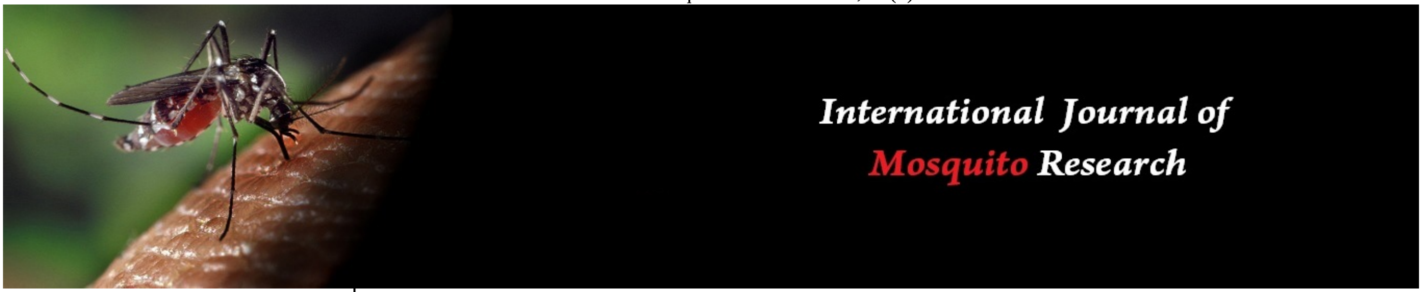

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Andrographis paniculata leaf extracts: A natural mosquito control agent in combating *Aedes Aegypti* and *Culex quinquefasciatus*

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Abstract

Mosquito-borne diseases are a major public health concern worldwide. Plant extracts from *Andrographis paniculata* have anti-mosquito properties. The study evaluated the extracts' larvicidal, pupicidal, and ovicidal activities, and their LC_{50} values were compared between the two mosquito species to determine their relative effectiveness in controlling mosquito populations. The study shows that methanol extracts have promising larvicidal, pupicidal, and ovicidal activities against *Aedes Aegypti* and *Culex quinquefasciatus* mosquito species. Methanol extracts showed promising larvicidal, pupicidal, and ovicidal activities against *Ae. Aegypti* and *Cx. quinquefasciatus* species with LC_{50} values of 86.74 and 84.41 ppm for larvicidal activity, 95.27 and 92.91 ppm for pupicidal activity, and 56.8 and 60.0 ppm for ovicidal activity, respectively. Hexane and chloroform extracts showed good larvicidal activity but not promising pupicidal and ovicidal activities. Further research is needed to determine the active compounds and evaluate their safety and efficacy in the field.

Keywords: *Andrographis paniculate* leaves, *Aedes Aegypti*, *Culex quinquefasciatus*, extracts

1. Introduction

Mosquitoes are vectors of several deadly diseases such as dengue, chikungunya, zika, and malaria [1]. Mosquito control is necessary due to the fact that mosquitoes are known vectors of several deadly diseases that cause millions of deaths and illnesses each year. For example, malaria accounts for over 229 million infections and 409,000 fatalities worldwide annually [2]. Additionally, dengue fever causes an estimated 100 million cases and 25,000 deaths each year [3]. Zika virus, which is transmitted by the *Aedes* mosquito, can cause serious birth defects in infants born to infected mothers [4]. Controlling mosquitoes can be achieved through several methods, including the use of chemical pesticides, biological control, and physical control. Chemical pesticides, such as synthetic insecticides, have been widely used for mosquito control, but their use has been associated with environmental pollution, toxicity to non-target organisms, and the development of resistance in mosquitoes [5]. Therefore, alternative and sustainable methods of mosquito control are needed. Biological control methods involve the use of natural predators and parasites to reduce mosquito populations, while physical control methods include the removal of breeding sites and the use of mosquito nets and screens [6]. In addition, plant-based extracts have been shown to have potent antimosquito activity and may be a promising alternative for controlling mosquito populations [7]. Several studies have reported on the use of crude extracts of different plant species, such as *Azadirachta indica*, *Ocimum sanctum*, and *Vitex negundo*, for the control of mosquito populations [8-9]. These extracts are environmentally friendly, biodegradable, and cost-effective, making them an attractive option for controlling mosquito-borne diseases. In this regard, we report the effectiveness of various extracts from *Andrographis paniculata* leaves in repelling mosquitoes, specifically *Ae. Aegypti* and *Cx. quinquefasciatus*.

