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Lethal efficacy of phytochemicals as sustainable sources of insecticidal formulations derived from the leaf extracts of Indian medicinal plants to control Dengue and Zika vector, *Aedes aegypti* (Dipetra: Culicide)

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Abstract

Aedes aegypti mosquitoes transmitted the Dengue and Zika viruses to humans, which have recently caused the high morbidity and mortality worldwide. Vaccine and antiviral therapies for dengue and Zika infections are not available, and control of mosquito vectors is a specific strategy that minimizes the occurrence of these arboviral infections. The present research was aimed at exploring the larvicide and pupicidal properties of leaf extracts of three medicinal plants (Lantana camara, Catharanthus roseus, and Ficus religiosa) with acetone solvent against the immature stages of Aedes aegypti. The powdered plant material (leaf) of each plant was extracted using acetone. At 24 h post-exposure, the aqueous leaf extract measured at concentrations of 100, 200, 300, 400 and 500 ppm against Aedes aegypti larvae and pupae. All the three medicinal plant species were evaluated had possessed a different range of larvicidal and pupicidal property. Highest larvicidal and pupicidal activities were exhibited by Catharanthus roseus (LC_{50} ranged from 78.56–228.63ppm and LC_{90} ranged from 132.88 – 288.61ppm) compared to Lantana camara (LC_{50} ranged from 198.52–309.64ppm and LC_{90} ranged from 256.24–392.27 ppm) and Ficus religiosa (LC_{50} ranged from 223.25–339.16ppm and LC_{90} ranged from 289.3–419.42ppm). Catharanthus roseus leaves showed the highest larvicidal and pupicidal activities have contained alkaloids (catharanthine, tabersonine and ajmalicine). It can be used as an eco-friendly, repellent or anti-feeding and target-specific approach to control dengue and zika vector, Aedes aegypti. Further, advanced monitoring of the mode of intervention by the phytoconstituents, synthetic analogues, and field-based research is important for preparing strategies in the Aedes vector management programs.

Keywords: *Lantana camara*, *Catharanthus roseus*, *Ficus religiosa*, Acetone solvent, Lethal concentration, *Aedes aegypti*.

Introduction

Dengue and Zika arboviral diseases transmitted by the *Aedes* mosquito genus, belonging to the Diptera family Culicidae have recently become a serious public health concern, with an increase in morbidity and mortality in tropical and subtropical regions around the world[1-3]. In India, the first case of dengue fever (DF) was documented in West Bengal in 1963, while Zika viral (ZIKV) infection was documented from Ahmedabad of Gujarat in 2016 and thereafter, two more cases of ZIKV were reported from Ahmedabad of Gujarat in 2017[4-5].

According to the report on the World Health Organization (WHO), India alone contributes to 15% of the global dengue burden[6], while the ZIKV burden has limited to India. In India, the state of Odisha has hyper-endemic to DF; and alone has reported more than 4.5 percent of DF cases in the country. In Odisha, the first case of DF reported in 1998 and thereafter, several epidemics were precipitated due to dengue viral (DENV) infection that causes an increment in the incidence and the case fatality rate (CFR). In 2011, there was a major dengue outbreak in the Angul district of Odisha, accounting for 33 cases of death[7].

Both DENV and ZIKV share similar symptomatology, which makes the diagnosis more difficult. The most common clinical symptoms of these infections are fever, headache, retroorbital pain, nausea, vomiting, muscle pain, arthralgia, diarrhea, and fatigue; besides a non-specific constitutional symptom exanthema (skin rashes) can be misdiagnosed as measles[8-9]. Proper treatment and control of these arboviral infections are very much complex because of the non-availability of vaccine and antiviral therapies. Several clinical attempts are under evaluation for development of the dengue and Zika vaccine candidates[10]. Thus, vector control remains the specific method of management and prevents the prevalence of these arboviral diseases. Multifarious approaches being adapted to control the vectors interrupt in the chain of arboviral disease transmission.

