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Repellent Activity of Origanum syriacum (Lamiaceae), Pergularia tomentosa (Apocynaceae), and Senna italica (Fabaceae) Leaves Extracts against Culex pipiens L. (Diptera: Culicidae)

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ABSTRACT

The present study aimed at investigating the repellent activity of methanol, ethyl acetate, chlorobenzene and hexane extracts from leaves of Origanum syriacum, Pergularia tomentosa and Senna italica against Culex pipiens starved females. Three doses were used (0.83, 1.67 and 3.33 mg/cm²) to test the repellent activity of selected extracts. The obtained results revealed that all tested extracts evoked a variable degree of repellent activity against C. pipiens starved females depending on the solvent used in the extraction and the dose. Potent repellent activity (91.53%) was recorded by hexane extract from leaves of O. syriacum at the dose of 3.33 mg/cm^2 , while, the lowest repellent activity (22.07%) was recorded by methanol extract from leaves of *O. syriacum* at the dose of 0.83 mg/cm², respectively. According to RD₅₀ and RD₉₀ values, hexane extraction from leaves of all tested plants was more effective against C. pipiens starved females than chlorobenzene, ethyl acetate and methanol extractions. Also, methanol, ethyl acetate, chlorobenzene and hexane extracts from leaves of O. syriacum were more effective against C. pipiens starved females than those of S. italica and P. tomentosa.

INTRODUCTION

Culex pipiens L. (Diptera: Culicidae) is considered one of the most widely distributed mosquitoes worldwide and can be found in urban and suburban areas and lives near people (Bernard *et al.*, 2001). *Culex pipiens* are vectors of many arbovirus encephalitides and lymphatic filariases (Cetin *et al.*, 2009). About 120 million people worldwide are infected with the *Wuchereria bancrofti* (Cao *et al.*, 1997; Turell *et al.*, 2000). Also, *C. pipiens* bites cause allergic responses and systemic reactions such as urticaria and angioedema (Peng *et al.*, 2004). In Egypt, *C. pipiens* is the main vector of the Rift Valley fever virus (Meagan *et al.*, 1980; Darwish and Hoogastrall, 1981), *Wuchereria bancrofti* (Khalil *et al.*, 1930; Gad *et al.*, 1996) and Western Nile virus (Pelah *et al.*, 2002). Preventing *C. pipiens* bites is an effective way for reducing the spread of disease transmission by minimizing the contact between humans and vectors (Pitasawat *et al.*, 2003; Shehata 2018).

Although Commercial chemical repellent products such as DEET (N, N-diethyl-3-methylbenzanmide) are good repellent agents against *C. pipiens* (Walker *et al.*, 1996), its side effects stimulated the researchers to look for new natural products that are derived from plant extracts and could act as repellent agents (Qiu *et al.*, 1998; Tawatsin *et al.*, 2001).

Origanum syriacum (Lamiaceae), Pergularia tomentosa (Apocynaceae) and Senna italica (Fabaceae) are curative plants found in south Sinai, Egypt and locally used in the medication of stomach wall inflammation, coughing, colic, expansion of the airways, intestinal disinfectant and constipation.

The present study was carried out to investigate the repellent activity of different extracts from leaves of *Origanum syriacum*, *Pergularia tomentosa* and *Senna italica* against *C. pipiens* starved females.

MATERIALS AND METHODS *Culex pipiens* Colonization:

A standard rearing procedure of Hassan *et al.*, (2014) was applied in order to rearing larvae of *Culex pipiens* L., which was obtained from the Medical Entomology institute, for several generations in Medical Entomology Insectary, Animal house, Department of Zoology, Faculty of Science, Al-Azhar University.

Preparation of Plant Materials:

Leaves of Origanum syriacum (Lamiaceae), Pergularia tomentosa (Apocynaceae) and *Senna italica* (Fabaceae) were collected from Saint Catherine South Sinai Protectorate, Governorate. Leaves were left to dry in shadow at room temperature $(27\pm3^{\circ}C)$ for ten days. After pulverizing leaves to powder, the extraction performed using methanol, ethyl was acetate, chlorobenzene and hexane solvents using the procedure of Bream et al., (2018).

