

ACUTE LARVICIDAL TOXICITY AND REPELLENCY EFFECT OF *OCTOPUS CYANEA* CRUDE EXTRACTS AGAINST THE FILARIASIS VECTOR, *CULEX PIPIENS*

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Abstract

This study evaluated the acute larval toxicity and repellent effect of solvent extracts of the big blue octopus, *Octopus cyanea* on the mosquito, *Culex pipiens*. Results showed that the highest effect of the acute larvicidal activities was recorded for ethanol extract ($LC_{50}=24.57\text{ppm}$), followed by methanol extract ($LC_{50}=35.85\text{ ppm}$). The repellency percentages increased with the increase of concentration producing the highest at 200 ppm and 50 ppm for methanol and ethanol extracts, respectively. GC-MS analysis revealed the presence of 19 compounds in ethanol extract and 5 compounds in methanol extract with insecticidal activities. The major identified bioactive compounds are Eugenol; Furoscrobiculin B; Hexadecanoic acid, methylester; Hexadecanoic acid, 1(hydroxymethyl)-1,2-ethanediylester; Octadecanoic acid, methylester; Oleic acid, eicosyl ester; 2-Methyl-5H-dibenz [b, f] azepine and many polysiloxanes compounds. Generally, results obtained indicated that *O. cyanea* ethanol extract induced remarkable effects on both acute larvicidal and repellent activities.

Keywords: *Culex pipiens*; toxicity; repellency; octopus; GC-MS.

Introduction

Mosquitoes play a considerable role as vectors of a wide variety of human pathogens and parasites. *Culex pipiens* is one of the prevalent mosquitoes in Egypt. It is the vector of filariasis (Fouda *et al*, 2013), Rift Valley fever virus and West Nile virus (El-Bahnasawy *et al*, 2013a,b) and other diseases like leishmaniasis, dengue and malaria that cause a considerable morbidity, mortality and economic load; (El-Kholy *et al*, 2017) reported possible role in the transmission of HC virus. Control of mosquitoes largely relies on chemical insecticides. Unfortunately, mosquitoes have developed resistance to most of synthetic insecticides (Chareonviriyaphap *et al*, 2013); thus the control of mosquito vector encounters a number of major difficulties, besides the adverse effects of chemical insecticides on the environment and health (Kumar *et al*, 2012). In the same context, searching for new compounds from natural origin for their mosquitocidal activities may replace conventional insecticides and these compounds may serve as insecticides, antifeedants as well as repellents (Reegan *et al*, 2013; Hasaballah and El-Naggar, 2017).

Marine organisms considered a source for numerous bioactive compounds and secondary metabolites that attract the attention of chemists and biologists due to their potential anticancer, antimicrobial, antiviral and antifungal activities (Zapata and Amemiya, 2000; Datta *et al*, 2015). Environmental challenges such as nutrition, space competition, predation and self-defence stimulate marine organisms to produce secondary metabolites. Marine invertebrates have the ability to live in halobiotic environment; special adaptation, metabolic activities and secretions propel them to produce bioactive substances to combat these environmental challenges. The whole body extract of several marine organisms showed a potential therapeutic source for various diseases (Datta *et al*, 2015).

An alternative insect and/or pest control method is the utilization of marine natural products to reduce the toxic insecticide usage and minimize harmful impact on insects and also on the environment (Dias *et al*, 2012; Hasaballah and El-Naggar, 2017). As a result, many scientists focused on isolation of compounds with insecticidal properties from marine algae (El-Sayed *et al*, 1997), sponge

(Hasaballah and El-Naggar, 2017; Elnagar *et al.*, 2018), soft corals (Momtaz, 2016) and marine microorganisms (Xiong *et al.*, 2004).

Marine organisms, including Molluscs proved their potential role as a source for various natural products with multiple bioactivities (Gnanambal *et al.*, 2005; Li *et al.*, 2009; Benkendorff 2010). Natural products research on the phylum Mollusca were primarily focused on the shell-less or soft-bodied molluscs, particularly cephalopoda, nudibranchs and opisthobranchs (Karuso, 1987; Faulkner, 1992; Babar *et al.*, 2012; Ióca *et al.*, 2018; Miller *et al.*, 2018). Cephalopods such as cuttlefishes, squids, nautilus and octopus are the main important sources of such bioactive compounds. As the majority of them lack an outer shell, they use a variety of defense ways, including venom to neutralize enemies and prey, and liquid-ink to evade predator attacks (Sudhakar and Nazeer, 2015).

