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LARVICIDAL ACTIVITIES OF 215 MEDICINAL PLANTS EXTRACTS AGAINST *AEDES AEGYPTI* FROM BANGLADESH

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Abstract

This study examined the effects of fresh crude extract from 215 plants on the mortality rate of third-instar larvae of *Aedes aegypti* after a 24-hour exposure. Twenty five plants demonstrated a high mortality rate (up to 80%) at five different concentrations. The LC_{50}, LC_{90}, and LC_{95} values of the tested plants showed that 15 plants were with 100% larvicidal potential. *Stemona tuberosa* showed the highest efficacy against *Aedes* larvae having LC_{50} = 3.78, LC_{90} = 7.51, and LC_{95} = 7.97, while *Jatropha gossypifolia* showed the lowest efficacy i.e., LC_{50} = 6.58, LC_{90} =11.23, and LC_{95} = 11.31. The extracts from *Acmella radicans*, *Allium sativum*, and *Oxalis violacea* exhibited a similar LC_{50} value of 3.99, indicating their high potency. Hence, the tested plants are a potential source of bioactive compounds that may work as a potential alternative to chemical mosquito larvicides.

Introduction

In the past few years, mosquito-borne illnesses have grown to be a concerning worldwide problem. They are the disease carriers that cause millions of deaths annually (Rahman *et al.* 2008). People can get the dengue virus by being bitten by an infected mosquito. Particularly in urban and semi-urban environments, it is most prevalent in tropical and subtropical countries (Rezza 2014). *Aedes* mosquitoes are a highly effective vector for several arboviruses, such as zika, chikungunya, and dengue. Dengue fever is a fatal mosquito-borne illness that strikes over 128 nations worldwide (Rahman *et al.* 2021). Fifty million cases of dengue fever are recorded annually, putting an estimated 2.5 billion individuals at risk of contracting the disease due to the extensive dispersion of *Aedes aegypti* (WHO 2009). The Ministry of Health and Family Welfare (MOHFW) recorded 69,483 dengue cases between January 1 and August 7, 2023, coupled with 327 associated deaths (case fatality rate = 0.47%) (Haider *et al.* 2023). To keep the mosquitoes under control, very toxic synthetic pesticides were used. Insect resistance problems are exacerbated by the use of foggers and aerial sprays of synthetic insecticides, such as pyrethroid compounds against adults. The environment and living things are both at risk from these products, which are nevertheless widely used (Silvério *et al.* 2020). To solve the problem, scientists have been investigating insecticides made from natural sources, like plants. Using plant extracts for pest control offers several advantages including increased biodegradability, fewer risks, and a wide range of biologically active compounds. As a result, there has been a noticeable increase in interest in using biologically active plant materials in mosquito control efforts recently. Although some earlier studies showed that numerous plants are used as insecticides (Faruque *et al.* 2018, 2019, Islam *et al.* 2021),

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however, the larvicidal activity of Bangladeshi medicinal herbs against dengue vectors have not yet been extensively studied except Hasan and Jannat (2023). Considering this, the present study aims to investigate the larvicidal effect of selected 215 plant species against dengue vectors.

Materials and Methods

Tested plants were collected from different locations of the University of Chittagong and Chattogram district. Collected plants were identified by consulting with experts and published literature. Voucher specimens of the tested plants were prepared following recommended techniques and were deposited in the Chittagong University herbarium with an accession number.

The *Aedes* mosquito larvae were collected from 57 suitable breeding places in various areas of the Chattogram region from March 2021 to May 2024, such as stagnant drains, ditches, derelict ponds, and containers containing dirty water.

Collections were usually done in the morning between 7 and 10 a.m. using a long-handled dipper. The larvae and the water were put into plastic jars with nets on top. They were brought in the lab and transferred in distilled water in an earthen bowl. The *Aedes* larvae were identified using appropriate taxonomic keys (Langat *et al*. 2012). The larvae were subjected to controlled environmental conditions, including a temperature of 27±2℃, relative humidity of 75-80%, and a light and dark cycle of 14:10. They were provided with a diet consisting of Brewer's yeast and dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively (Bagavan *et al.* 2008).

