EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

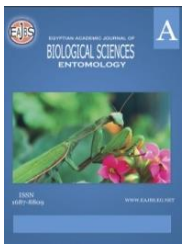
WWW.EAJBS.EG.NET

Vol. 13 No. 4 (2020)

Egypt. Acad. J. Biolog. Sci., 13(4):175-186(2020)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Activity of *Otostegia fruticosa* (Lamiaceae) Leaves Extracts Against Lymphatic Filariasis Vector, *Culex pipiens* L. (Diptera: Culicidae)

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ARTICLE INFO
Article History
Received:3/9/2020
Accepted:22/11/2020

Keywords:
Larvicidal activity;
Repellency;
Fecundity;
Culex pipiens;
Otostegia fruticosa.

ABSTRACT

The present study was carried out to evaluate the activity of methanol, ethyl acetate, chlorobenzene, and hexane extract from leaves of *Otostegia fruticosa* as larvicidal and repellent agents against *Culex pipiens.* The effect of tested extracts on the female reproductive potential treated as larvae was also investigated. The obtained results showed that hexane extract have potent larvicidal activity with LC_{50} and LC_{90} values equal to (126.27 and 236.84 ppm), respectively followed by chlorobenzene (242.14 and 501.17 ppm), ethyl acetate (578.07 and 856.29 ppm) and methanol (653.00 and 1127.10 ppm). All tested extracts of *O. fruticosa* induced significant ($P<0.01$) reduction in fecundity and increased the infertility percentages of *C. pipiens* females developed from treated larvae as compared with control and these effects were solvent- and concentration-dependent. In addition, the repellency effect of tested extracts was found to be varied among tested extracts. Four hours post-treatment, the dose of 3.33 mg/cm^2 recorded repellency percentages of 64.13 and 75.09 by methanol and ethyl acetate extracts, respectively. Meanwhile, chlorobenzene extract induced repellency percentages of 80.15, 75.94, and 65.84 for the doses of 3.33, 1.67 and 0.83 mg/cm^2, respectively. Moreover, repellent activity of hexane extract at doses of 3.33, 1.67 and 0.83 mg/cm^2 recorded 88.08, 79.01 and 75.50%, respectively.

INTRODUCTION

Culex pipiens L. is reported as one of the most widely distributed mosquitoes worldwide (Shehata 2019). *Culex pipiens* or the house mosquito is widely distributed in the urban and peri-urban areas (Bernard *et al.*, 2001; Cetin *et al.*, 2013). In Egypt, *C. pipiens* is widely distributed in the urban areas of Cairo governorate (Ammar *et al.*, 2013) and it is the main vector of Rift Valley fever virus (Meagan *et al*., 1980; Darwish and Hoogastrall, 1981), *Wuchereria bancrofti* (Khalil *et al*., 1930; Gad *et al*., 1996; Fouda *et al.*, 2013; Hassan *et al.*, 2013) and Western Nile virus (Pelah *et al*., 2002).

For mosquito control, immature stages (eggs, larvae, and pupae) are usually targeted by synthetic insecticides (Shehata 2019). Although highly efficacious of these insecticides, mosquito control is facing a threat due to the development of resistance to

these chemical insecticides resulting in rebounding vectorial capacity (Liu *et al.,* 2006; Bream *et al.,* 2018), besides its hazards on human health, non-target organisms, and the environment. (Hemingway and Ranson, 2000; Gold *et al.,* 2001). From this point of view, researchers diverted their attention towards the plant kingdom to find alternative agents that possess bioactive chemicals that may act as potential insecticides, antifeedants, oviposition deterrents, repellents as well as growth inhibitors (Murugan *et al*., 1996; Koul 2005; Hasaballah 2015; Bream *et al.,* 2018).

Additionally, botanical pesticides have abroad-spectrum activity, relative specificity in their mode of action, more easily degradable and easy to process and use as well as they tend to be safe for animals and the environment (Belmain *et al*., 2001). The present study aimed to evaluate the activity of different tested extracts from leaves of *Otostegia fruticosa* as larvicidal and repellent agents against *C. pipiens.* Also, to study the effect of tested extracts on the reproductive potential of females resulted from treated larvae.

