NATURAL LARVICIDES OF BOTANICAL ORIGIN AGAINST DENGUE VECTOR *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Abstract. Products of plant origin, with antimosquito potential are now considered as advantageous alternatives to conventional synthetic chemicals for management of mosquito vectors. The present study was, therefore, carried out to investigate botanical products extracted from eighteen indigenous plants as larvicidal agents against the dengue vector, *Aedes aegypti*. All plant materials were extracted with ethanol and provided yields ranging from 1.90% to 28.31% (w/w), whereas three plant species, namely, *Alpinia conchigera*, *Homalomena aromatica* and *Litsea petiolata*, produced liquid oils with yield of 0.19%, 0.20% and 2.63% (v/w), respectively. A discriminating dosage (200 mg/l) prepared from essential oil or ethanolic extract of each plant species was screened individually for larvicidal activity against early 4th instars of *Ae. aegypti*, resulting in five plant extracts with promising larvicidal potential (42-100% mortality). A dose-response larvicidal bioassay against *Ae. aegypti* established the essential oil of *L. petiolata* leaf as being the most effective, exhibiting an LC_{50} (50% lethal concentration) of 28.32 mg/l, while the ethanolic extract had an LC_{50} of 187.60 mg/l. This study demonstrates the promising potential of plant products, particularly of *L. petiolata* oil, in research and development of new natural larvicidal compounds for controlling *Ae. aegypti*.

Keywords: *Aedes aegypti*, *Alpinia conchigera*, *Homalomena aromatica*, *Litsea petiolata*, essential oil, ethanolic extract, larvicide

INTRODUCTION

Aedes (*Stegomyia*) *aegypti* (L.) is considered one of the most dangerous mosquito vectors because it can transmit a number of potentially serious arboviral diseases, not only dengue fever, but also chikungunya, yellow fever and Zika, which contribute significantly to human morbidity and mortality globally (WHO, 2012; Bhatt *et al*, 2013; Weaver *et al*, 2016). World Health Organization (WHO) estimated nearly half of the world's population is now at risk of being infected with at least one type of vector-borne pathogens (WHO, 2004, 2013). Furthermore, diverse

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concomitant factors, such as deforestation, migration and poor sanitation as well as ongoing climate changes, which contribute to widespread mosquito distribution, significantly increase the numbers of population at risk.

Mosquito-borne diseases are becoming highly prevalent worldwide, with a growing concern of global warming translating into an explosive growth of human infections (Molyneux, 2003; Mota *et al*, 2016). *Ae. aegypti* originated in Africa (Mousson *et al*, 2005), but is now found in tropical and subtropical regions throughout the world, including Thailand (Womack, 1993; Chareonviriyaphap *et al*, 2003). Currently, *Ae. aegypti* has also become a serious public health problem in Thailand because of the increasing threat from dengue and the recent emergence of chikungunya and Zika (Bureau of Epidemiology, 2012; Buathong *et al*, 2015; Ratanawong *et al*, 2016).

Unavailability of effective vaccines and specific antiviral therapies have meant that mosquito control is the most viable preventive measure against transmission of these mosquito-borne diseases. Reducing population abundance with conventional synthetic insecticides to target larval stages in breeding sites remains one of the main strategy for mosquito management (Dusfour *et al*, 2011). However, as mosquitoes are closely associated with humans and their dwellings, and have a number of breeding and behavioral quirks, it is extremely difficult to control or eliminate *Ae. aegypti* (CDC, 2016; WHO, 2016). Furthermore, several factors, such as mosquito resistance to insecticides, adaptive vector behavior and environmental health concerns, have limited the sustainable success of control strategies, particularly those based on conventional synthetic chemicals (Morrison *et al*, 2008; Eisen *et al*, 2009; Chareonviriyaphap *et al*, 2013; Achee *et al*, 2015). There is, therefore, a strong need to search and develop new approaches with high effectiveness and environmental safety to control mosquito vectors.

