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Evaluation of mosquitocidal activities of *Rauvolfia serpentina* leaf extract against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract
In order to develop an environment-friendly mosquitocides an alternative to the chemical mosquitocides, extracts were made from the leaves of *Rauvolfia serpentina* with three organic solvents such as methanol (ME), Acetone (AN), and petroleum ether (PE). The objective of the present research is to determine the mosquitocidal activity of *Rauvolfia serpentina* with three different solvents against *Culex quinquefasciatus.* Bioassay tests were carried out by WHO guidelines for determination of mosquitocidal activities. All the solvent extracts showed promising mosquitocidal effect, however the effectivity was found highest in the metholic leaf extract. The present investigation suggests that the possible medicinal use of plant extracts as it provided an excellent potential for controlling *Culex quinquefasciatus*.

Keywords: *Rauvolfia serpentina*, larvicidal, pupicidal, adulticidal, pupal duration, adult duration

1. Introduction
Mosquitoes are the most important single group of insects in terms of public health because they are vectors of serious human diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, and yellow fever. Mosquito-transmitted diseases are a major cause of illness and death in the world, particularly in tropical and subtropical countries [7]. These diseases are also responsible for huge economic losses, both in terms of health care costs and lost productivity [16]. It is estimated that more than 700 million people are infected with mosquito-transmitted diseases annually [27]. Among these diseases, elephantiasis, which is caused by parasites of the genus *Wuchereria* and transmitted by infected mosquitoes of the genus *Culex*, continues to be a major public health problem in tropical and subtropical countries. In 2017, the World Health Organization (WHO) estimated that there were 216 million cases of elephantiasis and 655 000 deaths worldwide.
Throughout the ages, humans have relied on nature for their basic needs for the production of food, shelter, clothing, and means of transportation, fertilizers, flavours, fragrances, and medicines [12]. Plants have been used as sources of medicines since ancient times [22]. A medicinal plant is defined as any plant which can be used to prevent or cure a disease. Fossil records date human use of plants as medicines at least to the middle Paleolithic age, some 60,000 years ago [9]. These medicines took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations [6]. Despite advances in pharmacology and synthetic organic chemistry, the reliance on plants, remains largely unchanged. According to the World Health Organisation (WHO), about 80% of the world's population, primarily those of developing countries rely on plant-derived medicines for their healthcare needs [12]. The specific plants to be used and the methods of application for particular ailments are handed down from generation to generation through oral communication [6].
Plants have been used to repel blood sucking insects since ancient times [13]. The use of plants against biting insects was first recorded among the ancient Greeks [20]. Plant products have been used traditionally to repel or kill mosquitoes in many parts of the world [24]. The repellent properties of plants to mosquitoes and other insects were well known before the advent of synthetic chemicals [14]. Man has used plants as repellents for thousands of years, most simply by hanging bruised plants in houses. Plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away mosquitoes and later as oil formulations applied to the skin or clothes [29]. In this study, we determined the larvicidal, pupicidal, adulticidal, larval, pupal and adult duration, reproductive activity, repellency, and biting deterrency activities of the soluble crude petroleum ether, acetone and methanolic fractions obtained from the leaves of *Rauvolfia serpentina*.

2. Material and Methods
2.1 Collection and preparation of plant extracts
Healthy leaves of *Rauvolfia serpentina*were collected from Nilgiri hills of Tamilnadu, India. The plants were taxonomically identified with the help of experts in the Department of Botany, Govt. Arts College, Udhagamandalam and standard books and voucher specimen deposited. The collected plant materials were washed in tap water, cut into small pieces and air dried. After the plants were completely dry, they have been ground into powder and then macerated in different solvents (acetone, petroleum ether and methanol) at room temperature for 3 days and filtered. The combined filtrate were concentrated to dryness by rotary evaporation at 50^{o}C and kept in a freezer. In preparing test concentrations, each plant extract were volumetrically diluted in different solvents.

2.2. Mosquito culture
Mosquito larvae/eggs of *Culex quinquefasciatus* have been

collected in an around Ooty. The mosquito colonies were maintained at 27 ± 2 ^{0}C, 75-85% relative humidity index a 14:10 light/dark photo period cycle [21].