Andrographis paniculata (Burm.f.) Wall. Ex Nees is an herbaceous plant that falls under the Acanthaceae family [10].

It is also commonly known as "green chireta" or "king of bitters". The plant is native to India, Sri Lanka, and some parts of Southeast Asia.

It grows up to a height of 30-110 cm and has a stem that is erect, branched, and quadrangular in shape [11]. The plant has been traditionally used for treating various ailments such as fever, sore throat, and respiratory infections. It is also known for its anti-inflammatory, hepatoprotective, and immuno-modulatory properties [12]. Andrographolide, the main bioactive constituent of the plant, has been extensively studied for its pharmacological activities and therapeutic potential [13]. *A. paniculata* has been reported to contain a wide range of bioactive compounds, including diterpenoids, flavonoids, xanthones, and polyphenols. These compounds have been linked to diverse pharmacological effects, including antimicrobial, cytotoxic, antidiabetic, antioxidant, hepatoprotective, and cardiotonic activities [14]. The active compounds responsible for the mosquito control properties of *A.paniculata* extracts have not been fully identified yet. However, some studies have suggested that the flavonoids and terpenoids present in the plant may be responsible for the observed larvicidal and pupicidal activities against mosquitoes [15-16]. Herbal plants have a long history of medicinal use, and modern research has confirmed their therapeutic potential. Numerous pharmacologically active compounds with therapeutic potential have been discovered from various herbal plants worldwide. The objective of the current research is to assess the mosquito larvicidal, pupicidal and ovicidal effects of consecutive extracts (hexane, chloroform, and methanol) obtained from the foliage of plants.

2. Materials and Methods

2.1 Collection of plant material

In April 2020, the A. Paniculata leaves were gathered from Palayamkottai, Thriunelveli, Tamil Nadu, and India. The accuracy and genuineness of the plant material were confirmed and validated by Dr. KN Sunil Kumar, a Research Officer at the Department of Pharmacognosy within the Siddha Central Research Institute in Chennai. A voucher sample was placed in the institute's herbarium (Authentication Code No: A24012304P).

2.2 Extraction of the plant material

A sequential extraction process was employed to extract bioactive compounds from 1 kg of shade-dried leaves of the plant using hexane, chloroform, and methanol as solvents in a Soxhlet apparatus. Successive extractions with each solvent were carried out to obtain a range of compounds with varying polarities. The resulting extracts were then filtered and concentrated using a vacuum rotary evaporator, with the hexane extract yielding 6.7 g, the chloroform extract yielding 34.8 g, and the methanol extract yielding 63.0 g. The desiccated extracts were subsequently preserved in sealed containers at 4 ºC until needed for subsequent utilization. This extraction method allows for the isolation of different bioactive compounds that can be used for a variety of applications.

2.3 Insect Rearing

The Animal Husbandry unit of the Department of Advanced Zoology and Biotechnology at Loyola College in Chennai provided the *Ae. Aegypti* and *Cx. quinquefasciatus* larvae. They were reared in tap water at a temperature of 27.4 °C, relative humidity (RH) of 75-85%, and a photoperiod cycle of 13 hours of light and 11 hours of darkness (L/D). The diet provided to the larvae comprised a mixture of dog biscuits and Brewer's yeast, with a proportion of 3 parts biscuits to 2 parts yeast. The experiment employed third instar larvae for the study [17].

2.4 Tests for evaluating larvae and pupa control methods

The larvicidal and pupicidal activities of different extracts were evaluated following the guidelines of the World Health Organization (WHO) [18]. The assays were conducted using concentrations of 500, 250, 125, and 62.5 ppm, with each concentration tested in quintuplicate for all three activities. The extracts were emulsified in 1.0% aqueous DMSO solution. For the assays, 20 larvae or pupae were introduced to 100 ml of the solution in 150 ml plastic containers. A negative control using 1% aqueous DMSO was included, while temephos was used as the positive control. After 24 hours of incubation, the mortality of the larvae or pupae was recorded. Larvae or pupae were deemed lifeless if they exhibited no noticeable motion upon contact with a glass rod. The percentages of mortality and adjusted mortality were computed utilizing the formulas provided. [19].

