvae against methanolic extract of *Azadirachta indica* leaves.

	24 h		48 h		72 h	
	Mean mortality	Percentage of mortality (%)	Mean mortality	Percentage of mortality (%)	Mean mortality	Percentage of mortality (%)
1.25 mg/ml	4.33 ± 1.15[a]	46.30[a]	6.33 ± 2.31[a]	44.81[a]	7.00 ± 1.73[a]	50.95[a]
2.50 mg/ml	8.00 ± 0.00[b]	74.82[b]	10.00±0.00[b]	100.00[b]	10.00 ± 0.00[b]	100.00[b]
3.75 mg/ml	9.67 ± 0.58[c]	96.30[c]	9.67 ± 0.58[b]	96.30[b]	9.67 ± 0.58[b]	95.24[b]
Abate	10.00 ± 0.00[c]	100.00[c]	10.00 ± 0.00[b]	100.00[b]	10.00 ± 0.00[b]	100.00[b]
1,8-cineole	10.00 ± 0.00[c]	100.00[c]	10.00 ± 0.00[b]	100.00[b]	10.00 ± 0.00[b]	100.00[b]

Means within the same column followed by the same letter are not significantly different by *t*-test. ($P<0.05$).

The mean and percentage of mortality of *Aedes albopictus* larvae against methanolic extract of *Murraya koenigii* leaves on 24, 48 and 72 hours of observation was summarized in Table 2. The concentration of 1.25mg/ml of the extract commences the larvacidal activity by providing the mean mortality of 4.33 ± 1.15 with the percentage of mortality of 30.37% in which it shows a significant

difference (P<0.05) compared to other concentrations that was used in these studies. The concentration of 2.50mg/ml of the extract exhibit mean mortality of 7.33 ± 0.58 with a percentage of mortality of 65.56% in which it shows an increase of 35.19% as compared 1.25mg/ml. The maximum concentration that was used in these studies was 3.75mg/ml with a mean mortality 8.67 ± 0.58 and the percentage of mortality was 83.70%. The increment of percentage of mortality in 3.75mg/ml was 18.14% compared to 2.50mg/ml. Whereas Abate and 1,8-cineole remain potent as they cause 100% of mortality. Similar effects were observed at 48 and 72 hours.

Table 2. Mean mortality and percentage of mortality of *Aedes albopictus* larvae against methanolic extract of *Murraya koenigii* leaves.

	24 h		48 h		72 h	
	Mean mortality	Percentage of mortality (%)	Mean mortality	Percentage of mortality (%)	Mean mortality	Percentage of mortality (%)
1.25 mg/ml	4.33±1.15[a]	30.37[a]	9.00±1.00[a]	89.26[a]	9.00±1.00[a]	87.14[a]
2.50 mg/ml	7.33±0.58[b]	65.56[b]	9.67±0.58[a]	96.67[a]	10.00±0.00[a]	100.00[a]
3.75 mg/ml	8.67±0.58[c]	83.70[c]	10.00±0.00[a]	100.00[a]	10.00±0.00[a]	100.00[a]
Abate	10.00±0.00[d]	100.00[d]	10.00±0.00[a]	100.00[a]	10.00±0.00[a]	100.00[a]
1,8-cineole	10.00±0.00[d]	100.00[d]	10.00±0.00[a]	100.00[a]	10.00±0.00[a]	100.00[a]

Means within the same column followed by the same letter are not significantly different by t-test (P<0.05).

The interaction between the two extracts was additivity with the co-toxicity factor of 11.11 (Table 3). *Azadirachta indica* was found to be more susceptible compared to *M. koenigii*. The effective concentration of *A. indica* extract that kills 50% population of the *Aedes albopictus* larvae was 1.45mg/ml. On the other hand, the LC_{50} for *M. koenigii* was 2.01mg/ml (Table 4).

Table 3. Joint action of mixtures of *Azadirachta indica* and *Murraya koenigii* extract against *Aedes albopictus* larvae.

	Observed mortality of larvae	Expected mortality of larvae	Co-toxicity factor	Joint action
A.indica + M.koenigii	100	90	11.11	Additive

Table 4. Determination of Median Lethal Concentration, LC_{50} of *Azadirachta indica* and *Murraya koenigii* against *Aedes albopictus* larvae.