2.3 Larvicidal and Pupicidal assays

Larvae tested for the present study was obtained from our laboratory culture. Freshly hatched/moulted larvae were used for the bioassay tests. The required quantity of different plant extract concentrations were mixed thoroughly with 200 ml of rearing water in 500ml plastic troughs.

One hundred early fourth instars mosquito larvae were released into each trough. Larvae food consisted of 1g of finely ground dog biscuits per day per trough. Dried coconut midribs were place over water as the substratum for pupation. The plastic trough containing 200 ml of rearing water with different solvents served as the control. Dead larvae and pupae was removed and counted at 24 h intervals. Observations on larval and pupal mortality were recorded. The experiment was replicated five times. Percentage mortality observed in the control was subtracted from that observed in the treatments [1].

LC_{50} and LC_{90} values and their 95% confidence limits were estimated for larval mortality by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each extract [10].

The day from moulting of the larvae to pupation and to adulthood was noted. Fecundity was assessed by counting the number of eggs laid during the life span by control and experimental mosquitoes. The larval and pupal duration of treated and control individuals were compared and developmental rates were determined.

2.4 Adulticidal assay

Culex quinquefasciatus fresh adults were exposing to filter paper treated with different concentration of plant extracts. The paper was keep inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to distilled water with different solvents treated paper and muslin cloth. Mortality count was taken after 24h [25].

2.5 Ovipositional assay

Different quantities of plant extracts from a stock solution were mixed thoroughly with 200 ml of rearing food in 250 ml glass jars to obtain the concentration desired for the tests with *Culex quinquefasciatus*. The gravid females were given a choice between treated and control jars. During the tests, the groups of females were kept separate for 48 h in cages measuring 25 x 25 x 30cm. After the eggs were counted the oviposition activity index (OAI) was calculated using the formula:

$$OAI = \frac{(NC - NT)}{(NC + NT)} \times 100$$

Where NC is the number of eggs in the control
NT is the number of eggs in the treatment

2.6 Ovicidal assay

Culex quinquefasciatus eggs were released in water. The test extracts were added in desired quantities and hatching were observed for one week. The eggs were then exposed to deoxygenated water and the numbers of hatching eggs were recorded. Percentage hatching was compared with the control in which only distilled water with different solvents were used [25].

2.7 Repellency activity

Different concentrations of plant extract were mixed thoroughly with 10ml of goat blood in glass plates. The untreated blood served as the control. Adult females were release into each cage. The number of females landing on the treated blood and untreated blood were recorded. The repellent index of the plant extracts were calculated [21].

2.8 Biting deterrency activity

The percentage protection in relation to dose method was used [28]. Blood starved female *Culex quinquefasciatus* (100 nos), 3 - 4 days old, was kept in a net cage (45x30x45 cm^2). The arm of the test person was cleaned with isopropanol. After air drying the arm, a 25 mc^2 area of the dorsal side of the skin was exposed, the remaining portion was covered by rubber gloves. The plant extracts were dissolved in methanol, distilled water with different solvent served as control. Different concentration of the plant extracts was applied. The control and treated arms was introduced simultaneously into the cage. The numbers of bites was count over 5 minute from 6 pm to 6 am. The experiment was conducted five times. The percentage protection was calculated by using formula:

$$\text{Percentage protection} = \frac{(\text{No of bites received by control arm}) - (\text{No of bites received by treated arm})}{(\text{No of bites received by control arm})}$$

2.9 Statistical analysis

All data was subject to analysis of variance and the treatment mean was separated by Duncan's Multiple Range Test [8]. Statistical analysis was carried out using the (Statistical Package Social Science) SPSS software, version 16.0.

3. Results

The results of leaf extracts of *Rauvolfia serpentina* were screened for their mosquitocidal activity. The larvicidal activity were obtained from bioassay of the crude extracts of acetone, petroleum ether and metholic solvent leaf extracts varied among the concentration and the susceptibility of larval varied with respect to their developmental stages. All the solvent showed moderate toxic effect on the fourth instar larval forms after 24 h of exposure; however the highest mortality was found in leaf metholic extracts with LC_{50} values of 1.17%, 1.35%, 1.58% and 1.84% for I, II, III and IV instars (table 3). The 95% confidence limits of LC_{50} and LC_{90}, chi-square and degree of freedom (df) values were also calculated. In control assays we did not find any significant mortality.