Percentage mortality:

$$\frac{\text{No. of dead larvae or pupae}}{\text{No. of larvae or pupae exposed}} \times 100$$

Corrected percentage mortality: $[1- nT/nC] \times 100$

If the mortality rate in the control group is less than 5%, it's advisable to use a formula that accounts for the control group mortality. This formula considers the counts of viable larvae or pupae after treatment (nT) and the number that are alive in the control group (nC) to calculate the percentage mortality. This approach prevents the exaggeration of treatment effectiveness due to low mortality rates in the control group.

2.5 Ovicidal activity

To assess the ovicidal activity, the method described Elango [20] was adapted with minor modifications. The study involved using twenty freshly laid eggs from both *Ae. Aegypti* and *Cx. quinquefasciatus*, and subjecting them to five different doses used in larvicidal and pupicidal activities. The eggs were observed under a compound microscope to evaluate their hatchability. After 120 hours of treatment, the percentage of ovicidal activity was determined by calculating the percentage reduction in the number of hatched eggs, employing the subsequent formula.

Percentage of Ovicidal activity

$$\frac{\text{No. of unhatched eggs}}{\text{Total number of eggs exposed}} \times 100$$

The findings were contrasted with those of the Temephos standard control.

2.6 Statistical Analysis

For the determination of LC_{50} and LC_{90} values, the corrected mortality percentages corresponding to each concentration of larvicidal, pupicidal, and ovicidal data were analyzed through probit analysis using US EPA probit analysis software version 1.5. Statistical significance was established at $p \leq 0.05$, and any observed distinctions were regarded as statistically noteworthy. [21].

3. Results

The results of our study showed that the methanol extract from the plant's leaves exhibited the highest efficacy against *Ae. Aegypti* and *Cx. quinquefasciatus* 3rd instar larvae, pupae, and eggs. Tables 1 and 2 showcase the findings related to the larvicidal and pupicidal activities, respectively.

Table 1: The recorded lethal concentration (measured in ppm) of crude leaf extracts from *A. paniculata* concerning larvae of *Ae. Aegypti* and *Cx. quinquefasciatus* is as follows

Species	Extract	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ^2
			LL	UL		LL	UL			
Ae. Aegypti Larvae	Hexane	126.14	109.58	143.37	401.24	330.96	520.93	2.5±0.2	-0.3±0.5	2.0*
	Chloroform	119.5	35.0	226.3	341.1	191.9	10319.7	2.8±0.5	-0.8±1.1	5.0*
	Methanol	86.74	74.17	98.74	241.34	205.15	300.60	2.8±0.2	-0.5±0.6	3.3*
Cx. quinquefasciatus Larvae	Hexane	120.31	105.44	135.66	344.93	290.10	434.40	2.8±0.2	-0.8±0.5	5.9*
	Chloroform	110.86	96.83	125.09	313.18	264.53	392.22	2.8±0.2	-0.8±0.5	5.9*
	Methanol	84.41	72.24	95.97	228.59	194.99	283.38	2.9±0.3	-0.7±0.6	2.3*

LC_{50} stands for the lethal concentration causing the demise of 50% of the larvae under examination; LC_{90} signifies the lethal concentration resulting in the demise of 90% of the larvae under examination. LL represents the lower limit within a 95% confidence interval, while UL represents the upper limit within the same confidence interval. The significance level for chi-square values is denoted by *$p \leq 0.05$.

Table 2. The lethal concentration (expressed in ppm) of unrefined leaf extracts derived from *A. paniculata* concerning pupae of *Ae. Aegypti* and *Cx. quinquefasciatus* is detailed below

Species	Extract	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope±SE	Intercept±SE	χ^2
			LL	UL		LL	UL			
Ae. Aegypti larvae	Hexane	214.92	182.08	257.61	1077.23	754.90	1852.34	1.8±0.2	0.7±0.4	2.0*
	Chloroform	338.63	169.56	3190.23	1392.01	738.38	19372.39	2.1±0.3	-0.1±0.8	5.8*
	Methanol	95.27	81.64	108.55	281.07	236.71	354.45	2.7±0.2	-0.3±0.5	5.2*
Cx. quinquefasciatus Larvae	Hexane	204.55	173.61	243.68	1002.73	712.33	1682.04	1.8±0.2	0.7±0.4	2.5*
	Chloroform	291.05	149.93	2921.88	1206.88	478.17	1615632.40	2.0±0.4	-0.1±0.9	5.5*
	Methanol	92.91	79.40	105.99	273.95	230.86	345.30	2.7±0.2	-0.3±0.5	4.8*
Cx. quinquefasciatus Larvae	Hexane	204.55	173.61	243.68	1002.73	712.33	1682.04	1.8±0.2	0.7±0.4	2.5*
	Chloroform	291.05	149.93	2921.88	1206.88	478.17	1615632.40	2.0±0.4	-0.1±0.9	5.5*
	Methanol	92.91	79.40	105.99	273.95	230.86	345.30	2.7±0.2	-0.3±0.5	4.8*

