International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 08-11-2024, Accepted: 09-12-2024, Published: 24-12-2024
Volume 9, Issue 12, 2024, Page No. 153-159

Phytochemical analysis and larvicidal activity of *Ruellia tuberosa* against the harmful mosquito vectors

Jeyanthi R[1], Kaleena P K[1*], Babu M[2], Velu K[1], Sudha Ravi[1], Janaki A[1]
[1] Department of Zoology, Presidency College (Autonomous), Chennai, Tamil Nadu, India
[2] Department of Microbiology and Biotechnology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

Abstract
There has been an urgent requirement for alternatives since the regular use of synthetic insecticides to control insect pests destabilizes the environment and increases pest and insecticide resistance. Furthermore, growing evidence of synthetic insecticides' detrimental effects on the environment and human health, along with stricter environmental regulations on pesticides, have sparked a renewed interest in the creation and application of bio-insect management products to control pests and mosquitoes. The larvicidal activity of *Ruellia tuberosa* methanol, ethanol, hexane, chloroform, and aqueous whole plant extracts against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciayus* is the main emphasis of this work. It was discovered that *Ruellia tuberosa* worked well against each of the three fourth-instar larvae that were tested. The larvicidal effect of whole plant extracts of *Ruellia tuberosa* on mosquitoes has never been documented before.

Keywords: *Ruellia tuberosa*, phytochemical profiling, mosquito larvicidal activity

Introduction

Worldwide, mosquitoes constitute the biggest threat to public health. More than 100 of the 3492-mosquito species known to exist globally are able to spread a variety of diseases to humans and other vertebrates [1]. Humans can contract chikungunya, dengue fever, yellow fever, filariasis, Japanese encephalitis, and malaria from mosquitoes [2]. Worldwide, but especially in tropical nations, mosquito-borne illnesses are a major cause of disease burden, mortality, poverty, and social debility. Among these diseases, malaria continues to be the most dangerous vector-borne disease, killing between 1.4 and 2.6 million people worldwide each year and impacting between 300 and 500 million people. Over 40% of the world's population resides in malaria-prone areas [3]. Dengue fever can present as either the hemorrhagic type, which frequently results in death, or the classic form, which incapacitates the patient for a week or more [4].

Widespread across the tropics and subtropics is *Aedes aegypti*, the main vector for the viruses that cause dengue fever, dengue hemorrhagic fever, chikungunya, and yellow fever. Since there is currently no viable dengue vaccine, controlling mosquitoes which frequently requires the use of traditional synthetic insecticides—is the only method to lower the disease's frequency. In Southeast Asian and African nations, the Chikungunya virus, a member of the alpha virus genus, poses a serious threat to public health [5].

In many tropical nations, *Culex quinquefasciatus* is the most common mosquito that rests indoors [5]. Reproducing in contaminated waterways, such as soak-age pools near residential areas, clogged sewers, or broken septic tanks. It is an urban vector of *Wuchereria bancrofti*, the causative agent of filarial fever, and a pantropical pest. Due to their ease of control in breeding environments and poor mobility, larval mosquitoes make an appealing target for control operations [6]. In underdeveloped nations, mosquito control measures are a crucial part of illness prevention initiatives. Since mosquitoes are becoming more and more resistant to pesticides. Alternative chemicals for mosquito control have drawn attention, according to Lima *et al*. [7].

Since they are a natural source of many different chemicals, plants are known to contain larvicidal substances that can act alone or in combination. Ghayal *et al*. [8] state that certain phytochemicals interfere with the growth and development, reproduction, and synthesis of olfactory cues that work as repellents or attractants, while others operate as general toxicants against both adult and larval stages of mosquitoes. Since they are less detrimental to the environment and non-target creatures, natural goods are the best choice. A number of substances and extracts from several plant families have been tested for novel and promising larvicides [9]. The requirement that a new insecticide be of plant origin and not negatively impact the ecology has become the main goal in recent years. Secondary chemicals originating from plants, including saponin [10], steroids [11], isoflavonoids [12], essential oil [13], alkaloids, and tannins [14], have been shown by researchers to be efficient mosquito larvicides. An other source of insect repellents is found in plant components and their essential oils [15]. R. tuberose, a member of the Acanthaceae family, is a significant medicinal plant with a variety of traditional therapeutic use.