This study was made as an attempt to investigate the acute larvicidal activity and repellent effect of ethanolic and methanolic extracts of the big blue octopus, *Octopus cyanea* against mosquito vector, *Culex pipiens*.

Materials and Methods

Mosquito, *C. pipiens* Linnaeus (L) was obtained from Medical Entomology Research Center. It was reared for several generations, in the insectary of medical entomology at the department of zoology, faculty of science, Al-Azhar University, under controlled conditions at a temperature of $27\pm 2^{\circ}\text{C}$, with relative humidity of $70\pm 10\%$ and 12-12 hr light-dark regimen. Adult mosquitoes were kept in wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs (Hasaballah 2015).

The octopus specimens were collected by hand from reef flat crevices during spring 2017 from Napq protected area at Aqaba Gulf coast of Egypt. After collection, the specimens were washed and preserved at -20°C in an icebox filled with ice cubes and some of table salt for late processing in the laboratory.

By using keys proposed in Vine (1986), Erwin and Picton (1990), and Lieske and Myers (2004), the specimens' identification was done on the basis of their morphological features.

The big blue octopus *O. cyanea*, belong to Phylum: Mollusca, Class: Cephalopoda, Order: Octopoda, Family: Octopodidae. It is commonly found in coral reefs and shallow water at depths of 1 to 100 meters in Pacific Ocean, Red Sea and Mediterranean Sea. It is a diurnally active predator searches for fish, crabs, shrimp and molluscs (Mather and Mather, 2004).

Preparation of crude extract: In the laboratory, the samples were washed under running tap water and chopped into small pits. To prepare the crude methanol and ethanol extracts, two samples of about 200 g were set in two bottles, the first was homogenized with 400 ml 70% aqueous ethanol and the second was homogenized with 400ml absolute methanol. The mixtures were continuously stirred and stored in dark to avoid photolysis and secondary metabolites degradation at room temperature. After a week with gentle shaking, mixtures were filtered via Whatman 542 filter paper. Solvents were evaporated with rotary evaporator (Ballantine *et al.*, 1987).

Different concentrations were prepared to study the acute larvicidal and repellent activities of tested extracts. The early 3rd larval instar was collected and placed in plastic cup its capacity 250mL containing the extract solution. Control larvae were placed in 250mL distilled water (25 of 3rd instar larvae/cup).

Acute larvicidal toxicity: The acute larvicidal toxicity that measured as mortality recorded 24 h post treatment was determined for the early 3rd larval instar of *Cx. pipiens*. Mosquito larvicidal toxicity assays were done (WHO, 1996) with some modifications (Pavella *et al.*, 2016). Dilution of extracts was carried out in dimethyl sulphoxide (DMSO) to prepare a serial dilution of test concentrations. Early 3rd larval instar was selected and transferred in 25mL-distilled water. For each treatment, 1mL of serial dilutions was added to 224mL of distilled water and lightly shak-

en to ensure a homogenous test solution. The selected larvae were transferred in distilled water into a cup of prepared test solution (25 larvae/cup). Three replicates were used in each experiment with at least 6 concentrations. During the experiments, food was not offered to the mosquito larvae.

Repellent activities: Selected concentrations from each extract were dissolved in 2mL water with a drop of Tween 8. The concentration was directly applied onto ventral surface of pigeon after abdominal feathers removal. Positive control tests were carried out using commercial repellent (Deet) purchased from Johnson Wax, Egypt, after (Uniyal *et al*, 2016). For each experimental treatment and after 10 min, pigeon was placed for 2 hr in cage containing fifty starved *C. pipiens* adult females. Alongside with these treatments control test was carried out using water. The number of fed and unfed females were counted and calculated after treatments.

