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Evaluation of larvicidal activity of the aerial extracts of a medicinal plant, *Ammannia baccifera* (Linn) against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*

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ABSTRACT

Objective: To analyze the larvicidal effect of the aerial extracts of *Ammannia baccifera* on two important mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*. **Methods:** The larval mortality of fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* after 24h and 48h of treatment were observed separately in control 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg/L concentrations of the aerial extracts (methanol, ethyl acetate, chloroform) of *A. baccifera*. **Results:** Based on the probit analysis, the 24h and 48h aerial methanol extract of *A. baccifera* LC_{50} value of *C. quinquefasciatus* was found to be in 164.00 mg/L and 107.00 mg/L and LC_{90} values for *C. quinquefasciatus* was found be in 310.00 and 261.00 mg/L. The 24h and 48h aerial part of methanol extract of *A. baccifera* LC_{50} value of *A. aegypti* was found be in 226.00 mg/L and 186.00 mg/L and LC_{90} values was found be in 476.00 and 309.00 mg/L. **Conclusions:** The results indicate that the *A. baccifera* could be effectively used for the control of mosquito larvae and the possibility of exploiting for the development of commercial larvicides a plant widely occurring in India.

1. Introduction

Blood-feeding female mosquitoes are responsible for the intolerable biting nuisance and transmission of a large number of diseases such as malaria, yellow fever, dengue, filariasis, chikengunya, and encephalitis. They cause serious health problems to humans and present obstacles to the socioeconomic development of developing countries, particularly in the tropical region[1]. In addition to mortality, vectorborne diseases cause morbidity of millions of persons resulting in loss of man-days and causing economic loss[2].

There are 3300 species of mosquitoes belonging to 41 genera; all contained in the family Culicidae. *Aedes aegypti*, a vector of dengue is now endemic and found to be widely distributed in the tropic and subtropics. Synthetic chemical larvicides continue to be applied for controlling mosquitoes in most parts of the world. But many of these chemicals are toxic to human, plant and animal life and insecticides resistance can be problematic in maintaining control, especially with organophosphate and pyrethroid larvicides[3]. Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the larvae.

Mosquitoes are ecologically important components of the aquatic and terrestrial food chain, then they are the most important group of insects in terms of public health importance, and thus, appropriate control programs are justified. Until a few years ago, only the adults were sprayed, but now, it is well known that a more efficient way to reduce mosquito populations is to target the larvae [4,5].Much effort has been focused on phytochemicals as potential sources of commercial control agents or as lead compounds, because many are selective and have few or no harmful effects on non-target organisms or the environment[6].

Plant extracts or oils in general have been recognized as an important natural resource for the control of parasites [3,7-10]. Mosquitoes belonging to the genera Anopheles, Culex and Aedes are the vectors for the pathogens of different diseases such as malaria, filariasis, Japanese encephalitis, dengue and

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dengue haemorrhagic fever, yellow fever and chickungunya [3,11].Many researchers have reported on the effectiveness of plant extract against mosquito larvae[12-14]. Even though they are effective they have created many problems like insecticide resistance, pollution and toxic side effects on human beings. This has necessitated the need for a research and development of environmentally safe, biodegradable indigenous method of vector control.

In this study, mosquito larvicidal activity was investigated using *Ammannia baccifera* (Blistering Ammannia) Blistering Ammannia is an erect, branched, smooth, slender, annual herb, found in open, damp, and waste places. It is more or less purplish herb 10-50 cm in height, with somewhat 4-angled stems. The plant has analgesic activity[15], anti-inflammatory and antiarthritic activity[16]. The present investigation was done to assess the larvicidal effect of *Ammannia baccifera* against early 3rd and 4th instar larvae of *A. aegypti* and *C. quinquefasciatus*. A botanical insecticide should be ecofriendly, biodegradable, and target-specific against pest. Furthermore, requisites for its market success should be low cost, easily available, easy utilization, and simple storage[17].

2. Material and methods

Matured plant of *Ammannia baccifera* were collected from Mahadevamalai, Gudiyattam, Vellore district, Tamilnadu, India. The *Ammannia baccifera* was identified by Dr. K. Murugesan CAS in botany, University of Madras, Guindy Campus, Chennai 600 025. The plants were washed and shade dried. Each sample was powdered with blender. The respective plant powder 70g was filled in the thimble and extracted successively with chloroform, ethyl acetate and methanol using soxhlet extractor for 10 h. All the extracts were concentrated using rotary flash evaporator and preserved at 5 °C in an airtight bottle until further use.

2.1. Selection of mosquito species

Two important vector species of mosquitoes such as *A. aegypti* (L) and *C. quinquefasciatus* (Say) were selected for the study. *A. aegypti* is the principal vector of dengue fever and dengue hemorrhagic fever and it is reported to infect more than hundred million people every year in more than 110 countries in the tropics[18].

