Journal of the Egyptian Society of Parasitology, Vol.44, No.1, April 2014
J. Egypt. Soc. Parasitol. (JESP), 44(1), 2014: 243 - 248

REPELLENT EFFECT OF *LAGENARIA SICERARIA* EXTRACTS AGAINST *CULEX PIPIENS*

By

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Abstract

Ethanolic, acetone and petroleum ether extracts from leaves and stems of *Lagenaria siceraria* (Cucurbitaceae) were screened for their repellency effect against *Culex pipiens* L. mosquito. The repellent action of the present plant extracts were varied depending on the plant parts and the dose of extract. The petroleum ether extract of leaves showed the same repellency percent (100%) of commercial formulation, N. N. diethyl toulamide (DEET) at the higher dose (3.33 mg/cm^2), while petroleum ether extract from stems exhibiting the repellent action (89.6%) at the same dose, respectively. Ethanolic extracts of leaves and stems exhibited the lowest repellent activity as it recorded (81.3% and 69.1%) at (6.67 mg/cm^2), respectively. Results of this study may contribute to design an alternative way to control mosquitoes currently based on applications of synthetic insecticides. These extracts could be developed commercially as an effective personal protection measure against mosquito bites and thus to control diseases caused by mosquito–borne pathogens.

Key words: Repellent, Ethanolic extract, Acetone extract, Petroleum ether extract, *Lagenaria siceraria*, *Culex pipiens*.

Introduction

Mosquitoes mainly *Culex*, *Anopheles* and *Aedes* are serious vectors threat to the public health, since they to transmit many zoonotic infectious diseases as filariasis, malaria, West Nile fever, St. Louis encephalitis, Japanese encephalitis, La Crosse encephalitis, Yellow fever, Dengue fever, Rift Valley fever…etc. (Mandal, 2011).

Unfortunately, in Egypt, the Rift Valley fever (Hanafi *et al*, 2011), filariasis and malaria are encountered (El Bahnasawy *et al*, 2013) and human cases of Dengue fever (El Bahnasawy *et al*, 2011) and re-emerged *Aedes aegypti* (Shoukry *et al*, 2012) were reported in Southern Egypt as well as Soliman *et al.* (2010) reported that West Nile fever was actively circulating in the different Egyptian areas examined causing febrile illness in a considerable proportion of individuals.

Besides, the mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticaria and angioedema (Peng *et al,* 2004). The insect repellents from natural medical plants and herbs are more or less safer to man and environment than those of chemical origins and thus, being broadly investigated (Chio and Yang, 2008). Anti-feeding and growth inhibiting molecules reduce damages produced even without killing the pest (Bream *et al,* 2009).

The commonest mosquito repellent product available in the market is DEET (N, N-diethyl-3 -toluamide) (Mc-Ca be *et al,* 1954; Schreck, 1985). However, DEET has had a remarkable safety profile for the last 40 years of worldwide use, there are a number of reports on its toxicity against the skin, nervous and immune systems, usually occurring when the product was used incorrectly or at long term (Fradin, 1998; Blackwell *et al,* 2003).

Other undesirable effects of this product are an unpleasant odor, uncomfortable oily or sticky feeling, and danger to plastics and synthetic rubber (Qiu, *et al,* 1998), due to these disadvantages, so many authors preferred to use alternatives such as repellents of plants origin (Fradin and Day, 2002)..

The present study aimed at the investigation of the repellent effect of the different extracts from leaves of *Lagenaria siceraria* against the mosquito vector, *Culex pipiens*.

Materials and Methods

Mosquito culture: The mosquitoes were reared and maintained continuously for several generations in an insectary using the standard procedures described by (Kasap and Demirhan, 1992).

Collection and extraction of the plant materials: The freshly leaves of *L. siceraria* (Family: Cucurbitaceae) were collected in the month of June 2013 from Sharkia. The leaves were washed and dried in the shade at room temperature (27-31°C) for 7 days till they become brittle, then pulverized to powder in a hammer mill. The extraction was performed using 70% ethanol, acetone and petroleum ether solvents. One hundred grams of powder for each solvent separately were extracted five times with 300 ml of aqueous 70% ethanol, acetone and petroleum ether at room temperature. After 24 hr., the supernatants were decanted, filtrated through Whatman filter paper No.5 and dried in a rotary evaporator at 40ºC for (-3) hours to ethanol and (40-60) minutes to other solvents. The dry extracts were kept in deep freezer (-4ºC) till experimentally used (El-Sheikh, *et al,* 2012).

