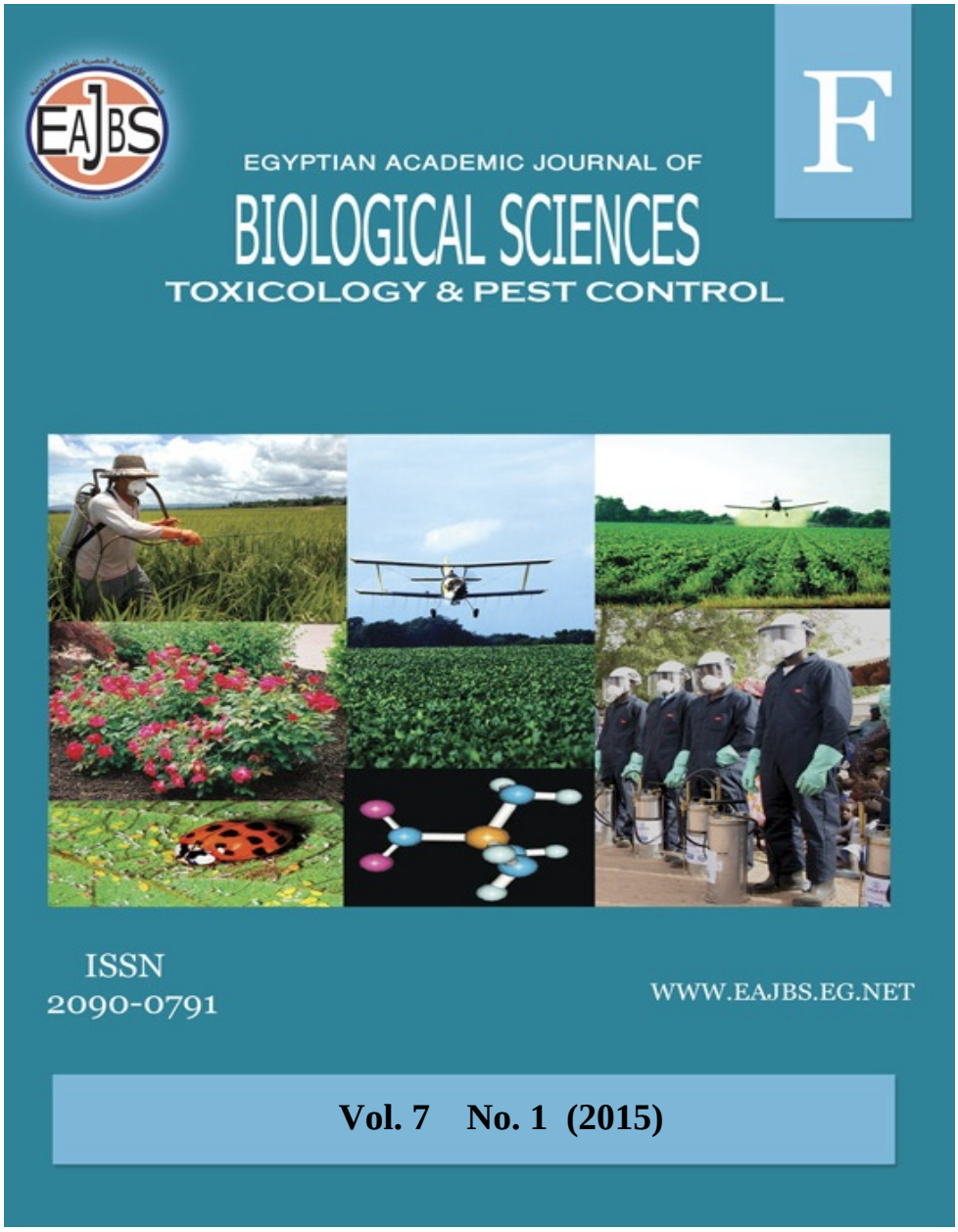

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www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 7(1): 65-70 (2015)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest control
ISSN: 2090 - 0791
www.eajbs.eg.net

Mosquito Larvicidal and Pupicidal Potential of *Heliotropium curassavicum* L. Against *Culex pipiens* (Diptera: Culicidae) and Their Chemical Composition in Kingdom of Saudi Arabia

