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LARVICIDAL AND PUPICIDAL ACTIVITY OF ARGYREIA POMACEA (CONVOLVULACEAE) LEAF EXTRACT AGAINST IN AEDES AEGYPTI (DIPTERA: CULICIDAE)

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ABSTRACT **Objective:** To determine the phytochemical, larvicidal and pupicidal activity of Argyreia pomacea choisy leaf extracts against fourth instar larvae and newly emerged pupae of Ae. aegypti. **Methods:** Analysis to determine the phytochemical composition of chloroform and aqueous leaf extract was performed. At concentrations of 0.625%, 1.25%, 2.5% and 5% (g/ml), larvicidal and pupicidal activity was determined against Ae. aegypti. After 24, 48, 72, and 96 hours, the mortality of larvae and pupae was evaluated, and after 24 and 48 hours, respectively. **Results:** The preliminary phytochemical results revealed the presence of steroids in the chloroform extract. The crude chloroform leaf extracts of A. pomacea showed the highest percentage of larval mortality with LC_{50} of 1.865, 1.384, 1.157 and 1.014 ppm and LC_{90} of 5.165, 3.678, 3.033 and 1.879 ppm and Pupal mortality with LC_{50} of 11.657 and 3.068 ppm and LC_{90} of 159.673 and 22.737 ppm than aqueous extracts larval mortality with LC_{50} of 16.938, 8.302, 6.950 and 5.656 ppm and LC_{90} of 166.711 , 48.444, 35.613 and 25.844 ppm and pupal mortality with LC_{50} of 40.677 and 9.516 ppm and LC_{90} of 876.260 and 120.062 ppm against Ae. aegypti. **Conclusion:** These results indicated that the leaf extracts of A. pomacea showed the potential to be an ideal, sustainable approach to the control of Ae. aegypti.

KEYWORDS : Phytochemical, Larvicidal, pupicidal, Steroids, Argyreia pomacea, Aedes aegypti.

INTRODUCTION

Mosquitoes are the foremost single group of insects well-known for their public health criticle which transmit a number of diseases, such as dengue fever, yellow fever, malaria, filariasis and encephalitis[1,2,3]. Dengue, the habitually mosquito-borne disease in human caused by the dengue virus is transmitted by the vector Ae. aegypti, There has been a extensive increase in mosquitoes and vector-borne diseases as a result of different anthropogenic activities such deforestation, industrialized farming, and inadequate drainage facilities[4]. The current mosquito control approach is based on synthetic insecticides. Even though they are forceful, they created many problem like insecticide defiance, pollution, toxic side effect on human being [5,6]. Hence, the plants are accordingly important sources for finding new natural products to substitute chemical ones[7,8]. Plants contain naturally befalling compounds that are derived from them which act quickly (in contradiction to biological controls), decomposition quickly, and often have low mammalian toxicity[9,10]. Phytochemicals extracted from various plant species have been tested for their larvicidal and pupicidal activity against mosquito[11,12].These natural derivatives may reduce the cost of control approaches, and plant-based solutions are not pernicious to the environment[13,14].

Convolvulaceae, the morning glory family of flowering plants (Order: Solanales), which comprise some 59 genera and about 1, 600 species. The family is extensive in both tropical and warm temperate areas, and its members are broadly cultivated for their colourful funnel-shaped flowers (Encyclopaedia Britannica). Convolvulaceae species are found all over the Indian subcontinent except the higher montane regions[15].

Plant extracts from the 13 species of woody climbers with pink flowers that are more continually found in Sri Lanka, A. pomacea is, have been used to treat jaundice for a week[16]. A.pomacea root extracts used to Fever and headache, tuberculosis, acidity, piles and rheumatism treatment and one of the Green leafy vegetable in market at Sri lanka [17,18]. As raw of A. pomacea was reported that to be effective in Ulcer treatment[19]. As per our literature survey was concerned, no information was available on the larvicidal and pupicidal activity against Ae. aegypti. Therefore, the objective of the present investigation was to determine whether A. pomacea extracts had any larvicidal or pupicidal effects on Ae. aegypti larvae during their fourth instar.