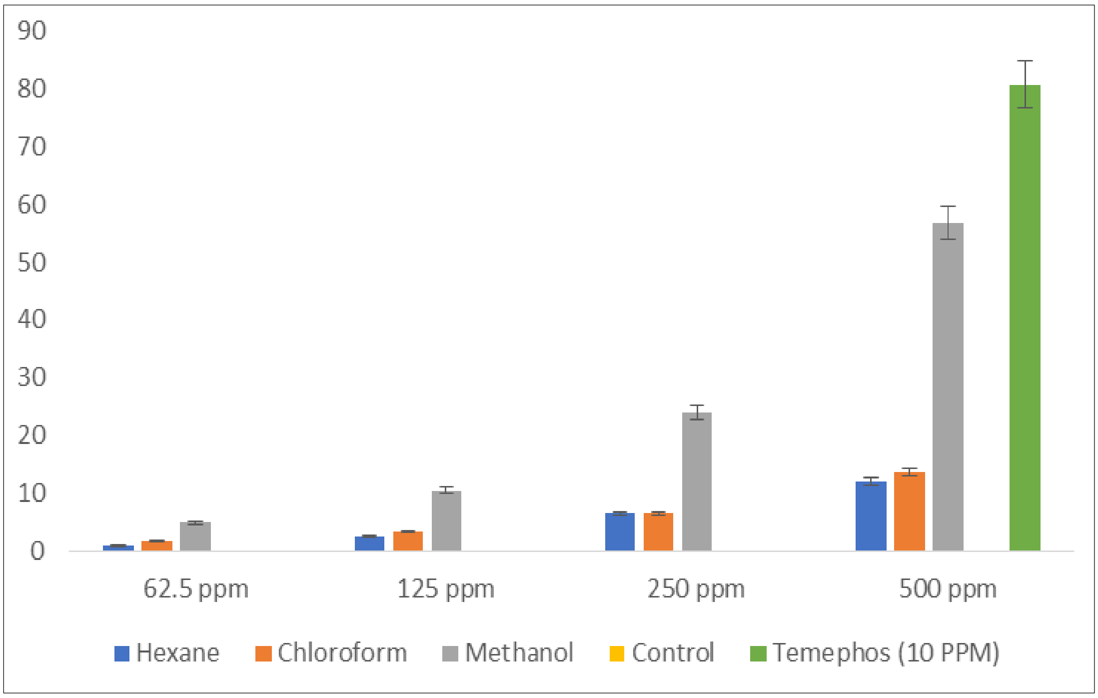

Fig 1: The percentage of ovicidal activity exhibited by crude leaf extracts of *A. paniculata* against eggs of *Ae. Aegypti* is as follows