The strategy of environmental management alongside larvicides and pupicides has been applied for the control of mosquito's (immature stages). Bacillus thuringiensis israelensis (Bti) is a biocontrol agent used for eliminating their habitat. Adulticides has applied either as a residual surface spraying or as space spraying in indoors or outdoors to control the adult mosquitoes with the use of chemical insecticides, including organophosphates, carbamates, and synthetic pyrethroids.

However, mosquitoes were developing genetic resistance to synthetic organic chemical insecticides[11] and also the synthetic insecticides and the biocontrol agent have affected the environment by contaminating air and water[12]. There is, therefore, a desperate perception that the new mosquito controls strategies can address insecticide resistance or reduce the use of such chemicals in mosquito species, which is more potent, eco-safe and low-cost.

In recent years, the natural insecticides derived from the plant products that may equivalent to the synthetic insecticides are biodegradable and delays in developing resistance of mosquito vectors because of its Phytochemicals structures and metabolism characteristics, which has none hazardous effect on the ecosystem and less toxic that safe for human health and can use in vector management programs[13]. Insecticidal properties of 2,000 species of sublunar plants have reported[14].

Among these, mosquitocidal activity of different products of 344 plant species has been reported and some of them have been used against different species of mosquitoes in field base trials[15]. However, systematic studies on evaluation of biological activities of the ethnomedicinal plants in dengue vector control in Odisha, India haven't been taking on till date.

The aim of this study was to explore the insecticidal properties (larvicidal and pupicidal) of extract derived from the leaves of *Lantana camara*, *Catharanthus roseus*, and *Ficus religiosa* against the immature stages of *Aedes aegypti* (Dipetra: Culicide) as the target species. These sublunary plants were selected based on their medicinal value since time immemorial and their easy availability across the Odisha state in eastern India throughout the year. It therefore presents the promising results of this study in a report that development of bioactive chemical compounds derived from the medicinal plant source as a new natural, sustainable and cheap mosquitocidal agent for the control of *Aedes* mosquito.

Materials and methods

This study was carried out as an operational research. The study population comprised the three sublunary plants, i.e., *L. camara*, *C. roseus*, and *F. religiosa*; and larvae and pupae of *Aedes aegypti*.

Plant species: The plants were selected for the study according to their availability, phytochemical properties, and practical usage by means of the conventional knowledge of local people and culture.

Lantana camara: *Lantana camara* is a shrub and herbaceous plant rising upto 1.6–6.6 ft in height (Figure-1). The common names of these are ' Shrub verbenas or Lantanas ' and locally called 'Puttu'. The shape of the leaf is ovate and has a distinctive smell when crushed. The flowers are small tubular in form, each of which has four petals, arranged in clusters of terminal stems. The flowers appear in many colors, along with red, yellow, green, pink and orange, depending on the location of the inflorescences, age, and maturity. Fruits are a berry-like drupe that turns from green to dark purple when ripe[16].

Catharanthus roseus: *Catharanthus roseus* is a perennial subshrub and herbaceous plant rising upto 3.3 ft height (Figure-2). The common names of these are 'Madagascar periwinkle or Rosy periwinkle' and locally called 'Sada Bihari'. The leaves are glossy green, hairless, with a light midrib and a small petiole, and elongated oval shape (2.5–9cm long and 1–3.5cm wide). The flowers have five spreading petal-like lobes in white to dark pink, with a basal tube and a corolla[17-18].

Ficus religiosa: *Ficus religiosa* is a large, dry seasonal, deciduous or semi-evergreen tree rising upto 98 ft height with a trunk diameter of upto 9.8 ft. (Figure-3). The common names of these is 'Peepal tree' and locally called 'Ashwattha'. The shape of the leaf is cordate (10–17cm long and 8–12cm wide) with a petiole (6–10cm long). The fruits are small figs of 1–1.5cm in diameters and the colour looks a green ripening to purple[19].