MATERIALS AND METHODS

Plant Collection and Extraction

Matured fresh leaves of A. pomacea were collected from Samathuvapuram, Coimbatore District, Tamil Nadu, India and brought to the laboratory at PG and Research Department of Zoology, Government Arts College, Coimbatore district, Tamil Nadu, India. The collected plant substance was washed methodically running tap water and dehydrated on a cotton cloth at room temperature; dehydrated plant substance was pulverized using an electrical mixer. A total of 30g of dried and pulverized leaves was subjected to sequential extraction using chloroform and aqueous in a soaking (3days). The extract was filtered through filter paper and residual solvent were evaporated by rotary vacuum evaporator. The extracts were concentrated at reduced pressure 22-26 mmHg at 45˚ C, and stored an amber vial were kept in cool and dark place at 4˚ C until testing for subsequent bioassays.

Phytochemical Screening

Qualitative analyses of the phytochemicals present in crude chloroform leaf extract of A. pomacea were carried out using standard methods, since it showed higher larvicidal and pupicidal activity. Phytochemical constituents like steroids were analyzed.

Test For Alkaloids

Dragendarff's Reagent Test: A few ml of extract was treated with 1-2ml of Drag reagent and observed of orange or yellow precipitate.

Mayer's Test: A fraction of extract was treated with Mayer's reagent by sides of test tube. Formation of white or creamy precipitate indicted the presence of alkaloids.

Flavonoids: 5% NaOH test: To 1 ml of extract was treated with few drops of 5% NaOH. The appearance of yellow colour which decolorizes while adding conc. HCL shows the presence of flavonoids.

Alkaline Reagent Test: few drops of extract treated with add few drops of NaOH and observed for formation of yellow colour that confirms the presence of flavonoids.

Tannins: Fed_3 test: 1 ml of extract was treated with few drops of neutral 5% Fed_3. The dark green colour developed indicates the presence of Tannins.

Steroids: To test the presence of steroids phytochemical, 1ml of extract with add 1 ml acetic Anhydride and 1ml of conc. H□SO□ added by side of test tube. The upper layer turns green. This indicated the presence of steroids.

Triterpenoids

Libermann's –Burchard Test: To 1 ml of extract treated with 1 ml of acetic anhydride and 1 ml of conc. H_2SO_4 added by side of test tube. Brown ring in junction of two layer and deep red colour in lower layer that confirm the presence of triterpenoids.

Salkowki's Test: 1 ml of chloroform was added with 1ml of extract followed by a few drops of concentrated hydrochloric acid. Shaken and allowed to stand after few second. The golden yellow developed

indicates the presence of triterpenoids.

Saponins
To 1 ml of extract was dissolved with some amount of distilled water and shaked for 15-30 Sec and left for 10 Sec. And observed for the formation of 1 or 2 cm foam that confirms the presence of saponins.

Glycosides
To 2 ml of extract treated with 1ml of glacial acetic acid and 3 drops of ironic chloride and few drops of conc. H□SO□. A greenish blue colour show the presence of Cardiac glycosides.

Detection Of Fixed Oil And Fat
2 piece of filter paper dip in small quantity of extract and oil stain on the filter paper that indicates the presence of fixed oil.

Mosquito Culture
The National Centre for Disease Control (NCDC), Mettupalayam, Tamil Nadu, India, provided larvae of Aedes aegypti for this bioassay. The larvae were kept in plastic cup containing tap water was used for rearing under laboratory conditions at 26±3°C and relative humidity of 80%–85%. The photophase was maintained at 9:15light and dark. The larvae of Ae.aegypti mosquito were maintained in plastic cups under the identical laboratory conditions and fed with dry yeast powder (0.2mg).The trays with pupae and larvae of Ae. aegypti were maintained in mosquito cages at 26±3°C and relative humidity of 85±5% for adult emergence. Cotton soaked with aqueous sucrose (10%) solution in a petri dish to feed adult mosquitoes was placed individually in each mosquito cage. We hand was placed for 20 min inside the cage in order to provide a blood meal especially for female mosquitoes.