MATERIALS AND METHODS

Culex pipiens Colonization:

The mosquito used in this study was *Culex pipiens* L., which was provided by the Medical Entomology institute. It was reared for six generations in the Medical Entomology Insectary, Animal house, Faculty of Science, Al-Azhar University, under controlled conditions at a temperature of 27±2 °C, relative humidity 70±10 % and 12-12 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 post-emergence. After this period, females were allowed to take a blood meal from a pigeon host which is necessary for laying eggs (Hassan *et al.*, 2014).

Preparation of Plant Materials:

Leaves of *Otostegia fruticosa* (Lamiaceae) were collected from Saint Catherine Protectorate, South Sinai Governorate, and left to dry away from sun rays at room temperature (27-31ºC) for 14 days, then it was pulverized to powder in a hammer mill. The extraction was performed using methanol, ethyl acetate, chlorobenzene, and hexane solvents according to the procedure described by Bream *et al.,* (2018).

Larvicidal Activity:

Larvicidal activity of different extracts from leaves of *O. fruticosa* was carried out using the procedure previously described by El-Sheikh *et al.* (2012) with minor modification. Tested material of the methanol extract was dissolved in 0.1ml of methanol, while those of ethyl acetate, chlorobenzene, and hexane extracts were dissolved in two drops of $Tween_{80}$ as an emulsifier to facilitate dissolving oils of tested materials in water.

The Reproductive Potential of Females:

Females that succeeded to emerge from the 3rd instar larvae treated with each concentration of tested extracts were collected and transferred with normal males obtained from the colony to the wooden cages (20×20×20 cm) by using an electric aspirator recommended by WHO and fed with 10% sugar solution for three days. Then, males and females left one day without sugar solution. On day five, the starved females were allowed to take a blood meal from a pigeon and allowed to lay egg rafts on clean water previously placed in oviposition traps within the cages. The number of eggs/rafts was counted by a binocular microscope and then the mean value was taken. Non-hatched embryonated eggs were determined by the apparent confirmation of the presence of an embryo under a dissecting microscope (Shehata 2018).

Repellent Activity:

A standard procedure described by Hassan *et al.,* (2014) was applied to test the

repellent activity of *O. fruticosa* extracts, different weights from each extract were dissolved in 2ml (methanol, ethyl acetate, chlorobenzene, and hexane with a drop of $Tween_{80}$ separately) in glass beaker 5×5 cm to prepare different concentrations, it was directly applied on the ventral surface of pigeon after feathers removal from the abdomen to evaluate repellency against tested mosquito compared with commercial repellent DEET (N. N. diethyl- meta- toluamide) that was purchased from Johnson Wax Egypt as a positive control. After 10 minutes, pigeons were placed in the cages containing 50 starved 5-7 day-old-females for three hours. Control tests were carried out alongside the treatments using the same amount of solvents without extracts. Each test was repeated three times to get a mean value of the repellent activity.

Statistical Analysis:

Data represented as Mean±SD. Statistical analysis of the data was carried out according to the method of Lentner *et al*., (1982). LC_{50} and LC_{90} values were calculated using multiple linear regressions (Finney 1971). One-way analysis of variance ANOVA was applied to find the differences between the activity of tested extracts using Tucky's HSD test at 5% probability level, where means with $P>0.05$ are not statistically significant. Statistical analysis was carried out using GraphPad InStat software.

RESULTS

Larvicidal Activity:

Obtained results revealed that, complete larval mortality 100% was obtained by methanol extract of *Otostegia fruticosa* at a concentration of 1400 ppm, meanwhile, for ethyl acetate it was achieved by the concentration of 1000 ppm, respectively compared with 0.0% for the control group (Table 1). The mean larval duration was significantly ($P<0.001$) prolonged by all tested concentrations of ethyl acetate as compared with the untreated group. At the highest concentration used, pupal mortality percentages reached 44.44 for methanol and 100.0 for ethyl acetate extracts. Mean pupal duration was significantly ($P<0.05$) prolonged at all tested concentrations of methanol and ethyl acetate extracts except the concentration of 200 ppm for methanol extract. The growth index recorded 7.27 versus 16.38 in control for methanol and 4.28 versus 16.35 for ethyl acetate at the highest tested concentrations (Table 1).