GC-MS analysis: Gas Chromatography Mass Spectrometry (GC-MS) analysis was performed using gas Hewlett-Packard HP-5890 series II equipped with split/splitless injector and a capillary column (30 m, 0.25 mm and 0.25 lm) fused with phenyl poly (silphenylenesiloxane). The temperature of both injector and detector was set at 280 and 300 °C, respectively; the oven temperature was kept at 80°C for 1min up to 300°C at 20°C/min. Helium was used as carrier gas at continuous and constant flow of 1.0 ml/min. A volume of 2 µl was injected into the splitless mode and 1min purge. The MS (Hewlett-Packard 5889B MS Engine) was used with selected ion monitoring (SIM). The mass spectrometer was operated at 70 eV and scan fragments from 50 to 650 m/z. The crude extract peak identification was performed depending on the obtained mass spectra with those available in the NIST library.

Statistical analysis: Abbott's formula (Abbott, 1925) was used to correct control mortality (which was <3%). The larval mortality data were subjected to probit analysis for calculating LC₅₀, LC₉₀, 95% confidence limits and Chi-square values were calculated using sta-

tistical package for social sciences (SPSS, ver. 15.0). The repellency percent was calculated by the following formula, Repellency % = [A% - B% /100 - B%]×100, where A was percent of unfed females in treatment and B percent of unfed females in control.

Results

The acute larvicidal toxicities of *O. cyanea* different extracts on the early 3rd instar *C. pipiens* larvae were tested and the LC₅₀, LC₉₀, confidence limits at (95%), regression equation and chi-square were also calculated (Tables 1, 2).

The acute larvicidal toxicity of the 3rd larval instar of *Cx. pipiens* treated with *O. cyanea* crude methanol extract increased gradually as the applied concentration increased when compared to the control group. The highest acute toxicity % recorded was 98.7 and 90.7 at concentrations 200 and 100 ppm, respectively compared to 2.7% for control (Tab. 1).

Data revealed that the acute larvicidal toxicity of the 3rd larval instar of *C. pipiens* treated with the crude ethanol extract of *O. cyanea* was concentration-dependent (i.e. increased as the concentration increased). The highest acute toxicity % recorded was 98.7 and 86.7 at concentrations 50 and 40 ppm, respectively compared to 0.0 % for the control group (Tab. 2).

Testing the effect of *O. cyanea* different crude extracts against the acute larvicidal toxicity of the 3rd instar *C. pipiens* larvae showed that the highest effect was recorded by *O. cyanea* ethanol extract. Based on the comparison of the lethal concentrations, ethanol extract was selected as the most effective, LC₅₀ recorded (24.57ppm), followed by methanol extract (35.85ppm) and LC₉₀ recorded (43.89 ppm), followed by methanol extract (145.23 ppm).

Repellent effects of *O. cyanea* crude methanol extract against starved *C. pipiens* adult females. The repellent effect was concentration-dependent (i.e. increased as the concentration increased). At concentrations 100 & 200 ppm, repellent effect was extremely induced as calculated in 84 & 87.94 %, respec-

tively, compared to 100% repellent for DEET at a concentration 10 ppm (Tab. 3).

Repellent effect of different concentrations of crude ethanol extract against starved *Cx. pipiens* females showed that effect was concentration-dependent, at >40 ppm, was extremely induced. Compared with ethanol extract was higher against *Cx. pipiens* followed by methanol extract that effect depended on extract concentration strength (Tab. 4).

The GC-MS analysis of *O. cyanea* ethanol extract recorded nineteen active compounds with insecticidal activities. The most predominant compounds are Furoscrobiculin B; Eugenol; Phenol, 2-methoxy-3- (2-propenyl); Hexadecanoic acid, methylester; Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester; Octadecanoic acid, methylester, Cyclo-decasiloxane, eicosamethyl; Oleic acid, eicosyl ester; trans-Isoeugenol; Phenol, 2-methoxy-5-(1-propenyl)-(E); Docosanoic acid, 1,2,3-propanetriyl ester; Octasiloxane, 1,1,3,3,5,