Larvicidal Bioassay
The larvicidal bioassay was conducted according to the standard WHO larval susceptibity test methods with slight modifications was adopted for the study [20]. In the larvicidal bioassay, Ae. aegypti mosquito larvae were exposed to 5%, 2.5%, 1.25% and 0.625% concentrations and were used to determine the lethal concentration of 50% (LC_{50}) and the lethal concentration of 90% (LC_{90}) values. Tween 80 (emulsifier) in water served as a control.The forth instar larvae of Ae. aegypti were introduced in 250ml plastic cups containing 200 ml of aqueous medium (199ml of dechlorinated tap water + 1ml of emulsifier) and the required amount of plant extract were added five replicates were kept for each test concentration as stated earlier. In each replicate 10 larvae were used, with five replicates of Control. The experiment was performed under laboratory conditions at 27±2˚C. Any larva was considered to be dead if its appendages did not move when prodded repeatedly with a soft brush. Mortality of larva was recorded after 24, 48, 72, and 96 h. Mortality was then calculated.

Corrected Mortality

$$= \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{X } 100$$

$$\text{Percentage mortality} = \frac{\text{Numbere of dead larvae}}{\text{No of larvea introduced}} \text{X}100$$

Pupicidal Bioassay
The pupicidal activity of the plant crude extract was evaluated as per the protocol previously described by WHO . Batches of ten pupae were introduced into 200 ml of the test medium (dechlorinated tap water) in a 250 ml plastic glass, containing a particular concentration of the selected solvent extracts of the plants with the same concentrations as mentioned in the previous experiments. In control, the same number of pupae was maintained at 200 ml of dechlorinated tap water containing appropriate volume of 1 ml tween 80. All containers were maintained at room temperature (27±2) C. Similarly test of mortality rate as stated in the previous experiments were monitored and the mortality of pupa was recorded after 24 and 48 h of exposure to the extract. Mortality was then calculated.

Corrected Mortality

$$= \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{X } 100$$

$$\text{Percentage mortality} = \frac{\text{Numbere of dead pupae}}{\text{No of pupae introduced}} \text{X}100$$

Statistical Analysis
The data analysis of profit was carried out with Microsoft Excel 2007. Lethal concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of all test organisms within the specified period of exposure. It was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract LC_{50} and LC_{90} were determined together with their fiducial limits at a 95% confidence level by probit analysis using SPSS 16.0 (Statistical Package of Social Sciences) software based on the test organism mortality reported in these bioassays. Results that had a 0.05 p value are regarded as statistically significant.

RESULT

Phytochemical Analysis
The phytochemical screening of A.pomacea of Chloroform extracts was assessed and the results pertaining to the experiments are shown in table 1.The plant A.pomacea showed the presence of steroids.

Table1: Preliminary Phytochemical Analysis Of Chloroform Extracts Of A. Pomacea

Constituents	Chloroform leaf extract
Alkoloids	-
Flavonoids	-
Tannins	-
Steroids	+
Triterpenoids	-
Saponins	-
Glycerides	-
Oil and fat	-

+ Presence of compound; - Absence of compound

The larvicidal and pupicidal activity of leaf extracts of A. pomacea against Ae. aegypti reported in the present study exhibit the mosquitocidal properties in the plant leaf extracts suggesting their use in mosquito population control (Table 2, 3, 4 & 5). The chloroform and aqueous leaf extracts of A. pomacea showed highest percentage of larval and pupal mortality against Ae. aegypti. The data pertaining to the chloroform extract of A. pomacea against the fourth instar larvae of Ae. aegypti with percentage of larvae mortality was assessed after 24, 48, 72 and 96 h ; pupal mortality was assessed after 24 and 48 h . The larval and pupal mortality of the Ae. aegypti was more prominent as evidenced from the Table 2 and 3, Which showed 100% mortality in 96 h at 5% concentration with the LC_{50} of 1.865, 1.384, 1.157 and 1.014 ppm and LC_{90} of 5.165, 3.678, 3.033 and 1.879 ppm and Pupal mortality with LC_{50} of 11.657 and 3.068 ppm and LC_{90} of 159.673 and 22.737 ppm respectively.