Table 1: Effect of methanol and ethyl acetate extracts from leaves of *Otostegia fruticosa* on some biological aspects of *Culex pipiens.*

Extract	Conc. (ppm)	Larval mort. (%)	Larval Period	Pupal Mort. (%)	Pupal Period	Adult Emergence (%)	Development Period	Growth Index
Methanol	Control	0.00	4.37±0.21^{a}	0.00	1.75±0.15^{a}	100.00±0.00	6.11±0.28^{a}	16.38±0.77^{a}
	200	10.67±4.62	4.43±0.06^{a}	0.00	1.97±0.15^{a}	100.00±0.00	6.40±0.17^{a}	15.63±0.43^{a}
	400	29.33±4.62	4.50±0.10^{a}	0.00	2.13±0.12^{b}	100.00±0.00	6.63±0.15^{b}	15.08±0.34^{a}
	600	45.33±4.62	4.70±0.10^{a}	0.00	2.23±0.15^{c}	100.00±0.00	6.93±0.15^{d}	14.43±0.32^{b}
	800	57.33±4.62	4.87±0.12^{c}	15.56±5.09	2.43±0.06^{d}	84.44±5.09	7.30±0.10^{d}	11.57±0.67^{d}
	1000	78.67±2.31	4.97±0.15^{c}	18.89±1.92	2.48±0.02^{d}	81.11±1.92	7.44±0.14^{d}	10.90±0.19^{d}
	1200	90.67±2.31	5.03±0.15^{d}	44.44±9.62	2.59±0.01^{d}	55.56±9.62	7.63±0.15^{d}	7.27±1.12^{d}
	1400	100.00±0.00	----	----	----	----	----	----
Ethyl Acetate	Control	0.00	4.07±0.07^{a}	0.00	2.05±0.08^{a}	100.00±0.00	6.12±0.06^{a}	16.35±0.17^{a}
	400	18.67±2.31	4.54±0.07^{d}	9.84±0.28	2.27±0.07^{c}	90.16±0.28	6.81±0.02^{d}	13.24±0.04^{c}
	500	37.33±2.31	4.61±0.02^{d}	21.25±3.31	2.45±0.05^{d}	78.75±3.31	7.05±0.06^{d}	11.17±0.53^{d}
	600	50.67±2.31	4.85±0.08^{d}	24.36±8.40	2.59±0.02^{d}	75.64±8.40	7.44±0.06^{d}	10.17±1.05^{d}
	700	74.67±2.31	5.01±0.09^{d}	42.06±8.36	2.69±0.04^{d}	57.94±8.36	7.71±0.10^{d}	7.52±1.04^{d}
	800	85.33±4.62	5.46±0.08^{d}	64.45±3.85	2.86±0.10^{d}	35.55±3.85	8.32±0.02^{d}	4.28±0.46^{d}
	900	97.33±2.31	5.67±0.04^{d}	100.00±0.00	----	----	----	----
	1000	100.00±0.00	----	----	----	----	----	----

No. of tested larvae = 25 per replicate; Conc. = Concentration; ppm = particle per million; SD = standard deviation; mort. = mortality; a = non-significant ($P>0.05$); b = significant ($P<0.05$); c = highly significant ($P<0.01$); d = very highly significant ($P<0.001$). Within each column and for each solvent, different letters denote means are significantly different. All data represented as Mean±SD. All periods are calculated as Days±SD.

The highest larval mortality 100% was attained at 600 and 300 ppm by chlorobenzene and hexane extracts, respectively, while the lowest mortality percentages 6.67 and 13.33 was reached by the concentrations 50 and 25 ppm for the same solvents, respectively compared with 0.0% for the control group (Table 2). The mean larval period was significantly (P<0.001) prolonged by each tested concentration of chlorobenzene and hexane extracts as compared with the untreated group. Also, the mean pupal period was significantly (P<0.01) prolonged at all concentrations used. A remarkable reduction in adult emergence percentages when high concentrations of chlorobenzene extract were applied, adult emergence percentages recorded 61.11, 72.70, and 78.18% at 500, 400 and 300 ppm, respectively, compared with 100.0% for the control group. A retarded effect on the growth of larvae, pupae and adults of *C. pipiens* was observed in particular at concentrations of 500 and 400 ppm for chlorobenzene extract, the growth index recorded 7.40 and 9.01, respectively versus 16.17 for the untreated group (Table 2).