The larvicidal bioassay was conducted in accordance with the standard protocols established by the World Health Organization, with appropriate modifications (WHO 1981). Five different concentrations and three replications were maintained. Sterilized distilled water was used to obtain five different concentrations (10, 5, 2.5, 1.25, and 0.625%) following Rawani *et al.* (2014) and three replications were maintained. For each replication, a previously prepared 100 ml solution for primary screening and 50 ml solutions for final screening were taken in a large Petridis, and 25 larvae were exposed therein. Larvae considered dead were those displaying no movement even after being lightly touched with a glass rod (Langat *et al.* 2012). The percentage of mortality was calculated by using the formula published by Abbott *et al.* 1925.

The percentage of mortality observed was corrected by Abbott's formula. Statistical analysis of the experimental data was performed using "ORIGIN LAB" and "MS Excel 2007" to find out the log probit analysis regression equations and mean percentage mortality.

Results and Discussion

Initially, aqueous fresh crude extracts of different parts i.e., leaves, stem, root, flower, bulb, bark, and fruit of 215 plants were screened to determine their effectiveness against *Aedes* larvae at 24 hours of exposure (Supplementary Table 1). The results of the plants showing more than 20% effectiveness are presented in Table 1. Based on the results of the preliminary screening, 25 plant species were subjected to further screening at different concentrations i.e., 10, 5, 2.5, 1.25 and 0.625%. Fifteen plants showed 100% mortality rate at 10% concentration after 24 h of exposure (Table 2). LC_{50}, LC_{90} and LC_{95} values along with their regression equations of tested plants were also calculated and summarized in Table 2. The findings from the regression analysis of fresh crude plant extracts indicate a positive correlation between the mortality rate (Y) and the concentration of exposure (X).

Table 1. List of medicinal plants with their mean mortality of mosquito larvae.