As a rich resource of chemicals, with less hazardous and readily biodegradable properties, products of plant origin with antimosquito potential have been considered attractive alternatives to conventional chemical insecticides for current and future vector control. A considerable amount of studies has been conducted on antimosquito properties of a variety of promising phytochemicals (Sukumar *et al*, 1991; Shaalan *et al*, 2005; Ghosh *et al*, 2012). In Thailand, there is a great diversity of medicinal and aromatic plants, with high potential to be developed as new bioinsecticides to replace synthetic chemicals. Fortunately, *Ae. aegypti* in Thailand still is susceptible to natural insecticidal products prepared from various plants, viz. *Carum carvi*, *Curcuma zedoaria*, *Apium graveolens*, *Illicium verum*, *Piper longum*, *Piper sarmentosum*, *Foeniculum vulgare*, *Myristica fragrans*, *Limnophila aromatica*, *Curcuma longa*, *Cinnamomum verum*, *Alpinia galanga* and *Cyperus rotundus*, even though some strains of this mosquito (larval or adult stage) already show significant resistance to conventional insecticides, such as deltamethrin, permethrin and temephos (Chaiyasit *et al*, 2006; Intirach *et al*, 2016; Chansang *et al*, 2017). These reports provide encouragement to the high probability of developing plant products as new mosquitocidal agents for controlling *Ae. aegypti*. However, despite much research efforts only a limited number of compounds of botanical origin have become new commercially successful bioinsecticides and products containing synthetic ingredients still dominate the

global insecticide market (Mann and Kaufman, 2012; Olson, 2015).

A low number of bioactive compounds available along with an increase of consumer awareness and mosquito resistance to conventional synthetic chemical insecticides have led to a continual exploration of plant-based products as alternatives in vector control. The present study investigated natural products extracted from eighteen indigenous plants for larvicidal activity against a Thai strain of the dengue vector, *Ae. aegypti*.

MATERIALS AND METHODS

Preparation of plant materials

A total of eighteen indigenous plants (Table 1) were selected based on the availability and literature survey for their larvicidal potential against *Ae. aegypti* (Sukumar *et al*, 1991; Shaalan *et al*, 2005; Ghosh *et al*, 2012). The plant materials were obtained from traditional herb suppliers or collected from different localities in Thailand. Taxonomic identification was performed by Ms Wannaree Charoensup, Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University (CMU), Chiang Mai Province, Thailand. A voucher specimen of each plant was deposited at the Department of Parasitology, Faculty of Medicine, CMU. After air-drying under shade at ambient temperature (30° ± 5°C daytime) for 1-2 weeks, each dried plant material was ground mechanically using an electrical blender into a fine powder.

Preparation of ethanolic extracts

The fine plant powder (0.5 kg) was extracted exhaustively by maceration with 3 liters of 95% ethanol with frequent agitation at room temperature for 2 days, and then filtered twice, first using a fine cloth and then Whatman number 1 filter paper. The extraction procedure was repeated twice with new ethanol solvent. The combined filtrates were concentrated to dryness under reduced pressure using a vacuum rotary evaporator (EYELA, Tokyo, Japan) at 65°C. The residues were freeze-dried at -55°C (Lyotrap Freeze Dryers, Oldham, UK) and stored at -20°C until used. Yield of ethanolic extract is expressed as percent (w/w) crude extract from dry plant powdered material.

Preparation of plant essential oils

Approximately 250 g of plant powder was extracted by steam distillation for at least three hours to ensure complete essential oil isolation. After removing the aqueous phase using a separating funnel, the essential oil was dried over anhydrous Na_2SO_4 to eliminate traces of moisture and stored in an amber-colored bottle at 4°C until used. Oil yield is expressed as percent (v/w) dry weight.

