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REPELLENT ACTIVITY OF *DEVERRA TRIRADIATA* (APIACEAE) EXTRACTS AGAINST *ANOPHELES SERGENTII* THEOBALD, *CULEX PIPIENS* LISTON AND *CULEX ANTENNATUS* BECKER MOSQUITOES

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Abstract

The present study evaluated the repellent activity of hexane, chloroform, methanol and ethyl acetate extracts from *Deverra triradiata* aerial parts against three mosquito species (*Anopheles sergentii, Culex pipiens* and *Culex antennatus*). At 3.33, 1.67, 0.83 & 0.42 mg/cm^2, all tested extracts showed a variable degree of repellency against tested mosquito species depending on solvent used in extraction. The highest repellent activity attained by hexane extract, with RD_{50} equal to 0.704, 1.122 & 0.92 mg/cm^2 against *An. sergentii*, *Cx. pipiens* and *Cx. antennatus* starved females, followed by ethyl acetate (0.904, 1.323 & 0.9 mg/cm^2), chloroform (1.101, 1.367 & 1.157 mg/cm^2) and methanol (1.183, 1.578 & 1.323 mg/cm^2) extracts. Also, RD_{90} of hexane, chloroform, methanol and ethyl acetate extracts recorded 2.567, 2.92, 3.067 and 2.88 mg/cm^2 against *An. sergentii*, 3.027, 3.317, 3.593 & 3.547mg/cm^2 against *Cx. pipiens*, 2.703, 3.09, 3.267 & 2.81mg/cm^2 against *Cx. antennatus* starved females, respectively. In addition, complete repellency time was varied according to solvent used in extraction. *D. triradiata* tested extracts showed a strong biting deterrency against tested mosquito species, where the highest complete repellency time (187.7min) a achieved by methanol extract against *An. sergentii* starved females at 3.33 mg/cm^2 and the lowest complete repellency time (57.7min) recorded by hexane extract against *Cx. pipiens* bites at 0.42 mg/cm^2, respectively.

Keys words: *Anopheles sergentii, Culex pipiens, Cx. antennatus*, repellency, *Deverra triradiata*.

Introduction

Mosquito bites causes allergic responses including local skeeter syndrome such as urticaria and angioedema (Abdel-Motagaly *et al*, 2017), and transmission of many diseases as malaria, lymphatic filariasis, dengue, Japanese encephalitis with annual millions of deaths (El-Bahnasawy *et al*, 2013). Huge numbers of mosquitoes were reported in Egypt (Mikhail *et al*, 2009). Malaria is still the important cause of infectious disease mortality in many parts of Africa, and some areas in Asia and Latin America (WHO, 2014). Egyptian cases of dengue fever and *Aedes aegypti* were reported (Morsy, 2018). Also, *Cx. antennatus* (Becker) was the vector of Rift Valley Fever virus during an outbreak in the Nile Delta of Egypt (Hanafi *et al*, 2011). Personal protection products, including repellents, are widely used to reduce the transmission of diseases by minimizing the contact between humans and vectors (Pitasawat *et al*, 2003). Commercial repellent products contain che-mical compounds as DEET (N, N-diethyl-3-methylbenzanmide), showed best repellency against mosquitoes (Walker *et al*, 1996). The side effects of these chemical products varied from mild to fatal (Qiu *et al*, 1998) which stimulated to get repellents derived from medicinal plants and herbs to alternate the DEET (Tawatsin *et al*, 2001). *Deverra triradiata* belongs to Apiaceae is a medicinal plant found in south Sinai, Egypt and locally used for get rid of dyspnea.

This study aimed to offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

Materials and Methods

Collection and rearing of mosquitoes: Larvae of *Anopheles sergentii* and *Culex pipiens* were collected from El-Fayoum Governorate, in March 2017, while *Cx. antennatus* larvae collected from Shubramunt, Giza in April 2017. All mosquitoes were kept for several generations, Medical Insectary, Animal House, Department of Zoology, Al-Azhar Faculty of Science; under controlled temperatue of (27±2^{o}C), RH (70±10%), and light and dark cycles (12-12). Larvae were provided with finely ground dog biscuit and adults were fed on 10% sucrose solution and were periodically allowed to take a blood meal from the pigeon (Haldar *et al*, 2014).

