

Evaluation of larvicidal, pupicidal and adulticidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

Pratima Karmakar¹, Shilpi Chakraborty¹, Jayeeta Khanrah¹ and Anjali Rawani^{2*}

¹Department of Zoology, The University of Gour Banga, Malda, West Bengal, India ² Assistant Professor, Department of Zoology, The University of Gour Banga, Malda, West Bengal, India Correspondence Author: Anjali Rawani Received 13 Jan 2023; Accepted 21 Feb 2023; Published 27 Feb 2023

Abstract

Mosquitoes are the most annoying creatures on the planet, causing the spread of deadly diseases such as malaria, filariasis, dengue fever, yellow fever, and Japanese encephalitis. The major purpose of the present finding is to evaluate the mosquito larvicidal activity of the flowers of *Senna sophera L.* and the leaves of *Mammea americana* and *Dieffenbachia seguine* against *Culex quinquefasciatus*. The fresh plant parts of three plants were collected and tested at different concentrations. All the graded concentrations of three plant parts, i.e., 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, and 1 ml, showed significant larval mortality. The lowest LC₅₀ value calculated was 0.45 ml, 0.53 ml, and 0.68 ml of flower extract of *S. sophera* and crude leaf extract of *M. americana and D. seguine*, respectively. The crude extracts of both leaves and flowers also showed pupicidal and adulticidal activity with LC₅₀ values of 5.83 ml, 6.67 ml, and 5.68 ml; 2.81 ml, 2.34 ml, and 1.14 ml, respectively. The qualitative phytochemical tests are done in the laboratory. The result of phytochemical analysis indicates the presence of secondary metabolites such as saponin, alkaloid, glycoside, carbohydrate, and protein in the crude extract of the tested plant parts of three plants. The dead larvae were also examined under a compound microscope to determine the possible reason for their deaths. The results support the idea that the tested plant extracts can be used for the control of larval and adult life forms of *Culex quinquifasciatus*.

Keywords: Senna sophera, Mammea americana, Dieffenbachia seguine, Culex quinquefasciatus, larvicidal activity, pupicidal activity, adulticidal activity, phytochemical analysis

Introduction

The most important arthropod vector for medical purposes is the mosquito. Mosquitoes are responsible for one of the greatest health issues in the majority of the world, which is vector-borne diseases. It is affecting the socio-economic status of many nations and it is also causing allergy too, that includes a local skin reaction (Taubes, 1997) [40]. They spread viruses and parasites, which continue to have negative effects on people. Some of the fatal diseases spread by mosquitoes include filariasis, dengue, yellow fever, malaria, Japanese encephalitis, and chikungunya. Culex quinquefasciatus is an important vector of Bancroftian filariasis in tropical and subtropical regions and the arboviral diseases such as dengue and chikungunya are also transmitted by this day biting mosquito species. Zika infection is an emerging viral infection which is transmitted by Ae. aegypti but there is an indication that other mosquito species including Cx. quinquefasciatus, transmit the virus (Banelli and Beier, 2017; Banelli and Romano, 2017; Guedes et al 2017; Banelli and Mehlhorn, 2016) [5, 3, 14, 4, 48]. According to WHO (2022), the lymphatic parasite Wuchereria bancrofti has infected roughly 90 million people globally at some point, and ten times as many additional people are at risk of contracting the disease. 25 million individuals in India alone are infected with microfilaria, and 19 million people have filarial illnesses. Despite its crippling effects, lymphatic filariasis is given a very low control priority (Ramaiah et al. 2000) [30]. Numerous characteristics of Culex quinquefasciatus encourage invasiveness. In contrast to 'clean' freshwater mosquitoes such as Aedes aegypti or Aedes albopictus, in 'filthy' eutrophic waterbodies rich in organic matter, such as stagnant water tainted with human or animal waste, Cx. quinquefasciatus can reproduce and achieve extremely high larval densities.