Plant	LC_{50}(mg/ml)	Regression equation	R^2	95% confidence Interval (lower to upper)
Azadirachta indica	1.45	y = 0.756x + 3.9067	0.9922	0.86 – 2.04
Murraya koenigii	2.01	y = 0.596x + 3.8	0.9839	1.26 – 2.76

Discussion

The result of this study reveals that the methanolic extract of *Azadirachta indica* leaves has larvacidal activity against *Aedes albopictus* larvae. The bioassay shows that 3.75mg/ml of *A. indica* is considered as optimum concentration because it has a similar effects as abate and 1,8-cineole which serve as positive control, in which the percentage of mortality was 96.30% for the first 24 hours. Similarly, Maragathavalli *et al.* (2012), stated that the methanolic extract of *A. indica* leaves shows 90% of mortality against *Aedes aegypti* larvae of third and fourth instar with the concentration of 200mg. In addition to that, the essential oil of *Ocimum americanum* were found to be effective against *Aedes albopictus* larvae of 4th instar by causing 100% of

mortality with the concentration of 6.6µg/ml at 24 hours of observation (Umesh *et al.*, 2014).

Murraya koenigii leaves extract also exhibit larvacidal activity against *A. albopictus* larvae in this study. At concentration of 3.75mg/ml, *M. koenigii* shows larvacidal activity with the percentage of mortality of 83.70% after 24 hours of observation. Whereas in previous research it was found that chloroform extract of *M. koenigii* leaves shows larvacidal activity against *A. aegypti* larvae (Hima and Manimegalai, 2014). Besides that, previous research has been done using essential oil from the seed of citrus cultivars shows that *Citrus sinensis* (Valencia late) shows highest mortality of 81% and *Citrus reticulate* (Feutrall early) shows 68% of mortality on 24 hours against third instar of *Aedes albopictus* larvae (Bilal *et al.*, 2012).

This study indicates that methanolic extract of *A. indica* is more potent than *M. koenigii*. The LC_{50} value for *A. indica* is 1.45mg/ml and for *M. koenigii* is 2.01mg/ml. In addition to that, previous study indicates that the ethanolic extract of *A. indica* leaves demonstrated a lethal concentration of 8.32mg/ml against 4^{th} instar of *Aedes aegypti* (Mgbemena, 2010). In a another study, it was found that the hexane extract of *M. koenigii* leaves shows LC_{50} value of 0.96mg/ml (963.53ppm) against 3^{rd} instar of *Culex quinquefasciatus* over 24 hours (Kovendan *et al.*, 2012).

The larvacidal activity exhibited by *M. koenigii* was probably due to the presence of 1,8-cineole. In the previous studies, 1,8-cineole was found as one of the active compound in the essential oil of *M. koenigii* leaves (Rajendran *et al.*, 2014). Study conducted by Liu *et al.* (2015), shows that 1,8-cineole exhibit larvacidal activity against *A. aegypti*. Thus, 1,8-cineole was used as a positive control in this studies.

The larvacidal activity of *A. indica* against *A. albopictus* possibly due to the presence of active compound, Azadiractin. The previous study done by Alouani *et al.* (2009), using Azadirachtin which is an active constituent of *Azadirachta* indica was found to be effective against fourth instar of *Culex pipiens* as a larvicide. Furthermore, it was also stated that these compound has major effects as growth regulators. In this study, it was qualitatively found that the living *A. albopictus* larvae treated with methanolic extract remain as third instar compared to the larvae in the control container which turns as pupae after certain days.

Conclusion

As a conclusion, *Azadirachta indica* and *Murraya koenigii* has shown the larvicidal potential against *Aedes albopictus* larvae. Future study should be conducted to find out the active constituents that responsible for larvacidal activity, mechanism of action and formulating the active constituent by using nanoparticle technology will be more effective in killing the larvae or adults without disturbing the ecological food chain.

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References

Abbott WS. 1925. A method of computing the effectiveness of insecticide. Journal of economic entomology **18,** 265-267.

Alouani A, Rehimi N, Soltani N. 2009. Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in the Republic of Algeria. Jordan Journal of Biological Sciences **2,** 15-22.

Aarati N, Ranganath NN, Soumya GB, Kishore B, Mithun K. 2011. Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extract of Neem (*AzadirachtaIndica)* an in vitro study. International Journal of Research in Ayurveda and

Pharmacy **2,** 230-235.

Ajay S, Rahul S, Sumit G, Paras M, Mishra A, Gaurav A. 2011. Comprehensive review: Murraya koenigii Linn. Asian Journal of Pharmacy and Life Sciences **1,** 417-425.

Arivoli S, Tennyson S. 2012. Larvicidal efficacy of *Strychnosnuxvomica* Linn. (Loganiaceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera :Culicidae). World Journal of Zoology **7,** 06-11.

Bansal P, Bansal R, Gupta V. 2010. Antifertility effects of *Azadirachta indica* (Neem) - A review. Annals of Biological Research **1,** 108-113.