Maintenance of mosquitoes

Free-mating laboratory *Ae. aegypti* established from specimens collected originally at clean stagnant water areas in Chiang Mai Province (Sutthanont *et al*, 2010) were maintained under controlled conditions (25° ± 2°C, 80% ± 10% relative humidity and 14:10 hour light:dark photoperiod cycle) at the Department of Parasitology, Faculty of Medicine, CMU. Larvae were reared in plastic trays containing tap water and fed twice daily on sterilized ground dog chow until the larvae transformed into pupal stage, whereupon they were transferred to humidified mosquito cages for adults to emerge. Adult mosquitoes were fed *ad libitum* with 10% sucrose solution containing10% multivitamin syrup, while females were periodically blood-fed for egg production. Plastic cups lined with filter-paper discs were placed

inside the mosquito cages for oviposition. Eggs were allowed to hatch into larvae and early 4th instar larvae were used in larvicidal bioassays.

Preliminary larvicidal bioassay

Plant products, essential oils and/or ethanolic extracts were screened individually for larvicidal activity at an initial concentration of 200 mg/l against *Ae. aegypti* 4th instars larvae according to the standard protocol (WHO, 1981), with slight modifications. In brief, a batch of 25 4th instar larvae of *Ae. aegypti* was transferred into a cup containing 249 ml of distilled water and 1 ml of the test plant solution [ethanol, acetone or dimethyl sulphoxide (DMSO)]. Control and untreated group of larvae were maintained in solvent-distilled water and distilled water, respectively. Four duplicate trials were performed for each test sample. All groups were incubated under the controlled conditions used for rearing. Mortality was determined after 24 hours of exposure, during which no food was provided to the larvae. Dead larvae (no signs of responding when probed with a tiny brush) were pooled and corrected for control mortality using Abbott's formula (Abbott, 1925). Plant samples producing mortality >40% were further assessed in a dose-response larvicidal bioassay.

Dose-response larvicidal bioassay

Essential oils and/or ethanolic extracts were subjected to a dose-response larvicidal bioassay (WHO, 1981). In short, a series of each plant solution was prepared by at least four sequential concentrations to obtain mortality ranging between 10% and 90%. Four groups of 25 *Ae. aegypti* 4th instar larvae were exposed simultaneously to each test concentration. Each experiment was performed in four replicates under controlled conditions as described above using mosquitoes from separately reared batches.

Data analysis

Corrected larval mortality rates were calculated as percentages, means and standard errors. Lethal values of 50%, 95% and 99% (LC_{50}, LC_{95} and LC_{99}, respectively) at the corresponding 95% confidence interval (95% CI) were calculated using probit analysis according to Finney (1971), with a statistical program SPSS Version 19.0 (IBM, Armonk, NY). Chi-square value was calculated for each bioassay to assess significance and measurement of difference between test samples.

RESULTS

Preparations of plant products using steam distillation and ethanol maceration provided essential oils (EOs) and/or ethanolic extracts (EEs) with various yields and different physical characteristics (Table 2). For isolation of EOs, only three plant species, namely, *Alpinia conchigera*, *Homalomena aromatica* and *Litsea petiolata* (Fig 1), furnished EOs with yield of 0.19%, 0.20% and 2.63% (w/w). These EOs were less dense than water, clear and colorless or pale yellow, with characteristic odors. EEs of the 18 plant species presented a large range of yields (w/w), from 1.90% for *Diospyros rhodcalyx* to 28.31% for *L. petiolata*. For the same plant species, it was that EE yield was greater than that of EO.

In the preliminary larvicidal screening experiments, a number of EO- or EE-treated *Ae. aegypti* 4th instar larvae exhibited abnormal behavior indicative of intoxication, such as excitation, restlessness, sluggishness, tremor and convulsion followed by paralysis at the bottom of the container. After 24 hours, moribund and dead larvae were found in these treated groups. On the other hand, larvae in the

Alpinia conchigera rhizomes

Homalomena aromatica rhizomes

Litsea petiolata leaves

Fig 1–Dried samples of *Alpinia conchigera*, *Homalomena aromatica* and *Litsea petiolata*.