Table 2: The LC_{50} and LC_{90} values of chloroform leaf extract A. pomacea against in the 4th larvae of Ae. aegypti after exposure of 24, 48, 72 and 96 hrs.

Hours	Concentration (%)	Ae. aegypti Larval mortality (%)	LC50 (LCL-UCL)	LC90 (LCL-UCL)	X2 (Df=3)
24 Hrs	CONTROL	0	1.865	5.165	0.162
	0.625	10			
	1.25	24			
	2.5	72			
	5	88			
48 Hrs	CONTROL	0	1.384	3.768	0.097
	0.625	26			
	1.25	32			
	2.5	80			
	5	96			
72 Hrs	CONTROL	0	1.157	3.033	0.069
	0.625	26			
	1.25	44			
	2.5	84			
	5	98			
96 Hrs	CONTROL	0	1.014	1.879	0.031
	0.625	30			
	1.25	52			
	2.5	88			
	5	100			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

Table 3: The LC_{50} and LC_{90} values of chloroform leaf extract of A. pomacea against newly emerged pupae of Ae. aegyti after exposure of 24 and 46 hrs.

Hours	Concentration (%)	Ae. Aegypti larval mortality (%)	LC50 (LCL-UCL)	LC90 (LCL-UCL)	X2 (Df=3)
24 Hrs	CONTROL	0	11.657	159.673	0.358
	0.625	8			
	1.25	14			
	2.5	20			
	5	36			
48 Hrs	CONTROL	0	3.068	32.737	0.203
	0.625	16			
	1.25	28			
	2.5	42			
	5	64			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

Similar trend of larval and pupal mortality observed in aqueous extract of A. pomacea against with Ae. aegypti with LC_{50} of 16.938, 8.302, 6.950 and 5.656 ppm and LC_{90} of 166.711 , 48.444, 35.613 and 25.844 ppm and pupal mortality with LC_{50} of 40.677 and 9.516 ppm and LC_{90} of 876.260 and 120.062 ppm respectively are shown in Table 3 and 4.In the present study, the chloroform extract of A. pomacea against Ae. aegypti showed the highest percentage of larval and pupal mortality rather than aqueous extract. The chloroform leaf extract of A. pomacea showed the presence of steroid.The screening of local medicinal plants for mosquito larvicidal and pupicidal activity may finally lead to their use in natural product- based mosquito decrement practices.

Table 4: The LC_{50} and LC_{90} values of aqueous leaf extract of A. pomacea against 4th instar larvae of Ae.aegypti after 24, 48, 72 and 96 h.

HOURS	Concentration (%)	Ae. aegypti Larval mortality (%)	LC50 (LCL-UCL)	LC90 (LCL-UCL)	X2 (Df=3)
24 Hrs	CONTROL	0	16.398	166.711	0.496
	0.625	4			
	1.25	6			
	2.5	12			
	5	28			
48 Hrs	CONTROL	0	8.302	48.444	0.463
	0.625	4			
	1.25	8			
	2.5	12			
	5	44			
72 Hrs	CONTROL	0	6.950	35.613	0.450
	0.625	4			
	1.25	8			
	2.5	14			
	5	48			
96 Hrs	CONTROL	0	5.656	25.844	0.419
	0.625	4			
	1.25	10			
	2.5	16			
	5	54			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

Table 5: The LC_{50} and LC_{90} values of aqueous leaf extract of A. pomacea against newly emerged pupae of Ae. aegypti after 24 and 48 h