Plant collection and preparation of crude extract: *Deverra triradiata* was collected in April 2017 from South Sinai Governorate, away from sun rays, left to dry at room temperature (25-30ºC) for 5 to 10days and pulverized to powder commercial electrical stainless steel blender. Extraction was performed using hexane, chloroform, methanol and ethyl acetate (El-Sheikh *et al*, 2016).

Repellent activity: For repellent activity of *D. triradiata* extracts, cages (60×60×60cm) were used. Different weights from each extract were dissolved in 2ml of the solvent with a drop of $Tween_{80}$ separately in glass 4×4cm to prepare different concentrations. After removing feathers from the pigeon abdomen, each concentration was directly applied onto 5×6cm of ventral surface to evaluate the repellency against *An. sergentii, Cx. pipiens* & *Cx. antennatus*. After 10min., the pigeons were placed in cages containing one hundred *An. sergentii, Cx. pipiens* & *Cx. antennatus* starved females (5-7d-old) for three hours. Control tests were carried out alongside with the treatments using hexane, chloroform, methanol & ethyl acetate with a drop of $Tween_{80}$ separately. Each test was repeated three times to get a mean value of repellent activity. The time in which mosquitoes began to descend on the pigeon for feeding has been recorded. After treatments, fed and unfed females were calculated (Abbott, 1925): Repellency %= [%A-%B/100-%B] ×100 (A = unfed treatment females% and B = unfed females control%. Statistical analysis: Data were tabulated and analyzed using Statistical Package Social Science software version 11.5 (SPSS, 2007).

Results

The maximum repellent activity was observed for *Deverra triradiata* hexane extract against tested mosquito species as compared with other extracts. At the highest concentration (3.33mg/cm^2) hexane extract recorded 91.8, 85.0 and 90.4% repellent activity against *Ano. sergentii, Cx. pipiens* and *Cx. antennatus* starved females, respectively.

The highest repellent activities achieved by chloroform, methanol and ethyl acetate extracts against *An. sergentii* were 86.8, 85.9 and 90.7% at 3.33mg/cm^2, respectively. Also, hexane extract at the lowest dose (0.42mg/cm^2) provided complete protection against *An. sergentii* bites for at least 116.3 min vs. 9.3 min for the control group, respectively (Tab.1).

D. triradiata chloroform, methanol & ethyl acetate extracts evoked variable repellent activities against *Cx. pipiens* starved females, where, at 3.33, 1.67, 0.83 & 0.42mg/cm^2 the repellent activities were 80.0, 68.5, 53.6, 35.6% for chloroform extract; 76.7, 60.6, 46.9, 32.8% for methanol extract and 80.5, 74.2, 49.1, 38.3% for ethyl acetate extract, respectively. *D. triradiata* hexane, chloroform, methanol & ethyl acetate extracts provided highest protection against *Cx. pipiens* bites (105.0, 117.0, 136.0 & 125.7min) at highest dose (3.33mg/cm^2), respectively (Tab. 2).

The highest and lowest repellent percentages recorded against *Cx. antennatus* starved females were 83.2 & 39.0 by chloroform extract; 82.7 & 38.1 by methanol extract; 89.0 & 56.4 by ethyl acetate extract at 3.33 & 0.42mg/cm^2, respectively at doses of 3.33, 1.67, 0.83 & 0.42mg/cm^2, the complete protection times against *Cx. antennatus* bites recorded by hexane and chloroform extracts were 158.0, 146.3, 136.0, 112.7 & 175.3, 155.3, 141.0, 121.7min, respectively. Complete protection times recorded by methanol and ethyl acetate extracts against *Cx. antennatus* bites were 169.0, 157.7, 149.3, 147.0 & 167.7, 166.7, 158.3, 146.7 min, respectively, compared with 8.3 & 9.7min for untreated ones (Tab. 3). Hexane extract from aerial parts gave the highest repellent activity against females as compared with other extracts, where, RD_{50} were 0.704, 1.122 &

$0.92mg/cm^2$ against *An. sergentii*, *Cx. pipiens* and *Cx. antennatus* starved females. The lowest repellent activity was by methanol extract against *An. sergentii*, *Cx. pipiens* and *Cx. antennatus* females with RD_{50} were 1.183, 1.578 & $1.387mg/cm^2$, respectively. RD_{90} of hexane, chloroform, methanol and ethyl acetate extracts gave 2.567, 2.92, 3.067 & $2.88mg/cm^2$ against *An. sergentii*, 3.027, 3.317, 3.593 & $3.547mg/cm^2$ against *Cx. pipiens*, 2.703, 3.09, 3.267 & 2.81mg/ cm^2 against *Cx. antennatus* starved females, respectively (Tab. 4).