Preparation of plant extracts: Disease free leaves of *L. camara*, *C. roseus*, and *F. religiosa* were collected for the study. They were washed with water and dry in the laboratory under normal environment condition. The dried leaf was grinded with the electric blender and filtered through the strainer.

Preparation of aqueous extraction: Leaf powder (500 gm) soaked in 1.5 liters of acetone (C_3H_6O) solvent for 9 hours and the mixture filtered using the filter paper. Filtrate kept evaporated under vacuum evaporator to get the extract from dried form. Dried residues obtained were 60gm (*L. camara*), 53 gm (*C. roseus*), and 55gm (*F. religiosa*) of acetone extracts; and stored at 4°C in an airtight desiccators for further analysis.

Mosquito culture (Immature stages): Larvae and pupae of the *Aedes aegypti* were morphological identified[20] (Figure-4, and 5, and Table-1). The larvae of *Aedes aegypti* were collected using standard dippers from different breeding habitats in and around the Angul district Odisha. Larvae were maintained for generations of the laboratory conditions, i.e. temperature of 27±2°C and relative humidity (RH) of 75%–85%. Pupae collected into a bowel containing water and placed in the mosquito cages. The larvae and pupae were maintained in the same condition as above and available for the experiments. The same condition as above was maintained for the larvae as well pupae and available for the experiments.

Larvicide and pupicidal bioassay: Based on the WHO protocol, the larvicide and pupicidal bioassay of leaf extracts of *L. camara*, *C. roseus*, and *F. religiosa* plants have been evaluated[21]. Larvicidal bioassay was performed with *Aedes aegypti* larvae inculcating of first, second, third and fourth instars; and pupicidal bioassays were performed with *Aedes aegypti* pupa. The acetone leaf extract of *L. camara*, *C. roseus*, and *F. religiosa* was tested at 100, 200, 300, 400 and 500ppm concentrations. Control test with acetone diluent to water was conducted. Twenty larvae and pupae were used at each concentration of treated samples. No food offered during the experimental period. Mortality and survival rates were recorded at 24 h post-exposure. Six replicates were maintained at a time for each experiment. The results of the experiment (mortality percentage) were corrected by Abbott's formula[22].

Mortality (%) = $\frac{X - Y}{100 - Y} x\, 100$

X is the mortality (%) in the treated sample and Y the mortality (%) in the control test.

Statistical Analysis: IBM-SPSS Statistical version 22 was used for probit analyses of average mortality of larvae and pupa *of Aedes aegypti* at 24 hours of post-exposure to the aqueous extract of three medicinal plants (leaf) with acetone solvent to get the lethal concentration of 50% (LC_{50}) and lethal concentration of 90% (LC_{90}). 95% fiducial limits including of the upper confidence limit (UCL) and lower confidence limit (LCL) values were calculated for rapid determination of lethal concentration.

Results and discussion

Table-2 presents the scientific classification and ethnobotanical data of the three medicinal plants, such as *L. camara*, *C. roseus*, and *F. religiosa* used for larvicide and pupicidal toxicity evaluation[16-19]. The preliminary screening results of phytochemical properties of the leaf extract from these selected plants showed that Quinine ($C_{20}H_{24}N_2O_2$), Lantadene ($C_{35}H_{52}O_2$) and Icterogenin ($C_{35}H_{52}O_6$)**;** and Catharanthine ($C_{21}H_{24}N_2O_2$), Tabersonine ($C_{21}H_{24}N_2O_2$) and Ajmalicine ($C_{21}H_{24}N_2O_3$) compounds are alkaloids present in *Lantana camara* and *Catharanthus roseus*, respectively; whereas, Quercetin ($C_{15}H_{10}O_7$), Myricetin ($C_{15}H_{10}O_8$) and Kaempeferol ($C_{15}H_{10}O_6$) compounds are flavonoids present in *Ficus religiosa* (Figure-1, 2, 3).