Around the world, it has been utilized to treat a wide range of medical issues. Additionally, the entire plant and its infusion are thought to have antimalarial, diaphoretic, and antipyretic properties [16]. In addition to being used as a diaphoretic and febrifuge to treat yellow fever, the leaves and flowering tops have been shown to contain lantamine, an alkaloid that resembles quinine [17]. Phenolic compounds have been found in the essential oils of various parts of *R. tuberosa*; the main phytochemical groups are flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpenes, sesquiterpenoides, and tannin. According to reports, the extract of *R. tuberosa* has ovicidal, larvicidal, and pupicidal properties against *A. aegypti* [18]. The current work examined the larvicidal

activity of whole plant extracts from *R. tuberosa* against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* larvae in their fourth instar.

Materials and methods

Selection of plant

The natural population of *R. tuberosa* was gathered from Chennai, Tamil Nadu, India. Prof. P. Jayaraman of the Plant Anatomy Research Centre (PARC/2017/3530), West Tamabaram, Chennai-45, identified and verified the plant. For roughly 20 days, the entire plant was allowed to dry at room temperature in the shade.

Fig 1: *Ruellia tuberosa*

Preparation of plant extract

Using an electric blender, the dried plant was ground into a fine powder and sieved. Using a Soxhlet extractor, 70g of the powder was placed in a thimble and extracted over the course of ten hours using ethanol, methanol, hexane, and chloroform. 50g of plant powder was steeped in 500ml of distilled water for 48 hours in order to prepare the aqueous extract. Using a rotary flash evaporator, all of the extracts were concentrated and stored at 5°C in an airtight bottle until they were needed.

Phytochemical screening

Standard protocol was used for phytochemical screening [19], and Table 1 lists the phytocompounds present in the solvent extracts.

Separation of bioactive compounds using TLC Preparation of extract

For TLC analysis, 10 mg/ml of the extract in ethanol solvent was utilized [20]. The aluminum sheets coated with silica gel 60 F 254 were cut to 1.5 x 5.5 cm. On a silica plate, the prepared methanol extract was loaded and allowed to air dry. The ratio of ethyl acetate to methanol (2: 1; 2:1:1; 0.5) was determined by standardizing the extracts in ethyl acetate with hexane and then with chloroform.

GC–MS analysis

GC-MS was used to identify the phytocompounds in the plant extracts. The following condition is used by a gas chromatography-mass spectrometer (GC–MS) instrument: 30 m x 250 m capillary column running in electron mode at 4.20 e7; helium was utilized as the carrier gas at a steady flow rate of 1.491 ml/min and injection volume of 1.0 ml; the injector was heated to 260 °C and the ion source was heated to 240 °C. The temperature of the oven was set to 60°C. At 4.2 eV, mass spectra were recorded.

Selection of mosquito species

A. aegypti, *A. stephensi*, and *C. quinquefasciatus* were the mosquito species used for this investigation. Dengue fever, chikungunya, and yellow fever are among the illnesses spread by the yellow fever mosquito, *A. aegypti* (Linnaeus). Only the female *A. aegypti* bites in order to obtain blood, which she needs to mature her eggs, making it a vector for the transmission of many trophical fevers. In India, *A. stephensi* is the malaria vector, and its larvae typically inhabit in diverse environments. They spread the filarial worm that causes filariasis and are nocturnal and crepuscular in nature [20]. The West Nile virus, which causes encephalitis or meningitis that damages brain tissue and results in irreversible neurological damage, is spread by *C. quinquefasciatus* [21].

Mosquito culture

A. aegypti, *A. stephansi*, and *C. quinquefasciatus* mosquito larvae in their fourth instar were obtained from the Entomological Research Institute (ERI) at Loyola College in Chennai. *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* raised in laboratories without exposure to pesticides or diseases were the subjects of all tests. The insectariums' cyclic vector mosquito production was kept between 25 and 29 degrees Celsius. Adult mosquitoes were given a 10% glucose solution, while larvae were fed a larval diet consisting of a 3:1 mixture of yeast and powdered dog biscuit. In order to produce eggs, adult female mosquitoes were occasionally blood-fed on confined albino mice [22].