Mosquito management programs depend on a routine of chemical insecticides. But, this synthetic chemical product for mosquito control contains many pesticides such as pyrethroid, organophosphates, carbamates etc. Organophosphates and carbamates are toxic to the nervous system (Blondell, 1997) [7] and some of the pyrethroids are believed to be toxic to the reproductive system and disruptive to endocrine function (Garey &Wolff, 1998) [12]. Mosquito coils which are easily available in the market containing synthetic pyrethroids and other organophosphorus compounds which cause so many side effects, such as breathing problem, asthma, eye irritation, headache, and sneezing to the users (Sharma, 2001) [37]. Beside these health problems these synthetic insecticides subsequently lead to the expansion of resistance among the vectors, along with other problems such as environmental pollution, bio magnification, and adversely affecting the quality of human and animal health, worldwide (Senthil-Nathan, 2020) [36]. This has made it necessary to do research and produce environmentally safe, biodegradable, and inexpensive indigenous vector control methods that may be employed by people and communities in certain situations with the least

amount of care. (Kumar et al., 2014) [22].

Today, environmental safety is considered to be of paramount importance. An insecticide should be environmentally friendly rather than having a high fatality rate among its target organisms (Ullah et al., 2018) [43]. Searching for new control agents from natural products such as plant secondary metabolites has gained popularity among researchers in countries with a strong herbal tradition and large numbers of plants have been reported to possess insecticidal activity (Komalamisra et al. 2005) [20]. Plant-based treatments are emerging as a possible replacement for synthetic chemical pest management/control agents. Phytochemicals belonging to different chemical classes such as steroids, alkaloids, terpenes, and phenolic constituents were investigated earlier for insect control potential and found promising efficacy against the vector mosquitoes (Pavela, 2015a; Pavela 2015b; Mathew et al. 2009) [29, 28, 23]. There is a need for an alternate source with minimum risk to environment and human health, and this study intends to identify potential plant-based mosquito larvicides against the mosquito vectors Cx. quinquefasciatus.

During the present study, an effort was made to establish the larvicidal, pupicidal and aduliticidal properties of crude extracts of three plants, viz. Senna sophera L., Mammea americana Juss. and Dieffenbachia seguine (Jacq.) Schott against Culex quinquefasciatus Say as target species. The study also includes a phytochemical analysis of the tested plant extracts to know the nature of the bioactive principle responsible for their insecticidal properties. A microscopic examination of dead larvae was also made to determine the possible mode of action of the tested plant parts.

Material and methods Study area

This study was conducted in the laboratory of parasitology, vector biology, and Nanotechnology, Department of Zoology, University of Gour Banga, Malda. The period of the study was from March 2022 to August 2022.

Collection of mosquito larvae

Mosquito larvae of species *Cx. quinquefasciatus* used during the project work were taken from drains of the neighbouring locality of University of Gour Banga, Malda. The larvae were collected in a 5 lit bucket and transferred into a plastic tray (45 cm×07cm ×30cm).

Maintenance of larvae and adults

To maintain the larvae 500 ml of water was added into the tray because the *Cx. quinquefasciatus* larvae's natural habitat is stagnant water and the temperature was 25°-27°C, humidity 75-85% and photo period 14L:10D for 24 hrs. After the larvae transformed into pupae, the pupae were collected in a glass beaker and placed it in a case until it transformed into adults (Kamaraj *et al.*, 2011) [18].

Collection of plant material and authentication

The flowers of *Senna sophera*, were collected from the plants grown in the different localities in the district of Dakshin Dinajpur, West Bengal, India. The fresh leaves of *Mammea americana* were collected from the University premise, University of Gour Banga, Malda, in the month of March, 2022. And the other plant leaves (*Dieffenbachia seguine*) were

collected from town Hili, Dakshin Dinajpur in the month of April, 2022. The plants were authenticated by Dr. Monoranjan Chowdhury, Assistant Professor, Department of Botany, University of Gour Banga, Darjeeling, India. A voucher herbarium specimen was deposited in the Department of Zoology, University of Gour Banga.

Preparation of crude extract

The fresh plant parts were washed by tap water several times to remove the filth and dust. All the three plant parts were grinded by an electric grinder machine to get a fine paste and the extract was squeezed out by a cotton cloth with mesh size of 20 µm. The extracts were collected in plastic containers with different concentration by pipette and the excess extracts were stored at 4°C in refrigerator for further use (Rawani *et al.* 2009) [32]

Larvicidal bioassay

The larvicidal activity was assessed by the procedure of WHO (2005) [46] with some suitable modification. 1st, 2nd, 3rd and 4th instar larvae of *Cx. quinquefasciatus* were used for this experiment. 25 larvae of each instar were separately transferred into different beaker containing 100 ml of water. Low to high gradation of concentration i.e. 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml concentration of crude extract of three plant parts were added to each beaker. Mortality rate were recorded at 24 hrs of post exposure. Dead larvae were recognized when they failed to move after piercing with a needle in a siphon or cervical region. A control set up was also there with 100 ml of water and larvae only. The experiment was repeated three times and mortality rate were recorded.