control and untreated groups were still active with normal zigzag motion and did not die or turned into pupae within 24 hours of exposure period. Due to zero larval mortality observed in both control and untreated groups, no correction of mortality rates in the treated groups was needed. Insecticidal potential of the plant products, EOs and EEs, at 200 mg/l against *Ae. aegypti* early 4th instars larvae was highest (100% mortality) for *A. conchigera*, *H. aromatica* and *L. petiolata* EOs, while that for *Cassia alata*, *H. aromatica*, *Moringa oleifera* and *Tacca chantrieri* EEs was lowest (0% mortality) (Table 2). All EOs produced greater mortality than the corresponding EEs.

EOs and/or EEs of *A. conchigera*, *Cissampelos pareira*, *H. aromatica*, and *L. petiolata*, which could cause 42-100% mortality in the preliminary screening, were further assessed for their efficacy in the dose-response larvicidal bioassay, revealing highest larvicidal activity from *L. petiolata* EO (LC_{50} value = 28.32 mg/l), followed by *A. conchigera* EO (84.97 mg/l), *H. aromatica* EO (95.77 mg/l), *C. pareira* EE (157.77 mg/l), and *L. petiolata* EE (187.60 mg/l) (Table 3).

DISCUSSION

Mosquito control by targeting aquatic stages, specifically larvae in breeding sites, still are a preferable strategy, being more localized in time and space compared to adult stages management (Ghosh *et al*, 2008; Mdoe *et al*, 2014). As an advantageous alternative to conventional chemical compounds, products of plant origin are now extensively explored in large scale for their larvicidal potential against different genera of mosquitoes (Ghosh

Table 1
Plant species selected for the preliminary screening of larvicidal activity against *Ae. aegypti*.

Family/Species	Common name	Voucher number	Part used
Amaryllidaceae			
Crinum asiaticum Linn.	Crinum lily	PARA-CR-003-St-Le/1	Leaf and stem
Araceae			
Homalomena aromatica Schott.	Colla aromatica	PARA-HO-001-Rh/1	Rhizome
Cruciferae			
Brassica pekinensis Lour.	Chinese cabbage	PARA-BR-001-Se/1	Seed
Brassica juncea (L.) Lour Czern.	Chinese mustard	PARA-BR-002-Se/1	Seed
Ebenaceae			
Diospyros rhodcalyx Kurz.	Ebony	PARA-DI-002-Ba/1	Bark
Euphobiaceae			
Vernicia fordii Hemsl.	Tung tree	PARA-VE-003-Se/1	Seed
Irvinggiaceae			
Irvingia malayana Oliv.	Wild almond	PARA-IR-001-Se/1	Seed
Lamiaceae			
Leonurus japonicus Houtt.	Honeyweed	PARA-LE-001-Le/1	Leaf
Lauraceae			
Litsea petiolata Hook.f.	Medang	PARA-LI-003-Le/2	Leaf
Leguminosae			
Cassia alata Linn.	Ringworm bush	PARA-CA-005-Se/1	Seed
Menispermaceae			
Cissampelos pareira Linn.	Velvet leaf	PARA-CI-010-Rh/1	Rhizome
Moraceae			
Artocarpus altilis Forsberg.	Breadfruit	PARA-AR-003-Fl/1	Flower
Moringaceae			
Moringa oleifera Lam.	Horse radish tree	PARA-MO-001-Se/1	Seed
Myrsinaceae			
Ardisia polycephala Wall.	Shoebutton ardisia	PARA-AR-004-Se/1	Seed
Smilacaceae			
Smilax peguana A.DC.	Smylax	PARA-SM-001-Wh/1	Whole plant
Solanaceae			
Lycium barbarum Linn.	Goji berry	PARA-LY-001-Fr/1	Fruit
Taccaceae			
Tacca chantrieri Andre.	Bat flower	PARA-TA-003-St/1	Stem
Zingiberaceae			
Alpinia conchigera Griff.	Lesser alpinia	PARA-AL-002-Rh/1	Rhizome