Repellent Activity:

To test the repellent activity, a procedure described by Hassan *et al.*, (2014) was applied with minor modifications. Each extract was dissolved in 2 ml of methanol, ethyl acetate, chlorobenzene and hexane with a drop of Tween⁸⁰. A commercial repellent DEET (N. N. diethyl- meta- toulamide) (Johnson Wax Egypt) was used as a positive control. All tests were carried out using 50 *C. pipiens* starved females (5- 7d-old) for three hours. Control tests were carried out alongside the treatments using solvents. Three replicates were usually used for each test and the mean value was taken.

Statistical Analysis:

All results represented as Mean±SD. Statistical analysis of the data was carried out according to the method of lentner *et al.*, (1982). RD₅₀ and RD₉₀ values were calculated using multiple linear regressions (Finney 1971) and 95% Confidence Limits calculated using GraphPad InStat software.

RESULTS

Data showed that, at the doses of 3.33 and 1.67 mg/cm², methanol extract from leaves of *Origanum syriacum* induced a degree of repellency equal to 42.76 and 33.09%, respectively. While, ethyl acetate extract from leaves of *O. syriacum* induced a higher degree of repellency (61.74 and 50.71%), respectively at the same doses compared with 100.0% repellency for DEET at the dose of 1.80 mg/cm² (Table 1).

In addition, hexane extract from leaves of *O. syriacum* had a more repellent activity against С. pipiens females than chlorobenzene extract. The repellent action for chlorobenzene and hexane extracts were 59.99 and 91.53% at the highest dose (3.33 mg/cm²), while; it recorded 37.94 and 42.93% at the lowest dose (0.83 mg/cm²), respectively, compared with 100.0% repellency for DEET at the dose of 1.80 mg/cm^2 (Table 1).

Extracts	Dose (mg/cm²)	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±1.16	0.00	4.17 (9.27)	3.08 (6.50)	5.26 (12.04)	7.9325
	0.83	24.67±3.06	22.07±3.14				
	1.67	35.33±5.03	33.09±5.39				
	3.33	44.67±1.16	42.76±1.04				
	Control	4.00±2.00	0.00	1.94 (6.51)	1.44 (3.91)	2.43 (9.10)	9.0344
Ethyl Acetate	0.83	40.67±1.16	38.18±1.78				
	1.67	52.67±2.31	50.71±1.58				
	3.33	63.33±4.16	61.74±5.08				
	Control	3.33±2.31	0.00	2.01 (6.77)	1.50 (6.35)	2.53 (7.20)	8.4045
Chlorabangana	0.83	40.00±2.00	37.94±1.07				
Chlorobenzene	1.67	52.00±3.46	50.27±4.84				
	3.33	61.33±1.16	59.99±1.08				
Hexane	Control	5.33±3.06	0.00	1.17 (3.26)	0.86 (2.53)	1.47 (3.98)	19.338
	0.83	46.00±3.46	42.93±3.84				
	1.67	62.67±6.11	60.47±7.09				
	3.33	92.00±3.46	91.53±3.73				
DEET	1.8	0.0	100.00±0.0				

Table 1: Repellent activity of tested extracts from leaves of *Origanum syriacum* against *Culex pipiens* starved females.

As recorded in table 2, methanol and ethyl acetate extracts from leaves of Pergularia tomentosa against C. pipiens starved females proved an efficacy as repellents depending on doses used; as the potent repellency (51.42 and 53.79%) attained by methanol and ethyl acetate extracts at 3.33 mg/cm², respectively during the entire testing period of 4h of treatment. The repellent action decreased to record 29.97 and 31.75% by methanol and ethyl acetate extracts at the lowest dose (0.83 mg/cm^2), respectively, compared with

100.0% repellency for DEET at the dose of 1.80 mg/cm^2 .

Also, both chlorobenzene and hexane extracts from leaves of *P. tomentosa* were found to possess a good repellency against *C. pipiens* starved females (Table 2). The previously mentioned extracts induced 68.28 and 77.48% repellency at the dose of 3.33 mg/cm²; 57.26 and 59.87% repellency at the dose of 1.67 mg/cm², respectively, compared with 100.0% repellency for DEET at the dose of 1.80 mg/cm² (Table 2).