Table 2: Effect of chlorobenzene and hexane extracts from leaves of *Otostegia fruticosa* on some biological aspects of *Culex pipiens.*

Extract	Conc. (ppm)	Larval mort. (%)	Larval Period	Pupal Mort. (%)	Pupal Period	Adult Emergence (%)	Development Period	Growth Index
Chlorobenzene	Control	0.00	4.24±0.07^{a}	0.00	1.95±0.08^{a}	100.00±0.00	6.18±0.02^{a}	16.17±0.04^{a}
	50	6.67±2.30	4.57±0.02^{d}	0.00	2.19±0.04^{c}	100.00±0.00	6.77±0.06^{d}	14.78±0.13^{a}
	100	28.00±0.00	4.67±0.03^{d}	0.00	2.35±0.07^{d}	100.00±0.00	7.02±0.04^{d}	14.24±0.09^{b}
	200	44.67±5.77	4.90±0.07^{d}	0.00	2.49±0.04^{d}	100.00±0.00	7.39±0.08^{d}	13.54±0.15^{c}
	300	57.33±2.31	5.16±0.06^{d}	21.82±4.81	2.65±0.07^{d}	78.18±4.81	7.81±0.11^{d}	10.01±0.73^{d}
	400	76.00±4.00	5.25±0.04^{d}	27.30±6.76	2.83±0.06^{d}	72.70±6.76	8.07±0.09^{d}	9.01±0.92^{d}
	500	89.33±2.31	5.31±0.02^{d}	38.89±9.62	2.95±0.07^{d}	61.11±9.62	8.26±0.07^{d}	7.40±1.22^{d}
	600	100.00±0.00	----	----	----	----	----	----
Hexane	Control	0.00	4.33±0.10^{a}	0.00	1.91±0.07^{a}	100.00±0.00	6.23±0.04^{a}	16.04±0.10^{a}
	25	13.33±4.62	4.76±0.04^{d}	0.00	2.49±0.04^{d}	100.00±0.00	7.25±0.07^{d}	13.79±0.14^{d}
	50	29.33±2.31	4.89±0.04^{d}	0.00	2.70±0.08^{d}	100.00±0.00	7.58±0.11^{d}	13.19±0.19^{d}
	100	38.67±2.31	5.18±0.04^{d}	0.00	2.84±0.05^{d}	100.00±0.00	8.01±0.07^{d}	12.48±0.10^{d}
	150	57.33±2.31	5.55±0.10^{d}	0.00	3.11±0.10^{d}	100.00±0.00	8.66±0.15^{d}	11.55±0.20^{d}
	200	76.00±4.00	5.82±0.04^{d}	0.00	3.28±0.05^{d}	100.00±0.00	9.10±0.08^{d}	10.99±0.09^{d}
	250	86.67±4.62	6.14±0.13^{d}	0.00	3.43±0.08^{d}	100.00±0.00	9.56±0.19^{d}	10.46±0.21^{d}
	300	100.00±0.00	----	----	----	----	----	----

See footnote of table (1).

From the aforementioned results, extraction from leaves of *O. fruticosa* by hexane revealed a potent larvicidal activity than those of chlorobenzene, ethyl acetate, and methanol (Table 3).

Table 3: Lethal concentrations (LC_{50} and LC_{90}) of tested extracts from leaves of *Otostegia fruticosa* against *Culex pipiens* larvae.