The pupal, adult mortality and adult emergence are reported inTable 4**.** The concentration of plant extracts was increased and the mortality also increased proportionally with dosage. Among the extracts, the highest activity effect was found in metholic extract. It was observed that at every concentration increase, significantly there is an increase in the pupal mortality**.** When screened for adult emergence, there existed

an inverse relation with respect the concentration tested in our study with values ranging from 71%, 56% and 30% for 1%, 2% and 4% concentration of leaf extract.

After the treatment of mosquito *Culex quinquefasciatus,* the treated mosquito's larvae were assessed for their larval duration and the data is represented in Table 5. Summatively, it was observed that the larvae took more time to develop into pupae in all the treatments when compared to the untreated groups - control. Directly we can say that the developmental duration has been extended. Dose – response relationship was determined for plants applied to *Culex quinquefasciatus* in our study. Increase in the concentration of the extracts, increase in the developmental duration, which clearly reveals the dose – response relationship. The duration of larval instars and the total developmental time were prolonged. The possible reason could be a harmonic mimic as per the literature but, on the other hand we are not aware of the exact mechanism to reveal this effect. However, the effect between the solvents follows the order as follows: acetone < petroleum ether < methanol.

The developmental metamorphosis for pupae and adult developmental duration (days) was recorded and presented in Table 6**.** Analysis of the adult *Culex quinquefasciatus* developmental metamorphosis against plant extracts of acetone, petroleum ether and methanol, time taken for total larval and pupal developmental periods (in days) were significantly inhibited. Analysis of acetone extracts of plants with respect to pupal and adult duration (days) signifies that it is concentration dependent (Table 6). Increase in the concentration from 1%, 2% and 4%, results was increase the total pupal duration which signifies a direct relationship with the concentration, at the same time increase in the concentration of plant extracts the total adult duration (days) was decreased which shows an inverse relationship with the concentration gradient.

The data in Table 7 reveals that exposure to plant extracts inhibited overall oviposition in treated bowels and the number of eggs laid were comparatively lower in treated bowels than those in untreated bowls irrespective of the total number of eggs laid both on treated or untreated bowls. At the highest concentrations methanol extracts reduced egg lying when compared to acetone and petroleum ether. Results revealed that the significant difference between the numbers of egg lay in treated and control bowl. Our selected plant had a promising effect on *Culex quinquefasciatus*. When analysing the number of eggs laid after the treatment of plant extracts with acetone, it was observed that at all the concentration, the plant exhibited promising efficient effect on the fecundity (Table 7). To the extent of increase in concentration from 1%, 2% and 4% of *Calendula arvensis* extracts recorded 186, 166 and 129 numbers of eggs respectivelyand the obtained result is presented in Table 7.

Laboratory test results of stages of development of the biological parameters like adult repellency activity (%) and ovipositional deterrency (%) with all the three solvents had a promising effect (Table 8). It was very interesting to note that in comparison with all our treated groups, between the solvents, methanolic extract showed the highest adult repellency (%) with values ranging from 28%, 31% and 64% repellency with 1%, 2% and 4% concentration of leaf extract and ovipositional deterrency (%) with 41%, 57% and 80% for 1%, 2% and 4% concentration. Methanolic extracts of plant represented strong repellent activity against *Culex quinquefasciatus* at 4% concentration. It was found that between the concentration and also between the solvent there exhibited significant differences in all treatments.

In our present study, we also enumerated the larval-pupal intermediate (%) of *Culex quinquefasciatus* with various increasing concentration (1%, 2% and 4%) of acetone, petroleum ether and methanolic extracts (Table 9). Analysis of larval – pupal intermediate (%) of *Culex quinquefasciatus* with acetone extracts showed promising determinable effect. The larval-pupal intermediate of *Calendula arvensis* registered for acetone leaf extracts of 15%, 27% and 38% intermediates, with increasing concentration of 1%, 2% and 4% concentration, similarly for petroleum ether 20%, 33% and 49%, and metholic leaf extract with 26%, 41% and 53%. The analysis clearly indicates that lower concentration of the plants with solvents effectively produced clear morphological growth disruption in the treated mosquito larvae, pupae and adults compared to controls, showed normal structural features.