Repellency test: The standard cages (20 ×20×20 cm) were used to test the repellent activity of the extracts. Different amounts from each extract were dissolved in 2 ml (70% ethanol or water with a drop of Tween 80)in 4×4 cm cups to obtain the different concentrations. The concentration was directly applied onto 5×6 cm of the ventral surface of pigeon after removed feathers from the abdomen. After 10 min of treatment, pigeons were placed for 3 hr. in cages containing the Egyptian strain of *C. pipiens* starved females. Control tests were carried out using ethanol or water. Each test was repeated three times to get a mean value of repellent activity (El-Shei-kh *et al,* 2012). Post-treatment, the number of fed and unfed females was counted and mortality calculated according to Abbott (1925).

The repellency % = (% A - % B/100 - %B) x 100; where A was the percentage of unfed females in treatment and B the percentage of unfed females in control.

Results

Ethanolic extract of the leaves and stems: The data in (Tab. 1) indicated that the leaves extract of *L. siceraria* had a more repellent activity against *C. pipiens* fema-les than stems extract. The repellent action of leaves recorded 81.3, 72.0, 60.4 and 44.0% at 6.67, 3.33, 1.67 & 0.833 mg/cm^2, while the stems extracts recorded 69.1 and 50.5% at doses 6.67 and 3.33 mg/cm^2, respectively.

The acetone extract of leaves and stems: (Tab. 2) showed that acetone extracts of leaves and stems gave 96.3 and 72.8% protection from the bites of starved *C. pipiens* females at 6.67 mg/cm^2. However, at the lowest doses (3.33 and 1.67 mg/cm^2) the two extracts provided 83.3, 63.7 and 65.9, 55.2% protection, respectively as compared to100% repellency of Deet at a dose1. 8mg/cm^2.

Petroleum ether extract of leaves and stems: Both leaves and stems of the petroleum ether extracts of *L. siceraria* were found to possess a good repellency against the tested insect (Tab. 3). Its extracts induced a complete protection (100%) and 89.6% repellency at dose 3.33 mg/ cm^2; 95.1 and 77.4% repellency at 1.67 mg/cm^2; 83.2 and 58.7% repellency at 0.833 mg/cm^2; respectively. Moreover, the repellent activity at the dose 0.417 mg/cm^2 was 68.7 and 44.8% for leaves and stems extracts, respectively as compared to 100% repellency for Deet at 1.8 mg/cm^2.

Discussion

Bottle gourd (*Lagenaria siceraria*) was one of the first domesticated plants, and the only one with a global distribution during pre-Columbian times. However, native to Africa, bottle gourd was in use by humans in East Asia, as early as 11,000 y ago (BP) and in the Americas by 10,000 BP (Kistler *et al*, 2014).

This plant is well known eco-friendly and is not toxic to plants, animals and human. Moreover, it is clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts (Jang *et al,* 2002; Cavalcanti *et al,* 2004; Maurya *et al,* 2009; El-Sheikh *et al,* 2012).

All doses of the plant extracts used in the present study exhibited some repellency activity against the starved female adults of *C. pipiens*. The repellent activity of the plant extracts tested varied depending on the plant part used and the dose of the extract.

The present study indicated that the petroleum ether extract of leaves was more effective in exhibiting the repellent action against the mosquito tested as compared with the stems extract and showed same repellency percent (100%) at the higher dose (3.33mg/cm^2) as compared with a commercial formulation, N. N. diethyl toulamide (DEET) at (1.8 mg/cm^2).

A large number of plant extracts and the essential oils manifest repellency activity against different mosquito species. The present results are in accordance with such results obtained by Govere *et al*. (2000) used extracts of fever tea *Lippia javanica*
Rose, geranium *Pelargonium reniforme* & the lemon grass *Cymbopogon excavatus* against *A. arabiensis*.

Jeyabalan *et al.* (2003) reported that the methanol extracts of *Pelargonium citrosa* leaf exhibited repellency activity (36, 51, 78 & 100%) against the adult mosquito of *A. stephensi* at the concentrations (0.5, 1.0, 2.0, and 4.0%). Yang, *et al*. (2004) found that the repellent activity of methanol extracts of *Cinnamomum cassia* bark, *Nardostachys chinensis* rhizome, *Paeonia suffruticosa* root bark and *Cinnamomum camphora* at the dose of 0.1 mg/cm^2 was (91%), (81%), (80%) and (94%) res-pectively as comparable to DEET (82%) against starved *A. aegypti*. Choochote *et al.* (2007) used repellent activity of the selected essential oils from ten plant species against *A. aegypti*. Mullai *et al.* (2008) applied the leaf extract of *Citrullus vulgaris* against the malarial vector, *A. stephensi*. Singh *et al.* (2008) studied the effect of acetone leaves extract of *T. terrestris* against *A. stephensi*. Also, El-Sheikh (2009) evaluated sixteen ethanolic and petroleum ether extracts of 4 indigenous plants as repellent in the field against the wild mosquitoes.