The lethal efficacy of *L. camara*, *C. roseus*, and *F. religiosa* evaluated at 100, 200, 300, 400 and 500 ppm concentrations against the larvae and pupa *of Aedes aegypti* at 24 hours of post-exposure. The larval mortality rate increased as the treated concentration increased. Mortality of 100% observed at 500 ppm concentrations of *L. camara*, *C. roseus* and *F. religiosa* against the larvae (Different instars) and pupa of *Aedes aegypti*. No mortality observed in control experiment (Table-3).

Conspicuous morphological deformities were occurring among the larvae and pupa of *Aedes aegypti* exposed to the leaf extracts of *L. camara*, *C. roseus* and *F. religiosa* with acetone solvents. larvae and pupa of *Aedes aegypti* survived through treatment showed significant behavioral changes were observed inability to come to the surface, viz., Plate-1a: Dechitinized larvae with a damaged digestive tract; Plate-1b: Exuvia of the proceeding instar attached to the dead larvae; Plate 1c: Death of larval stage with no intiation of pupation and Plate 2a: Malformations like demelanized pupae with a straight abdomen; Plate 2b: Dwarf pupae with a retarded abdomen; Plate 2c: Pupae with some melanization (brown pupa) (Figure-6).

Table-4 presents the bioassay values of these three plant extracts against larvae and pupa of *Aedes aegypti*. LC_{50} and LC_{90} values leaf extrat of *L. camara*, *C. roseus* and *F. religiosa* against larvae (I^{st}, II^{nd}, III^{rd} and IV^{th} instar) of *Aedes aegypti* were 198.52, 78.56, 223.25; 230.12, 90.28, 258.43; 258.07, 138.42, 297.77; 284.18, 180.11, 313.86; and 256.24, 132.88, 289.34; 302.40, 158.18, 328.56; 338.18, 192.96, 368.78; 355.21, 240.42, 406.01ppm, respectively. LC_{50} and L C_{90} values leaf extract of *L. camara*, *C. roseus* and *F. religiosa* against pupae of *Aedes aegypti* were 309.64, 228.63, 339.16; and 392.27, 288.61, 419.42ppm, respectively. Of these three plant extracts, *C. Roseus* was found to have the most effective larvicide and pupicidal activity in relation to *L. camara* and *F. religiosa* against the dengue and Zika vector, *Aedes aegypti*.

Discussion: Mosquitoes are the vector for numerous human pathogens. These are the deadliest carriers of these diseases that cause life; and it causes the high morbidity and mortality rates[23,24]. *Aedes aegypti* mosquito is the etiologic agent of agent of vector-borne diseases, especially dengue, and Zika. Vaccine or specific treatment for dengue and Zika hasn't been available till date. Therefore, vector control is the main strategy to minimize dengue incidence. But the vector control strategy becomes a failure because of development of resistance to synthetic insecticides in adult mosquitoes. The advantage of controlling mosquitoes at immature stages is, however, that they cannot escape from their breeding sites until the adult emerges. Nowadays, the 24-hour bioassay is a key tool for assessing the toxicity of phytotoxins, and plant extracts of different plant species have been evaluated for their toxicity to mosquitoes[25-27]. Phytochemical compounds of plants include alkaloids and flavonoids which possess herbal and pesticidal characteristics and are also responsible for insecticidal and toxic effects on other organisms[28]. It may be used as an effective alternative to synthetic insecticides, since they are safe, inexpensive and widely available[13]. Screening local mosquito control plants will minimize dependency on costly imported products[29]. The present study discovered the toxicity of leaf extracts of three medicinal plants using acetone solvent that shown larvicide and pupicidal activity against dengue and Zika vector *Aedes aegypti*.