Table 2: Repellent activity of tested extracts from leaves of *Pergularia tomentosa* against *Culex pipiens* starved females.

Extracts	Dose (mg/cm²)	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	6.67±1.16	0.00	3.09 (7.90)	2.83 (6.81)	3.35 (8.99)	8.3391
	0.83	34.67±2.31	29.97±3.36				
	1.67	44.00±3.46	40.03±2.99				
	3.33	54.67±1.16	51.42±1.63				
Ethyl Acetate	Control	3.33±1.16	0.00	2.85 (6.79)	1.65 (5.88)	4.05 (7.70)	8.396
	0.83	34.00±3.46	31.75±1.67				
	1.67	46.00±2.00	44.13±1.64				
	3.33	55.33±5.03	53.79±5.14				
	Control	3.33±1.16	0.00	1.22 (5.58)	0.66 (4.86)	1.78 (6.31)	9.2365
Chlorobenzene	0.83	46.00±2.00	44.13±2.18				
	1.67	58.67±3.06	57.26±2.80				
	3.33	69.33±1.16	68.28±0.81				
Hexane	Control	5.33±2.31	0.00	0.65 (4.59)	0.42 (3.80)	0.88 (5.39)	10.213
	0.83	54.67±1.16	52.11±0.05				
	1.67	62.00±2.00	59.87±1.36				
	3.33	78.67±2.31	77.48±2.19				
DEET	1.8	0.0	100.00±0.0				

On the other hand, methanol extract from leaves of *Senna italica* induced repellency effect equal to 57.85, 47.12 and 38.57% at the doses of 3.33, 1.67, and 0.83 mg/ cm², respectively. While, the repellent activity of ethyl acetate extract from leaves of *S. italica* recorded 67.82, 58.90 and 43.82% at the same doses, respectively, compared with 100.0% repellency for the positive control (DEET) at the dose of 1.80 mg/cm² (Table 3).

Moreover, hexane extract from leaves of *S. italica* proved higher efficacy as a repellent agent against *C. pipiens* starved females than chlorobenzene extract. Hexane extract from leaves of *S. italica* at the of dose 3.33 mg/cm^2 produced the highest protection 81.97% during the entire testing period of 4h of treatment. While, the lowest repellent percentages (48.55 and 59.72) were recorded by chlorobenzene and hexane extracts from leaves of *S. italica* at the lowest dose (0.83 mg/cm²), respectively. Meanwhile, at the dose of 1.67 mg/cm², chlorobenzene and hexane extracts gave 57.79 and 67.37% repellency against the tested females (Table 3).

Table 3: Repellent activity of tested extracts from leaves of Senna italica against Culex pipiens starved females.

Extracts	Dose (mg/cm²)	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	6.67±1.16	0.00	2.21 (7.88)	1.67 (5.64)	2.76 (10.13)	7.5371
	0.83	42.67±4.16	38.57±4.28				
	1.67	50.67±3.06	47.12±3.80				
	3.33	60.67±3.06	57.85±3.39				
Ethyl Acetate	Control	2.67±1.16	0.00	1.17 (5.65)	0.75 (4.79)	1.60 (6.51)	9.0076
	0.83	45.33±1.16	43.82±1.87				
	1.67	60.00±2.00	58.90±2.10				
	3.33	68.67±1.16	67.82±0.81				
Chlorobenzene	Control	5.33±2.31	0.00	0.92 (5.28)	0.82 (4.94)	1.02 (5.62)	9.1794
	0.83	51.33±1.16	48.55±2.51				
	1.67	60.00±3.46	57.79±2.67				
	3.33	73.33±1.16	71.80±1.93				
Hexane	Control	4.00±2.00	0.00	0.82 (4.66)	0.64 (4.03)	0.99 (5.29)	8.8853
	0.83	61.33±1.16	59.72±0.63				
	1.67	68.67±3.06	67.37±3.02				
	3.33	82.67±3.06	81.97±2.99				
DEET	1.8	0.0	100.00±0.0				