Botanical name (Voucher number)	Habit	Part used	Mean mortality (%)	Mean mortality ± S. E
Acalypha hispida Burm.f. (EFL0042)	Shrub	Leaves	56	56±0.00
A. indica L. (EFL0073)	Herb	Leaves	90.67	90.67±0.33
Acmella radicans (Jacq.) R.K.Jansen (EFL0133)	Herb	Flower	100	100 ±0.00
		Leaves	72	72±0.00
A. uliginosa (Sw.) Cass. (EFL0075)	Herb	Whole plant	100	100±0.00
Ageratum conyzoides L. (EFL0097)	Herb	Whole plant	100	100±0.00
Allamanda cathartica L. (EFL0474)	Shrub	Leaves and Flower	37.33	37.33±0.33
Allium cepa L. (EFL0443)	Herb	Bulb	28	28±0.57
A. sativum L. (EFL0012)	Herb	Bulb	100	100±0.00
Amaranthus gangeticus L. (EFL0371)	Herb	Whole plant	30.67	30.67±0.67
A. spinosus L. (EFL0741)	Herb	Root	76	76±0.00
Andrographis paniculata (Burm.f.) Wall. ex Nees (EFL0026)	Herb	Leaves	58.67	58.67±0.33
Aphanamixis polystachya (Wall.) R.Parker (EFL0431)	Tree	Fruits	78.67	78.67±0.33
		Leaves	50.67	50.67±1.20
Araucaria araucana (Molina) K.Koch (EFL0627)	Tree	Leaves	40	40±0.57
Artabotrys hexapetalus (L.f.) Bhandari (EFL0126)	Shrub	Fruits	100	100±0.00
		Leaves	77.33	77.33±0.33
Asclepias curassavica L. (EFL0063)	Herb	Leaves	90.67	90.67±0.33
Azadirachta indica A.Juss. (EFL0067)	Tree	Leaves	94.67	94.67±0.33
Baccaurea motleyana (Müll.Arg.) Müll.Arg. (EFL0456)	Tree	Leaves	38.67	38.67±1.20
Blumea lacera (Burm.f.) DC. (EFL0122)	Herb	Leaves	49.33	49.33±0.33
Brassica oleracea L. (EFL1311)	Shrub	Leaves	21.33	21.33±0.33
Cajanus kerstingii Harms (EFL0459)	Shrub	Leaves	38.67	38.67±0.67
Calliandra tergemina (L.) Benth. (EFL1232)	Shrub	Leaves	20	20±0.57
Camellia sinensis (L.) Kuntze (EFL1126)	Shrub	Leaves	20	20±0.57
Capsicum frutescens L. (EFL0700)	Herb	Fruit	22.67	22.67±0.33
Carallia brachiata (Lour.) Merr. (EFL0319)	Tree	Leaves	68	68±0.57
Cinnamomum tamala (Buch.-Ham.) T.Nees & C.H.Eberm. (EFL1221)	Tree	Leaves	21.33	21.33±0.88
C. verum J.Presl (EFL0156)	Tree	Leaves	100	100±0.00
		Bark	100	100±0.00
Citrus limon (L.) Osbeck (EFL0433)	Shrub	Leaves	60	600±0.57
C. maxima (Burm.) Merr. (EFL0659)	Tree	Leaves	24	24±0.00
Clerodendrum indicum (L.) Kuntze (EFL0201)	Shrub	Leaves	22.67	22.67±0.88
C. viscosum Vent. (EFL0619)	Shrub	Leaves	72	72±0.57
Clitoria ternatea L. (EFL0201)	Herb	Leaves	64	64±0.57
Cordyline fruticosa (L.) A.Chev. (EFL0995)	Shrub	Leaves	33.33	33.33±0.33
Coriandrum sativum L. (EFL0017)	Herb	Leaves	58.67	58.67±0.33
Crassocephalum crepidioides (Benth.) S.Moore (EFL0048)	Herb	Stem	60	60±0.00
Cymbopogon schoenanthus (L.) Spreng. (EFL0085)	Herb	Leaves	50.67	50.67±0.33
Cynodon dactylon (L.) Pers. (EFL0221)	Herb	Whole plant	22.67	22.67±0.67
Delonix regia (Bojer ex Hook.) Raf. (EFL0643)	Tree	Leaves	28	28±0.58
Diospyros montana Roxb. (EFL0310)	Tree	Leaves	22.67	22.67±0.67
Elaeocarpus serratus L. (EFL0233)	Tree	Leaves	46.67	46.67±0.67
Elettaria cardamomum (L.) Maton (EFL0399)	Herb	Seeds	24	24±0.57
Eucalyptus obliqua Decne. (EFL0310)	Tree	Leaves	61.33	61.33±0.67
Fatsia japonica (Thunb.) Decne. & Planch. (EFL0174)	Shrub	Leaves	100	100±0.00
Hedychium coronarium J.Koenig (EFL1421)	Herb	Leaves	21.33	21.33±0.88
Heliotropium indicum L. (EFL0385)	Herb	Leaves and Flower	49.33	49.33±0.88
Hibiscus cannabinus L. (EFL1433)	Shrub	Leaves	20	20±0.57
H. sabdariffa L. (EFL0037)	Shrub	Fruit	100	100±0.00

Table 1 Contd.