et al, 2012; George *et al*, 2014; Zoubiri and Baaliouamer, 2014). In this study, from an initial screening of EEs and EOs of 18 local plant species in Thailand for insecticidal activity against *Ae. aegypti* early 4th instar larvae, EOs of *A. conchigera*, *H. aromatica* and *L. petiolata* (LC_{50} values of 28.32- 95.77 mg/l), and EEs of *C. pareira* EE (LC_{50} = 157.77 mg/l), and *L. petiolata* (LC_{50} = 187.60 mg/l) yielded the best results. Herbal

Table 2
Percent yield, physical characteristics of essential oils (EOs) and larvicidal activity of eighteen plant products, EOs and ethanolic extracts (EEs) against *Ae. aegypti*.

Plant*	Plant product								
	EO					EE			
	% yield	Color	Phase	Density (g/ml)	% mortality	% yield	Color	Phase	% mortality
C. asiaticum	0	NA	NA	NA	NA	7.07	Brown	Powder	2
H. aromatica	0.20	Colorless	Liquid	0.89	100	2.73	Brown	Semi-solid	0
B. pekinensis	0	NA	NA	NA	NA	8.05	Yellow	Semi-solid	3
B. juncea	0	NA	NA	NA	NA	5.73	Yellow	Semi-solid	2
D. rhodcalyx	0	NA	NA	NA	NA	1.90	Black	Powder	2
V. fordii	0	NA	NA	NA	NA	10.73	Yellow	Semi-solid	22
I. malayana	0	NA	NA	NA	NA	18.46	White	Powder	1
L. japonicus	0	NA	NA	NA	NA	2.70	Brown	Powder	16
L. petiolata	2.63	Colorless	Liquid	0.85	100	28.31	Dark green	Powder	42
C. alata	0	NA	NA	NA	NA	5.31	Brown	Semi-solid	0
C. pareira	0	NA	NA	NA	NA	13.32	Brown	Powder	63
A. altilis	0	NA	NA	NA	NA	12.80	Yellow	Powder	1
M. oleifera	0	NA	NA	NA	NA	13.78	Dark yellow	Semi-solid	0
A. polycephala	0	NA	NA	NA	NA	6.50	Black	Semi-solid	2
S. peguana	0	NA	NA	NA	NA	3.40	Brown	Powder	1
L. barbarum	0	NA	NA	NA	NA	17.36	Orange	Semi-solid	1
T. chantrieri	0	NA	NA	NA	NA	5.66	Black	Powder	0
A. conchigera	0.19	Pale yellow	Liquid	0.83	100	2.90	Brown	Semi-solid	12

*From Table 1. NA, not applicable.

Table 3
Larvicidal activity of effective plant essential oils (EOs) and ethanolic extracts (EEs) against *Ae. aegypti*.

Plant product (mg/l)	% mortality (mean ± SE)	Larvicidal activity (95% CI, mg/l)			χ^2	df	SE	Regression coefficient
		LC_{50}	LC_{95}	LC_{99}				
H. aromatica (EO)		95.77	107.29	112.07	10.85	2	0.007	0.14
89.00	13.25 ± 1.78	(92.96-98.58)	(102.89-120.11)	(106.17-129.86)				
93.45	41.25 ± 2.57							
97.79	64.50 ± 3.38							
102.35	79.50 ± 1.86							
L. petiolata (EO)		28.32	73.63	92.40	5.85	3	0.002	0.04
17.00	30.50 ± 2.36	(23.70-31.92)	(63.69-91.83)	(78.23-118.69)				
25.50	50.00 ± 2.10							
34.00	59.00 ± 2.11							
42.50	70.25 ± 1.86							
51.00	77.75 ± 1.15							
L. petiolata (EE)		187.60	274.30	310.21	12.44	3	0.001	0.02
160.00	33.50 ± 1.20	(174.28-197.76)	(251.57-322.13)	(278.24-379.01)				
180.00	43.00 ± 0.77							
200.00	52.50 ± 1.26							
220.00	76.00 ± 0.73							
240.00	85.25 ± 1.14							
C. pareira (EE)		157.77	247.45	284.61	9.33	2	0.001	0.02
120.00	26.50 ± 4.14	(135.29-176.23)	(214.85-347.71)	(239.99-426.58)				
150.00	43.75 ± 4.37							
180.00	60.50 ± 4.14							
210.00	86.50 ± 2.16							
A. conchigera (EO)		84.97	95.36	99.67	1.68	2	0.008	0.16
78.85	16.50 ± 2.19	(84.54-85.40)	(94.35-96.57)	(98.30-101.32)				
83.00	36.75 ± 3.54							
87.15	66.00 ± 2.99							
91.30	83.00 ± 2.38							