In this study, leaves of three selected medicinal plants, i.e., *Lantana camara*, *Catharanthus roseus* and *Ficus religiosa* for screening were found to possess phytochemical properties, such as alkaloids and flavonoids[16-19]. The lab-based assesment report of Remiya and Logaswamy during 2010 revealed that, the LC_{50} value leaf extracts of *C. roseus* and *L. camara* against larvae (IInd and IVth instars) and pupa of *Aedes aegypti* were found 75.31, 203.49; 156.85, 230.76; and 207.83, 281.35 ppm, respectively[30]; and the report of Hemalatha *et al.* during 2014 revealed that, the LC_{50} and LC_{90} value leaf extracts of *L. camara* plant using acetone solvents against larvae (IV^{th} in star) and pupa of *Aedes aegypti* were found 60.64 and 184.72ppm, respectively[31]. The toxicity results of these studies did not corroborate the results our present study and there was a mild variation in the lethal concentration of leaf extracts *C. roseus* and *L. camara* species using acetone solvent against the larvae (II^{nd} and IV^{th} instars) and pupa of *Aedes aegypti* mosquito at 24 hr post-exposure. This is due to the behavioral changes of vector mosquitoes and variation in the ecological condition.

The results of present study showed that the highest rates of larval and pupal mortality were observed in *C. roseus* (LC_{50} ranged from 78.56 – 228.63ppm and LC_{90} ranged from 132.88 – 288.61ppm) compared to *L. camara* (LC_{50} ranged from 198.52 – 309.64ppm and LC_{90} ranged from 256.24 – 392.27ppm) and *F. religiosa* (LC_{50} ranged from 223.25 – 339.16ppm and LC_{90} ranged from 289.34 – 419.42ppm), consistent with the results of the study carried out in Tamil Nadu in 2010, it was found that larvae (II and IV instars) and pupae mortality were higher when exposed to *C. roseus* in relation to *L. camara*[29]; and the reports of Deppa et al. in 2015, it was found that the leaf extract of *F. religiosa* with methanol solvent exhibited highest larvicidal activity with LC_{50} and LC_{95} value of 111.14 and 155.42, respectively against larvae (IIIrd instar) of *Aedes aegypti*[32]. Toxic effects from phytochemical compounds to mosquito larvae depend on factors such as plant quality, plant material quality, ecological condition and chemical solvents, which may be the source of variability in the lethal response[33].

The findings got in the present investigation point out that, the highest potency natural mosquito larvicidal and pupicidal efficacy was noticed in the leaf extracts of *C. roseus* species. This is because of the presence of catharanthine, tabersonine, and ajmalicine compounds, which are alkaloids with their anti-juvenile hormonal activity[17, 34]. Our findings are consistent with the findings of the study conducted in Mexico in 2015, which found that alkaloids and flavonoids were overwhelming in hexanic and ethyl acetate seed extracts of *Argemone mexicana* and hexanic stem-bark extracts of *Pseudosmodingium perniciosum* are good candidates for dengue vector control as they were highly toxic to larvae[35].

To prevent the deleterious effects on non-target organisms caused by chemicals used to control Zika and dengue vectors, natural products of plant origin may be used as an alternative control measure[36]. This study proposed a new, promising alternative biopesticide derived from leaf extracts of medicinal plants, which is developed year-round from local flora and can be incorporated into ongoing mosquito control programmes. Biological control of plant extracts is cheaper than traditional chemical control by mosquito management. The biological control comprising plant extracts is cheaper than conventional chemical control using for mosquito management. The medicinal plant extracts act as bio-friendly, elevated-cost phytochemicals, more adequate and specific targets for mosquito control[37].

Table-1: Morphology and morphometry of *Aedes aegypti* Larvae.

Size (width and length) of the head, neck, thorax and abdomen of larvae instars (I-IV) of *Aedes aegypti*.