Botanical name (Voucher number)	Habit	Part used	Mean mortality (%)	Mean mortality ± S. E
Hopea odorata Roxb. (EFL0531)	Tree	Leaves	29.33	29.33±0.33
Hyptis suaveolens (L.) Kuntze (EFL0167)	Herb	Leaves	33.33	33.33±0.33
Ipomoea aquatica Forssk. (EFL0719)	Hydrophyte	Leaves	25.33	25.33±0.33
Jatropha gossypiifolia L. (EFL0076)	Shrub	Stem and fruit	82.67	82.67±0.33
		Leaves	53.33	53.33±0.33
Justicia adhatoda L. (EFL1148)	Shrub	Leaves	20	20±0.00
Lawsonia inermis L. (EFL0081)	Tree	Leaves	100	100±0.00
Leea indica (Burm.f.) Merr. (EFL0421)	Shrub	Leaves	61.33	61.33±0.88
Leucas aspera (Willd.) Link (EFL0232)	Herb	Leaves	61.33	61.33±0.88
Limnocharis flava (L.) Buchenau (EFL0234)	Herb	Leaves	46.67	46.67±0.33
Lonicera caprifolium L. (EFL0221)	Climber	Leaves	62.67	62.67±0.67
Lophopetalum fimbriatum Wight (EFL0173)	Tree	Leaves	32	32±0.57
Magnolia champaca L. Baill. ex Pierre (EFL0315)	Tree	Fruit	68	68±0.00
Manihot esculenta Crantz (EFL0218)	Shrub	Leaves	70.67	70.67±0.33
Melaleuca viminalis (Sol. ex Gaertn.) Byrnes (EFL0197)	Tree	Flowers	33.33	33.33±0.33
		Leaves	29.33	29.33±0.88
Mimosa pudica L. (EFL0625)	Herb	Leaves	42.67	42.67±0.67
Mussaenda roxburghii Hook.f. (EFL0423)	Shrub	Leaves	41.33	41.33±0.33
Nerium oleander L. (EFL0071)	Shrub	Leaves	52	52±0.57
Oroxylum indicum (L.) Kurz (EFL1211)	Tree	Leaves	21.33	21.33±0.33
Oxalis violacea L. (EFL0095)	Herb	Whole plant	100	100±0.00
O. corniculata L. (EFL 0038)	Herb	Whole plant	26.67	26.67±0.33
Parthenium hysterophorus L. (EFL0135)	Herb	Leaves	50.67	50±0.67
Passiflora foetida L. (EFL0576)	Climber	Leaves	32	32±0.57
Pilea peperomioides Diels (EFL0047)	Herb	Leaves	53.33	53.33±0.33
Piper longum L. (EFL0165)	Climber	Leaves	100	100±0.00
		Stem and Root	100	100±0.00
P. nigrum L. (EFL0185)	Climber	Leaves	100	100±0.00
		Fruits	100	100±0.00
Plumbago zeylanica L. (EFL0318)	Herb	Flower and Leaves	81.33	81.33±0.33
Pongamia pinnata (L.) Pierre (EFL0133)	Tree	Fruits	100	100±0.00
Portulaca oleracea L. (EFL0418)	Herb	Whole	30.67	30.67±0.33
Rauvolfia vomitoria Wennberg (EFL0134)	Shrub	Stem	100	100±0.00
		Leaves	98.67	98.67±0.33
Rorippa indica (L.) Hiern (EFL0786)	Herb	Flower and Fruit	74.67	74.67±0.33
		Stem	69.33	69.33±0.33
Salvia splendens Blue Ribbon Sellow ex Nees (EFL0139)	Herb	Leaves	100	100±0.00
Salvia splendens Scarlet Sage Red Sellow ex Nees (EFL0125)	Herb	Leaves	97.33	97.33±0.33
Scoparia dulcis L. (EFL0796)	Herb	Leaves	26.67	26.67±0.33
Sida acuta Burm.f. (EFL0393)	Shrub	Leaves	37.33	37.33±0.88
S. cordifolia L. (EFL0626)	Shrub	Leaves	41.33	41.33±0.88
Solanum lycopersicon L. (EFL0711)	Herb	Leaves	64	64±0.00
S. nigrum L. (EFL0193)	Herb	Fruit	96	96±0.57
		Root	90.67	90.67±0.33
Spilanthes acmella (L.) L. (EFL0221)	Herb	Leaves and flower	100	100 ±0.00
		Stem	100	100 ±0.00
Synedrella nodiflora (L.) Gaertn. (EFL0258)	Whole plant	Herb	24	24±0.57
Stemona tuberos Lour. (EFL0301)	Climber	Leaves, Stem, and Root	100	100 ±0.00
Syzygium aromaticum (L.) Merr. & L.M.Perry (EFL0123)	Tree	Unopened flower	100	100±0.00
S. cumini (L.) Skeels (EFL0355)	Tree	Leaves	24	24±0.57
Swietenia mahagoni (L.) Jacq. (EFL0358)	Tree	Leaves	48	48±0.00
Tadehagi triquetrum (L.) H.Ohashi (EFL0539)	Shrub	Leaves	42.67	42.67±0.67
Tamarindus indica L. (EFL0335)	Tree	Leaves	45.33	45.33±0.88
Vitex negundo L. (EFL1163)	Shrub	Leaves	90.67	90.67±0.33
Xanthium strumarium L. (EFL0141)	Herb	Leaves	28	28±0.57
Zingiber officinale Roscoe (EFL0097)	Herb	Leaves	94.67	94.67±0.33