5,7,7,9,9,11, 11,13,13,15,15-hexadecamethyl; 2-Methyl-5H-dibenz[b,f]azepine; Cyclotetra-siloxane, octamethyl; Pyrazole[4,5-b]imidazole, 1-formyl-3-ethyl-6-á-d-ribofuranosyl; Cyclohexasiloxane, dodecamethyl; Cycloheptasiloxane, tetradecamethyl; Hexasiloxane, 1,1,3-,3,5,5,7,7,9,9,11,11-dodecamethyl; Cyclo-nonasiloxane and octadecamethyl with peak areas of 11.78; 11.09; 11.09; 5.37; 5.17; 4.76; 4.29; 3.17; 1.86; 1.86; 1.17; 1.23; 0.7; 0.42; 0.19, 0.06, 0.6, 0.6 and 0.22 %, respectively (Tab. 5).

The GC-MS analysis of *O. cyanea* methanol extract revealed five active compounds with insecticidal activities. These compounds are, Cyclodecasiloxane, eicosamethyl; Cycloheptasiloxane, tetradecamethyl; Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11, 11,13,13,15,15-hexadecamethyl; Cyclooctasiloxane, hexadecamethyl; Cyclohexasiloxane, dodecamethyl, with low peak areas of 1.50; 0.67; 0.67; 0.35 and 0.25%, respectively (Tab. 6).

Table 1: Larvicidal toxicity of *O. cyanea* crude methanol extract against 3rd larval instar of *C. pipiens*

Concentrations ppm	Acute larvicidal toxicity %	LC ₅₀ ppm (Its limits at 95%)	LC ₉₀ ppm (Its limits at 95%)	Regression equation	χ ²
200	98.7	35.85 (34.54 - 38.21)	145.23 (143.53 - 150.42)	Y = 0.3657x + 36.889	2.241
100	90.7				
50	65.3				
25	54.7				
12.5	38.7				
6.25	17.3				
Control	2.7				

Chi-Square (χ²) was calculated for LC₅₀ values.

Table 2: Larvicidal toxicity of *O. cyanea* crude ethanol extract against 3rd larval instar of *Cx. pipiens*

Concentrations ppm	Acute larvicidal toxicity %	LC ₅₀ ppm (Its limits at 95%)	LC ₉₀ ppm (Its limits at 95%)	Regression equation	χ ²
50	98.7	24.57 (22.40 - 28.08)	43.89 (41.71 - 47.96)	Y = 2.0696 x - 0.8518	1.193
40	86.7				
30	69.3				
20	30.7				
10	14.7				
5	9.3				
2.5	10.7				
Control	0.0				

Table 3: Repellency effect of *O. cyanea* crude methanol extract against *Cx. pipiens* females

Concentrations ppm	Females	Fed No.	Fed%	Unfed No.	Unfed %	Repellency %
200	50	3.0	6.0	47	94	87.94
100	50	8.0	16	42	84	84
50	50	21	42	29	58	58
25	50	30	60	20	40	40
12.5	50	42	84	8	16	16
6.25	50	45	90	5.0	10	10
Deet (10 ppm)	50	0.0	0.0	50	100	100
Control	50	47	94	3.0	6.0	6.0

Table 4: Repellency effect of *O. cyanea* crude ethanol extract against *Cx. pipiens* females

Concentrations ppm	Females	Fed No.	Fed%	Unfed No.	Unfed %	Repellency %
50	50	1.0	2.0	49	98	93.96
40	50	3.0	6.0	47	94	94
30	50	9.0	18	41	82	82
20	50	19	38	31	62	62
10	50	32	64	18	36	36
5.0	50	41	82	9.0	18	18
2.5	50	48	96	2.0	4.0	4.0
Deet (10 ppm)	50	0.0	0.0	50	100	100
Control	50	48	96	2.0	4.0	4.0

Table 5: Compounds with insecticidal activities from GC-MS analysis of *O. cyanea* ethanol extract