Extracts	**LC_{50} (LC_{90}) ppm**	**Slope**	**95% Confidence Limits LC_{50} (LC_{90})**		**χ^2**
			Lower	Upper	
Methanol	683.28 (1212.68)	0.0757	653.00 (1127.10)	713.57 (1298.80)	6.53$^{n.s.}$
Ethyl Acetate	585.48 (866.50)	0.1424	578.07 (856.29)	592.90 (876.73)	5.07$^{n.s.}$
Chlorobenzene	261.13 (508.12)	0.1621	242.14 (501.17)	280.09 (515.07)	3.11$^{n.s.}$
Hexane	129.92 (259.57)	0.3094	126.27 (236.84)	133.57 (282.310)	1.46$^{n.s.}$

χ^2*:* Chi square; (n.s.) nonsignificant at *P* value = 0.05

The Reproductive Potential of Females:

Data in table (4) revealed that fecundity was decreased by increasing the concentrations of methanol and ethyl acetate extracts from leaves of *O. fruticosa* and the statistical analysis showed a significant (P<0.001) decrease in the mean number of eggs laid by females resulted from treated larvae. Also, there was a reduction in the

hatchability percentages of tested extracts, where it was reduced to 84.89% with a concentration of 1200 ppm for methanol and 84.26% with a concentration of 900 ppm for ethyl acetate, as compared with the control. The percent of non-hatched embryonated eggs was 7.55% for the control group, while this percent reached about 17.98 when treated with 200 ppm raised up to 23.53 at a concentration of 1200 ppm for methanol extract. For ethyl acetate, the same pattern with a higher reduction in the percent of non-hatched embryonated eggs reached about 29.41 with a concentration of 900 ppm. A positive correlation between the infertility index and concentration was observed, where it reached 56.41% and 59.36% ppm when the highest concentration of methanol and ethyl acetate applied, respectively, compared with 0.0% for the control group (Table 4).

Table 4: Effect of methanol and ethyl acetate extracts from leaves of *Otostegia fruticosa* on the reproductive potential of *Culex pipiens* resulted female.

Extract	Con. (ppm)	No. of tested females	Eggs laid		Hatched eggs		Non-Hatched embryonated eggs		Unfertility Index (UI %)
			Total	Mean±SD	Total	%	Total	%	
Methanol	Control	12	2682	223.50±2.68^{a}	2629	98.02	4	7.55	0.00
	200	10	1287	128.70±2.41^{d}	1198	93.08	16	17.98	45.32
	400	9	1115	123.89±2.71^{d}	1023	91.75	18	19.57	48.11
	600	8	981	122.63±2.67^{d}	887	90.42	19	20.21	49.39
	800	6	710	118.33±2.50^{d}	631	88.87	17	21.52	52.00
	1000	5	578	115.60±2.70^{d}	502	86.85	17	22.37	54.17
	1200	2	225	112.50±2.12^{d}	191	84.89	8	23.53	56.41
Ethyl Acetate	Control	12	2714	226.17±3.41^{a}	2687	99.01	4	14.81	0.00
	400	10	1221	122.10±2.23^{d}	1123	91.97	18	19.39	49.85
	500	8	972	121.50±2.45^{d}	881	90.64	20	21.98	50.82
	600	6	712	118.67±1.97^{d}	634	89.04	18	23.08	52.81
	700	6	686	114.33±2.16^{d}	607	88.48	19	24.05	54.83
	800	4	448	112.00±2.16^{d}	394	87.95	14	25.93	56.01
	900	1	108	108.00±0.00	91	84.26	5	29.41	59.36

See footnote of table (1).

On the other hand, the fecundity of females resulted from larvae treated with chlorobenzene extract was significantly decreased from 120.20±2.94 eggs/♀ at the lowest concentration (50 ppm) to 106.33±3.06 eggs/♀ at the highest concentration applied (500 ppm), compared with 226.23±3.17 eggs/♀ for the control. The infertility index of females resulted from larvae treated with chlorobenzene extract recorded 57.26, 59.09 and 60.72% at 300, 400 and 500 ppm, respectively compared with 0.0% for control (Table 5). In addition, a significant decrease in the mean number of eggs laid by females resulted from treated larvae by hexane extract, where fecundity recorded (109.83±1.72, 101.50±2.89 and 99.50±2.12 eggs/♀ for concentrations 300, 200 and 100ppm, respectively vs. 229.82±2.48 eggs/♀ for the control. The infertility index for females resulted from larvae treated with hexane extract recorded 66.69% at the highest concentration used (250 ppm) decreased to 52.35% at the lowest concentration (25 ppm), compared with 0.0% for the control (Table 5).

Table 5: Effect of chlorobenzene and hexane extracts from leaves of *Otostegia fruticosa* on the reproductive potential of *Culex pipiens* resulted female.

