



Original Research Article

doi: <http://dx.doi.org/10.20546/ijcrbp.2016.310.018>

Phytochemical Screening, Larvicidal and Pupicidal Activity of *Murraya paniculata* (L.) Jack (Rutaceae) Leaf Extracts against Three Important Vector Mosquitoes (Diptera: Culicidae)

G. Ramar¹ and A. Jeyasankar^{2*}

¹PG & Research Department of Zoology, Arignar Anna Government Arts College, Musiri-621 211, Tamil Nadu, India

²Entomology Unit, PG & Research Department of Zoology, Government Arts College (Autonomous), Coimbatore-641 018, Tamil Nadu, India

*Corresponding author.

Abstract

Phytochemical represents a rich resource for the discovery of novel pesticides that are effective, cheap and environmentally safe. The main target mosquito vectors, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* cause serious human diseases. The petroleum ether, chloroform and ethyl acetate extracts of *Murraya paniculata* were studied for larvicidal and pupicidal activity against the mosquitoes, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The results of the present study showed that the chloroform extracts of *M. paniculata* leaves against the larvae and pupae of the mosquito, *An. stephensi* with high mortality and LC₅₀ values of 44.72 and 48.01ppm respectively. The results suggest that the chloroform leaf extract of *M. paniculata* have the potential to be used as good larvicidal and pupicidal agent.

Article Info

Accepted: 28 September 2016

Available Online: 06 October 2016

Keywords

Larvicidal activity
Leaf extract
Murraya paniculata
Phytochemical analysis
Pupicidal activity
Vector mosquitoes

Introduction

Three mosquito species of the genera *Anopheles*, *Culex* and *Aedes* are vectors responsible for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue fever and yellow fever. Most parasitic disease is the tropical, indentifying globalization and climatic changes are increasing the danger of contracting arthropod borne diseases (Ansari, 2000; Tawatsin et al., 2001). Most popular centuries, the vector-borne diseases are imposing a serious public health problem in human's deaths worldwide. Besides their negative public health impact, these diseases are also posing a serious obstacle to socio-economic development in countries wherever they are endemic in nature (Karunamoorthi and Sabesan, 2010). *Ae. aegypti* is vector of Dengue and Chikungunya.

Approximately 3.5 billion people live in dengue endemic countries which are located in the tropical and subtropical regions of the world (WHO, 2011a). Lymphatic filariasis, commonly known as elephantiasis is so far a neglected tropical disease. The infection occurs when filarial parasites are transmitted to humankind through *Cx. quinquefasciatus* (WHO, 2011b). Anopheline adults reset with their abdomens positioned at a discrete angle to the surface, whereas other species keep their bodies parallel to the surface, which makes them easy to identify when sitting on the skin (Rutledge et al., 2005). The larvae lie horizontally at the surface of the water where they filter feed on organic material. They do not possess the breathing siphon present on other mosquito genera. They obtain oxygen through palmate hairs along the abdomen. The food sources include a variety of plant and animal matter

suspended at the surface of the water and small enough to eat (O'Malley, 1992). According to WHO data, *Plasmodium falciparum* malaria is exclusively transmitted by *Anopheles arabiensis* Patton and *Anopheles gambiae* Giles in Djibouti (WHO, 2013). Although the Horn of Africa is known to be highly susceptible to mosquito-borne infectious diseases, Djibouti was formerly thought to be a meso- to hypo-endemic country with unstable malaria transmission (Carteron et al., 1978).

Medicinal plant is *Murraya paniculata* (L.) Jack, otherwise known as *Murraya exotica* L., belonging to the Rutaceae family. The folk medicinal practitioners of Jessore district in Bangladesh advise boiling the leaves of the plant in water and then gargling with the water (to which a little table salt has been added) three to four times daily for three days (Islam et al., 2011). Stems of the plant are also used for toothache and oral care in India (Kumar, 2014), where traditional practitioners advise brushing teeth with stems to get relief from toothache and for maintaining healthy gums and teeth. Therefore the present study was carried out to determine the larvicidal and pupicidal activity of *M. paniculata* leaf extracts against important vectors *An. stephensi*.