Table 1: Repellent activity of *D. triradiata* different extracts against *An. sergentii*.

Extract	Dose (mg/cm^2)	Fed females (%)	Unfed females (%)	Average repellency (%)	Complete repellency time (min.)
Hexane	3.33	8.0±2.6	92.0±2.6	91.8±2.5	174.0±10.4
	1.67	14.3±2.1	85.7±2.1	85.3±1.7	164.0±7.9
	0.83	21.0±4.0	79.0±4.0	78.5±3.6	126.0±7.0
	0.42	43.7±3.8	56.3±3.8	55.2±3.2	116.3±6.5
	Control	97.3±2.1	2.7±2.1	0.0	9.3±1.5
Chloroform	3.33	12.7±2.5	87.3±2.5	86.8±2.8	175.0±6.0
	1.67	20.3±3.1	79.7±3.1	78.9±3.0	174.3±7.2
	0.83	31.7±2.9	68.3±2.9	67.1±3.8	142.0±6.6
	0.42	55.0±2.6	45.0±2.6	42.9±3.2	122.0±3.0
	Control	96.3±2.5	3.7±2.5	0.0	9.0±2.0
Methanol	3.33	13.7±1.5	86.3±1.5	85.9±1.8	187.7±6.7
	1.67	28.3±2.1	71.7±2.1	70.7±1.7	175.7±3.7
	0.83	44.3±3.5	55.7±3.5	54.1±3.9	167.3±8.2
	0.42	51.3±1.5	48.7±1.5	46.9±1.4	138.7±5.5
	Control	96.7±1.5	3.3±1.5	0.0	8.7±1.2
Ethyl Acetate	3.33	9.0±2.0	91.0±2.0	90.7±2.3	179.3±6.5
	1.67	30.3±2.5	69.7±2.5	68.6±3.3	174.7±7.1
	0.83	31.3±2.5	68.7±2.5	67.6±2.0	164.0±5.6
	0.42	40.3±4.2	59.7±4.2	58.3±3.6	150.0±2.6
	Control	96.7±2.3	3.3±2.3	0.0	9.3±4.2

Table 2: Repellent activity of *D. triradiata* different extracts against *Cx. pipiens*.

Extract	Dose (mg/cm^2)	Fed females (%)	Unfed females (%)	Average repellency (%)	Complete repellency time (min.)
Hexane	3.33	14.3±1.5	85.7±1.5	85.0±1.7	105.0±6.0
	1.67	26.3±3.5	73.7±3.3	72.4±3.7	92.7±8.5
	0.83	34.0±3.5	66.0±3.5	64.3±3.8	75.7±4.5
	0.42	55.3±2.9	44.7±2.9	41.9±3.3	57.7±4.2
	Control	95.3±0.6	4.7±0.6	0.0	6.3±0.6
Chloroform	3.33	19.3±2.1	80.7±2.1	80.0±1.9	117.0±4.2
	1.67	30.3±2.5	69.7±2.5	68.5±1.8	94.7±5.0
	0.83	44.7±2.1	55.3±2.1	53.6±3.1	89.0±5.6
	0.42	62.0±6.6	38.0±6.6	35.6±7.6	64.7±3.2
	Control	96.3±1.2	3.7±1.2	0.0	7.3±1.5
Methanol	3.33	22.7±2.5	77.3±2.5	76.7±3.0	136.0±3.6
	1.67	38.3±2.1	61.7±2.1	60.6±2.9	130.0±3.5
	0.83	51.7±2.1	48.3±2.1	46.9±1.8	109.0±7.0
	0.42	65.3±3.5	34.7±3.5	32.8±5.1	91.7±4.0
	Control	79.3±2.1	2.7±2.1	0.0	7.3±3.5
Ethyl Acetate	3.33	18.7±3.2	81.3±3.2	80.5±3.1	125.7±4.7
	1.67	24.7±4.0	75.3±4.0	74.2±4.6	112.7±4.7
	0.83	48.7±3.5	51.3±3.5	49.1±3.2	101.0±4.4
	0.42	59.0±3.6	41.0±3.6	38.3±4.3	90.3±0.6
	Control	95.7±1.5	4.3±1.5	0.0	6.7±2.1

Table 3: Repellent activity of *D. triradiata* different extracts against *Cx. antennatus*.