Larvicidal bioassay

For the following bioassays, three trials were conducted against vector mosquitoes, each with five replicates. The larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* in their fourth instar were used in separate toxicity tests of the crude extract. 100 mg of crude extract was dissolved in 1 ml of hexane to create a stock solution (1000 ppm), which was then topped up with 100 ml of distilled water. Twenty fourth-instar larvae were released from various dilutions of 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm made in 200 ml of deionized water, and mortality was measured after 24 hours. The larvae were exposed to 200 ml of water containing 0.1 ml of hexane, which acted as a control, while the beakers were maintained at 28ºC ± 2 oC. Five replications of each treatment were conducted [23].

Larval susceptibility tests

The normal WHO technique was used while conducting the larval susceptibility tests [24]. In order to observe the larvicidal property, larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* in their fourth instar were introduced in each test solution containing extracts of varying quantities. Twenty larvae per group were added to 200 milliliters of the extract solution. Parallel control tests were conducted without extract. Following a 24-hour exposure period, the number of dead larvae in each solution was counted, and the average of five replicates was used to calculate the % mortality. Mortality was calculated using Abbott's [25] model

and adjusted when control mortality was between 5 and 20%.

Statistical analysis

In order to determine LC50, LC90, and other statistics at 95% confidence limits of upper and lower confidence limits, the average larval mortality data was subjected to probit analysis. SPSS 11.5 was used to calculate the chi-square values.

Results

Phytochemical profiling

Table 1 shows the findings of the phytochemical characterisation of *R. tuberosa*. Triterpenoids, saponins, flavonoids, alkaloids, and terpenoids were found to be highly present in aqueous, methanol, and ethanol extracts, according to the preliminary phytochemical screening. Steroids, glycosides, phenols, coumarins, and tannins were among the other phytocompounds found.

Table 1: Phytochemical Screening of whole plant extracts of *R. tuberosa*

S.No.	Secondary Metabolism	Aqueous	Chloroform	Ethanol	Methanol	Hexane
1.	Carbohydrates	+++	++	++	-	++
2.	Tannins	-	-	+	+	-
3.	Saponins	++	+	++	+++	+
4.	Flavonoids	++	+	-	-	-
5.	Alkaloids	-	++	+	+	-
6.	Anthocyanin	+	++	++	+++	-
7.	Quinones	+	-	-	-	+
8.	Glycosides	++	+	-	-	-
9.	Cardiac Glycosides	++	+	+	++	-
10.	Terpenoids	++	+	+	+++	+
11.	Triterpenoids	+	++	+++	++	-
12.	Phenols	-	-	++	+++	-
13.	Coumarins	+	+	-	++	-
14.	Acids	-	-	-	-	-
15.	Proteins	-	-	-	-	-
16.	Steroids	+	+	++	+++	-

+++ Strongly Positive; ++ Positive; + Trace; - Not detected

Mosquito larvicidal activity

R. tuberosa methanolic extract demonstrated 100% mortality at 250 ppm against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* larvae in their fourth instar. All three mosquito species' fourth instar larvae were shown to be similarly susceptible to the effects of ethanol whole plant extract (Table 2, 3, and 4). After 24 hours of exposure, the methanol extract of R tuberosa showed 81% death of *A. aegypti* at a 200-ppm concentration, whereas the ethanol extract demonstrated 68% mortality. In comparison to *A. aegypti* (99.33 ppm and 250.77 ppm) and *A. stephensi* (84.10 ppm and 182.16 ppm), the LC_{50} and LC_{90} values against *C. quinquefasciatus* were 79.59 ppm and 171.30 ppm, respectively. Comparing the methanol extract to the other examined species, it demonstrated strong larvicidal efficacy against *C. quinquefasciatus*. In comparison to methanol and ethanol plant extracts, all other examined extracts exhibited mosquito larvicidal action at a comparatively high concentration. Table 2 and Figure 2 provided the LC_{50} and LC_{90} values.