Based on the LC_{50}, LC_{90}, and LC_{95} values obtained from the extracts of 25 plant species against *Aedes aegypti* larvae, it was observed that *Stemona tuberosa* exhibited the highest toxicity against *Aedes* larvae, recording the lowest lethal concentration values (LC_{50} = 3.79%, LC_{90} = 7.51%, LC_{95} = 7.98%). Likewise, *Acmella radicans*, *Allium sativum*, and *Oxalis violaceae* extracts had the same LC_{50} value (3.99%) and also exhibited notable potency. *Artabotrys hexapetalus*, *Pongamia pinnata*, *Cinnamomum verum*, *Syzygium aromaticum*, *Piper nigrum*, *Aegeratum conyzoides*, *Rauvolfia vomitoria*, *Salvia splendens*, *Spilanthes acmella*, *Lawsonia inermis*, and *Piper longum* also showed moderate larvicidal activity (Table 2) with LC_{50} values ranging between 4.04 and 4.78 %. Conversely, *Jatropha gossypifolia* exhibited the lowest efficacy against *Aedes aegypti*. Notably, the *Stemona tuberosa* root, leaf, and stem extract had the most anti-*Aedes* larvae activity among the selected plant parts.

Table 2. Larvicidal bioassay of selected medicinal plants against *Aedes aegypti* larvae at different concentrations.

Botanical names	Family	Plant parts	Lethal concentration (%)			Regression equation
			LC_{50}	LC_{90}	LC_{95}	
Acmella radicans	Asteraceae	Flower	3.99	7.94	8.43	y=10.128x + 9.5546
		Leaves, Stem	8.21	14.15	14.89	y = 6.6838x - 4.8338
A. uliginosa	Asteraceae	Whole plant	4.03	7.71	8.135	y = 10.865x + 6.1654
Aegeratum conyzoides	Asteraceae	Whole plant	4.12	8.14	8.62	y = 9.9556x + 8.8879
Allium sativum	Amaryllidaceae	Bulb	3.991	7.836	8.319	y = 10.403x + 8.4746
Artabotrys hexapetalus	Annonaceae	Leaves	6.12	11.17	11.80	y = 7.9109x + 1.6092
		Fruits	4.16	7.93	8.40	y = 10.621x + 5.7788
Asclepias curassavica	Apocynaceae	Leaves	5.70	10.35	10.935	y = 8.5465x + 1.5425
Azadirachta indica	Meliaceae	Leaves	5.174	9.276	9.783	y = 9.7498x - 0.4483
Cinnamomum verum	Lauraceae	Leaves	5.59	10.23	10.83	y = 8.5966x + 1.8862
		Bark	4.23	7.86	8.32	y = 11.002x + 3.5004
Fatsia japonica	Araliaceae	Leaves	4.53	8.41	8.89	y = 10.317x + 3.2238
Hibiscus sabdariffa	Malvaceae	Fruit	5.92	10.17	10.70	y = 9.4201x - 5.835
Jatropha gossypifolia	Euphorbiaceae	Fruit, Stem	6.58	11.23	11.81	y = 8.6021x - 6.6671
Lawsonia inermis	Lythraceae	Leaves	4.60	8.40	8.87	y = 10.511x + 1.7346
Oxalis violacea	Oxalidaceae	Whole plant	3.913	7.731	8.208	y = 10.477x + 9
Piper nigrum	Piperaceae	Leaves	4.56	8.43	8.92	y = 10.32x + 2.9442
		Fruits	4.31	7.99	8.46	y = 10.839x + 3.3338
P. longum	Piperaceae	Leaves	4.46	8.41	8.91	y = 10.136x + 4.7233
		Stem, Root	4.04	7.92	8.40	y = 10.319x + 8.28
Pongamia pinnata	Fabaceae	Fruits	4.16	7.88	8.34	y = 10.764x + 5.2208
Plumbago zeylanica	Plumbaginaceae	Stem, Flower	5.69	10.06	10.61	y = 9.1479x - 2.1163
Rauvolfia vomitoria	Apocynaceae	Leaves	4.49	8.42	8.90	y = 10.196x + 4.2213
		Stem	4.20	7.86	8.32	y = 10.942x + 4.0008
Salvia splendens	Lamiaceae	Scarlet Sage red (leaves)	4.38	8.30	8.75	y = 10.299x + 4.89
		Blue Ribbon (leaves)	4.20	7.90	8.40	y = 10.741x + 4.7775
Solanum nigrum	Solanaceae	Root	5.64	10.15	10.70	y = 8.8632x + 0.055
		Fruits	5.10	9.20	9.70	y = 9.8224x + 0.0583
Spilanthes acmella	Asteraceae	Leaves	4.42	8.43	8.88	y = 11.102x – 3.5542
Stemona tuberosa	Stemonaceae	Root	3.78	7.51	7.98	y = 10.745x + 9.2883
		Stem	4.48	8.22	8.68	y = 10.716x + 1.94
		Leaves	4.85	8.44	8.89	y = 11.134x - 3.9463
Syzygium aromaticum	Myrtaceae	Unopened flower buds	4.13	7.79	8.26	y = 10.905x + 4.9442
Vitex negundo	Lamiaceae	Leaves	5.360	9.688	10.229	y = 9.2438x + 0.4442
Zingiber officinale	Zingiberaceae	Rhizome	4.959	8.976	9.478	y = 9.9588x + 0.6058