Extract	Dose (mg/cm^2)	Fed females (%)	Unfed females (%)	Average repellency (%)	Complete repellency time (min.)
Hexane	3.33	9.3±1.5	90.7±1.5	90.4±1.6	158.0±7.9
	1.67	17.7±2.5	82.3±2.5	81.8±2.6	146.3±6.1
	0.83	29.3±0.6	70.7±0.6	69.9±0.4	136.0±9.8
	0.42	53.7±5.7	46.3±5.7	44.8±6.1	112.7±5.7
	Control	97.3±0.6	2.7±0.6	0.0	6.7±2.3
Chloroform	3.33	16.3±2.5	83.7±2.5	83.2±2.9	175.3±2.1
	1.67	28.3±2.1	71.7±2.1	72.2±2.1	155.3±4.2
	0.83	33.7±1.5	66.3±1.5	65.4±1.1	141.0±3.6
	0.42	59.3±4.7	40.7±4.7	39.0±5.8	121.7±3.2
	Control	97.3±1.5	2.7±1.5	0.0	7.7±1.2
Methanol	3.33	16.3±4.0	83.7±4.0	82.7±4.2	169.0±1.7
	1.67	31.7±3.5	68.3±3.5	66.4±4.2	157.7±4.6
	0.83	49.0±2.6	51.0±2.6	48.0±3.6	149.3±2.5
	0.42	58.3±4.2	41.7±4.2	38.1±5.1	147.0±10.1
	Control	94.3±1.5	5.7±1.5	0.0	8.3±2.5
Ethyl Ace-tate	3.33	10.7±4.0	89.3±4.0	89.0±4.0	167.7±5.9
	1.67	20.3±2.5	79.7±2.5	79.1±2.5	166.7±3.8
	0.83	36.7±4.9	63.3±4.9	62.3±4.5	158.3±4.2
	0.42	42.3±2.1	57.7±2.1	56.4±1.3	146.7±8.3
	Control	97.0±2.0	3.0±2.0	0.0	9.7±2.1

Table 4: RD_{50} & RD_{90} mean values of *D. triradiata* different extracts against mosquito strains used.

Mosquito Species	Extract used	RD_{50} (mg/cm^2)	95% Confidence Limits		RD_{90} (mg/cm^2)	95% Confidence Limits	
			LCL	UCL		LCL	UCL
Anopheles sergentii	Hexane	0.704	0.4703	0.9377	2.567	2.423	2.710
	Chloroform	1.101	0.8516	1.348	2.92	2.741	3.099
	Methanol	1.183	1.105	1.225	3.067	2.923	3.210
	Ethyl Acetate	0.904	0.8242	0.9832	2.88	2.579	3.181
Culex pipiens	Hexane	1.122	0.9513	1.282	3.027	2.883	3.170
	Chloroform	1.367	1.249	1.484	3.317	3.058	3.575
	Methanol	1.578	1.311	1.849	3.593	3.245	3.941
	Ethyl Acetate	1.323	1.198	1.448	3.547	2.050	5.044
Culex antennatus	Hexane	0.92	0.8062	1.034	2.703	2.527	2.880
	Chloroform	1.157	0.9312	1.382	3.09	2.773	3.407
	Methanol	1.387	1.178	1.595	3.267	2.890	3.643
	Ethyl Acetate	0.9	0.7060	1.094	2.81	2.508	3.112

Discussion

Mosquito repellents are one of the most effective strategies in reducing the spread of diseases transmitted by different mosquito species. There are ongoing efforts in searching for a safer, better, and cheaper repellent agents against mosquito vectors, plant extracts providing a potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties. The present study showed that *Deverra triradiata* tested extracts displayed variable repellent activities against different mosquitoes (*An. sergentii, Cx. pipiens* & *Cx. antennatus*) reflected the complexity of the chemical composition of their constituents (Bisseleua *et al,* 2008). The repellent effect of tested extracts may be due to the presence of various compounds, including phenolics, terpenoids and alkaloids, which exist in *D. triradiata*; these compounds may jointly or independently contribute to produce a repellent activity Rajkumar and Jebanesan, (2005). Also, the repellent activity varied according to solvent used in extraction and the dose of the extract, as hexane extract was more effective in exhibiting the repellent action against three tested mosquito species than chloroform, methanol and ethyl acetate extracts. The present repellent activity exhibited by