Materials and methods

Plant material

The leaves of *M. paniculata* were collected from Arignar Anna Government Arts College, Musiri, Trichy District, Tamil Nadu, India during the July 2014. The collected plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India and The Voucher specimen (IPH 39) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

Extraction method

The dried leaves (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with petroleum ether, chloroform, and ethyl acetate (500ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22-26mm hg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C in an amber vial. Then the vials were named and covered with silver foil. Until use those vials were kept in cool and dark place at 4°C.

Phytochemical screening

The phytochemical screening of chloroform extract of *M. paniculata* leaves was carried out with the following standard procedures, since it showed higher larvicidal and pupicidal activity.

Test for alkaloids (Wagner's reagent): A fraction of extract was treated with 3-5 drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

Anthraquinones: About 0.5 g of each extract was boiled with 10 % HCl for few minutes in water bath, filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrates. Few drops of 10% ammonia was added to the mixtures and heated. Formation of rose-pink color indicated the presence of anthraquinones.

Catechin: Few mg of the substrate in alcohol is treated with a few drops of Ehrlich reagent and a few drops of concentrated HCl. The pink colour developed indicates the presence of catechin.

Coumarins: 10mg of the extract is dissolved in methanol and alcoholic KOH was added. The appearance of yellow colour which decolorizes while adding conc. HCL shows the presence of coumarin.

Flavonoids: 10mg of the extract was dissolved in methanol. Magnesium turnings were added into this followed by conc. HCL. A magenta colour shows the presence of flavonoids.

Test for phenols (ferric chloride test): A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

Test for quinines: A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

Test for saponins (foam test): To 2mls of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Steroids: To test the presence of steroid phytochemicals, 1 ml of extract dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid (H₂SO₄) added by sides of test tube. The upper layer turns red and sulphuric

acid layer shown yellow with green fluorescence. This indicated the presence of steroids.

Test for tannins (Braymer's test): 2ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for terpenoids (Salkowki's test): 1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Vector rearing

The larvae of the mosquitoes, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were collected from National centre for disease control, Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Larvicidal bioassay

The larvicidal activity of selected plants extracts were evaluated as per the protocol previously described WHO (2005), Based on the wide range and narrow range tests, all extracts tested ranging 30-200ppm were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of selected mosquito species. The plants oils were dissolved in 2 drop twin-20 and then diluted in 100ml of dechlorinated tap water to obtain each of the desired concentrations. The control was prepared using 2 drops of tween-20 in 100ml of dechlorinated water. The larvae of test species (10) were introduced in 250-ml plastic cups containing 100ml of aqueous medium (100ml of dechlorinated + 2 drops tween-20) and the required amount of chemical compositions was added. The larval mortality was observed and recorded after 24 hrs of post treatment. For each experiment, five replicates were maintained at a time. The LC₅₀ value was calculated by using Probit analysis (Finney, 1971). The average mortality data were subjected to Probit analysis for calculating LC₅₀, LC₉₀ and other statistics chi-square

values were calculated by using software, Statistical Package of Social Sciences (SPSS) version 16.0 for Windows and the significance level was set at $p \leq 0.05$.

Pupicidal bioassay

The pupicidal activity of plant crude extract will be assessed by using the standard method as prescribed by WHO (2005). Similar test concentrations as stated in the previous experiments were prepared and tested against the pupae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Tween-20 (emulsifier) in water was treated as control. The pupae of these mosquito species (10 pupae) were introduced in 250-ml plastic cups containing 100 ml of aqueous medium (100 ml of dechlorinated water + 2 drops of tween-20) and the required amount of plant extract was added. The pupal mortality will be observed and recorded after 24 hrs of post treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (Abbott, 1925).

Results and discussion

The present study was carried out on the plant samples revealed the presence of medicinally important bioactive compounds. Since the activity studied in the present study showed good results in the chloroform leaf extracts of *M. paniculata*, the phytochemical screening of chloroform extracts was carried out. The plant *M. paniculata* showed the presence of alkaloids, coumarin, quinines, steroids, tannins, terpenoids from chloroform extract (Table 1).

Table 1. Preliminary phytochemical analysis of leaf crude extracts of *M. paniculata*.

Phytochemical compounds	Leaf crude extract
Alkaloids	+
Anthraquinones	-
Catechin	-
Coumarin	+
Flavonoids	-
Phenols	-
Quinines	+
Saponins	-
Steroids	+
Tannins	+
Terpenoids	+

+ Presence of compound; - Absence of compound.