Regions	Parameters (mm)	Larval stage (instars)			
		Ist	IInd	IIIrd	IVth
Head	Width	0.235	0.392	0.640	0.987
	Length	0.260	0.380	0.608	0.868
Neck	Width	0.096	0.151	0.254	0.464
	Length	0.064	0.066	0.087	0.108
Thorax	Width	0.248	0.491	0.749	1.401
	Length	0.222	0.409	0.639	1.107
Abdomen	Width	0.152	0.293	0.487	0.858
	Length	1.199	0.080	3.009	5.119
Total	Length	1.745	2.935	4.343	7.202

Size (width and length) of various larval organs of larvae instars (I-IV) of *Aedes aegypti*.

Organs	Parameters (mm)	Larval stage (instars)			
		I^{st}	II^{nd}	III^{rd}	IV^{th}
Siphon	Width	0.127	0.178	0.277	0.389
	Length	0.217	0.452	0.645	0.792
Anal papilla	Width	0.021	0.042	0.076	0.135
	Length	0.148	0.222	0.389	0.649
Pecten teeth	Number	4.68	10.79	14.11	19.42
	Length	0.011	0.015	0.021	0.061
Comb	Number	4.4	9.05	9.8	10.6
Spine	Length	0.012	0.018	0.036	0.132
Antenna	Length	0.078	0.116	0.166	0.258
Median brush	Width	0.026	0.056	0.108	0.166
Lateral brush	Length	0.081	0.149	0.191	0.274

Table-2: Scientific classification and ethno botanical data's of the three medicinal plants studied.

Species	Genus, Family and Order	Plants part use	Local name	Common name	Medicinal values
L. camara	*Lantana*, Verbenaceae, Lamiales	Leaves	Puttu	Shrub verbenas or Lantanas	Leaves are used for antimicrobial, fungicidal and insecticidal properties. It's used as herbal medicines for treating a variety of ailments, including cancer, skin itches, leprosy, rabies, chicken pox, measles, asthma and ulcers.
C. roseus	*Catharanthus*, Apocynaceae, Gentianales	Leaves	Sada bihari	Madagascar periwinkle or Rosy periwinkle	Leaves, roots and shoots are used for antimicrobial, fungicidal and insecticidal properties. It's used as herbal medicines for treating a variety of diabetes, malaria, Hodgkin's lymphoma and leukemia.
F. religiosa	*Ficus*, Moraceae, Rosales	Leaves	Ashwattha	Peepal tree	It's management of various types of disease like respiratory disorders, sexual disorders, central nervous system disorders, cardiovascular, gastric problems, skin infections and diabetes etc.

Table-3: Larvicidal and pupicidal efficacy of the leaf extracts of three medicinal plants were preservation in acetone solvent against the immature stages of Zika and dengue vector, *Aedes aegypti*.

Plant	Immature stages of *Ae. aegypti*	Larval/ pupal mortality					
		Control	Effective Concentrations (ppm)				
			100	200	300	400	500
L. camara	Ist instar larvae	0	5	10	21	20	20
	IInd instar larvae	0	4	9	18	20	20
	IIIrd instar larvae	0	4	8	16	20	20
	IVth instar larvae	0	4	7	15	20	20
	Pupae	0	3	6	14	18	20
C. roseus	Ist instar larvae	0	13	20	20	20	20
	IInd instar larvae	0	11	20	20	20	20
	IIIrd instar larvae	0	7	14	20	20	20
	IVth instar larvae	0	6	11	20	20	20
	Pupae	0	4	9	19	20	20
F. religiosa	Ist instar larvae	0	4	9	19	20	20
	IInd instar larvae	0	4	8	16	20	20
	IIIrd instar larvae	0	3	7	15	20	20
	IVth instar larvae	0	3	6	13	18	20
	Pupae	0	3	6	13	17	20

Table-4: LC_{50} and LC_{90} values of the leaf extracts of three medicinal plants were preservation in acetone solvent against the immature stages of Zika and dengue vector, *Aedes aegypti*.