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ARTICLE INFO

Article History
Received: 1/8/2015
Accepted: 5/9/2015

Key words:
larvicidal
Pupicidal
Heliotropium curassavicum
Culex pipiens

ABSTRACT

Curassavine was isolated as a yellowish viscous liquid and identified as (ester of trachelanthamidine and 3-carboxy-4-methylhexane-2,3-diol) by the aid of mass spectrometry (MS) and Nuclear Magnetic Resonance spectroscopy (^{1}H and ^{13}C-NMR).Considerable mortality was evident after the treatment. With regard to the larvicidal activity, the obtained data showed mortality percentages between 4 and 24 % using concentrations between 100 and 500 mg/ml after 3 days. The mortality increased to reach 8 and 86 % for the same concentrations within 5 days. LC_{50} was 288.4 mg/ml. Concerning the pupicidal activity, no effect using concentrations between 100 and 500 mg/ml within 2 days; while they showed mortality percentages between 8 and 60 % within 5 days. LC_{50} was 512.9 mg/ml. On the other hand, Curassavine caused mortality percentages among larvae and pupae of *Culex pipiens* between 12 and 84 % using concentrations between 100 and 500 µg/ml after one day. While they showed mortality percentages between 16 and 100 % within 2 days; Value of LC_{50} was 147.9 µg/ml.

INTRODUCTION

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis (JE), etc., causing millions of deaths every year (James, 1992). Filariasis is transmitted by *Culex pipiens* (*Bancroftian filariasis*).

Continuous uses of chemical insecticides have led to the control failures and disease resurgence, owing mainly to the development of resistance in the vectors (Raghavendra and Subbarao, 2002). *Wuchereria* species, causing lymphatic filariasis, is widely distributed in tropical regions with around 120 million people infected and 44 million people under clinical manifestation (Bernhard *et al*., 2003).

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organ chlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control program has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale (Russell *et al*., 2009). The most effective alternative approaches under the biological control program is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavior and physiological processes. Thus there is very little chance of pests developing resistance to such substances. Botanicals work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases. Sukumar *et al*. (1991) listed and reported 344 plant species that only exhibited mosquitocidal activity. Shallan *et al.* (2005) showed the current state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effects on non-target organisms, resistance and screening methodologies, and discussed some promising advances made in phytochemical research.

In the present investigation we have a compound was isolated as a yellowish viscous liquid and identified as Curassavine (ester of trachelanthamidine and 3-carboxy-4-methylhexane-2,3-diol) have the potential to be used as an ideal Ecofriendly approach for the control of mosquito.

MATERIALS AND METHODS

Plant material

Heliotropium curassavicum L. (Fig. 1) was collected in January 2014, from desert of Sakaka, Aljouf, Kingdom of Saudi Arabia and was kindly identified by Mr. Hamdan Ogereef Al-Hassan M.Sc. (Camel and Range Research Center), Aljouf, Kingdom of Saudi Arabia. A voucher specimen was kept and deposited at the herbarium museum at the Department of Pharmacognosy, College of Pharmacy, Aljouf University.

Fig. 1: *Heliotropium curassavicum*

Mosquitoes

The mosquitoes, *Culex pipiens*, was reared in Department of Biology, College of Science, Aljouf University, Kingdom of Saudi Arabia. The larvae were fed with biscuits and yeast powder in a 3:1 ratio. Adults were fed on the sugar syrup concentration of 7%, and in case of need to lay eggs we introduced inside the cage a pigeon to enable the breeding females taking of blood meal for 12 hours. Mosquitoes were held at 27±2°C temperature, 70–80% relative humidity, with a photoperiod of 12-h. light/12-h. dark.

Preparation of plant extract

Experimental

Hewlett Packard 5989 A Mass Spectrometer with 59980 B Particle Beam LC/MS Interface, at 70 eV or by chemical ionization with methane as reactant gas (Agilent technologies, Palo Alto, USA). Jeol JNMR-GX400 (400 MHz) operating at 400 MHz (for ^{1}H) and 100 MHz (For ^{13}C), NMR Spectra were in $CDCl_3$ solvent.

Alkaloid extraction and identification

One kilogram of the air-dried aerial parts of *Heliotropium curassavicum* L. was powdered and exhaustively extracted with70% ethanol (3x5L) at room temperature by maceration. Combined ethanol extracts were concentrated under vacuum at 40 °C till dryness to yield 135 g crude extract. The extract was suspended in 200 ml of distilled water, acidified with 2.5% Sulphuric acid, then stirred overnight at room temperature with Zn powder to reduce pyrrolizidine alkaloids (PA) N-oxides into tertiary PA. The aqueous acidic extract was filtered to remove excess Zn, and rendered alkaline with 25% NH_4OH solution till pH10 and then re-extracted with CH_2CI_2 until no more alkaloids could be detected in the aqueous phase (TLC; silica gel; eluent CH_2Cl_2/MeOH/NH_4OH85:14:1;detection by Dragendorff's reagent) Tundis *et al*. (2007) and El-Shazly (2002). The organic portions were combined, dried over anhydrous sodium sulphate and then evaporated to dryness to obtain the total alkaloid extracts (1.55 g). Mohanraj *et al*. (1978).