Table 2. Larvicidal activity of *M. paniculata* leaf extracts against three important vector mosquitoes.

Leaf extract used	Larval mortality (%) in different concentration of extracts (ppm)					LC ₅₀ (ppm)	95% of Confidence limit (ppm)		LC ₉₀ (ppm)	95% of Confidence limit (ppm)		χ^2 (df =4)	Chi-square test
	50	100	150	200	250		LCL	UCL		LCL	UCL		
Larvicidal activity of <i>M. paniculata</i> against <i>Ae. aegypti</i>													
Pet-Eth LE	42.2 ±1.6	53.6 ±2.0	66.2 ±2.4	75.8 ±2.6	85.6 ±2.9	83.58	53.07	104.79	289.26	253.20	349.43	3.311	0.082
Chloroform LE	46.8 ±1.6	63.2 ±3.4	73.6 ±3.8	82.2 ±3.2	90.8 ±1.9	57.58	22.73	80.64	246.20	217.83	291.48	2.383	0.307
Eth-Acet LE	35.4 ±2.3	45.6 ±3.3	56.8 ±2.1	69.8 ±2.5	82.2 ±2.9	115.21	92.69	133.40	315.49	276.60	379.44	5.426	0.489
Larvicidal activity of <i>M. paniculata</i> against <i>An. stephensi</i>													
Pet-Eth LE	46.4 ±2.5	66.8 ±3.5	76.2 ±3.6	83.8 ±2.9	91.6 ±3.2	52.57	17.62	75.69	234.27	208.04	275.27	1.278	1.088
Chloroform LE	52.8 ±2.2	65.8 ±1.3	84.4 ±3.2	90.2 ±1.0	96.4 ±1.3	44.72	14.61	65.32	192.30	172.98	219.98	5.498	0.870
Eth-Acet LE	45.4 ±1.1	53.6 ±2.7	66.2 ±3.3	75.2 ±2.7	84.8 ±3.8	76.74	40.60	100.45	301.00	260.30	372.41	3.877	0.294
Larvicidal activity of <i>M. paniculata</i> against <i>Cx. quinquefasciatus</i>													
Pet-Eth LE	44.8 ±1.4	62.6 ±2.0	72.8 ±1.9	83.4 ±3.6	90.4 ±2.5	62.96	31.37	84.48	243.98	216.86	286.37	2.059	0.327
Chloroform LE	49.4 ±3.3	65.9 ±2.9	76.4 ±2.4	85.4 ±2.0	94.4 ±1.8	52.06	19.63	73.92	221.21	197.56	257.11	4.511	0.454
Eth-Acet LE	44.4 ±3.4	57.2 ±1.5	66.4 ±2.4	75.2 ±3.2	86.2 ±3.1	74.05	38.10	97.63	292.85	254.21	359.72	3.216	0.397

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 24h of exposure period. Chi square test, LC50=Lethal Concentration brings out 50% mortality and LC95 = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit. Pet-Eth LE= petroleum ether leaf extract; Chloroform LE= chloroform leaf extract; Eth-Acet LE= ethyl acetate leaf extract.

As shown in Table 2 above, the efficacy of *M. paniculata* against the 4th instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* showed moderate to good larvicidal activity. The chloroform extract of *M. paniculata* showed highest larvicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* with the LC₅₀ values of 57.58, 44.72 and 52.06ppm respectively with the respective LC₉₀ values of 246.20, 192.30 and 221.21ppm followed by petroleum ether of larvicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (LC₅₀ values of 83.58, 52.57 and 62.96 ppm respectively; LC₉₀ values of 289.26, 234.27 and 243.98ppm respectively). Ethyl acetate extract of *M. paniculata* showed least

larvicidal activity among the extracts used (Table 2). The pupicidal activity of the leaf extracts of *M. paniculata* is provided in Tables 3, 4 and 5. The chloroform extract of *M. paniculata* showed highest pupicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (LC₅₀ values=53.06, 48.01 and 53.34 respectively; and LC₉₀ values 234.85, 187.32 and 210.77ppm respectively), followed by petroleum ether leaf extract (Table 3). Ethyl acetate leaf extract of *M. paniculata* showed a pupicidal activity in terms of LC₅₀ values, 149.50, 83.67 and 81.44 ppm respectively against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (Table 5). The Chi-square values were significant at $p < 0.05$ level.