Bilal H, Akram W, Hassan SA. 2012. Larvicidal activity of Citrus Limonoids against *Aedes albopictus* larvae. Journal of Arthropod- Borne Diseases **6,** 104-111.

Bhandri PR. 2012. Curry leaf (*Murraya koenigii*) or cure leaf: Review of its curative properties. Journal of Medical Nutrition and Nutraceuticals **1,** 92-97.

Caminade C, Medlock JM, Ducheyne E, McIntre KM, Leach S, Baylis M, Morse AP. 2012. Suitability of European climate for the Asian tiger mosquito *Aedes albopictus*: recent trends and future scenarios. Journal of The Royal Society Interface **9,** 1-10. http://dx.doi.org/10.1098/rsif.2012.0138

Chandrawathani P, Shariman ZY, Premaalatha B, Rahimah H, Norhafiza NH, NurulAini R, NorAndilla I, Wahab AR. 2013. Evaluation of neem leaf (*Azadirachta* indica) product for worm control on goats. Malaysian Journal of Veterinary Research **4,** 5-12.

Cheah WK, Ng KS, Marzilawati AR, Lum LCS. 2014. A review of dengue research in Malaysia. Medical Journal of Malaysia **69,** 59-67.

Darvekar VM, Patil VR, Choudhari AB. 2011. Anti-inflammatory activity of *Murraya koenigii* Spreng on experimental animals. Journal of Natural Product and Plant Resources **1,** 65-69.

Gomase PV, Rangari VD, Verma PR. 2011. Phytochemical evaluation and hepatoprotective activity of fresh juice of young stem (tender) bark of *Azadirachta indica* A. Juss.International Journal of Pharmacy and Pharmaceutical Sciences **3,** 55-59.

Hima CR, Manimegalai M. 2014. Studies on the control of mosquito, *Aedes aegypti* (Culicidae: Diptera) using the chloroform leaf extract of *Murraya koenigii* as biocide. World Journal of Pharmacy and Pharmaceutical Sciences **3,** 766-773.

Kaplan L , Kendell D, Robertson D, Livdahl T, Khatchikian C. 2010. *Aedes aegypti* and *Aedes albopictus* in Bermuda: extinction, invasion and extinction. Biological invasions **12**, 3277-3288. http://dx.doi.org/10.1007/s10530-010-9721-z

Kovendan K, Arivoli S, Maheshwaran R, Baskar K, Vincent S. 2012. Larvicidal efficacy of *Sphaeranthus indicus, Cleistanthus collinus* and *Murraya koenigii* leaf extracts against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research **111,** 1025-1035.

Kumar S, Agrawal D, Patnaik J, Patnaik S. 2012. Analgesic effect of neem (*Azadirachta indica)* seed oil on albino rats. International Journal of Pharma and Bio Sciences **3,** 222-225.

Lokanadhan S, Muthukrishnan P, Jeyaraman S. 2012. Neem products and their agricultural applications. Journal of Biopesticide **5,** 72-76.

Liu Y, Liu XC, Liu QY, Niu C, Liu ZL. 2015. Larvicidal activity of *Illicium difengpi*BN Chang (Schisandraceae) stem bark and its constituent compounds against *Aedes aegypti* L. Tropical Journal of Pharmaceutical Research **14,** 103-109.

http://dx.doi.org/10.3390/molecules180910733

Musabyimana T, Saxena RC, Kairu EW, Ogol CPKO, Khan ZR. 2001.Effects of neem seed derivatives on behavioral and physiological responses of the *Cosmopolites sordidus* (Coleoptera: Curculionidae). Journal of Economic Entomology **94,** 449-454.

Murthy JM, Rani PU. 2009. Biological activity of certain botanical extracts as larvicides against the yellow fever mosquito, *Aedes aegypti*. L. *Journal of* Biopesticides **2,** 72-76.

Mgbemena IC. 2010. Comparative evaluation of larvacidal potentials of three plant extracts on *Aedes aegypti*.Journal of American Science **6,** 435-440.

Mansour SA, Bakr RF, Hamouda LS, Mohamed RI. 2010. Toxic and synergistic properties of several botanical extracts against larval and adult stages of the mosquito, *Anopheles pharoensis*. Biopesticides International **6,** 129-145.

Malwal M, Sarin R. 2011. Antimicrobial efficacy of *Murraya koenigii* (Linn.) Spreng.root extracts. Indian Journal of Natural Products and Resources**2,** 48-51.

Maragathavalli S, Brindha S, Kaviyarasi NS, Annadurai B, Gangwar SK. 2012. Mosquitoes larvacidal activity of leaf extract of neem (*Azadirachta indica*). International Journal of Advanced Biological Research **2,** 138-142.