The alkaloidal residue (1.55g) was subjected to a series of silica gel column chromatography and gel filtration (Sephadex LH-20) for separation and purification in order to obtain the major compounds in a pure form by using dichloromethane, methanol and traces of ammonia solution as mobile phase for silica gel column chromatography and methanol for gel chromatography, the major compound A was eluted as a pure oily form by (CH_2Cl_2: CH_3OH: NH_3 82: 17: 1), detected as single spot on TLC plate and was further purified on Sephadex LH-20 column chromatography.

Biological activity of the extract and the Alkaloid

The study was conducted on both larvae and pupae of *Culex pipiens* separately for crude extract and together for curassavine. Five replicates were performed for each test and each tested level. Twenty individuals of different larval instars and pupae were placed in a plastic boxes (9 cm x 4.5cm) separately. While, 100 cm^3 tap water and food were added . The dose Added in 1 ml of water, the doses used are zero, 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml for crude extract and curassavine was tested at 100µg/ml, 200µg /ml, 300µg /ml,400µg/ml and 500µg /ml. Mortality counts were recorded daily for five days from the initiation of the experiment. Percent mortality was calculated for each concentration level. Probit analysis was used for determining the LC_{50} and slope (b) values (Finny, 1977). The treated and untreated replicates were incubated under constant conditions of 27±2 °C and 70–80 % RH.

Statistical analysis

Mortality data were subjected to probit analysis in order to determine the LC_{50} and slope (b) values (Finny 1977). The tested concentrations were compared for their efficiency to the *Culex pipiens* according to their LC_{50}.

RESULTS AND DISCUSSION

Extraction and identification

Compound A (32 mg)was isolated as a yellowish viscous liquid and identified as Curassavine (ester of trachelanthamidine and 3-carboxy-4-methylhexane-2,3-diol) Fig. 2 by the aid of mass spectrometry (MS) and Nuclear Magnetic Resonance spectroscopy (^{1}H and ^{13}C-NMR).

The positive ion CI/MS of compound A showed the presence of quasi-molecular ion peak at *m/e* 300 $[M+H]^{+}$ which is compatible with the formula $C_{16}H_{29}NO_4$ indicating that m/e to be 299 $[M]^{+}$.^{13}CNMR (100 MHz, $CDC1_3$): δ 45.1 (C-l), 30.7 (C-2), 54.4 (C-3), 54.8 (C-5), 25.9 (C-6), 32.1 (C-7), 68.0 (C-8), 68.1 (C-9), 176.7 (C-10), 84.3 (C-ll), 70.9 (C-12), 17.3 (C-13), 39.2 (C-14), 24.9 (C-15), 12.4 (C-16), 12.4 (C-17).^{1}H NMR (400 MHz, $CDC1_3$): δ 2.23 (IH, m,H-1), 2.11 (IH, m, H-2ß), 1.79 (IH, m, H-2a), 3.22 (IH, m, H-3a), 2.61 (IH, m, H-3b), 3.02 (IH, m, H-5a), 2.73 (IH, m, H-5b), 1.91 (2H, m, H-6), 2.03 (IH, m, H-7a), 1.59 (IH, m, H-7b), 3.33 (IH, m, H-8), 4.33 (IH, dd, *J*=11.0 and 6.5 Hz, H-9a), 4.19 (IH, dd, *J* = 11.0 and 6.5 Hz, H-9b),1.89 (IH, m, H-12), 0.91 (3H, d, *J* = 7Hz, H-13), 3.96 (IH, q, *J* = 6.0 Hz, H-14), 1.28 (3H, d, *J* = 6.5 Hz, H-15), 1.25 (2H, m, H-16), 0.87 (3H, t, *J* = 7Hz, H-17). These data was found in great accordance with those reported in literature for Curassavine alkaloid. Roeder *et al.*(1991). Mohanraj and Herz (1982).