Table 3. Pupicidal activity of *M. paniculata* leaf extracts against *Ae. aegypti*.

Leaf extract used	Concentration (ppm)	Adult emergence (%)	Pupal mortality (%)	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	χ^2 (df=4)	Chi-square test
Pet-Eth LE	Control	95.2 ± 3.8	3.4 ± 1.9	90.22	311.138	4.141	0.245
	50	53.8 ± 1.0	42.2 ± 1.6	(58.85-112.01)	(269.44-383.55)		
	100	46.4 ± 1.5	51.2 ± 1.6				
	150	34.2 ± 1.9	62.4 ± 2.8				
	200	26.2 ± 2.6	73.6 ± 3.2				
	250	15.8 ± 2.5	83.2 ± 2.5				
Chloroform LE	Control	94.2 ± 2.6	3.6 ± 3.0	53.06	234.85	6.955	1.354
	50	43.8 ± 1.3	51.6 ± 1.8	(18.32-76.05)	(208.45-276.23)		
	100	36.6 ± 2.8	60.2 ± 2.8				
	150	25.4 ± 1.5	73.2 ± 2.3				
	200	11.8 ± 1.0	87.6 ± 2.1				
	250	7.6 ± 2.0	91.4 ± 1.6				
Eth-Acet LE	Control	96.4 ± 2.3	2.6 ± 2.3	149.50	346.26	6.247	1.034
	50	68.8 ± 1.4	26.8 ± 3.1	(131.38-167.61)	(303.33-416.57)		
	100	59.4 ± 3.9	37.8 ± 1.6				
	150	53.6 ± 3.5	49.2 ± 2.2				
	200	37.6 ± 3.2	59.4 ± 3.7				
	250	21.4 ± 2.7	77.2 ± 1.6				

Values represent mean ± S.D. of five replications. *Mortality of the pupae observed after 24 hrs of exposure period. Chi square test, LC₅₀=Lethal Concentration brings out 50% mortality and LC₉₅ = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit. Pet-Eth LE= petroleum ether leaf extract; Chloroform LE= chloroform leaf extract; Eth-Acet LE= ethyl acetate leaf extract.

The results of present study are comparable with similar reports of earlier workers. The toxicity to the third instar larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* by the ethyl acetate leaf extract of *Breyenia vitis-idaea* showed the LC₅₀ value of 98.2, 107.79 and 115.8ppm respectively (Jeyasankar and Ramar, 2014a). The investigation by Ramar et al. (2014) was designed to determine the larvicidal activity of silver nanoparticles synthesized from aqueous leaf extract of *Cleistanthus collinus* against the larvae of *Ae. aegypti*, and the synthesized nanoparticles exhibited significant larvicidal activity. This shows that the nanoparticles synthesized from plant extracts also possess mosquito larvicidal

activity. The toxicity to the third instar larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* by the ethyl acetate leaf extract of *Andrographis paniculata* showed the least LC₅₀ value of 20.85 (Jeyasankar and Ramar, 2015a). The toxicity study of *Tragia involucrata* leaf extract showed good larvicidal activity against the dengue vector mosquito *Ae. aegypti* (Jeyasankar and Ramar, 2014b). The petroleum ether extract of *Andrographis paniculata* against mosquito vectors exhibited more than 85% pupal mortality and 100% ovicidal activity at 250ppm (Jeyasankar and Ramar, 2015b). The methanol extracts of *Solanum trilobatum* was found to be susceptible to the larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* at 250ppm

with the LC₅₀ values of 125.43, 127.77 and 116.64ppm respectively (Premalatha et al., 2013). The results of the present study showed that the chloroform extracts of *M. paniculata* against larvicidal and pupicidal activity with

high mortality with LC₅₀ value of 44.72 and 48.01ppm respectively. The results suggest that the chloroform leaf extract of *M. paniculata* have the potential to be used as good larvicidal and pupicidal agent.

Table 4. Pupicidal activity of *M. paniculata* leaf extracts against *An. stephensi*.

