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Research Article

SCREENING OF LARVICIDAL ACTIVITY OF *CROSSANDRA INFUNDIBULIFORMIS* EXTRACTS AGAINST *ANOPHELES STEPHENSI*, *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

The aim of this study is to investigate the larvicidal activity of crude extracts obtained from petroleum ether (**A**), ethyl acetate (**B**) and methanol (**C**) of *C. infundibuliformis* against *Anopheles Stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Six fourth-instar mosquito larvae were used for the study. The experimental results demonstrated that, petroleum ether extract (**A**) have significantly reduced the mortality of the larvae when compared with other two extracts. The results showed that the extracts of *C. infundibuliformis* may be considered as a potent source for new drug. The bioactivity-guided fractionation, isolation and identification will bring out potential drug to mankind, especially with anti-infective properties.

Keywords: *Crossandra infundibuliformis,* Larvicidal activity, *Anopheles Stephensi, Aedes aegypti, Culex quinquefasciatus*

INTRODUCTION

Mosquitoes are the most important single group of insects well-known for their public health importance, since they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, malaria, filariasis and encephalitis of different types including, japanese encephalitis[1]. *Anopheles stephensi*, *Aedes aegypti and Culex quinquefasciatus* are the major urban vectors of malaria, dengue and lymphatic filariasis respectively. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides[2].

Control of the mosquito larvae is frequently dependent on continued applications of organophosphates (chlorpyrifos, temephos, and fenthion) and insect growth regulators (diflubenzuron and methoprene)[3]. Effective, repeated use of controlling agents has disrupted natural biological control systems and led to outbreaks of insect species showing pesticide resistance[4]. It has also provoked undesirable effects, including toxicity to nontarget organisms and fostered environmental and human health concerns[5-7]. These side effects have initiated a search for alternative control measures. Larviciding is a successful way of reducing mosquito densities in their breeding laces before they emerge into adults. These problems have warranted the need for developing alternative strategies using eco-friendly products, more importantly the natural products.

Natural products of plant origin with insecticidal properties have been tested in the recent past for the control of a variety of insect pests and vectors. Plants are known to provide a rich source of botanical antihelmentic, antibacterial and insecticides[8-9]. Phytochemicals derived from plant sources can act as larvicidal, insect growth regulators, repellents and ovipositor attractants as observed by many researchers[10-11].

Plants constitute a rich source of bioactive compounds such as phenolics, terpenoids, coumarins and alkaloids. These natural compounds which are often active against a limited number of species including specific target insects are biodegradable leading to non toxic products and potentially suitable for use in integrated pest management programs. They could lead to the development of new classes of safer insect control agents[12-13]. Our earlier work on phytochemical and pharmacological screening of *C.infundibuliformis* reveals the pharmacological activities such as hepatoprotective[14], antibacterial, antifungal, and anticandidal activities[15]. Here we present our investigation on use the larvicidal activity of crude extracts obtained from dried leaves of *Crossandra infundibuliformis* against three species of mosquito vectors, *An. Stephens*, *Ae. aegypti* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Plant materials

The leaves of Crossandra infundibuliformis (Acanthaceae) were collected from Mettuvanam village, Vellore district, Tamil Nadu, India in August 2009 and were authenticated by Botanical Survey of India, Coimbatore, India (No: BSI/SC/5/23/09-10/Tech.-1718). A voucher specimen has been deposited in the Research laboratory of Pharmaceutical Chemistry Division, VIT University, Vellore.

Preparation of *C. infundibuliformis* crude extracts

The dried leaves (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with petroleum ether (600 ml), ethyl acetate (700 ml) and methanol (650 ml) in a Soxhlet apparatus separately until exhaustion. The extracts were concentrated under reduced pressure using rotary evaporator and the residue obtained was stored at 4 °C.

Preliminary phytochemical screening

Preliminary screening on *C.infundibuliformis* revealed the presence of alkaloids, saponins, phytosterols, phenolic compounds, flavanoids, tannins, fatty acids, carbohydrates, terpenoids, oils and fats. The study was carried out by following the standard protocol[16].

Rearing of Larvae

The larvae were collected from unused wells near VIT University, Vellore. Huge numbers of larvae were available in the unused wells, which made it possible for the entire larvicidal assay. Preliminarily, the identification of the collected larvae was done in Zonal Entomological Research Centre, Vellore. Further the larvae were separated in to different genera based on the macroscopic and microscopic morphological studies, based on the "Identification of the U.S mosquito larvae -manual".