Extract	Con. (ppm)	No. of tested females	Eggs laid		Hatched eggs		Non-Hatched embryonated eggs		Unfertility Index (UI %)
			Total	Mean±SD	Total	%	Total	%	
Chlorobenzene	Control	13	2710	226.23±3.17[a]	2653	97.90	6	10.53	0.00
	50	10	1202	120.20±2.94[d]	1081	89.93	28	23.14	51.19
	100	9	1063	118.11±2.98[d]	925	87.02	33	23.91	53.59
	200	7	799	114.14±2.19[d]	687	85.98	28	25.00	55.69
	300	6	668	111.33±2.07[d]	568	85.03	26	26.00	57.26
	400	5	544	108.80±1.92[d]	453	83.27	25	27.47	59.09
	500	3	319	106.33±3.06[d]	261	81.82	17	29.31	60.72
Hexane	Control	11	2528	229.82±2.48[a]	2460	97.31	7	10.29	0.00
	25	9	1090	121.11±2.80[d]	959	87.98	38	29.01	52.35
	50	8	933	116.63±2.26[d]	793	84.99	42	30.00	55.68
	100	8	912	114.00±2.27[d]	758	83.11	48	31.17	57.63
	150	6	659	109.83±1.72[d]	540	81.94	38	31.93	59.76
	200	4	406	101.50±2.89[d]	320	78.82	28	36.84	64.23
	250	2	199	99.50±2.12[d]	149	74.87	21	42.00	66.69

See footnote of table (1).

Repellent Activity:

Generally, the repellency effect of tested extracts from leaves of *O. fruticosa* was found to be varied among tested extracts as shown in table (6). At the dose of 3.33 mg/cm^2, repellent activity recorded 64.13 and 75.09% by methanol and ethyl acetate extracts, respectively. Meanwhile, chlorobenzene extract induced a repellency effect that reached 80.15, 75.94, and 65.84% for doses 3.33, 1.67, and 0.83 mg/cm^2, respectively. Moreover, the repellent activity of hexane extract recorded 88.08, 79.01 and 75.50% when doses 3.33, 1.67 and 0.83 mg/cm^2 were applied, respectively compared with 100.0% repellency for DEET at the dose of 1.80 mg/cm^2 (Table 6).

Table 6: Repellent activity of tested extracts from leaves of *Otostegia fruticosa* against *Culex pipiens* starved females.

Extracts	Dose (mg/cm^2)	Unfed Females (%)	Repellency (%)	RD_{50} (RD_{90}) mg/cm^2	95% Confidence Limits RD_{50} (RD_{90}) mg/cm^2		Slope
					Lower	Upper	
Methanol	Control	3.33±2.31	0.00	1.49 (6.86)	1.38 (5.13)	1.61 (8.60)	6.9343
	0.83	46.67±2.31	44.83±2.04				
	1.67	62.00±5.29	60.62±6.14				
	3.33	66.00±3.46	64.13±3.08				
Ethyl Acetate	Control	6.00±3.46	0.00	0.76 (5.81)	0.60 (4.69)	0.92 (6.93)	7.8694
	0.83	57.33±2.31	54.63±0.80				
	1.67	67.33±3.06	65.23±3.20				
	3.33	76.67±2.31	75.09±3.45				
Chlorobenzene	Control	6.00±3.46	0.00	0.70 (5.50)	0.45 (4.22)	0.95 (6.78)	5.2772
	0.83	68.00±4.00	65.84±5.31				
	1.67	77.33±3.06	75.94±2.40				
	3.33	81.33±2.31	80.15±2.23				
Hexane	Control	4.67±1.16	0.00	0.61 (4.74)	0.55 (3.61)	0.68 (5.88)	5.401
	0.83	76.67±2.31	75.50±2.74				
	1.67	80.00±2.00	79.01±2.33				
	3.33	88.67±3.06	88.08±2.49				
DEET	1.8	0.0	100.00±0.0	------	------	------	------

DISCUSSION

Control of *Culex pipiens* mosquito is a very important issue in order to prevent or decrease the prevalence of transmitted diseases and also decreasing the rate of transmission of re-emerging diseases (Elango *et al.*, 2009). The increased use of chemical insecticides during the past few decades to control mosquito vectors have resulted in

serious consequences such as insect resistance, mammalian toxicity, bioaccumulation through food chains, environmental contamination as well as the toxicity of insecticides to non-target organisms (Wattanachai and Tintanon, 1999; Rohani *et al.,* 2001). *Otostegia fruticosa* extracts used in the present study and others from the natural origin are known to be eco-friendly and are non-toxic substances.