Pupicidal bioassay

The pupicidal activity was carried out by the procedure of WHO, (1975) [47] with some suitable modification. In pupicidal bioassay ten pupae were collected in five different beaker containing 100 ml of water. Different concentration of crude extracts (0.5, 0.8 1, 2, and 3 ml) were added to it. After 24 hours' mortality of pupae were recorded and assessed.

Adulticidal bioassay

Adult mosquitoes of the species *Cx. quinquefasciatus* were selected for adulticidal bioassay. 1, 2, 3, 4 and 5 ml leave extracts were taken in different petridishes and the extracts were impregnated on filter papers. The filter papers were left to dry at room temperature. A cotton bed soaked with 10 % glucose solution was prepared and attached with cello tape on net. A 500 ml beaker was taken for this experiment. The soaked filtered paper was placed at the bottom of the beaker. 25 adult mosquitoes were taken for each concentration of extract. The mosquitoes were transferred to the beaker from the cage and the top of the beaker was surrounded by the cotton bed. The set up was held for 24 hours and the percentage of mortality of the adult mosquitoes were counted (Saini *et al.*,1986) [35].

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

 $Percentage\ mortality = \frac{Number\ of\ dead\ larvae\ /pupae}{Number\ of\ introduced\ larvae\ /pupae} \times 10$

Qualitative phytochemical analysis

The flowers of *Senna sophera*, leaves of *Mammea americana* and *Dieffenbachia seguine* were collected and then washed properly with tap water. The plant parts were then shade dried at room temperature for two weeks and grinded to powder. The resultant crude fine powder was used for phytochemical investigations according to Trease and Evans, 1989, [42] Choudhury *et al.* 2012 [8], Mallick *et al.* 2016 [24] & Rawani 2022 [34].

Examination of dead larvae

The treated and untreated dead larvae were examined in a compound microscopic 10 x view image to determine the possible cause of the death of the larvae.

Statistical analysis

The percentage of larval mortality (M %) was corrected using Abbott's formula. Statistical analysis of the experimental data was performed using the computer software Stat plus 2009 and MS Excel 2013.

Result

In the present study, the details of the plants such as common name, family, and parts used for bioassay and botanical description were given in Table 1. The result of larvicidal activity against all the instars of *Cx. quinquefasciatus* in different concentrations ranges from 0.5 to 1 % of crude extract of different plant parts of *M. americana*, *D. seguine*, and *S. sophera* presented in table 2. Among all the larval instar, the highest mortality was observed in 1st instar larvae that showed 96.67 % mortality in leaves crude extract of *M. Americana* followed by 94.67 % in flower crude extract of *S. sophera* and 83.33 % in crude leaves extract of *D. seguine* after 24 hours of exposure. The mean pupal percentage mortality of crude leaf

extract of M. americana, D. seguine, and flower extract of S. sophera against Culex quenquefasiatus pupae was presented in table 3. Here highest pupal mortality was observed in 3 ml concentration of leaves crude extract of M. americana (46.67 %) followed by crude leaves extract of *D. seguine* (26.67 %) and flower crude extract of S. sophera (16.66 %) after 24 hours of exposure. Table 4 showed the percentage mortality of leaf extract of M. americana, D. seguine, and flower extract of S. sophera against adults of Culex quinquefasciatus. In adulticidal activity, 100 % mortality was observed in crude leaves extract of D. seguine, 99 % in leaves crude extract of M. americana, and 98.67% in flower crude extract of S. sophera after 2h h of contact in 5ml concentration. Table 5 presented the LC₅₀ value of the larvicidal activity, pupicidal activity, and adulticidal activity of crude leaf extract of M. Americana, D. seguine, and flower extract of S. sophera. The results of the phytochemical analysis of all three plant parts are presented in table 6. Among 12 phytochemicals, flavonoid, phlobatannin, and saponin were present while anthraquinone was not found in all three planttested parts. A general behavioural change in the larvae of the tested mosquito species was observed and it was seen that larvae slowly became inactive within few hours of treatment. Figure A is the microscopic view of normal dead larvae. The microscopic examination of dead larvae showed disintegration of integument probably due to removal of chitin and abnormal stretching of body specially the neck region was also observed (Figure B & D). These symptoms suggested both growth regulating and probably neurotoxic action of the leaf extract of M. americana (Figure D) and in case of D. seguine, the extract penetrate the integument of larvae and broke the gut (Figure B), Figure C revealed that the flower extract of S. sophera has blocked the spiracles and the larva died due to suffocation, but further research is needed to substantiate this view.