Compound Name	RT	Area %	Formula	Mo. Weight	Compound Nature	Activity
Furoserobiculin B	38.17	11.78	C15H20O2	232	Lactarane sesquiterpenes	Antifeedant
Eugenol	24.93	11.09	C10H12O2	164	Phenolic compound	Insecticide, pesticide, larvicide, repellent
Phenol, 2-methoxy-3-(2-propenyl)	24.93	11.09	C10H12O2	164	Phenolic compound	Insecticide pesticide, larvicide, repellent
Hexadecanoic acid, methylester	38.01	5.37	C17H34O2	270	Palmitic acid ester	Pesticide
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyil ester	39.53	5.17	C35H68O5	568	Fatty acid esters	Pesticide
Octadecanoic acid, methylester	42.03	4.76	C19H38O2	298	Fatty acid esters	Repellent
Cyclodecasiloxane, eicosamethyl	47.55	4.29	C20H60O10Si10	740	Silicone polymers	Surfactant in certain pesticide products
Oleic acid, eicosyl ester	41.55	3.17	C38H74O2	562	Ester derivatives of fatty acids	Insecticide, repellent, larvicide
Trans-Isoeugenol	26.54	1.86	C10H12O2	164	Phenolic compound	Insecticide, pesticide, larvicide, repellent
Phenol, 2-methoxy-5-(1-propenyl)-(E)	26.54	1.86	C10H12O2	164	Phenolic compound	Insecticide, pesticide, larvicide, repellent
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl	40.36	1.17	C16H50O7Si8	578	Silicone polymers	Surfactant in certain pesticide products
Docosanoic acid, 1,2,3- propanetriyl ester	41.68	1.23	C69H134O6	1058	Fatty acid esters	Pesticide
2-Methyl-5H-dibenz[b,f]azepine	9.62	0.7	C15H13N	207	Azepine derivatives	Insecticide
Cyclotetrasiloxane, octamethyl	25.68	0.42	C10H12O2	164	Silicone polymers	Surfactants in certain pesticide products
Pyrazole[4,5-b]imidazole, 1-formyl-3-ethyl-6-4-d-ribofuranosyl	18.07	0.19	C12H16N4O5	296	Pyrazole Derivatives	Insecticide, pesticide, larvicide
Cyclohexasiloxane, dodecamethyl	22.17	0.06	C12H36O6Si6	444	Silicone polymers	Surfactant in certain pesticide products
Cycloheptasiloxane, tetradecamethyl	26.74	0.6	C14H42O7Si7	518	Silicone polymers	Mosquitocide, surfactant in certain pesticide products
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	26.74	0.6	C12H38O5Si6	430	Silicone polymers	Surfactant in certain pesticide products
Cyclononasiloxane, octadecamethyl	34.37	0.22	C18H54O9Si9	666	Silicone polymers	Surfactant in certain pesticide products

Table 6: Compounds with insecticidal activities from GC-MS analysis of *O. cyanea* methanol extract

Compound Name	RT	Area %	Formula	Mo. Weight	Compound Nature	Activity
Cyclodecasiloxane, eicosamethyl	49.38	1.50	C20H60O10Si10	740	Silicone polymers	Surfactant in certain pesticide products
Cycloheptasiloxane, tetradecamethyl	26.71	0.67	C14H42O7Si7	518	Silicone polymers	Mosquitocide, surfactants in certain pesticides
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	26.71	0.67	C16H50O7Si8	578	Silicone polymers	Surfactant in certain pesticides
Cyclooctasiloxane, hexadecamethyl	30.82	0.35	C16H48O8Si8	592	Silicone polymers	Surfactant in certain pesticides
Cyclohexasiloxane, dodecamethyl	22.11	0.25	C12H36O6Si6	444	Silicone polymers	Surfactant in certain pesticides

Discussion

Mosquito control at the larval stage proved an effective tool for mosquito-borne diseases (Nandita *et al*, 2008). Also, environmental safety is the most important issue in mosquito control programs (Wafa *et al*, 2014). The use of natural products in mosquito control to prevent the proliferation of mosquito-borne

diseases and to protect the environment from the application of chemical insecticides and pesticides is the main goal for scientists.