DISCUSSION

Preventing Culex pipiens bites is an effective way for reducing the spread of disease transmission (Shehata 2018). The results of the present study revealed that all tested extracts from leaves of Origanum syriacum, Pergularia tomentosa and Senna *italica* recorded a variable repellent activity against C. pipiens starved females reflecting the chemical composition of their constituents. The repellent activity was varied according to the solvent used in extraction and dose. Generally, hexane extraction from leaves of all tested plants was more effective against C. pipiens starved females than chlorobenzene, ethyl acetate

and methanol extractions. Also, methanol, ethyl acetate, chlorobenzene and hexane extracts from leaves of *O. syriacum* were more effective against *C. pipiens* starved females than those of *S. italica* and *P. tomentosa.* Rajkumar and Jebanesan, (2005) and Shehata 2018 attributed the repellent activity of botanical extracts to the presence of several compounds, including phenolics, terpenoids and alkaloids.

The present results of repellent activity of tested extracts against *C. pipiens* are in accordance with earlier results of El-Sheikh *et al.*, (2012) who tested the repellent activities of methanolic extract (leaves, seeds) of *Tribulus terrestris* against the malarial vector, Anopheles arabiensis and they concluded that, the seeds extract was more effective in exhibiting the repellent action (100.0%) against the mosquito tested as compared with the leaves extract (79.5%) at the dose 1.0 and 2.0 mg/cm², while, the seeds extract showed the same repellency percent (100.0%) of commercial formulation, N. N. diethyl toulamide (DEET) but at the lower dose (1.0mg/cm²), Rajeswary et al., (2013) who tested the repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of Pithecellobium dulce leaf and seed against Aedes aegypti and they found that, among the tested solvents the maximum efficacy was observed in the leaf and seed methanol extract, where, the highest concentration (5.0 mg/cm²) provided over 150 and 120 min. protection, Adhikari and Chandra, (2014) where, crude and petroleum ether extracts from leaves of Swietenia mahagoni showed repellency up to 2 h. after treatment against A. stephensi, Deepalakshmi and Jeyabalan, (2017) who tested the repellent activity of Glochidion neilgherrense, Cinnamomum wightii and Leucas linifolia methanol leaf extracts quinquefasciatus against С. and they reported that, all the concentration of plant extracts hared promising mosquito properties С. repellency against quinquefasciatus and increasing in the concentration of plant extracts from 1 to 4% was found to increase the biting deterrency percentage, Shehata (2018) where, hexane, chloroform, methanol and ethyl acetate extracts from Deverra triradiata (aerial parts) recorded a variable repellent activity against three mosquito species (A. sergentii, C. pipiens and C. antennatus) at 3.33, 1.67, 0.83 and 0.42 mg/cm^2 as, the highest repellent activity attained by hexane extract with RD₅₀ equal to 0.704, 1.122 and 0.92 mg/cm² against A. sergentii, C. pipiens and C. antennatus starved females and Shehata (2019) where, the highest repellent activity (97.3 and 90.2%) was recorded by petroleum ether extract of Prunus domestica at the highest doses $(3.33 \text{ and } 5.0 \text{ mg/cm}^2)$, while petroleum ether extract of Rhamnus

cathartica recorded 89.8 and 80.0% repellency at the same doses, respectively.

Similar observations were also reported by Benelli *et al.*, (2020) using the essential oils (EOs) from stem bark and wood of *Hazomalania voyronii* against the mosquito vectors, *Aedes aegypti* and *C. quinquefasciatus*. They recorded a wide spectrum repellent activity of *H. voyronii* EOs, suggesting a possible traditional use of the bark EO to repel mosquitoes.

Conclusion:

D50 and RD90 values, hexane extraction from leaves of all tested plants were more effective against Culex pipiens starved females than chlorobenzene, ethyl acetate and methanol extractions. Also, methanol, ethyl acetate, chlorobenzene and hexane extracts from leaves of Origanum syriacum were more effective against C. pipiens starved females than those of Senna italica and Pergularia tomentosa. Thus, Origanum syriacum, Pergularia tomentosa, and Senna italica tested extracts are new promising repellent agents against the mosquito vector, C. pipiens. However, we need to initiate more studies to test the repellent activity of other eco-friendly plant extracts against different mosquito species.

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