Table 1: Plants tested for their larvicidal, pupicidal and adulticidal activity in this study

Sl No	Botanical name	Local name	Family	Parts used	Botanical description
1.	Mammea americana	Mamey apple	Calophylla ceae	Leaf	It is an evergreen tree whose fruit is edible. It is an 18 - 21 m tall tree with a short trunk that reaches a diameter of 1.2 m. It is heavily branched and has a highly dense oval crown. The leaves are dark green, opposite, and leathery. The flowers are white and fragrant, with 4 or 6 petals. fruit is a round berry, with a brown or grey brown thick rind. (Huxley, 1992; Barwick, 2004) [17, 2]
2.	Dieffenbachi a seguine	Dumbcane	Araceae	Leaf	It is an herbaceous plant also known as zebra plant or spotted dieffenbachia. It is widely cultivated as a houseplant and used as an outdoor ornamental plant in tropical areas (Kaufman, 2017; Croat, 2004) [19, 9]. The leaves are 15-40 cm long, and have patterns of many irregular yellowish or cream-green splotches or white spots and flecks (Stone, 1970) [38].
3.	Senna sophera	Kasondi	Caesalpinia ceae	HOWER	It is an annual undershrub found throughout India and in most tropical countries. It is common in waste lands, on roadsides and in the forests. It grows up to 0.5-2 m high, soon becoming woody. The larger leaves are 7-18 cm long, with 4-10 pairs of leaflets, the largest blades are lance like or ovate-acuminate and up to 7 x 2 cm. The 4-10-flowered racemes have yellow flowers with roundish petals. The pods are erect, flattened and slightly thick at first, nearly cylindrical, swollen when ripe, 6-10 x 0.7-1 cm (USDA, NRCS. 2015) [44].

Table 2: Mean larval mortality of 25 larvae of Culex quinquefasciatus (1st to 4th instar larvae) of tested plants

Larval instar	Concentration (%)	Mean mortality (%) ± SE (after 24 h)			
Larvai ilistar	Concentration (76)	Mammea americana	Dieffenbachia seguine	Senna sophera	
	0.5	60.00 ± 3.34	23.33±3.33	50.00±11.5	
1 st instar	0.6	66.67± 2,02	33.33±3.33	60.00±5.77	
1 Histar	0.7	70.00 ± 5.27	46.67±3.33	70.00±5.77	
	0.8	76.67±3.34	50.00±5.77	80.00±5.77	

	0.9	80.00±5.77	53.33±3.33	86.67±3.33
	1.0	96.67±3.34	63.33±6.67	94.67±3.33
	Control	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	33.33 ± 2.02	30.00±5.77	40.00±5.77
	0.6	36.66 ± 3.33	33.33±3.33	46.66±8.81
	0.7	60.00 ± 5.27	40.00±0.88	73.33±3.33
2 nd instar	0.8	73.33 ± 4.53	50.00±5.77	76.67±2.02
	0.9	76.67± 2.02	60.00±3.33	80.00±5.77
	1.0	83.33± 0.81	70.00±5.77	90.00±3.33
	Control	0.00±0.00	0.00 ± 0.00	0.00±0.00
	0.5	30.00±1.08	36.67±3.33	60.00±5.77
	0.6	40.00±1.55	46.67±8.81	70.00±5.77
	0.7	50.00±1.55	53.33±3.33	80.66±3.33
3 rd instar	0.8	66.67± 6.67	66.67±8.81	86.67±5.77
	0.9	76.67±7.64	73.67±6.67	90.00±5.77
	1.0	86.67± 8.51	83.33±3.33	92.33±5.77
	Control	0.00±0.00	0.00 ± 0.00	0.00±0.00
	0.5	13.34±3.33	13.33±3.33	50.66±3.33
	0.6	16.67±3.33	23.33±3.33	56.67±6.66
	0.7	23.33±8.81	36.67±6.66	60.00±5.77
4 th instar	0.8	26.67±8.81	40.00±5.77	62.33±5.77
	0.9	30.00±0.88	45.67±2.02	66.67±3.33
	1.0	46.67±8.81	56.67±6.66	73.33±3.33
	Control	0.00±0.00	0.00±0.00	0.00±0.00