The larvicidal activity of *O. fruticosa* tested extracts was found to be solvent- and concentration-dependent. Hexane extraction was more effective against 3rd instar larvae of *C. pipiens* than those of chlorobenzene, ethyl acetate, and methanol, those arranged in descending order. These results are in agreement with the previous results recorded by Prabakar and Jebanesan, (2004) using *Momordica charantia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera,* and *Citrullus vulgaris* extracts against *C. quinquefasciatus* third larval instar, where the LC_{50} values after 24 h recorded 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm; Maurya *et al.* (2009) using petroleum ether extract from leaves of *Ocimum basilicum* against *Anopheles stephensi* and *C. quinquefasciatus,* where petroleum ether extract from leaves of *Oc. basilicum* was found to be the most effective against the larvae of both tested mosquitoes with LC_{50} values of 8.29, 4.57; 87.68, 47.25 ppm and LC_{90} values of 10.06, 6.06; 129.32, 65.58 ppm against *An. stephensi* and *C. quinquefasciatus*. Similar results were also recorded by Sakthivadivel *et al.* (2014) for aqueous extract from *Wrightia tinctoria* (fruit) which exhibited the highest larvicidal activity against *C. quinquefasciatus* followed by aqueous leaf extract with LC_{50} values of 0.17 and 0.09%; 0.21 and 0.11% after 24 and 48 h.

These results are also consistent with those obtained by Ullah *et al.* (2018) where, LC_{50} and LC_{90} values of *Cassia fistula* and *Nicotiana tabacum* extracts recorded 50.27, 203.99 and 17.77 and 206.49 ppm against larvae of *C. quinquefasciatus*; Hassanain *et al,* (2019) who used petroleum ether extract from leaves of *Lantana camara* against larvae of *An. Multicolor,* where the highest larval mortality 100.0% achieved at 140 ppm; Shehata (2019) where petroleum ether extract from leaves of *Prunus domestica* and *Rhamnus cathartica* was more effective against *C. pipiens* (LC_{50} 33.3 and 63.4 ppm) than chloroform (LC_{50} 70.8 and 192.1 ppm) and methanolic extracts (LC_{50} 132.7 and 273.5 ppm); Dey *et al.* (2020) who recorded that, aqueous extract of *Piper longum* showed the highest larval mortality after 24 h of treatment against *Ae. aegypti, An. stephensi* and *C. quinquefasciatus.*

Methanol, ethyl acetate, chlorobenzene, and hexane extracts from leaves of *O. fruticosa* induced a prolongation in both larval and pupal periods depending on the solvent used in the extraction and concentration of the extract. High concentrations of all tested extracts were found to significantly ($P<0.001$) extend the larval and pupal durations. These results are consistent with those of Sharma *et al.* (2006a&b) using petroleum ether extract of *Artemisia annua* against *An. stephensi* and *C. quinquefasciatus* larvae Similar observation was also reported by Coria *et al*. (2008) using ethanolic extract of *Melia azedarach* leaves on *Ae. aegypti* larvae; Juliene *et al.* (2009) using *Moringa oleifera* lectin against *Aedes aegypti* larvae; Hassanain *et al.* (2019) using petroleum ether extract from leaves of *L. camara* against larvae of *An. Multicolor*; Shehata (2019) using methanol, chloroform and petroleum ether extracts from leaves of *Pr. domestica* and *R. cathartica* against *C. pipiens*.