products with LC_{50} values <100 mg/l, such as oils of *A. conchigera*, *H. aromatica* and *L. petiolata*, and LC_{50} values of 100-200 mg/l, such as EEs of *C. pareira* and *L. petiolata*, are considered as strongly and moderately effective larvicides, respectively (Dias and Moraes, 2014; Ahmad *et al*, 2016; Gnankiné and Bassolé, 2017).

Similar screening studies by others also clearly demonstrated products of plant origin as effective mosquito larvicides, resulting in 80-100% mortality at 1,000 mg/l (Kumar *et al*, 2012; Warikoo and Kumar, 2013; Sharma *et al*, 2016). Plant products with LC_{50} values <100 mg/l against mosquito larvae such as *Aedes* spp are considered active (Dias and Moraes, 2014; Gnankiné and Bassolé, 2017). Sakthivadivel and Daniel (2008), evaluating larvicidal potential of petroleum ether extracts of 63 plant species, reported six crude extracts derived from different plant parts of *Acacia nilotica*, *A. mexicana*, *Citrullus colocynthis*, *Jatropha curcas* and *Withania somnifera* exhibiting toxic effects against 3rd instar of *Ae. aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*, with LC_{50} values of 20.17-89.19 ppm. Significant toxicity against 4th instar of *Ae. aegypti* larvae, with >75% mortality at 250 µg/ml, were observed from19/94 solvent extracts prepared from ten plant species widely found in northeast Brazil (Oliveira *et al*, 2010). The effective extracts with LD_{50} values of 12.1-97.7 µg/ml are those of *Spermacoce verticillata* aerial parts and roots, stems of *Guettarda grazielae* and *Rourea doniana*, and roots of *Triplaris americana*. Pronounced larvicidal activity against an Indian strain of *Ae. aegypti*, with LC_{50} values of 30.00-74.67 ppm were obtained from solvent extracts of *Achyranthes aspera*, *Cassia occidentalis*, *Lantana camara*, *Ricinus communis*, *Trachyspermum ammi* and *Zingiber officinale* (Kumar *et al*, 2012). Evaluation of larvicidal efficacy of five weeds, namely, *A. aspera*, *C. occidentalis*, *Catharanthus roseus*, *L. camara* and *Xanthium strumarium*, demonstrated highest larvicidal activity with *A. aspera* stem and leaf hexane extract, with LC_{50} value of 68.133 and 82.555 ppm, respectively (Sharma *et al*, 2016). Larvicidal potency of the abovementioned solvent extracts with LC_{50} values of 100 ppm are comparable to or lower than those obtained from EOs reported herein.