Mazzacano C, Black SH. 2013. Mosquito control methods. Ecologically sound mosquito management in wetlands. An overview of mosquito control practices, the risks, benefits, and nontarget impacts, and recommendations on effective practices that control mosquitoes, reduce pesticide use, and protect wetlands (pp 7-12). Portland. The Xerces Society for Invertebrate Conservation.

Mohan S, Abdelwahab SI, Cheah SC, Sukari MA, Syam S, Shamsuddin N, Mustafa MR. 2013. Apoptosis effect of girinimbine isolated from Murrayakoenigii on lung cancer cells in vitro. Evidence- Based Complementary and Alternative Medicine **2013,** 1-12.
http://dx.doi.org/10.1155/2013/689865

Parveen M. 2013. The bioactivity of neem (*Azadirachta indica* A. Juss.) based products against various animal systems. Indian Journal of Applied and Pure Biology **28,** 287-289.

Patil P, Patil S, Mane A, Verma S. 2013. Antidiabetic activity of alcoholic extract of neem (*Azadirachta indica*) root bark. National Journal of Physiology, Pharmacy and Pharmacology **3,** 142-146.

Parimi BN, Mopuri R, Meriga B. 2014. The protective effect of *Murraya koenigii* leaves against carbon tetrachloride- induced hepatic damage in rats. Journal of Costal Life Medicine **2,** 313-318.
http://dx.doi.org/10.12980/JCLM.2.201414J9

Randhawa MA. 2009. Calculation of LD_{50} values from the method of Miller and Tainter, 1944.Journal of Ayub Medical College Abbottabad **21,** 184-185.

Rageeb MD, Usman MD, Barhate SD. 2012. Phytochemical evaluation and effect of antipyretic activity on *Murraya koenigii* Spreng. Leaves extract. International Journal of Pharmaceutical and Chemical Sciences **1,** 231-236.

Rajendran MP, Pallaiyan BB, Selvaraj N. 2014. Chemical composition, antibacterial and antioxidant profile of essential oil from *Murraya koenigii* (L.) leaves. Avicenna Journal of Phytomedicine **4,** 200-214.

Sun YP, Johnson ER. 1960. Analysis of joint action of insecticide against house flies. Journal of economic entomology **53,** 887-892.
http://dx.doi.org/10.1093/jee/53.5.887

Shah KJ, Juvekar AR. 2006. Positive inotropic effect of *Murraya koenigii* (Linn.) spreng extract on an isolated perfused frog heart. Indian Journal of Experimental Biology **44,** 481-484.

Sharma US, Sharma UK, Singh A, Sutar N, Singh PJ. 2010. In vitro antihelminth activity of *Murraya koenigii* Linn. Leaves extracts. International Journal of Pharma and Bio Science **1,** 1-4.

Swati D, Pathak A, Burande MD. 2012. Antigenotoxic effect of *Murraya koenigii* towards cyclophosphamide induced cytogenetic damage in mouse bone marrow cells. International Journal of Pharmacognosy and Phytochemical Research **4,** 59-63.

Subramaniam J, Kovendan K, Kumar PM, Murugan K, Walton W. 2012. Mosquito larvacidal activity of *Aloe vera* (Family: Liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector, *Aedes aegypti*. Saudi Journal of Biological Sciences **19,** 503-509.
http://dx.doi.org/10.1016/j.sjbs.2012.07.003

Tiwari V, Darmani NA, Yue BY, Shukla D. 2010. In vitro antiviral activity of neem (*Azadirachta indica* L.) bark extract against herpes simplex virus type-1 infection. Phytotherapy Research **24,** 1132-40.
http://dx.doi.org/10.1002/ptr.3085

Tembhurne SV, Sakarkar DM. 2012. Anti-obesity and hypoglycemic effect of ethanolic extract of *Murraya koenigii* (L) leaves in high fatty diet rats. Asian Pacific Journal of Tropical Disease **2,** 166-168.

Umesh BT, Leeja L, Thoppil JE. 2014. A study on mosquito larvicidal activity of essential oil of four species of *Ocimum* against *Aedes albopictus*. Skuse. International Journal of Institutional Pharmacy and Life Sciences **4,** 1-4.

World Health Organization. 2015. Update on the dengue situation in the Western Pacific Region. 1-5 (Assessed on 31/3/2015).

Zaki AHM, Brett J, Ismail E, Azou ML. 2014. Epidemiology of dengue disease in Malaysia (2000-2012): A systemic literature review. PLOS Neglected Tropical Diseases **8,** 1-9.