Table 2: Larvicidal activity of *R tuberosa* against the fourth instar larvae of *A. aegypti*

Extract	Concentratio (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
Aqueous	50	15	141.319 127.155±157.266	437.766 353.067±598.450	4.288
	100	32			
	150	46			
	200	66			
	250	79			
Chloroform	50	12	219.787 188.918±272.124	947.595 628.509±1897.103	1.926
	100	21			
	150	35			
	200	46			
	250	58			
Ethanol	50	18	124.533 85.562±169.641	369.229 241.667±1297.776	8.998
	100	37			
	150	51			
	200	68			
	250	88			
Hexane	50	13	203.538 176.424±246.810	870.805 590.103±1665.923	1.920
	100	24			
	150	36			
	200	49			
	250	61			

Methanol	50	24	99.333 37.734±151.090	250.775 161.680±2105.198	21.638
	100	43			
	150	62			
	200	81			
	250	100			

Control - nil mortality
Significant at $p < 0.05$ level
LC_{50} - Lethal concentration that kills 50 of the exposed larvae
LC_{90} - Lethal concentration that kills 90 of the exposed larvae
UCL - Upper confidence limit
LCL - Lower confidence limit

Table 3: Larvicidal activity of *R. tuberosa* against the fourth instar larvae of *A. stephensi*

Extract	Concentration (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	r^2
Aqueous	50	13	184.961 161.780±219.059	773.410 539.988±1391.337	0.318
	100	28			
	150	41			
	200	53			
	250	62			
Chloroform	50	19	115.861 56.502±178.223	304.505 192.224±2871.915	18.926
	100	37			
	150	54			
	200	72			
	250	96			
Ethanol	50	23	94.550 45.016±135.599	213.127 146.417±776.811	20.283
	100	44			
	150	67			
	200	91			
	250	100			
Hexane	50	10	228.897 196.756±284.129	936.966 627.031±1840.750	1.543
	100	21			
	150	32			
	200	44			
	250	57			
Methanol	50	27	84.109 20.523±131.166	182.160 118.790±1556.645	29.638
	100	52			
	150	71			
	200	100			
	250	100			

Control - nil mortality
Significant at $p < 0.05$ level
LC_{50} - Lethal concentration that kills 50 of the exposed larvae
LC_{90} - Lethal concentration that kills 90 of the exposed larvae
UCL - Upper confidence limit
LCL - Lower co0nfidence limit

Table 4: Larvicidal activity of *R. tuberosa* against the fourth instar larvae of *C. quinquefasciatus*

Extract	Concentration (ppm)	24hr % Mortality	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	$_r2$
Aqueous	50	17	140.725 125.395±158.283	490.904 382.749 ±713.445	4.574
	100	35			
	150	47			
	200	61			
	250	79			
Chloroform	50	14	168.479 149.427±193.705	625.138 463.447±997.968	1.987
	100	28			
	150	42			
	200	56			
	250	69			
Ethanol	50	26	84.622 50.777±112.560	188.144 138.526±384.246	12.911
	100	52			
	150	76			
	200	94			
	250	100			
Hexane	50	7	372.778 285.710±607.630	1961.472 1020.539±7106.519	0.942
	100	15			
	150	22			

	200	30			
	250	41			
Methanol	50	29	79.597 36.881±112.559	171.130 120.226±475.975	19.145
	100	54			
	150	79			
	200	99			
	250	100			

Control - nil mortality
Significant at $p < 0.05$ level
LC_{50} - Lethal concentration that kills 50% of the exposed larvae
LC_{90} - Lethal concentration that kills 90% of the exposed larvae
UCL - Upper confidence limit
LCL - Lower co0nfidence limit