Mosquito larvicidal bioassay

The method of Rafikali and Nair, 2001[17] was modified and employed to conduct mosquito larvicidal activity test. Six fourth-instar mosquito larvae were placed in 24.5 ml of degassed distilled water in a 30 ml cup, followed by addition of 500 µl DMSO solution. The content was gently shaken to ensure homogeneity and each cup was left at ambient temperature. The concentrations used for the tests were 100, 80, 60, 40, and 20 ppm. A control was prepared with 24.5

ml of degassed distilled water and 500 µl of DMSO solution. Each treatment was done in three replicates.

Larvicidal activity was evaluated after 24 h. Larvae were considered to be dead if appendages did not move when prodded with a wooden dowel. The percentage of mortality was corrected for control mortality using Abbott's formula and the results were plotted on log/probability paper using the reported method[18]. Toxicity and effectiveness were reported as LC_{50} and LC_{90}, which represent the concentrations in ppm with 50 % and 90 % larvae mortality in 24 h of exposure.

Statistical Analysis

All the results are expressed as mean ± SD (n = 3). The percentages of mortality were determined and transformed to descriptive statistics using Sigma plot 11.0. Mortality data were collected after 24 h exposure in different concentration of crude extracts (A, B, C) and reported in Table 2. The LC_{50} and LC_{90} values of the respective species against the extracts (A, B, C) are given in Fig. 1 & 2.

RESULTS AND DISCUSSION

The preliminary phytochemical screening has been done for petroleum ether (**A**), ethyl acetate (**B**) and methanol (**C**) extracts for the presence of phytochemical constituents. The phytochemicals present in all the extracts are given in **Table 1**.

The larvicidal activity of crude extracts (**A**, **B**, **C**) from *C. infundibuliformis* that were carried out against *An. Stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* are presented in **Table 2**. The Fig. 1 & 2 shows the LC_{50} and LC_{90} values of crude extracts against the selected larvae. The extracts showed a good toxic effect on the selected larvae after 24 hr of exposure.

Table 1: Summary of preliminary phytochemical analysis of crude extracts, A-C

Phytoconstituents	*A**	*B**	*C**
Alkaloids	-	+	+
Carbohydrates	-	-	-
Saponins	-	+	-
Phenolics & Tannins	+	+	+
Flavanoids	+	+	+
Phytosterols	+	+	-
Terpenoids	+	-	-
Fatty acids	+	+	+
Oils & Fats	-	-	-

* A = Petroleum ether extract, B – Ethylacetate extract, C = Methanol extract

* (+) = presence of compound, (-) = absence of compound

Table 2: Larvicidal activity of crude extracts

Species	Extracts	% Mortality[a] ±SD				
		20 ppm	40 ppm	60 ppm	80 ppm	100 ppm
	A	83±0.000	88±7.857	88±15.714	100±0.000	100±0.000
An. stephensi	B	77±7.857	83±13.608	94±7.857	100±0.000	100±0.000
	C	83±7.857	94±7.857	100±0.000	100±0.000	100±0.000
	A	77±7.857	83±7.857	88±7.857	94±7.857	100±0.000
Ae. aegypti	B	77±7.857	83±13.608	88±7.857	94±7.857	100±0.000
	C	77±7.857	77±7.857	83±13.609	94±7.857	100±0.000
	A	72±7.857	83±0.000	88±7.857	100±0.000	100±0.000
Cx. quinque fasciatus	B	72±15.713	83±13.608	83±0.000	88±15.713	100±0.000
	C	66±13.608	72±7.857	83±13.608	94±7.857	100±0.000

Notes: [a]mean value of three replicates; Control – nil mortality

The activity of petroleum ether extracts (**A**) against *Anopheles stephensi* showed good mortality level of 83 ± 0.000 when 20 ppm of the extract were used, it increased to 100±0.000 with 100 ppm of extract. With 40 and 60 ppm, petroleum ether extract showed the toxic level 88 ± 7.857 mortality. But, the petroleum ether extract showed 72 ± 7.857 to 77 ± 7.857 mortality against the other two species *Aedes aegypti* and *Culex quinquefasciatus* larvae. However the percentage mortality increases with increase in the concentration of three extracts and in all three larvae.

In case of ethyl acetate extract (**B**), the mortality rate was found to be 77±7.857 and it increases, as the dosage of the extract is increased with respect to the larvae *Anopheles stephensi*. Similar observation is noted with respect to *Aedes aegypti* and *Culex quinquefasciatus*. But the amount of extract dosage required for good larvicidal activity is found to be 40 ppm only.