D. triradiata extracts agreed with results of Yang *et al,* (2004), where methanolic extract of *Cinnamomum cassia* (bark), *Nardostachys chinensis* (rhizome), *Paeonia suffruticosa* (root bark) and *Cinnamomum camphora* gave 91.0, 81.0, 80.0 & 94.0% repellent activities against starved *Ae. aegypti* at 0.1mg / cm^2, Mullai *et al.* (2008) using benzene, petroleum ether, ethyl acetate & methanol extracts of *Citrullus vulgaris* leaf for *An. stephensi*, Govnidarajan and Sivakumar (2011) using crude hexane, ethyl acetate, benzene, chloroform and methanol extracts of *Eclipta alba* and *Andrographis paniculata* leaf against *Ae. aegypti* at 1.0, 2.5, & 5.0mg/cm^2 and they suggested that the leaf solvent plant extracts have the potential to be used as an ideal eco-friendly approach for the mosquitoes control and El-Sheikh *et al.* (2012) using methanolic extract of *Tribulus terrestris* (leaves & seeds) against *An. arabiensis*, where the seeds extract recorded 100% repellent action at 1.0mg/cm^2 against females compared with 79.5% repellent activity caused by leaves extract at 2.0mg/cm^2.

Similar results were recorded by Hassan *et al,* (2014) for ethanol, acetone and petroleum ether extracts from *Lagenaria siceraria* (leaves & stems) against *Cx. pipiens,* Sabiha *et al.* (2017) for petroleum ether, chloroform and methanol extracts of *Melia azedarach* leaf which offered repellent activity at 5% level of significance (P<0.05) against *Cx. quinquefasciatus* and Bream *et al.* (2018) for ethanol 70%, acetone, chloroform and petroleum ether extracts from *Musca acuminata* leaves which evoked a variable degree of repellency against *Cx. pipiens* starved females.

In the present study, the tested extracts showed a strong biting deterrency against tested mosquito species according to solvent used in extraction. In general, the tested extracts provided a complete protection time ranging from 57.7 to 187.7min against *An. sergentii, Cx. pipiens* and *Cx. antennatus* bites, which agreed with Venkatachalam and Jebanesan (2001) who used methanol extract of *Fredonia elephantum* leaves against *Ae. aegypti* at 1.0 & 2.5mg/cm^2 concentrations and reported 100.0% protection up to 2.14 & 4.0 h, Rajkumar and Jebanesan (2004) used *Moschosma polystachyum* crude leaf extract showed 85.2 & 54.6min protection against *Cx. quinquefasciatus* bites at 1.0 & 2.5mg/ cm^2, Rajkumar and Jebanesan (2005) using volatile oils extracted from leaves of *Moschosma polystachyum* & *Solanum xanthocarpum* against *Cx. quinquefasciatus*, where the oil from *M. polystachyum* & *S. xanthocarpum* gave 332.2 & 311.4min protection against mosquito bites at 4 & 8% vs. 4.4 min protection in controls. The volatile oils of these two plant species were effective as repellents and gave more than 300min (>5 hour) protection against *Cx. quinquefasciatus* bite. Mullai *et al,* (2008) found that benzene, petroleum ether, ethyl acetate and methanol extracts of *Cx. vulgaris* (leaf) at 1.0, 2.5 & 5.0mg/cm^2 gave mean complete protection time against *An. stephensi* ranged from 119.17 to 387.83 min. Adhikari and Chandra (2014) found that petroleum ether leaf extract of *Swietenia mahagoni* showed repellency up to 2h against *An. stephensi*.

Conclusion

Deverra triradiata extracts proved to have a good repellent activity against *Anopheles sergentii*, *Culex pipiens* and *Culex antennatus*. Extensive studies are ongoing to identify the bioactive compound(s) responsible for repellent activity to be prepared as commercial product /formulation.

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