Table 3: Mean pupal mortality (%) of tested plant parts against Culex quenquefasiatus pupae

Concentration (0/)	Mean	mortality (%) ± SE 24 hours	
Concentration (%)	Mammea americana	Dieffenbachia seguine	Senna sophera
0.5	6.67±3.33	6.67±3.33	3.33±3.33
0.8	10.00±0.00	6.67±3.33	13.33±3.33
1	16.67±3.33	13.33±3.33	13.33±3.33
2	26.67±3.33	20.00±0.00	20.00±0.00
3	46.67±3.33	26.67±3.33	16.66±3.33
Control	0.00±0.00	0.00±0.00	0.00±0.00

(Mean of 3 experiments; SE=Standard error)

Table 4: Percentage mortality of adults of Culex quinquefasciatus against tested plant parts

Concentration (%)	Mean mortality (%) ± SE 24 hours			
Concentration (%)	Mammea americana	Dieffenbachia seguine	Senna sophera	
1	24.33± 2.33	28.67 ± 2.03	45.33± 2.02	
2	40.33± 1.45	43.67 ± 2.33	65.67 ± 1.76	
3	60.67 ±1.76	74.00 ± 2.30	87.00 ± 1.15	
4	80.87 ± 1.76	91.00 ±2.08	95.00 ± 1.73	
5	99.00± 0.58	100.00 ± 0.00	98.67 ± 0.88	
Control	0.00±0.00	0.00±0.00	0.00±0.00	

(Mean of 3 experiments; SE=Standard error)

 $\textbf{Table 5: } LC_{50} \text{ value of larvicidal, pupicidal and adulticidal activity of tested plant parts}$

Larval instar	Mammea americana	Dieffenbachia seguine	Senna sophera
1 st	0.54 ml	0.86 ml	0.53 ml
2 nd	0.53 ml	0.82 ml	0.63 ml
3 rd	0.64 ml	0.68 ml	0.45 ml
4 th	1.72 ml	1.22 ml	0.62 ml
Pupa	5.83 ml	6.67 ml	5.68 ml
Adult	2.91 ml	2.34 ml	1.14 ml

Table 6: Result of qualitative phytochemical analysis of the crude extract of the tested plants

Phytochemicals	Mammea Americana	Dieffenbachia seguine	Senna sophera
Alkaloid	+	-	-
Anthraquinone	-	-	-

Carbohydrate	-	-	+
Cardiac glycoside	-	-	+
Flavonoid	+	+	+
Phenolic compound	+	+	-
Phlobatannin	+	+	+
Protein	-	-	+
Saponin	+	+	+
Steroid	+	-	-
Tannin	+	-	+
Terpenoid	+	+	-



Fig A-D: A: Normal larvae, B: Dead larvae from the leaves extract of *D. seguine*, C: Dead larvae from the extract of flower extract of *S. sophera*, D: Dead larvae from the leaves extract of *M. americana*

Discussion

Globally, there is a prompt awareness going on, and always desired to use natural, eco-friendly compounds for larvicidal activity. Recent years have seen an increase in mosquito risk, and the annual mortality toll from diseases spread by mosquitoes has led to a loss of socioeconomic prosperity in many nations. Because of pesticide resistance in vectors and environmental imbalance, mosquito control by chemical insecticides is now not safe. The application of chemical or synthetic insecticides does not provide absolute results and also leads to deleterious effects in the long term. That is why alternative mosquito control methods are needed. The crude extract, which is obtained from plant parts like leaves, roots, flowers, bark, seeds, and fruits, has been used as a conventional larvicide. The secondary compounds of plants are a vast repository of compounds with a wide range of biological activities.