These findings also are in agreement with those of previous studies of botanical larvicides against many genera of mosquitoes. Row and Ho (2009) reported that while essential oil of *Piper betle* exhibits significant larvicidal potential against *Ae. aegypti* (LD_{50} = 48 ppm), methanolic and aqueous extracts of this plant show lower effectiveness (LD_{50} values <100 ppm). Essential oils of *Lavandula gibsoni* and *Plectranthus mollis* provide excellent larvicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (LC_{50} values of 25.4-62.8 mg/l), while acetone extracts of both plants show less activity against all three species of mosquitoes (LC_{50} values of 118.5-213.8 mg/l) (Kulkarni *et al*, 2013). Larvicidal evaluation against dengue vectors, *Ae. aegypti* and *Aedes albopictus*, revealed remarkable efficacy from wood and leaf essential oil of *Cunninghamia konishii* (LC_{50} = 85.7 and 194.4 µg/ml, respectively) but ethanolic extracts of these plant parts do not show significant larval toxicity towards these vectors (Cheng *et al*, 2013). Similarly, Intirach *et al* (2016) reported all ethanolic extracts having weaker larvicidal efficacy (LC_{50} ≥75.45 ppm) against *Ae. aegypti* than EOs from the same plants. Among 17 plant species investigated, the highest larvicidal efficacy was obtained from *Petroselinum crispum* fruit oil, followed by oils of *Foe-*

niculum vulgare, *Myristica fragrans*, *Limnophila aromatica*, *Piper sarmentosum* and *Curcuma longa* (LC_{50} values of 43.22-65.51 ppm) (Intirach *et al*, 2016).

A few contrary results of larvicidal efficacy of solvent extracts and essential oils were, however, reported against a number of mosquito species. Larvicidal bioassays of essential oils and crude solvent extracts from *Annona squamosa* seed and *Tagetes minuta* flower against *Anopheles* mosquitoes carried out under laboratory and semi-field conditions showed larvicidal LC_{50} value of 13.3, 23.3, 23.7, 45.8 and 574.9 ppm with acetone extract, hexane extract, essential oil, ethanol extract, and water extract, respectively of *A. squamosa* seed against a laboratory strain of *Anopheles arabiensis*, whereas *T. minuta* larvicidal LC_{50} value of essential oil, hexane extract, acetone extract, ethanol extract, and water extract is 29.4, 42.5, 79.9, 286.4 and 382.5 ppm, respectively (Assefa, 2011). Under semi-field conditions, the strongest larvicidal activity against *Anopheles* spp was detected in acetone extract of *A. squamosa* seed, followed by hexane extracts of *A. squamosa* seed and *T. minuta* flower, and essential oils of *A. squamosa* seed and *T. minuta* flower, exhibiting LC_{50} value of 28.0, 32.2, 32.3, 41.5 and 48.8 ppm, respectively. Likewise, methanolic extract of *Nepeta menthoides* (LC_{50} = 69.5 ppm) is more active than essential oil (LC_{50} = 234.3 ppm) against *An. stephensi* (Mahnaz *et al*, 2012).

Herbal essential oils are oily aromatica liquids constituting a variety of volatile compounds, *viz*. terpenes, terpenoids, phenol-derived aromatic components and aliphatic components, at quite different concentrations and with significant insecticidal potency, specifically ovicidal, larvicidal, adulticidal, repellency, antifeedant and growth and reproduction inhibition (Gnankiné and Bassolé, 2017). The insecticidal activity of essential oils depends on their chemical compositions and interactions among the various chemical components, both major and minor (Abagli and Alavo, 2011; Gnankiné and Bassolé, 2017). Nevertheless, except for the toxic effect of bioactive ingredients, higher larvicidal activities of essential oils are, expectedly, attributed to their oily physical property that possibly causes harmful effects, such as cuticle irritation and/or larvae suffocation, resulting in enhanced mosquito mortality. However, additional studies on the mechanisms of action and the target sites of essential oil components as well as symptomatic and morphological changes of the treated larvae will be required to prove this notion.

In summary, the present study establishes the larvicidal potential of plant-derived products, particularly essential oils of *A. conchigera*, *H. aromatica* and *L. petiolata*, against *Ae. aegypti* and their possible development and production as botanical larvicides for mosquito management. However, further investigations to determine the active components responsible for their bioactivity and methods to enhance efficacy as well as safety to users and the environment are necessary future research.

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