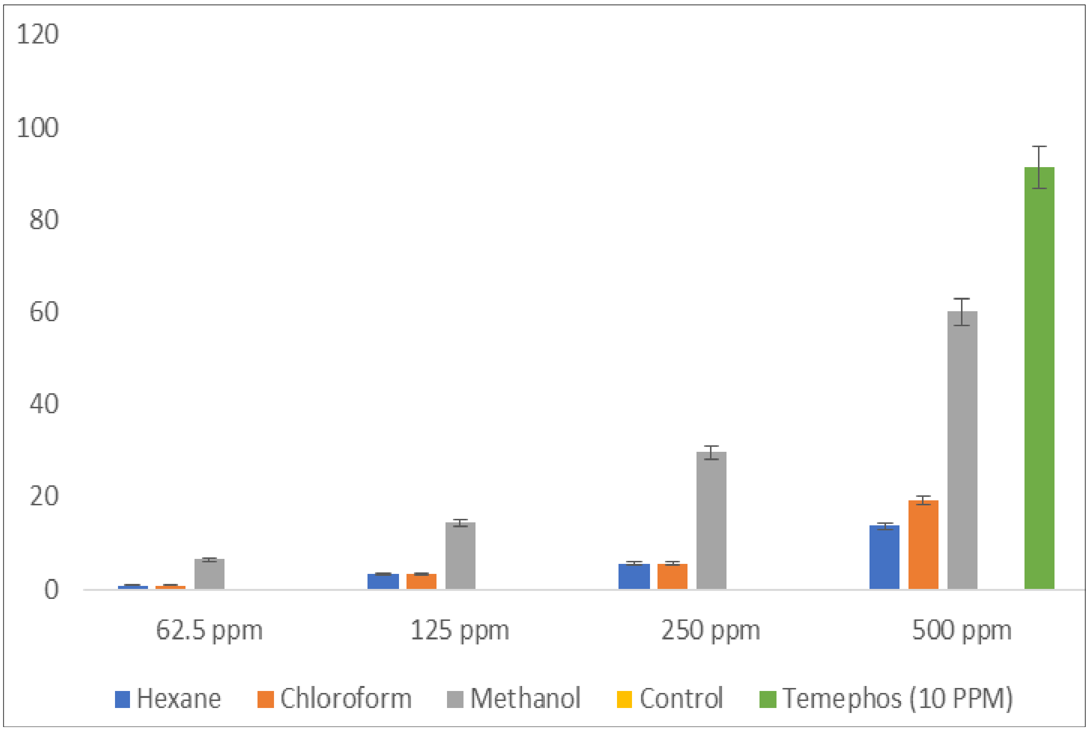

Fig 2: The percentage of ovicidal activity exhibited by crude leaf extracts of *A. paniculata* against eggs of *Cx. quinquefasciatus* is as follows

LC_{50} stands for the lethal concentration causing the demise of 50% of the larvae under examination; LC_{90} signifies the lethal concentration resulting in the demise of 90% of the larvae under examination. LL represents the lower limit within a 95% confidence interval, while UL represents the upper limit within the same confidence interval. The significance level for chi-square values is denoted by $*p \leq 0.05$.

The LC_{50} values for larvicidal activity were 86.74 ppm and 84.41 ppm for *Ae. Aegypti* and *Cx. quinquefasciatus*, respectively, while the values for pupicidal activity were 95.27 ppm and 92.91 ppm for *Ae. Aegypti* and *Cx. quinquefasciatus*, respectively, at 500 ppm concentration. Additionally, the methanol extract also exhibited the highest ovicidal activity, with LC_{50} values of 56.8 ppm and 60.0 ppm for *Ae. Aegypti* and *Cx. quinquefasciatus*, respectively, at 500 ppm concentration. In contrast, the chloroform and n-hexane extracts showed lower activity against the mosquito larvae, pupae, and eggs.

These findings are consistent with the graphical representation in Figures 1 and 2, which further highlight the superior efficacy of the methanol extract compared to the other extracts. Overall, our study suggests that the methanol extract from the plant's leaves holds promise as a natural insecticidal agent for proficiently managing populations of *Ae. Aegypti* and *Cx. quinquefasciatus* populations, while further research is required to uncover the active compounds accountable for these larvicidal, pupicidal, and ovicidal activities.