Effects of plant extracts with experimental solvents against *Culex quinquefasciatus* was examined for biting deterrency and represented in table 9. In this observation, all the crude extracts of solvents gave protection against mosquito bites without any allergic reaction to the test persons and also the biting deterrency activity is dependent on the concentration of the plant extracts. When analysed the effects of acetone extracts against *Culex quinquefasciatus* on biting deterrency, against 1%, 2% and 4% concentrations, it was observed that *Calendula arvensis* extracts recorded that the biting deterrency in all concentration (1%, 2% and 4%) with values of 27%, 38% and 70% for methanolic leaf extract.

4. Discussion

The efficacy of phytochemicals as mosquitocides can vary significantly depending on the type of plant species, plant parts used, age of plant parts, solvent used during extraction as well as upon the available vector species. Sukumar *et al*. (1991) [26] have described the existence of variations in the levels of effectiveness of phytochemicals compound on target mosquito species vs plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, effect on growth and reproduction. Changes in the larvicidal efficacy of the plant extract occurs due to geographical origin of plant as described [5, 30, 23].

It has been shown that the extraction of active biochemical from plants depends upon the polarity of the sovents used. Polar solvent will extract the polar molecules and a non-polar solvent extracts the non-polar molecules. This was achived by using mainly solvents ranging from petroleum ether, acetone and methanolic extracts as done by Anupam Ghosh *et al.* (2012)[3] and has observed the similar result like us following the order of activity as Acetone<Petoleum Ether<Methanol on the leaff extract. Thus our result compromises with his observations.

Maria Ruth *et al.* (2018) [19] worked on larvicidal and ovicidal activities of *Artocarpus blancoi* extracts against mosquito vectors. In their observation they found out that the phytochemical screening of the fractions revealed the presence of aromatic secondary metabolites which include sterols, saponins, glycosides, tannins, flavonoids and alkaloids. Thus further study on the purification of biochemical may help us the screen the phytochemicals of similarity in *Rauvolfia serpentina* on the applications to

assess its effectiveness in the community.

Plant based natural bioinsecticides have been proven to be useful for the control of mosquitoes. Vector control using plant based pesticides is highly preferred than conventional pesticides as their rapid environmental degradation and toxicity is extremely low in non-target organism [4]. The traditional sources of natural pesticides are plants since they have undergone evolutions and adaptations to improve their survival and reproduction against predators [18]. In our study, *Rauvolfia serpentina*leaf extract showed significant dose-dependent larvicidal and ovicidal activities against filarial vector *Culex quinquefasciatus* mosquitos, with methanolic leaf extract having highest and acetone having the least efficacy compared with the solvent extracts, but still acetone extract is able to show promising mosquitocidal activity

Yu (2015) [31] worked with insecticidal properties through production of hydrogen cyanide when metabolized causes the inhibition cyto-chrome-oxidase of mitochondria and other respiratory enzymes. Larvicidal, ovicidal and repellent activities of *Calotropis gigantean* by Kumar *et al.* (2012) [17] also revealed the same result as obtained by us. In the present findings, even though the compounds are not isolated but the oil may have a mixture of different bioactive molecules that may responsible to kill the adult mosquitoes. The present results are in agreement with the report of Andemo *et al.* (2014) [2] has reported 100% adult mortality of *An. Arabiensis* treated with methanol extracts of *M. ferruginea* seeds. Present study percentage mortality of *Culex quinquefasciatus* exposed to solvent extracts may be associated with the nature of phytochemicals and dissolving nature of those chemicals in respective solvents. Earlier the toxic compound rotenone was isolated from the roots of *Rauvolfia vomitoria* and reported by Kimbaris *et al.* (2012) [16]. The rotenoids have been used to prepare insecticides since 1848 Geroge (1980) [11]. The present findings also agreed with the earlier reports that the percentage mortality of *M. ferruginea* may be associated with rotenone. Furthermore, the varying polarities of the plant constituents may have also contributed to the low activity of the acetoned. This may be due to the possibility that the most bioactive compound which demonstrated the highest larvicidal and ovicidal properties is semi polarin nature, thereby rendering the methanolic leaf fractionmost effective.