Based on literature review, other studies have tested 10 of the tested plant species, and the results of this study are in line with their findings. The ethanolic extract of *Ageratum coyzoides* showed significant mortality against *A. aegypti* with the LC_{50} (4.30 mg/ml) (Chude *et al.* 2020). The effects of ether extract of *Piper nigrum* and *Syzygium aromaticum* on *Aedes albopictus* larvae were investigated by Bilal *et al.* (2012). Komalamisra *et al.* (2005) tested *Stemona tuberosa's* ethanolic extract for its larvicidal activity against *Aedes aegypti*. According to Swargiary *et al. (*2019), *Spilanthes acmella's* methanolic crude extract demonstrated remarkable efficacy. The efficacy of an ethanolic extract from *Allium sativum* bulbs against *C. quinquefasciatus* mosquito larvae was also shown by Sharma and Riat (2021). Nakasen *et al.* (2021) studied the larvicidal effect of the oil of *Cinnamomum verum* against the *C. quinquefasciatus* larvae. Likewise, the larvicidal activities of Z*ingiber officinale* against *Culex quinquefasciatus* larvae exhibited to be toxic after a 24 hrs exposure (Rahman *et al.* 2009). However, the larvicidal activity of four species, namely fresh aqueous flower extract of *Acmella radicans*, whole plant extract of *A. uliginosa*, leaf extract of *Oxalis violacea*, and *Rauvolfia vomitoria*'s stem extract against *Aedes* larvae or any mosquito larvae, had not been reported previously. It is also noteworthy that *Spilanthes acmella*, *A. radicans*, and *A. uliginosa*, had 100% larvicidal activity at 10% concentration (Table 2).

Most studies used different chemical solvents to prepare plant extracts. Nonetheless, using synthetic chemicals to control dengue vectors poses serious threats to nature and human health. However, this study employed water as a solvent to protect the environment and human health from chemical pollution. Literature survey illustrated that these 15 plants possess many types of phytochemicals like alliin, allicin, flavonoids, alkaloids, carotenoids, phytosterol, fatty acid, 3-isobutylamides, spilanthol, acmellonate, N-(2-phenylethyl)-2Z,4E-octadienamide, -phenyl-N-(2-phenylethyl)-2-propenamide, coumarin, naphthoquinone, anthracene derivative, saponin, lignan, triterpene, tannin, karanjin, pongamol, lignans, tuberostemonine, stemofolin, cinnamaldehyde, benzyl benzoate, terpenes, chromenes, sterol, steroids, anthraquinones, saponins, and phenolic acids which might be responsible for such larvicidal activity(Mittal *et al.* 2023, Lagnika *et al.* 2016, Farias *et al.* 2020,Yadav *et al.* 2019).

Among the tested plants, *Stemona tuberosa, Oxalis violaceae*, and *Acmella radicans* extracts appeared to be the most effective in terms of LC_{50}, LC_{90}, and LC95 values. There is no previous report on the larvicidal activity of extracts from *A. radicans, Rauvolfia vomitoria, A. uliginosa, Oxalis violacea*, *Artabotrys hexapetalus* and *Pongamia pinnata* against *Aedes.* Thus, the tested plants are a possible source of bioactive compounds and are the potential alternative to mosquito larvicides. These botanical derivatives could potentially reduce mosquito control costs and their environmental impact by substituting synthetic insecticides. However, to achieve this, additional studies are necessary to isolate active compound and *in vivo* studies.

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