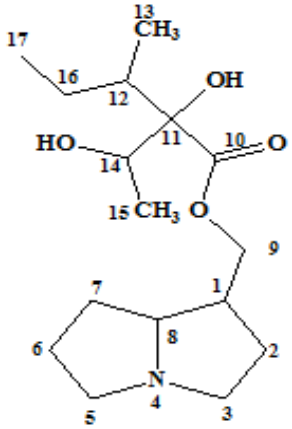

Fig. 2: Curassavine

Larvicidal and pupicidal activity

The results of larvicidal and pupicidal activity of Ethanol extract of *Heliotropium curassavicum* against *Culex pipiens* was noted and presented in Table 1. Considerable mortality was evident after the treatment. With regard to the Larvicidal activity, the obtained data showed no effect using concentrations between 100 and 400 mg/ml within 2 days; while they showed mortality percentages between 8 and 48 % within 5 days. It showed mortality percentages between 4 and 24 % using concentrations between 100 and 500 mg/ml after 3 days. The mortality increased to reach 8 and 86 % for the same concentrations within 5 days. LC_{50} was 288.4 mg/ml. Concerning the pupicidal activity, no

effect using concentrations between 100 and 500 mg/ml within 2 days; while they showed mortality percentages between 8 and 60 % within5 days. LC_{50} was 512.9 mg/ml.

Table 1: Laboratory evaluation of Ethanol extract of *Heliotropium curassavicum* against larvae and pupae of *Culex pipiens*

Concentration (mg/ml)	% Cumulative mean mortality after 5days									
	Ethanol extract of *Heliotropium curassavicum* against larvae					Ethanol extract of *Heliotropium curassavicum* against pupae				
	1day	2day	3day	4day	5day	1day	2day	3day	4day	5day
100 mg/ml	0.0	0.0	4	8	8	0.0	0.0	4	4	8
200 mg/ml	0.0	0.0	4	8	24	0.0	0.0	4	4	8
300 mg/ml	0.0	0.0	16	48	48	0.0	0.0	12	24	24
400 mg/ml	0.0	12	24	60	60	0.0	0.0	12	24	48
500 mg/ml	0.0	20	24	70	86	0.0	0.0	24	32	60
LC_{50} (%)	288.4 mg/ml					512.9 mg/ml				

Control mortality was zero % throughout the period of experiment

On the other hand, Curassavine caused mortality percentages among larvae and pupae of *Culex pipiens* between 12 and 84 % using concentrations between 100 and 500 µg/ml after one day. While they showed mortality percentages between 16 and 100 % within 2 days; Value of LC_{50} was 147.9 µg/ml (Table 2). Minjas and Sarda (1986) reported variations in toxicological efficacy with three mosquito species to the crude aqueous extract of fruit pods of *Swartzia madagascariensis* to which *Cx. Quinquefasciatus* was completely susceptible while *An. gambiae* was relatively more susceptible to the extract than *Ae. Aegypti*. Pathak *et al*. (2000) also reported variations in larvicidal efficacy of essential oil extracts from four plants *Tagetes erecta, Ocimum sanctum, Mentha piperita* and *Murraya koenigii*a gainst three species of mosquitoes, *An. stephensi, Ae. Aegypti* and *Cx. quinquefasciatus.*

Table 2: Laboratory evaluation of Alkaloidal fraction of *Heliotropium curassavicum* (Curassavine) against larvae and pupae of *Culex pipiens*

Concentration (µg/ml)	% Cumulative mean mortality after 5days		
	1day	2day	3day
100 µg/ml	12	16	20
200 µg/ml	16	84	100
300 µg/ml	52	100	-
400 µg/ml	84	100	-
500 µg/ml	84	100	-
LC_{50} (%)	147.9 µg/ml		

Control mortality was zero % throughout the period of experiment.

In this study the larvicidal activity of the curassavine has been tested against the larvae of filariasis vector *Culex pipiens*. The results suggest that the *Heliotropium curassavicum* have the potential to be used as an ideal ecofriendly approach for the control of mosquito.LC_{50} was 147.9 µg/ml.

CONCLUSION

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friendly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily

available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. According to Bowers *et al*. (1995) the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system.

The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and chromatography together with new pharmacological testing have led to an interest in plants as the source of new larvicidal compounds. Synergestic approaches such as application of mosquito predators with botanical blends and microbial pesticides will provide a better effect in reducing the vector population and the magnitude of epidemiology.

ACKNOWLEDGMENTS

The Authors are very grateful to Aljouf University for the facilities and financial support that made this work possible through a local research grant (No. 221/34).

Researchers are also grateful to the College of Science, Department of Biology and College of Pharmacy, Department of Pharmacognosy for providing the research facilities and space for the completion of this work.

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