Table 1: LC_{50} and LC_{90} values of acetone leaf extracts of *Rauvolfia serpentina* against larvae of *Culex quinquefasciatus*

Larval stages	LC_{50} (%)	LC_{90} (%)	95% confidence limit				$\chi^2(df)$	Regression equation
			LC_{50}		LC_{90}			
			LCL	UCL	LCL	UCL		
1st Instar	1.45	5.97	0.99	2.02	3.80	14.39	6.25(3)	Y=-.335+2.083X
2nd Instar	1.70	6.96	1.47	1.95	5.56	15.36	5.21(3)	Y=-.480+2.090X
3rd Instar	2.00	8.72	1.74	2.30	6.85	17.97	5.03(3)	Y=-.361+2.300
4th Instar	2.33	9.11	1.73	3.23	5.88	20.88	5.40(3)	Y=-.798+2.167X

LC_{50}, LC_{90} = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, χ^2 = *Chi*-square value, *DF= degree of freedom,* Significant at P≤0.05, PROBIT = Intercept + BX (Covariates X are transformed using the base 10.00 logarithm).

Table 2: LC_{50} and LC_{90} values of petroleum ether leaf extracts of *Rauvolfia serpentina* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC_{50} (%)	LC_{90} (%)	95% confidence limit				$\chi^2(df)$	Regression equation
			LC_{50}		LC_{90}			
			LCL	UCL	LCL	UCL		
1st Instar	1.34	5.30	0.82	2.00	3.21	16.45	9.01(3)	Y=-.273+2.147X
2nd Instar	1.57	6.28	1.06	2.24	3.92	16.34	7.09(3)	Y=-.415+20125X
3rd Instar	1.85	7.50	1.29	2.62	4.67	18.97	6.44(3)	Y=-.561+2.106X
4th Instar	2.16	8.50	1.54	3.07	5.29	21.47	6.35(3)	Y=-.718+2.152X

LC_{50}, LC_{90} = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, χ^2 = *Chi*-square value, *df= degree of freedom,* Significant at P≤0.05, PROBIT = Intercept + BX (Covariates X are transformed using the base 10.00 logarithm).

Table 3: LC_{50} and LC_{90} values of methanol leaf extracts of *Rauvolfia serpentina* against larvae of *Culex quinquefasciatus*

Mosquito Instar stages	LC_{50} (%)	LC_{90} (%)	95% confidence limit				$\chi^2(df)$	Regression equation
			LC_{50}		LC_{90}			
			LCL	UCL	LCL	UCL		
1st Instar	1.17	4.56	0.64	1.81	2.71	16.80	10.70(3)	Y=-.150+2.171X
2nd Instar	1.35	5.17	0.76	2.15	2.99	21.51	11.94(3)	Y=-.289+2.201X
3rd Instar	1.58	6.15	0.93	2.53	3.51	25.76	11.55(3)	Y=-.429+2.168X
4th Instar	1.84	7.21	1.17	2.89	4.17	26.47	10.09(3)	Y=-.574+2.162X

LC_{50}, LC_{90} = Lethal Concentration, LCL = Lower Confidence Limit, UCL = Upper confidence Limit, χ^2 = *Chi*-square value, *df= degree of freedom,* Significant at P≤0.05, PROBIT = Intercept + BX (Covariates X are transformed using the base 10.00 logarithm).

Table 4: Effect of *Calendula arvensis* leaf extract on *Culex quinquefasciatus*

Mosquito stages	Control	Acetone			Petroleum Ether			Methanol		
		1 %	2%	4%	1 %	2%	4%	1 %	2%	4%
Pupal Mortality (%)	00^{h}	27^{g}	40^{ef}	58^{c}	33^{fg}	45^{de}	60^{b}	38^{f}	50^{d}	77^{a}
Adult Mortality (%)	00^{f}	24^{e}	37^{de}	55^{b}	30^{e}	42^{cd}	65^{a}	35^{d}	48^{bc}	72^{a}
Adult Emergence (%)	100^{a}	71^{b}	56^{de}	30^{f}	66^{bc}	47^{e}	26^{e}	61^{cd}	40^{e}	21^{g}

Within a row means followed by the same letters are not significantly different at 5% level by DMRT

Table 5: Developmental duration of *Culex quinquefasciatus* after the treatment of solvent extracts of *Rauvolfia serpentina*