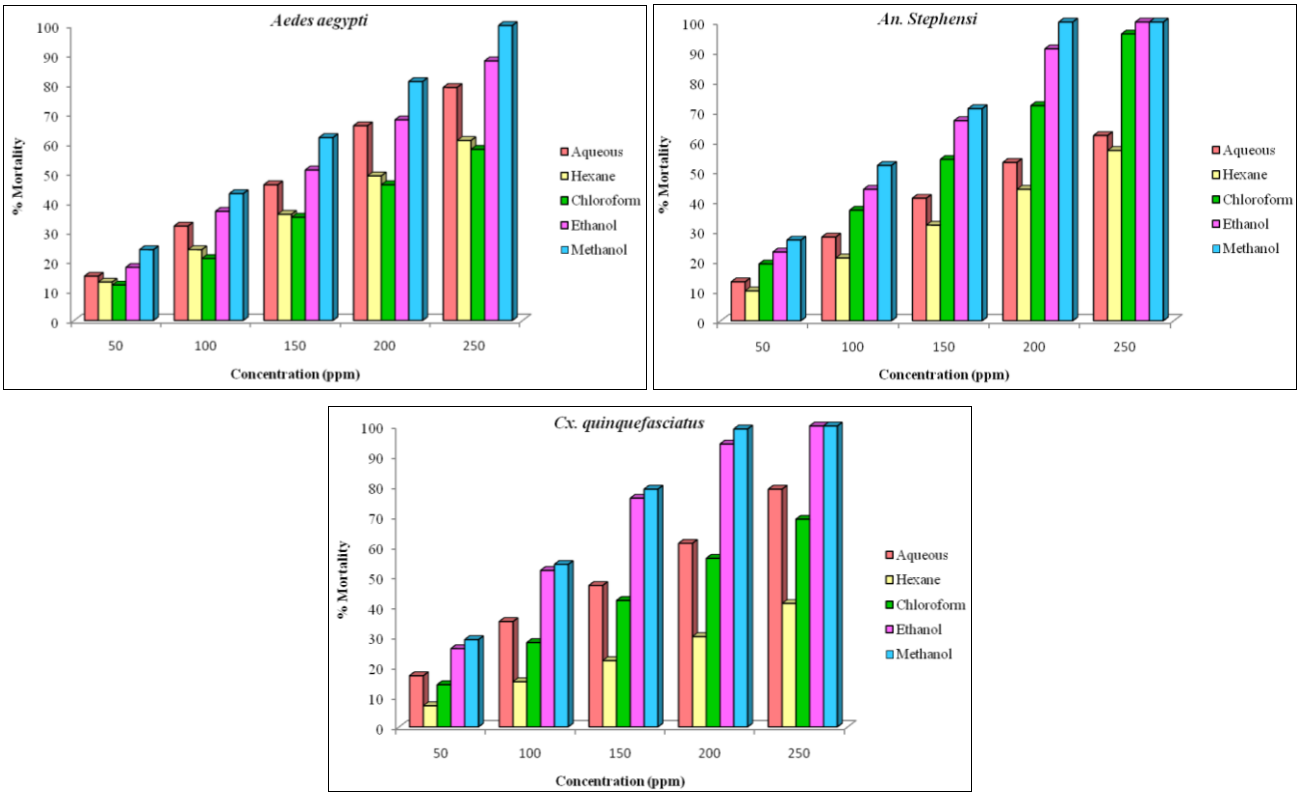

Fig 2: Larvicidal activity of *R. tuberosa* against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*

Table 2 and Figure 3 show the GC-MS characterizations of *R. tuberosa* methanolic extracts. The methanol extract of *R. tuberosa* included 15 components, of which 7 main compounds were identified, including octadecanoic acid, cyclopropyl, hexadecanoic acid, caryophyllene, lycoxanthin, pentol, cholestan, and others (Fig. 3).

Table 5: GC-MS analysis of methanol extracts of *R. tuberosa*

S. No	Retention Time	Compounds	Molecular Formula	Molecular Weight
1	8.39	3-[4-Acetoxybutyl]-2-Oxazolidinone	$C_9H_{15}O_4N$	201
2	8.51	1,3,2-Dioxaborinane, 2-Ethyl-5,5-Dimethyl	$C_7H_{15}O_2B$	142
3	8.56	Propanenitrile, 2-Hydroxy	C_3H_5ON	71
4	8.60	Cyclohexanecarboxylic Acid, 2-Amino-, Trans	$C_7H_{13}O_2N$	143
5	8.69	Cyclohexanecarboxylic Acid, 2-Amino-, Cis	$C_7H_{13}O_2N$	143
6	8.73	3-[N-Aziridyl] Propionaldehyde Semicarbazone	$C_6H_{12}ON_4$	156
7	8.85	2-Acetylamino-2-Cyano-Acetamide	$C_5H_7O_2N_3$	141
8	9.02	5-Methyl-2-Hexene, C&T	C_7H_{14}	98
9	9.14	Cyclohexanamine	$C_6H_{13}N$	99
10	9.19	Cis-3-Ethylidene-1-Vinyl-2-Pyrrolidone	$C_8H_{11}ON$	137
11	9.22	Cyclohexanamine	$C_6H_{13}N$	99
12	9.27	Oxirane, 2-(1,1-Dimethylethyl)-3-Ethyl-, CIS-	$C_8H_{16}O$	128
13	9.39	2-Methyl-2-Hexyl Methylphosphonofluoridate	$C_8H_{18}O2FP$	196
14	9.56	3-[N-Aziridyl] Propionaldehyde Semicarbazone	$C_6H_{12}ON_4$	156
15	9.59	Cyclohexane, (1,1-Dimethylethyl)-	$C_{10}H_{20}$	140