In the present results, GC-MS analysis revealed 19 active compounds in ethanol extract, while methanol extract possesses 5 active compounds. GC-MS results confirmed the higher efficacy of ethanol extract against

mosquito vector. The identified compounds in ethanol extract recorded activity against insects; some as pesticides; hexadecanoic acid, methylester (Jemimma *et al*, 2017), hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (Niyogi *et al*, 2016) and docosanoic acid, 1,2,3-propanetriyl ester (Geetha *et al*, 2013), Others act as insecticide; 2-Methyl-5 H-dibenz [b, f] azepine (Panneerselvam *et al*, 2012), or as repellents; octadecanoic acid, methylester (Abubakar and Majinda, 2016) and others have many activities; eugenol; transisoeugenol; phenol, 2-methoxy-5-(1-propen-yl)- and phenol, 2-methoxy-3-(2-propenyl) (Kegley *et al*, 2010), and others have insecticide, pesticide, larvicide and repellent properties; oleic acid, eicosyl ester (Gurunathan *et al*, 2016). Ethanol extract was rich with compounds act as surfactants in certain pesticides; cyclodecasiloxane, eicosamethyl; cyclotetrasiloxane, octamethyl; cyclohexasiloxane, dodecamethyl; cycloheptasiloxane, tetradecamethyl; cyclononasiloxane, octadecamethyl; octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15,15 hexadecamethyl & hexasiloxane, 1,1,3, 3,5,5,7,7,9,9,11,11 dodecamethyl. But, methanol extract produces compounds act as surfactants in certain pesticide products and only cycloheptasiloxane and tetradecamethyl with insecticidal activities (Jayashree *et al*, 2015).

One of the commonest compounds with insecticidal, pesticidal, larvicidal and repellent activities is eugenol (phenylpropene) is a potential alternative to synthetic insecticides due to lower mammalian toxicity, rapid degradation in environment and complex mixture of bioactive constituents with multi-modal activities against target insects (Kegley *et al*, 2010). Phenolic compounds widely scattered in ethanol extract have substantial allelopathic applications in agriculture and forestry as herbicides, insecticides and fungicides (Zhao *et al*, 2010; Bhawan and Nagar, 2016). The second major compound is Furoscrobiculin-B, a lactarane sesquiterpenes compound possesses antifeedant activity (Ogino *et al*, 1994; Catelan *et al*, 2015).

The biological activity of oleic acid, eicosyl ester uncovered the reasonable larvicidal and

repellent activities due to the presence of certain chitinase inhibitors and ecdysone-20-monooxygenase. This effect may be attributed to the dechitinization of body wall and inhibition of ecdysone-20-monooxygenase, an enzyme required to promote cell membrane development. Gurunathan *et al*. (2016) confirmed that these compounds could be considered as a potent source for their mosquito larvicidal properties. Among the identified photo-chemicals, Hexadecanoic acid; methylester; Hexadecanoic acid; 1-(hydroxymethyl)-1,2-ethanediyl ester; Octadecanoic acid; methylester and docosanoic acid, 1,2,3-propanetriyl ester have antioxidant, nematocide and pesticide activities (Gopalakrishnan and Vadivel, 2011; Geetha *et al*, 2013).

Pyrazole derivatives are intermediate in biological materials. They have a history of application in agrochemical industry as herbicides, insecticides & acaricides. GC-MS analysis showed that ethanol extract contains pyrazole [4,5-b] imidazole, 1-formyl-3-ethyl-6- β -d-ribofuranosyl. Song *et al*. (2013) showed that pyrazole derivatives possess promise activities against many insects as bean aphid *Aphis craccivora*, *Cx. pipiens* and moth *Plutella xylostella*. Dibenzazepine derivative proved effective against several diseases and pathogens. The 2-Methyl-5H-dibenz [b, f] azepine only derivative was found in ethanol extract. Azepine derivatives have some activities as antiviral, anticancer & insecticidal activity (Panneerselvam *et al*, 2012).

Inhibitory effect of ethanol extract against *Cx. pipiens* was related to excessive amount of polysiloxanes. Silicone polymers are widely used as surfactants in certain pesticides. Many polysiloxanes were found in tested extracts, but in ethanol extract in massive amounts. Polysiloxanes are important bioactive compounds with insecticidal properties (Singh and Handique, 1997).

Conclusion

The highest acute larvicidal activity was the ethanol extract followed by methanol extract. The repellency percentages increased as the applied concentration increased. These results showed that extracts of new naturally comp-

ounds and their derivatives proved to be effective, cheap and environmental friendly insecticides against mosquito-borne diseases.

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