2.2. Preparation of stock solution

One gram of the concentrated extract of dried aerial part of *A. baccifera* was dissolved in 1 mL of acetone and 99 mL of distilled H_2O (100mL) which was kept as stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

2.3. Procurement of eggs and rearing of mosquito larvae

The egg rafts of *A. aegypti* and *C. quinquefasciatus* were procured from Department of Entomology, Loyola College in Chennai, India during (2011). The eggs of *A. aegypti* were collected and brought to the laboratory (Presidency College, Chennai) and kept in tray containing tap water (as culture medium) at laboratory condition (26±2)℃. On the next day, eggs were observed to hatch out into first instar larvae. Appropriate amount of nutrients (yeast powder and glucose) were added to the culture medium. On the third day after hatching, the first instar larvae moulted into second instar larvae. On the fifth day, third instar larvae were observed which moulted into fourth instar larvae on the seventh day. The durations of first to fourth instar larval periods of *C. quinquefasciatus* were observed to be similar to that of *A. aegypti*. The fourth instar larvae which molted on the seventh day were allowed to grow in the medium up to eighth day. The fourth instar larvae of both *A. aegypti* and *C. quinquefasciatus* were used for treatment experiments in the present study.

2.4. Treatment of larvae with A. baccifera

In the present study, for treatment of larvae with the aerial extract of *A. baccifera*, 100 mL of tap water was kept in a series of glass beakers (of 200 mL capacity). Required quantity of stock solution (containing 10 mg/mL) was added into each beaker (containing 100mL of tap water) to obtain a particular concentration of the extract. Control medium was also maintained with 100 mL of tap water added with the maximum quantity of acetone present in the stock solution of the extract. Separate series of exposure medium with desired concentrations of extract were kept for *A. aegypti* and *C. quinquefasciatus*. The larval mortality of fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* were observed separately in control, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg/L concentrations of the aerial extract of *A. baccifera* (methanol, ethyl acetate and chloroform). Twenty numbers of 4th instar larvae of both *A. aegypti* and *C. quinquefasciatus* were separately introduced into control and different concentrations of aerial extract. The number of larvae surviving at the end of 24h and 48h was recorded and the per cent mortality values were calculated.Based on the per cent mortality values, LC_{50} and LC_{90} value of aerial extract of *A. baccifera* for *A. aegypti* and *C. quinquefasciatus* were obtained separately by calculating the regression line employing Probit analysis[19].

2.5. Phytochemical screening

Phytochemical analysis of the aerial part of *Ammannia baccifera* methanol solvent extract was conducted following the procedure of Cisneros *et al*[20] by this analysis, the presence of several phytochemicals listed in Table 1 was tested.

Table 1
Phytochemical screening of methanol plant extract of *A. baccifera*.

Phytochemical test	Methanol
Carbohydrate test	+
Tannins test	+
Saponin test	+
Flavonoid test	++++
Alkaloids test	+++
Triterpenoids test	+
Steroids test	++
Anthraquinones test	+++
Glycocides test	+++
Fatty acid test	+
Protein test	+

3. Results

The preliminary screening is a good means of evaluating

the potential larvacidal activity of plant. The results of the phytochemical charactersation of aerial part of methanol extract *Ammannia baccifera* are presented in Table.1. This gives a clue for the class of compounds present in the plant under investigation, namely compounds like flavonoids, cardiac glycosides, terpenoids, steroids, anthraquinones and alkaloids. The per cent mortality values of 4th instar larvae treated with different concentration (ranging from 50 to 500 mg/L) of the aerial extract of *A .baccifera* at the end of 24h and 48h are represented in Table 2 for *C. quinquefasciatus* and those for *A. aegypti* in Table 3. The regression equation (based on probit analysis) between the concentration of aerial parts extract (methanol, ethyl acetate, chloroform) and 24 h and 48 h per cent mortality of 4th instar larvae of *C. quinquefasciatus* and *A. aegypti* are represented in Table 4 and Table 5 respectively. Based on the Probit analysis, the 24h and 48h aerial methanol extract of *Ammannia baccifera* LC_{50} value of *C. quinquefasciatus* was found in 164.00 mg/L and 107.00 mg/L and LC_{90} values *C. quinquefasciatus* was 310.00 and 261.00 mg/L. The 24h and 48h aerial methanol extract of *Ammannia baccifera* LC50 value of *A. aegypti* was found in 226.11 mg/L and 186.00 mg/L and LC_{90} values was 476.00 and 309.00 mg/L. Comparing with ethyl acetate and chloroform extracts methanol extract showed better larvicidal activity against *C. quinquefasciatus* and *A. aegypti*.

4. Discussion

Mosquito borne diseases are one of the most public health problems in the developing countries. It can be controlled by using repellent, causing larval mortality and killing mosquitos. Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides[21]. However

Table 2

Larval mortality percentage of 4th instar larvae of *Culex quinquefasciatus* exposed for 24 and 48h to different concentrations of aerial methanol extract of *A. baccifera*.