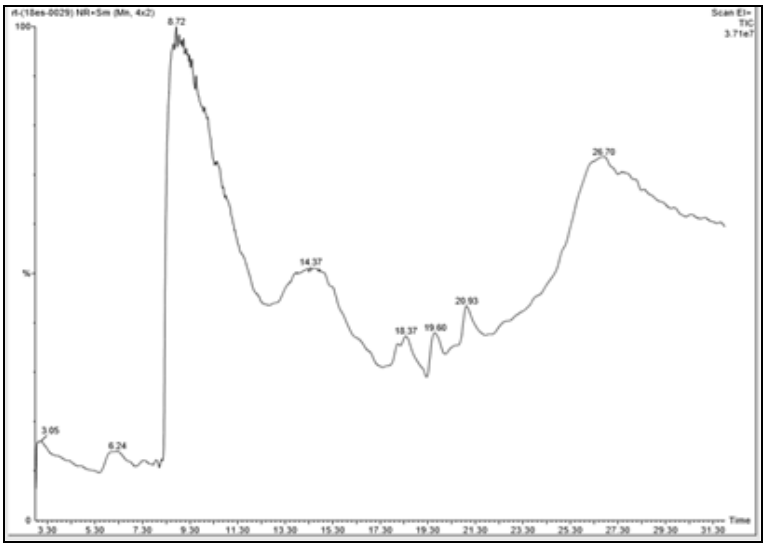

Fig 3: GC-MS analysis of methanol extract of *R tuberosa*

TLC was used to further analyze the phytocompounds found in the plant extracts (Fig. 4). *R. tuberosa* methanolic extract had seven bands when seen with iodine. R*f* values of 0.67, 0.62, 0.50, 0.46, 0.39, 0.31, and 0.24 for the chemicals found in bands 1 through 7 indicated the presence of saponins, quinones, and steroids, respectively.

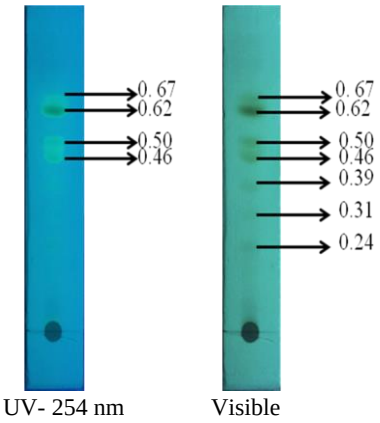

Fig 4: Separation of bioactive compound from methanol extract of *R. tuberosa* by TLC

Tables 2, 3, and 4 shows the results of a probit analysis comparing the quantities of plant extracts against the fourth instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* following a 24-hour exposure. The findings unequivocally show that *R. tuberosa* plant extract was harmful to all three tested mosquito species at extremely low doses. In comparison to *A. aegypti*, which had LC_{50} and LC_{90} values of 92.55 ppm and 194.73 ppm, respectively, the methanolic plant extract was shown to be more effective against *C. quinquefasciatus* and *A. stephensi*, with LC_{50} and LC_{90} values of 87.35 ppm and 182.68 ppm and 99.97 ppm and 214.10 ppm (Fig 3).

Discussion

One important aspect of controlling vector-borne illnesses is the use of larvicidal chemicals to control mosquito larvae. When it comes to controlling mosquito species at the community level, plants are seen to be a viable and preferable alternative to larvicides. Although several plant extracts have been proven to have mosquitocidal or repellent properties against mosquito vectors, only a small number of plant items have demonstrated usefulness in controlling mosquitoes [26]. Comparing *R. tuberosa*'s methanolic extract to those of *A. aegypti* and *A. stephensi*, the former demonstrated 100% larvicidal action against *C. quinquefasciatus* larvae in their fourth instar [23].