On the other hand, obtained results revealed a decreased percentage of adult emergence with tested extracts especially when higher concentrations were applied. The reduction in the adult emergence percentages was similar to that recorded previously by Nathan *et al*. (2006b) using methanolic extracts of leaves and seeds of *Melia azedarach* against *A. stephensi* larvae; Sharma *et al*. (2006a) using petroleum ether extract of *Ar. annua* against *An. stephensi* and *C. quinquefasciatus* larvae; Wiesman and Chapagain,

(2006) using one fraction obtained from the silica gel column chromatography of the methanol extract against *Aedes aegypti* mosquito larvae; Asiry *et al.* (2017) using ethanolic leaf extracts of *Citrullus colocynthis* (bitter apple), *Artemisia annua* (sweet wormwood), *Pergularia tomentosa* (Fattaka) and *Rhanterium epapposum* (Arfaj) against the larval stages of *Ae. Aegypti*, Nasir *et al.* (2017) using essential oils from some medicinal plants against *Ae. albopictus*; Shehata (2019) using methanol, chloroform, and petroleum ether extracts from leaves of *Pr. domestica* and *R. cathartica* against *C. pipiens* larvae.

The growth index of *C. pipiens* was decreased as the concentration of the tested extract increased. Such results are in agreement with previous studies using different plant extracts against some dipteran species such as those of Sharma *et al.* (2006b) using *Artemisia annua* extract against *C. autnauetesctetus*; Bream *et al.* (2010) using *Echinochloa stagninum* extracts against *C. pipiens*; El-Sheikh *et al.* (2012) using methanol extract of *Tribulus terrestris* against *An. Arabiensis*; Fouda *et al.* (2017) using *L. camara* (leaves and stems) extracts against the house fly, *Musca domestica* and Hassanain *et al.* (2019) using petroleum ether extract from leaves of *L. camara* against larvae of *An. Multicolor*.

Tested extracts from leaves of *O. fruticosa* induced a significant ($P<0.01$) reduction in fecundity and increased the infertility percentage of *C. pipiens* females developed from treated larvae as compared with control and this effect was depends on the solvent used in the extraction and the strength of tested concentration. A remarkable decrease in the hatchability percentages of eggs laid by females resulted from treated larvae was also noticed. These results are consistent with those obtained by many authors using different plant extracts against different mosquito species by Jeyabalan *et al.* (2003), Nathan *et al.* (2006a&b), Pavela (2007), Coria *et al.* (2008), Hasaballah (2018) and Shehata (2019).

At the doses of 0.83, 1.67 and 3.33 mg/cm^2, tested extracts exhibited repellency activity against *C. pipiens* starved females. Based on calculated RD_{50} and RD_{90}, hexane extract from leaves of *O. fruticosa* was more effective in exhibiting the repellent action against tested females than those of chlorobenzene, ethyl acetate and methanol. A potent repellency or biting deterrence with 88.08 % was attained by hexane extract at dose 3.33 mg/cm^2. The obtained results are in harmony with those obtained by Hassan *et al.* (2014) using ethanolic, acetone, and petroleum ether extracts from leaves of *Lagenaria siceraria* against *C. pipiens*; Deepalakshmi and Jeyabalan, (2017) using *Glochidion neilgherrense*, *Cinnamomum wightii,* and *Leucas linifolia* methanol leaf extracts against *C. quinquefasciatus*; Shehata (2018) using hexane, chloroform, methanol and ethyl acetate extracts from *Deverra triradiata* (aerial parts) against *An. sergentii, C. pipiens* and *C. antennatus*; Shehata (2019) using methanol, chloroform, and petroleum ether extracts from leaves of *Pr. domestica* and *R. cathartica* against *C. pipiens* females; Benelli *et al.* (2020) using essential oils from stem bark and wood of *Hazomalania voyronii* against *Ae. aegypti* and *C. quinquefasciatus*.

Conclusion

Based on obtained results, hexane extraction from leaves of *Otostegia fruticosa* was more effective against 3rd instar larvae of *C. pipiens* than those of chlorobenzene, ethyl acetate and methanol. Tested extracts from leaves of *O. fruticosa* reduces the fecundity and fertility of females resulted from treated larvae as compared with untreated control. The hexane extract was more effective in exhibiting the repellent action against starved females than chlorobenzene, ethyl acetate, and methanol extracts. Generally, *O. fruticosa* extracts used in the present study can be considered as new promising control agents against the mosquito vector, *C. pipiens.* Further, more studies are needed to reach

the bioactive ingredients in *O. fruticosa* extracts which responsible for the larvicidal and repellent activities.

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