Today's mosquito control program emphasis more on using plant extracts to control the immature stages of mosquitoes. Tennyson et al., 2012 [41] studied the larvicidal activity of twenty-five plant extracts against the 3rd larval instar stages of Culex quinquefasciatus. Likewise, Pavela (2008) [27] tested 56 species of plants in the Euro-Asiatic region for larvicidal activity against the fourth larval instar of the mosquito Culex quinquefasciatus Say. The result obtained from the study of Edwin et al. 2013 [10], evaluated the larvicidal activities of aqueous and ethanolic leaf and stem extracts of S. alata on fourth instar larvae of Anopheles gambiae, Culex quinquefasciatus, and Aedes aegypti, at extract concentrations of 0.15, 0.30, 0.45, 0.60 and 0.75 % w/v, for 72 hours in static bioassays. Ravindran et al. (2020) [31] studied that the larvicidal activity of Clitoria ternatea flower extracts affected the early 4th instar larvae of Aedes mosquito vectors. The highest larvicidal activity of the Clitoria flower was observed against the early 4th instar larvae of Aedes aegypti with LC50 values of 1056 mg/L. Rawani, 2021 [33] studied the role of crude and ethyl acetate solvent extracts of mature leaf of Callistemon viminalis as larvicidal, pupicidal, adulticidal, ovicidal, and

activities against filarial vector quinquefasciatus. At 1 % concentration, the mortality rate of 3rd larval instars of Cx. quinquefasciatus was significantly higher (p<0.05). In pupicidal and adulticidal bioassay, the observed LC₅₀ values were 1.12 ml and 1.8 ml respectively. Gope and Rawani, 2022 [13] carried out larvicidal, pupicidal, adulticidal, and repellent activities of crude extracts of mature leaf and fruit extract of Phyllanthus acidus against the filarial vector Culex quinquefasciatus. The LC50 value observed was lowest having 0.26 ml and 0.34 ml against the first instar larval stages of Cx. quinquefasciatus in crude leaf and fruit extract of P. acidus, respectively. The crude leaves extract of P. acidus exhibited pupicidal and adulticidal potency with LC50 values of 7.463 ml and 2.869 ml respectively after 24 h of

The present study results marked that the crude extracts of flowers of S. sophera, M. americana, and D. seguine leaves showed good efficacy against all the larval stages of the mosquito species tested. The highest mortality 94.67%, 96.67 %, and 83.33 % were observed in crude flower extract of S. sophera, leave extract of M. americana, and D. seguine against 1st and 2nd instar larvae of Cx. quinquefasciatus respectively in 1 ml concentration. The crude extracts of both leave and flowers also showed pupicidal activity and adulticidal activity with LC₅₀ values of 5.83 ml, 6.67 ml, and 5.68 ml; 2.81 ml, 2.34 ml, and 1.14 ml respectively. The qualitative phytochemical analysis indicates the presence of several phytochemicals such as flavonoids, phlobatannin, and saponins in leaves of M. americana, D. segiune, and flowers of S. sophera. Among the three plant parts, the leaf extract of M. americana showed better results.

The findings of this study have shown that phytochemical compounds extracted from *S. sophera*, *M. americana*, and *D. seguine* might be innovative and an alternative to synthetic mosquitocides in the future. Chemical compounds extracted from different parts of the plant have different active ingredients with different activities against mosquito larvae and adults. Because of synergistic ingredients and synergisms that can be effective in managing a resistant mosquito population, plant crude extracts may be more effective than individual active compounds.

Conclusion

Based on the above results, it is concluded that Senna sophera, M. americana, and D. seguine have paramount larvicidal importance. The pharmacological, medicinal, and traditional importance reported in the present review confirms the therapeutic value of these three plants. The plant reported here is widely used in the traditional medicinal system as hepatoprotective, anti-inflammatory, antimicrobial, antioxidant activity and also it has a wide spectrum of pharmacological activities. The synthesized chemical is fairly inexpensive and readily available. However, using plants to control larvae also provides a more secure option. Additionally, these findings might be helpful in the hunt for novel chemicals. In conclusion, the study revealed that these three plants have potent mosquito larvicidal properties and could be selected for further studies particularly those about their effect on the growth and development of mosquitoes.

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