The majority of individuals agree that triterpenoids have larvicidal properties against mosquitoes [27]. Terpenoids and triterpenoids are hydrocarbons found in the extract that block the developmental phases of insects, which may be the cause of the significant death rate seen in this study [28, 29]. Numerous studies have noted the various effects of phytochemicals produced from plants, which can function as larvicides, insect development regulators, repellents, and ovipositor attractants. Appetitiser, anemia, aphrodisiac, anti-inflammatory, brain-tonic, cardiotonic, cerebral disorders, diabetes, expectorant, obesity, syphilis, tuberculosis, ulcers and wounds, astringent, sweet, appetizer, digestive, carminative aphrodisiac, antiphlogistic, antiseptic, tonic, and emollient are among the properties mentioned by Dravyaguna [30, 31, 32]. rubifacient, brain tonic, diuretic, and used to treat rheumatism, gonorrhea, nervous system disorders, and the mosquito-repelling properties of *R. tuberosa*.

The findings of the current study provide more proof that agricultural weeds have a major impact on mosquito population reduction. To sum up, the plant extracts from *R. tuberosa* that have been investigated have the potential to be used in conjunction with the present methods of controlling *Aedes aegypti* and *Anopheles stephensi*. The extract might be sprayed in areas of stagnant water, which serve as mosquito breeding grounds and are known to transmit a wide range of infectious illnesses. Therefore, in the future, the plant extracts may be employed as a strong insecticidal agent.

Conclusion

Plant products are clearly becoming a viable source for controlling the number of mosquito larvae. It is therefore possible to employ the plant *R. tuberosa*'s crude extracts in stagnant water bodies, which are known to serve as mosquito breeding grounds. Extracts from *R. tuberosa* weed demonstrated encouraging results in controlling mosquitoes, and commercial use is definitely possible.