4. Discussion

A. paniculata is a medicinal plant traditionally used in Asian countries for various ailments. Recently, researchers have shown interest in its potential as a natural mosquito control agent [22]. Mosquito-borne diseases, such as dengue, chikungunya, and Zika, are a significant public health concern worldwide. The use of synthetic insecticides for mosquito control has raised several environmental and health issues, such as the development of insecticide resistance, ecological imbalance, and toxicity to non-target organisms. Therefore, researchers have been exploring alternative methods for mosquito control, such as natural plant-based products [23]. Several studies have investigated the efficacy of *A. Paniculata* leaf extracts as a mosquito control agent against two mosquito species, *Ae. Aegypti* and *Cx. quinquefasciatus*. In vitro and *in vivo* studies have demonstrated the potential of *A. Paniculata* leaf extracts as a natural mosquito control agent [24]. *In vitro* studies have shown that *A. Paniculata* leaf extracts have larvicidal, pupicidal, and adulticidal activity against *Ae. Aegypti* and *Cx. quinquefasciatus*. The extracts have been shown to disrupt the development of mosquito larvae and pupae, leading to their mortality [25]. *In vivo* studies have also confirmed the efficacy of *A. Paniculata* leaf extracts as a mosquito control agent. A study conducted in India found that *A. Paniculata* leaf extracts significantly reduced the larval population of *Ae. Aegypti* and *Cx. quinquefasciatus* in treated areas compared to untreated areas [26]. Furthermore, leaf extracts have been shown to have low toxicity to non-target organisms, such as fish and mammals, making it a safer alternative to synthetic insecticides. The use of leaf extracts as a natural mosquito control agent can also promote sustainable and eco-friendly mosquito control [27]. So, the *A. Paniclaita* leaf extracts have demonstrated larvicidal, pupicidal, and adulticidal activity against *Ae. Aegypti* and *Cx. quinquefasciatus*, making it a potential natural mosquito control agent. Further studies are needed to explore its efficacy in different environmental conditions and its long-term effects on non-target organisms [28].

The results of this study indicate that the methanol extract from the plant's leaves is highly effective against *Ae. Aegypti* and *Cx. quinquefasciatus* 3rd instar larvae, pupae, and eggs, with LC_{50} values of 86.74 ppm and 84.41 ppm for larvicidal activity and 95.27 ppm and 92.91 ppm for pupicidal activity, respectively. These findings are consistent with previous studies that have reported the larvicidal and pupicidal activity of various plant extracts against these two mosquito species [28-29]. Moreover, the methanol extract also showed high ovicidal activity, with LC_{50} values of 56.8 ppm and 60.0 ppm for *Ae. Aegypti* and *Cx. quinquefasciatus*, respectively, at 500 ppm concentration. This is in line with previous studies that have reported the ovicidal activity of plant extracts against these mosquito species [30-31]. In contrast, the chloroform and n-hexane extracts exhibited lower activity against the mosquito larvae, pupae, and eggs, indicating that the active compounds responsible for the larvicidal, pupicidal, and ovicidal activities are more soluble in methanol. This is in line with earlier research that has reported the higher efficacy of methanol extracts compared to chloroform and n-hexane extracts against mosquito larvae [32-33]. From the plant's leaves, the methanol extract showcased actions that were larvicidal, pupicidal, and ovicidal against the mosquito species *Ae. Aegypti* and *Cx. quinquefasciatu*s, outperforming the chloroform and hexane extracts in terms of effectiveness. [34-35].The LC_{50} values obtained for the methanol extract were relatively low, indicating its effectiveness in controlling both the larval and pupal stages of the mosquitoes [36-37]. The observed activities suggest the presence of bioactive compounds in *A. paniculata* that possess insecticidal properties. The effectiveness of *A. paniculata* against mosquitoes can be attributed to the presence of various bioactive compounds such as andrographolide, neoandrographolide, and andrographiside, which have been reported to possess insecticidal properties [38]. Furthermore, the plant has been found to be safe and non-toxic to non-target organisms, making it a promising alternative for mosquito control [39]. Overall, these results suggest that the methanol extract from the plant's leaves has the potential to be used as a natural insecticide for effective control of *Ae. Aegypti* and *Cx. quinquefasciatus* populations. However, further research is needed to identify the active compounds responsible for the observed larvicidal, pupicidal, and ovicidal activities and to evaluate the safety and efficacy of this extract in field trials.

5. Conclusion
The research indicates that methanol extracts sourced from *A. paniculata* exhibit potential in terms of their larvicidal, pupicidal, and ovicidal effects against both *Ae. Aegypti* and *Cx quinquefasciatus* mosquito species. The hexane and chloroform extracts showed good larvicidal activity but not promising pupicidal and ovicidal activities. The results suggest that *A. paniculata* extracts could be a potential source for developing effective and eco-friendly mosquito control agents.

6. Recommendations for future studies
Additional investigation is required to pinpoint the specific active components accountable for the observed effects and to elucidate their mechanism of action. Additionally, safety and efficacy evaluations of the extracts in the field are needed. Investigating the synergistic effect of different plant extracts or combining them with conventional insecticides could be another area for future research. Moreover, the study could be expanded to include other mosquito species of medical importance.

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8. Conflicts of interest
The authors declare that they have no conflict of interest.

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