Hassan *et al*. (2014) tested extracts from *Cupressus sempervirens* and *Cestrum nocturnum* against *C. pipiens*, the results indicated that the effective plant extract that evoked 100 % repellency or biting deterrence was the petroleum ether extract of *C. sempervirens* leaves at a dose of 1.5 mg/cm^2. Elango *et al.* (2011) used methanol extract of *Andrographis paniculata* against *A. Subpictus.*

Prabhu *et al.* (2011) used *Moringa oleifera* methanolic extracts against *A. stephensi* and Govnidarajan and Sivakumar (2011) tested the repellent activities of hexane, ethyl acetate, benzene, chloroform, and methanol extracts from the leaves of *Eclipta alba* and *Andrographis paniculata* at three different concentrations of 1.0, 2.5, and 5.0 mg/cm^2 against important vector of Yellow fever, *A. aegypti* and they reported that the leaf solvent plant extracts have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes and El-Sheikh *et al.* (2012) concluded that the seeds extract of *Tribulus terrestris* L. was more effective in exhibiting the 100% repellent action against malarial vector, *A. arabiensis* as compared with the leaves extract (79.5%) at the dose 1.0 and 2.0 mg/cm^2, also the seeds extract showed the same 100% repellency of the commercial

formulation, N. N. diethyl toulamide (DEET) but at the lower dose (1.0mg/cm^2). Sfara *et al*. (2013) demonstrated that insects with decreased locomotor activity were repelled to the same extent than control insects by the same concentration of DEET, and that the repellency and modification of locomotor activity elicited by DEET are non-associated phenomena.

Conclusion

The efficacy of petroleum ether extract from leaves of *L. siceraria* at the dose (3.33 mg/cm^2) gave the highest protection (100%) during the entire research period of 3hr post-treatment.

No doubt, mosquitoes are of wide distribution not only in Egypt but also world-wide. The outcome findings recommended the use of the environmental friend and safe mosquito repellent *Lagenaria sicera-ria*, which is one of the Egyptian floras of economic and medical importance.

Extensive studies on this plant against the immature stages and mode of action, synergism are ongoing and will be published in due time elsewhere.

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Table 1: Repellency effect of ethanolic extracts of leaves and stem of *Lagenaria siceraria* against *Culex pipiens* females.

Plant parts	Dose (mg/cm^2)	Tested females	No. of fed	% of fed	No. of unfed	% of unfed	Repellency %
Leaves	6.67	41	7	17.1	34	82.9	81.3
	3.33	39	10	25.6	29	74.4	72.0
	1.67	58	21	36.2	37	63.8	60.4
	0.833	43	22	51.2	21	48.8	44.0
Stem	6.67	46	13	28.3	33	71.7	69.1
	3.33	53	24	45.3	29	54.7	50.5
DEET	1.8	25	0.0	0.0	25	0.0	100.0
Control	0.0	47	43	91.5	4	8.5	0.0

Table 2: Repellency effect of acetone extracts of leaves and stems of *Lagenaria siceraria* against *Culex pipiens* females.

Plant parts	Dose (mg/cm^2)	Tested females	No. of fed	% of fed	No. of unfed	% of unfed	Repellency %
Leaves	6.67	58	2	3.4	56	96.6	96.3
	3.33	59	9	15.3	50	84.7	83.3
	1.67	64	20	31.3	44	68.7	65.9
Stem	6.67	44	11	25.0	33	75.0	72.8
	3.33	48	16	33.3	32	66.7	63.7
	1.67	56	23	41.1	33	58.9	55.2
DEET	1.8	25	0.0	0.0	25	0.0	100.0
Control	0.0	61	56	91.8	5	8.2	0.0

Table 3: Repellency effect of petroleum ether extracts from leaves and stems of *Lagenaria siceraria* against *Culex pipiens* females.

Plant parts	Dose (mg/cm^2)	Tested females	No. of fed	% of fed	No. of unfed	% of unfed	Repellency %
Leaves	3.33	72	0	0.0	72	100.0	100.0
	1.67	66	3	4.5	63	95.5	95.1
	0.833	78	12	15.4	66	84.6	83.2
	0.417	80	23	28.8	57	71.2	68.7
Stem	3.33	83	8	9.6	75	90.4	89.6
	1.67	77	16	20.8	61	79.2	77.4
	0.833	71	27	38.0	44	62.0	58.7
	0.417	69	35	50.7	34	49.3	44.8
DEET	1.8	25	0.0	0.0	25	0.0	100.0
Control	0.0	86	79	91.9	7	8.1	0.0