Larval mortality percentage	Experimental concentration (mg/L)										
	control	50	100	150	200	250	300	350	400	450	500
24 h	0	25	40	50	60	70	80	80	90	100	100
48 h	0	50	60	70	80	90	100	100	100	100	100

Table 3

Larval mortality percentage of 4th instar larvae of *Aedes aegypti* exposed for 24 and 48h to different concentrations of aerial methanol extract of *A. baccifera*.

Larval mortality percentage	Experimental concentration (mg/L)										
	control	50	100	150	200	250	300	350	400	450	500
24 h	0	10	20	40	50	60	70	80	80	90	100
48 h	0	20	30	40	60	70	80	100	100	100	100

Table 4

Lethal concentration of plant extract of *Ammania baccifera* against *Culex quinquefasciatus*.

Solvents used	Hours	LC_{50} (mg/L)	LC_{90} (mg/L)	Regression equation		95% Confidence limits			
						UCL (mg/L)		LCL (mg/L)	
				LC_{50} (mg/L)	LC_{90} (mg/L)	LC_{50} (mg/L)	LC_{90} (mg/L	LC_{50} (mg/L)	LC_{90} (mg/L)
Chloroform	24	259. 64	434. 21	Y=-2.613+0.010X	Y=-2.252+0.008X	264.823	464.018	253.872	413.114
	48	234. 15	328. 67	Y=-3.909+0.016X	Y=-3.935+0.015X	241.984	337.267	223.822	321. 859
Ethyl acetate	24	210. 88	391. 92	Y=-1.744+0.008X	Y=-1.439+0.006X	218.845	441.747	200.586	358.767
	48	198. 07	234. 43	Y=-3.192+0.016X	Y=-2.279+0.015X	203.359	243.102	191.793	227. 508
Methanol	24	164. 25	310. 44	Y=-1.448+0.008X	Y=-2.076+0.010X	172.523	329.147	157.121	297. 004
	48	107. 00	260. 70	Y=-1.346+0.012X	Y=-2.596+0.014X	166.807	274.440	93. 221	251. 096

UCL – Upper confidence limit. LCL –Lower confidence limit.

Table 5

Lethal concentration of plant extract of *Ammania baccifera* against Ades agypti.

Solvents used	Hours	LC_{50} (mg/L)	LC_{90} (mg/L)	Regression equation		95% Confidence limits			
						UCL (mg/L)		LCL (mg/L)	
				LC_{50} (mg/L)	LC_{90} (mg/L)	LC_{50} (mg/L)	LC_{90} (mg/L	LC_{50} (mg/L)	LC_{90} (mg/L)
Chloroform	24	318. 30	467. 19	Y=-3.010+0.009X	Y=-3.442+0.010X	324.626	493.247	313.747	448. 656
	48	284. 59	373. 59	Y=-5.199+0.018X	Y=-5.800+0.081X	290.583	380.874	277.238	367. 657
Ethyl acetate	24	277. 36	449. 57	Y=-3.203+0.011X	Y=-2.286+0.007X	281.827	483.271	272.973	425. 932
	48	235. 19	328. 14	Y=-4.117+0.017X	Y=-4.560+0.017X	240.678	333.766	228.592	323. 353
Methanol	24	226. 11	476.28	Y=-2.105+0.009X	Y=-1.065+0.004X	231.594	551.184	220.697	434. 888
	48	185. 65	308. 91	Y=-3.034+0.016X	Y=-3.420+0.015X	191.897	321.041	177.948	300. 056

UCL – Upper confidence limit. LCL – Lower confidence limit.

due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides better alternative means are sought[22]. An insecticide does not have to cause high mortality on target organisms in order to be acceptable[23]. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. According to the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health[24]. The botanical insecticides are generally pest-specific and are relatively harmLess to non-target organisms including man, easily biodegradable and also harmless to the environment[25]. Though several plant species from different families have been reported for mosquitocidal activity, only a few botanicals have moved from laboratory to field use which might be due to the presence of phytochemicals when compared to synthetic insecticides. To the best of our knowledge this is the first report of effective larvicidal activity of different solvent extracts of *A. baccifera* against *C. quinquefasciatus* and *A. aegypti*. Methanol extract of *A. baccifera* showed potential larvicidal activity. In short, our findings suggested that aerial part of *A. baccifera* and its effective constituents may be explored as a potent natural larvicide. Further investigations for the mode of the constituents' actions, effects on non-target organisms and field evaluation are necessary. The extracts of the aerial part of *A. baccifera* has been found to possessed larvicidal activity against the mosquito *A. aegypti* and *C. quinquefasciatus*. The biological activity of the plant extract might be due to the presence of various compounds, including phenolics, terpenoids, and alkaloids in plants, these compounds may jointly or independently contribute to produce larvicidal and adult emergence inhibition activity against *A. aegypti* and *C. quinquefasciatus*. The findings of the present investigation revealed that the aerial extract of *A. baccifera* possess remarkable larvicidal inhibition activity against mosquito *A. aegypti* and *Culex quinquefasciatus*. Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal and adult emergence inhibition activity should be identified and utilized, if possible, in preparing a commercial product to be used as a mosquitocidal.

Conflict of interest statement

We declare that we have no conflict of interest.

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