S. No	Treatment	Concentration (%)	Total larval duration (days)			
			1st Instar	2nd Instar	3rd Instar	4th Instar
1	Control		1.6^{e}	2.9^{g}	3.1^{f}	5.6^{e}
2	Acetone	1	3.2^{d}	3.9^{f}	5.3^{e}	6.0^{d}
		2	3.5^{d}	5.0^{d}	5.4^{e}	7.2^{b}
		4	4.0^{c}	6.4^{b}	6.9^{b}	7.6^{ab}
3	Petroleum ether	1	3.8^{c}	4.3^{ef}	5.5^{de}	5.9^{b}
		2	4.1^{c}	5.4^{d}	5.7^{de}	6.2^{cd}
		4	4.6^{b}	6.8^{a}	7.2^{b}	7.8^{a}
4	Methanol	1	4.2^{bc}	4.7^{e}	5.9^{d}	6.3^{cd}
		2	4.6^{b}	5.9^{c}	6.3^{c}	6.6^{c}
		4	5.2^{a}	7.0^{a}	7.7^{a}	8.0^{a}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

Table 6: Pupal and adult duration of *Culex quinquefasciatus* after the treatment of *Calendula arvensis*

S. No	Treatment	Concentration (%)	Total Pupal duration (days)	Total Adult duration (days)
1.	Control		3.1^{g}	71^{a}
2.	Acetone	1	3.3^{fg}	65^{b}
		2	4.1^{e}	53^{cd}
		4	5.6^{c}	40^{e}
3.	Petroleum Ether	1	3.6^{f}	61^{b}
		2	4.7^{d}	50^{cd}
		4	6.0^{b}	37^{e}
4.	Methanol	1	4.0^{e}	56^{c}
		2	5.2^{c}	47^{d}
		4	6.7^{a}	28^{f}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

Table 7: Effect of solvent extract of *Rauvolfia serpentina* on fecundity and egg hatchability of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Fecundity (days)	Egg Hatchability (days)
1.	Control		248^{a}	99^{a}
2.	Acetone	1	201^{b}	76^{b}
		2	190^{c}	55^{cd}
		4	157^{f}	43^{de}
3.	Petroleum Ether	1	191^{c}	69^{bc}
		2	180^{d}	49^{d}
		4	146^{g}	38^{e}
4.	Methanol	1	186^{cd}	62^{c}
		2	166^{e}	41^{e}
		4	129^{h}	31^{f}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

Table 8: Effect of solvent extract of *Rauvolfia serpentina* on the adult repellency and ovipositional deterrency of *Culex quinquefasciatus*.

S. No	Treatment	Concentration (%)	Adult Repellency (%)	Ovipositional deterrency (%)
1.	Control		00^{g}	00^{f}
2.	Acetone	1	19^{f}	32^{e}
		2	21^{ef}	47^{d}
		4	48^{c}	71^{b}
3.	Petroleum Ether	1	24^{de}	37^{e}
		2	26^{de}	52^{c}
		4	58^{b}	78^{ab}
4.	Methanol	1	28^{de}	41^{d}
		2	31^{d}	57^{c}
		4	64^{a}	80^{a}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

Table 9: Effect of solvent extract of *Rauvolfia serpentina* on larval pupal intermediate and biting deterrency *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Larval - Pupal intermediate (%)	Biting Deterrency (%)
1.	Control		00^{g}	00^{h}
2.	Acetone	1	15^{f}	18^{g}
		2	27^{d}	28^{e}
		4	38^{b}	53^{c}
3.	Petroleum Ether	1	20^{e}	23^{f}
		2	33^{c}	33^{d}
		4	49^{a}	61^{b}
4.	Methanol	1	26^{d}	27^{ef}
		2	41^{b}	38^{d}
		4	53^{a}	70^{a}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

5. Conclusion

In conclusion, the findings of this present study have shown a great effectiveness of *Rauvolfia serpentina*l leaf extracts by different solvents (acetone, petroleum ether and methanol) against the *Culex quinquefasciatus* mosquito species in aquatic stages and adults. Our findings showed that leaf extract of *Calendula arvensis* bioactive molecules to be an effective and can be developed as an eco-friendly larvicidal, pupicidal and adulticidal activities for mosquito vector control. This study suggests that future research work on the use of individual active ingredient to evaluate its mosquitocidal effect on the mosquito species used in this study in semi field and small scale field trials for invention of environmentally safe botanical insecticide for the control of mosquito vectors.

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