HOURS	Concentration (%)	Ae. aegypti Larval mortality (%)	LC50 (LCL-UCL)	LC90 (LCL-UCL)	X2 (Df=3)
24 Hrs	Control	0	40.677	876.260	0.488
	0.625	4			
	1.25	7			
	2.5	14			
	5	18			
48 Hrs	Control	0	9.516	120.062	0.335
	0.625	8			
	1.25	16			
	2.5	26			
	5	36			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

DISCUSSION

The results obtained in the present study are in accordance with earlier works. Pratheeba et al[21] reported that, chloroform extracts of Ocimum gratissimum leaves exhibited larvicidal activity with LC_{50} and LC_{90} value of 2.8916 and 5.4521 ppm after 24 h exposure than better pupal activity of mosquito was noticed in the same extract exposure at 24 h with LC_{50} and LC_{90} values of 2.6916 and 4.6521 ppm, respectively . Premalatha et al[22] have reported that methanol extracts of S. trilobatum was found to be more susceptible against the larvae of Ae. aegypti, Cu. quinquefaciatus and An. Stephensiat 250 ppm with LC_{50} value were of 125.43, 127.77 and 116.64 ppm after 24 and 48 h of exposure .The LC_{50} and LC_{90} adulticide values of G. pentaphylla leaf extract in acetone, methanol, chloroform and ethyl acetate were as follows for Cu. quinquefaciatus, An. Stephensi and Ae. aegypti: 2.957, 5.458, 2. 708 and 4.777, 3.449, 6.676 ppm respectively. Acetone extracts had the highest larvicidal effects against three species of major mosquitoes vectors with LC_{50} and LC_{90} values of 0.0004, 138.54; 0.2669, 73.7413 and 0.0585, 303.746 ppm, respectively that was reported by Govindaraju et al[23].The Larval mortality rate in chloroform: methanol (1:1 v/v) extract was significantly higher ($P<0.05$) than other extracts. LC_{90} value of petroleum ether, chloroform:methal (1:1 v/v) and ethyl acetate extracts were 195.33 ppm, 27.28 ppm and 74.19 ppm respectively, 48h of exposure [23]._Dr. A. Jeyasankar et al[25] have reported that ethyl acytate extracts of Phyllanthus emblica showed highest larval mortality against Ae. agyptiand C. quinquefaciatus with LC_{50} value were of 80.04 and 78.89 ppm and LC_{90} value 323.53 and 502.10 ppm after 24 h exposure.

Many such plant extracts, essential oils and their metabolites, which have been tested for their mosquitocidal activity against different life cycle forms Ae. aegypti, as well as for their efficacy in controlling mosquitoes was reported by Silverio et al[26] Ramar and Jeyasankar[27] * have also reported that chloroform extracts of M. Paniculata sleaves against the larvae and pupae of the mosquito, An. Stephensi with high mortality and LC_{50} value of 44.72 and 48.01 ppm repectively . Phytochemical derived from plant sources can act as larvicidal, pupicidal, insect growth regulator, repellent and ovipositor attractant and have different activities observed by many researchers[28].

Reducing mosquito borne diseases remains a big challenge even at the most advancement of modern science. But synthetics insecticides have consequence of several drawbacks that are mostly non biodegradable with having harmful residual hazards as well as expensive [29]. The findings of the present investigation revealed that the leaf chloroform and aqueous extracts of A. pomacea possess larvicidal and pupicidal activities against Ae. aegypti. It may concluded that plant origin chemicals from the A. pomacea leaf extracts showed insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal and pupicidal toxicity. Further studies on these screening, isolation and purification of bioactive phytochemical constituents followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use A. pomacea leaf extracts to control the larval and pupal stages of Ae. aegypti.

CONCLUSION

Aedes aegypti is significantly affected by the larvicidal and pupicidal properties of A. pomacea . It is an appealing option that can be evaluated in the search for a more effective and accessible larvicide and pupicide alternative, its interesting that this type of plant naturally occurs in tropical and warm- temperate climates, where mosquito-borne illnesses are prevalent. In order to generate a more advanced larvicide and pupicide, we suggest doing further molecular level investigation.

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