The effect of methanol extract (**C**) on *Anopheles stephensi* is found to be very good, showing the mortality rate of 100 % with 60 ppm concentration onwards where as the other two larvae, *Aedes aegypti* and *Culex quinquefasciatus* showed 100 % mortality on 100 ppm dosage. Hence, the methanol extract (**C**) has good mortality against *Anopheles stephensi* than the other two species *Aedes aegypti* and *Culex quinquefasciatus* larvae.

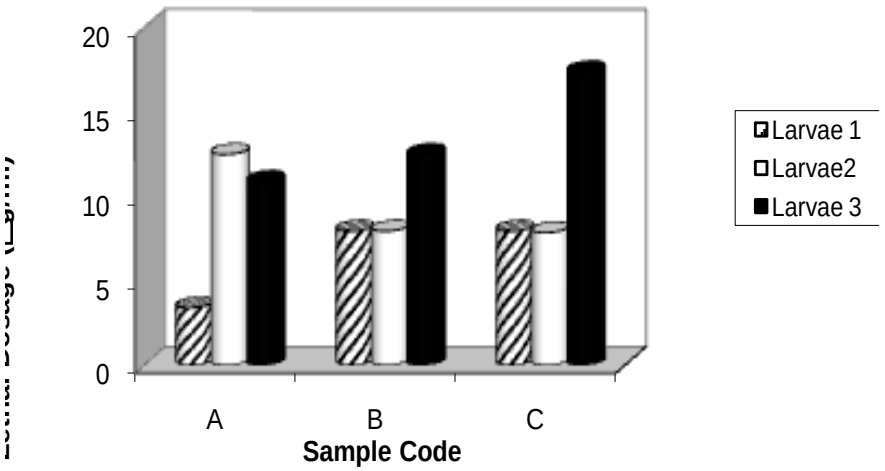

Fig. 1: LC_{50} values of *C. infundibuliformis* extracts against *An. Stephensi*, *Ae. aegypti* and *Cx. Quinquefasciatus*

The highest mortality was found to be petroleum ether (**A**) extract having LC_{50} of 3.4 µg/ml, 12.5 µg/ml and 10.9 µg/ml and the methanol extract (**C**) has a good inhibitory effect which can be found from its LC_{50} 7.95 µg/ml, 7.97 µg/ml and 12.5 µg/ml.

The LC_{90} values of petroleum ether extract (**A**) are 47.5 µg/ml, 34 µg/ml and 65 µg/ml, but contradictory to LC_{50} values, the methanol extract (**C**) has LC_{90} values of 24.8 µg/ml, 23.8 µg/ml, 84.3 µg/ml respectively Fig. 1 & 2. Ethyl acetate extract (**B**) has a moderate susceptility against all the selected larvae Fig. 1 & 2.

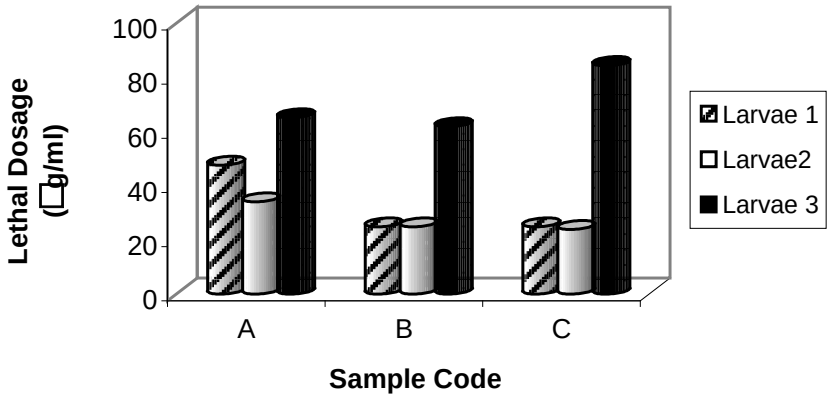

Fig. 2: Graph of LC_{90} of *Crossandra infundibuliformis* extracts against *Anopheles Stephensi*, *Aedes aegypti* and *Culex Quinquefasciatus*

CONCLUSION

The above study on determination of $LC_{50\ and}$ LC_{90} values makes us to conclude that all the three extracts showed a significant larvicidal activity. The petroleum ether extract (**A**) has a good mortality against *Anopheles stephensi*. The methanolic extract (**C**), can inhibit *Aedes aegypti* to a higher level when compared to the other extracts. The ethyl acetate extract (**B**) are effective against *Culex quinquefasciatus* larvae. The result of larvicidal activity of crude extracts from *C. infundibuliformis* infers that each extracts have effect on three different larvae. Hence it can be concluded that all the three extracts were very effective larvicidal agents, further isolation and identification of compounds from this species would bring a novel potent drug for the human kind.

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