Leaf extract used	Concentration (ppm)	Adult emergence (%)	Pupal mortality (%)	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	χ ² (df=4)	Chi-square test
Pet-Eth LE	Control	92.4 ±3.9	5.2 ±1.3	60.98	239.39	1.039	0.790
	50	53.4±1.9	44.6 ±2.7	(29.37-82.52)	(213.12-280.09)		
	100	35.4 ±1.5	62.4 ±2.5				
	150	23.4 ±3.2	76.4 ±1.5				
	200	13.8 ±1.6	83.7 ±3.7				
	250	8.4 ±1.1	90.2 ±2.2				
Chloroform LE	Control	94.2 ±2.5	2.4 ±2.7	48.01	187.32	4.006	2.999
	50	50.6 ±2.8	47.4±2.8	(20.41-67.26)	(169.11-212.96)		
	100	23.2 ±2.5	74.4±2.3				
	150	15.8±2.7	81.4±2.9				
	200	6.8 ±2.1	89.8±3.9				
	250	2.2 ±1.3	97.6±1.6				
Eth-Acet LE	Control	95.6 ±1.3	3.4 ±2.5	83.67	286.52	5.557	0.593
	50	53.4 ±1.9	43.6±2.9	(53.74-104.60)	(251.21-345.01)		
	100	45.6 ±2.5	52.8±2.1				
	150	33.2 ±1.6	63.8±2.9				
	200	21.8 ±3.2	77.6±3.2				
	250	12.4 ±1.6	86.2±2.9				

Values represent mean ± S.D. of five replications. *Mortality of the pupae observed after 24 hrs of exposure period. Chi square test, LC50=Lethal Concentration brings out 50% mortality and LC95 = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit. Pet-Eth LE= petroleum ether leaf extract; Chloroform LE= chloroform leaf extract; Eth-Acet LE= ethyl acetate leaf extract.

Table 5. Pupicidal activity of *M. paniculata* leaf extracts against *Cx. quinquefasciatus*.

Leaf extract used	Concentration (ppm)	Adult emergence (%)	Pupal mortality (%)	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	χ ² (df=4)	Chi-square test
Pet-Eth LE	Control	95.8 ±2.4	3.4 ±2.1	62.20	234.14	2.512	0.790
	50	54.2±2.0	44.4±2.7	(32.18-82.91)	(209.10-272.40)		
	100	33.4 ±1.1	64.6±2.7				
	150	25.6 ±2.6	73.6±2.9				
	200	10.2 ±1.9	83.8 ±3.7				
	250	5.6 ±1.3	92.2 ±3.3				
Chloroform LE	Control	94.8 ±2.3	4.6 ±2.1	53.34	210.77	3.264	0.965
	50	50.2±2.4	47.2±3.1	(23.71-73.78)	(189.29-242.39)		
	100	29.8 ±1.7	68.2±1.3				
	150	20.4±1.8	77.6±1.5				
	200	11.4±1.6	86.9±3.4				
	250	4.6 ±2.4	95.2±3.2				
Eth-Acet LE	Control	94.6 ±3.5	2.8 ±1.6	81.44	266.25	4.957	0.572
	50	57.8 ±2.3	42.2±3.1	(54.09-101.00)	(236.14-313.865)		
	100	42.6 ±3.0	54.6±3.2				
	150	29.2 ±1.0	68.6±1.3				
	200	23.8 ±1.9	77.2±2.1				
	250	7.8±2.9	89.4±1.5				

Values represent mean ± S.D. of five replications. *Mortality of the pupae observed after 24 hrs of exposure period. Chi square test, LC50=Lethal Concentration brings out 50% mortality and LC95 = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit. Pet-Eth LE= petroleum ether leaf extract; Chloroform LE= chloroform leaf extract; Eth-Acet LE= ethyl acetate leaf extract.

Conclusion

Phytochemicals are relatively safe, inexpensive and readily available in plants elsewhere. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. The results suggest that the chloroform leaf extract of *M. paniculata* have the potential to be used as good larvicidal and pupicidal agents.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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How to cite this article:

Ramar, G., Jeyasankar, A., 2016. Phytochemical screening, larvicidal and pupicidal activity of *Murraya paniculata* (L.) Jack (Rutaceae) leaf extracts against three important vector mosquitoes (Diptera: Culicidae). *Int. J. Curr. Res. Biosci. Plant Biol.* 3(10), 150-156. doi: <http://dx.doi.org/10.20546/ijcrbp.2016.310.018>