References

1. Rueda LM. Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in fresh water. Dev. Hydrobiol,2008:595:477-487.
2. Nour AH, Elhussein SA, Osman NA, Nour AH, Yusoff MM. A study of the essential oils of four Sudanese accessions of basil (*Oimum basilium* L.) against *Anopheles* mosquito larvae. American Journal of Applied science,2009:6:1359-1363.
3. Ghai OP, Gupta P. Essential preventive Medicine: a clinical and applied orientation.5, Ansari Road, New Delhi, 2000, 969.
4. Neves-Filho RAW, Silva CA, Silva CSB, Navarro DMAF, Santos FAB *et al*. Improved microwave-mediated synthesis of 3-(3-aryl-1,2,4-oxadiazol-5-yl) propionic acids and their larvicidal and fungal growth inhibitory properties. Chemical Pharmaceutical Bulletin 2009; 57:819-825.
5. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a Taqman RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses. Journal of virology methods,2005:124(1-2):65-71.
6. Howard AFB, Zhou G, Omlin FX. Malaria mosquito control using edible fish in Western Kenya: preliminary findings of a controlled study. BMC Public Health,2007:7:199-204.
7. Lima EP, Oliveira Filho AM, Lima JWO, Ramos-Junior AN, Cavalcanti LPG, Pontes RJS. Resistencia do *Aedes aegypti* ao temefos em municipios do Estado do ceara. Journal of the Brazilian Society of Tropical Medicine,2006:39:259-263.
8. Ghayal N, Padhye A, Dhumal K. Larvicidal activity of invasive weeds *Cassia uniflora* and *Syndrella nodiflora*. International Journal of Pharmaceutical and Bio Sciences,2010:1:3.
9. Ester Innocent, Cosam CJ, Nicholus KG, Manien JM, Mayunga HH, Nkunya Ahmed H. Mosquito-larvicidal constituents from *Lantana* Viburnoides varkisi (A. rich) verde (*Verbenacea*). Journal of Vector Borne Diseases,2008:45:240-244.
10. Wiseman Z, Chapagain BP. Larvicidal effects of aqueous extracts of *Balanties aegyptiaca* (desert date) against the larvae of *Culex pipens* mosquitoes. African Journal of Biotechnology,2005:4(11):1351-1354.
11. Chowdhurry N, Ghosh A, Chandra G. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. BMC Compement Altern Med,2008:8:10.
12. Joseph CC, Ndoile MM, Malima RC, Nkunya MH. Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from *Neorautanenia mitis*. Transactions of the Royal Society of Tropical Medicine and hygiene,2004:98(8):451-455.
13. Cavalcanti ESB, Morais SM, Lima MAA, Santana EWP. Larvicidal activity of essential oils from Brazilian plants against *Aedesaegypti* L. Memórias do Instituto Oswaldo Cruz,2004:99:541-544.
14. Khana VG, Kannabiran K. Larvicidal effect of *Hemidesmus indicus, Gymnema sylvestre* and *Eclipta prostrate* against *Culex quinquefasciatus* mosquito larvae. African Journal of Biotechnology,2007:3:307-311.
15. Yang YC, Le EH, Lee HS, Lee DK, Ahn YJ. Repellency of aromatic medicinal plant extracts *Aedes aegypti*. Journal of American Mosquito Control Association,2004:20(2):146-149.
16. Watt JM, Breyer-Brandwijk MG. The Medicinal and poisonous plants of southern and Eastern Africa (2nd Ed). Livingstone, London, 1962, 1049-1050.
17. Burkill IH. A dictionary of the economic products of the Malay Peninsula, Kuala Lumpur: Ministry of Agriculture and Co-operatives,1966:I-II:2444.
18. Weenen H, Nkunya MH, Brag DH. Anti-malarial activity of Tanzanian medicinal plants. Planta Medica,1990:56:368-370.
19. Harborne JB. Phytochemical Methods a Guide to Modern Technique of Plant Analysis. 2nd Edn, Chapman and Hall, London, 1984, 282-311.
20. Azevedo NA, Campos LFP, Ferreira HD. Chemical variability in the Burkill, H.M., 1995. The useful plants of West Tropical Africa. (families J-L) Rayol Botanic Gardens: Kew,2001:3:6-8:14-16:562-564.
21. Hubalek Z, Halouzka J. West Nile Fever a reemerging mosquito-borne viral disease in Europe. Emer. Infect. Dis,1999:2:519-529.
22. Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthus* (L.) Schrad (cucurbitaceae) whole plant extracts against *Anopheless stephensi, Aedesaegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Current Research,2011:3(4):296-304.
23. Tonk S, Bartarya R, Maharaj Kumari K, Bhatnagar VP, Srivastava SS. Effective method for extraction of larvicidal component from leaves of *Azadirachta indica* and *Artemisia annua* Linn. Journal of Environmental Biology,2006:27(1):103-105.
24. World Health Organization. Guideliness for laboratory and field testing of mosquito larvicdes. WHO, Geneva, 2005.
25. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology,1925:18:265-267.
26. Sun R, Sacalis JN, Chin CK, Still CC. Bioactive aromatic compounds from leaves and stems of Vanilla fragrans. Journal of Agricultural and Food Chemistry,2006:49:51-61.
27. Gbolade AA. Plant derived insecticides in the control of malaria vector. In: Phytomedicines in malaria and sexually transmitted diseases: Challenges for new millennium, edited by C.O. Adewunmi and S.K. Adesina, Drug research and production unit, Faculty of Pharmacy, Obafemi, Awolowo University, lle-lfe, Nigeria, 2000, 48-50.
28. Peerzada N. Chemical composition of the essential oil of *Hyptis suaveoluns*. Molecules,1997:2:165-168.
29. Azevedo NA, Campos LFP, Ferreira HD. Chemical variability in the Burkill, H.M., 1995. The useful plants of West Tropical Africa. (families J-L) Rayol Botanic Gardens: Kew,2001:3:6-8:14-16:562-564.
30. Dravyaguna Department. Ayurveda College, Thiruvananthapuram, Kerala, India.
31. Krishnaveni A, Santh RT, Pharmacognostical and Preliminary Phytochemical Studies of *Argyreia nervosa* Burm, Ethnobotanical Leaflets,2009:13:293.
32. Das PN, A Hand Book of Medicinal Plants, Jodpur: Agrobios, 2003.