Plant	Immature stages of *Aedes aegypti*	LC_{50} ppm	95% Fiducial limit		LC_{90} ppm	95% Fiducial limit		Slope
			LFL	UFL		LFL	UFL	
L. camara	Ist instar larvae	198.52	172.31	227.58	256.24	226.53	289.35	1.392475
	IInd instar larvae	230.12	202.16	261.72	302.40	269.83	338.05	1.127973
	IIIrd instar larvae	258.07	228.41	291.47	338.18	303.86	376.02	1.120609
	IVth instar larvae	284.18	252.86	319.02	355.21	319.97	393.91	1.122620
	Pupae	309.64	276.44	345.44	392.27	355.10	432.79	1.110475
C. roseus	Ist instar larvae	78.56	62.54	97.34	132.88	111.35	156.79	1.212661
	IInd instar larvae	90.28	73.26	110.62	158.18	135.24	184.64	1.194256
	IIIrd instar larvae	138.42	116.85	163.03	192.96	166.73	221.16	1.165396
	IVth instar larvae	180.11	155.59	208.30	240.42	211.53	272.36	1.145155
	Pupae	228.63	200.29	259.59	288.61	256.63	323.25	1.129925
F. religiosa	Ist instar larvae	223.25	195.62	254.26	289.34	257.58	324.31	1.130568
	IInd instar larvae	258.43	228.41	291.47	328.56	294.40	365.48	1.121533
	IIIrd instar larvae	297.77	265.12	332.77	368.78	332.30	407.58	1.113575
	IVth instar larvae	313.86	280.22	349.66	406.01	368.41	447.47	1.109082
	Pupae	339.16	304.81	377.07	419.42	380.78	461.10	1.106094

LC = Lethal Concentration, LFL = Lower Fiducial limit, UFL = Upper Fiducial limit.

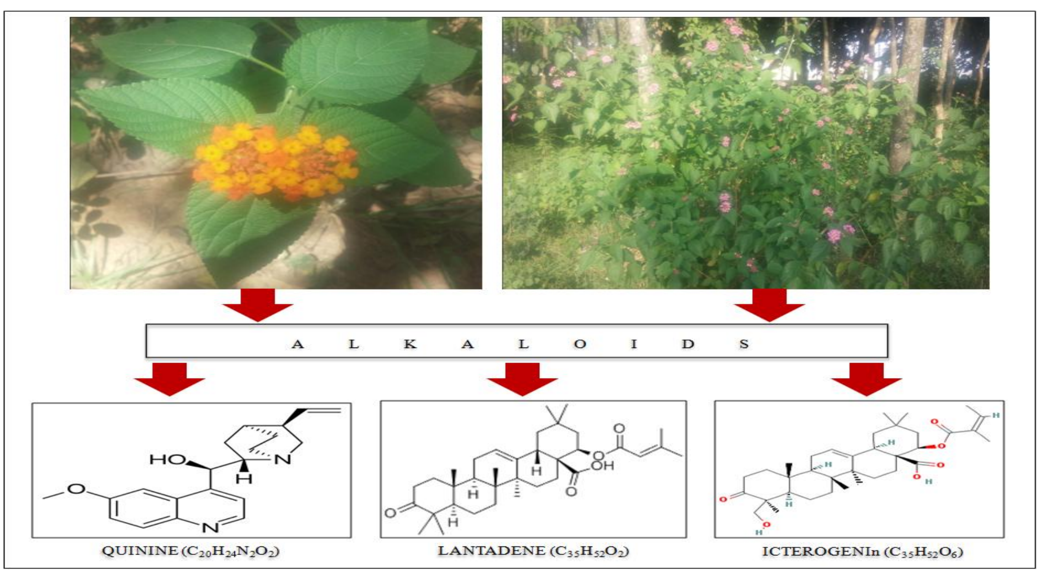

Figure-1: *Lantana camara.*

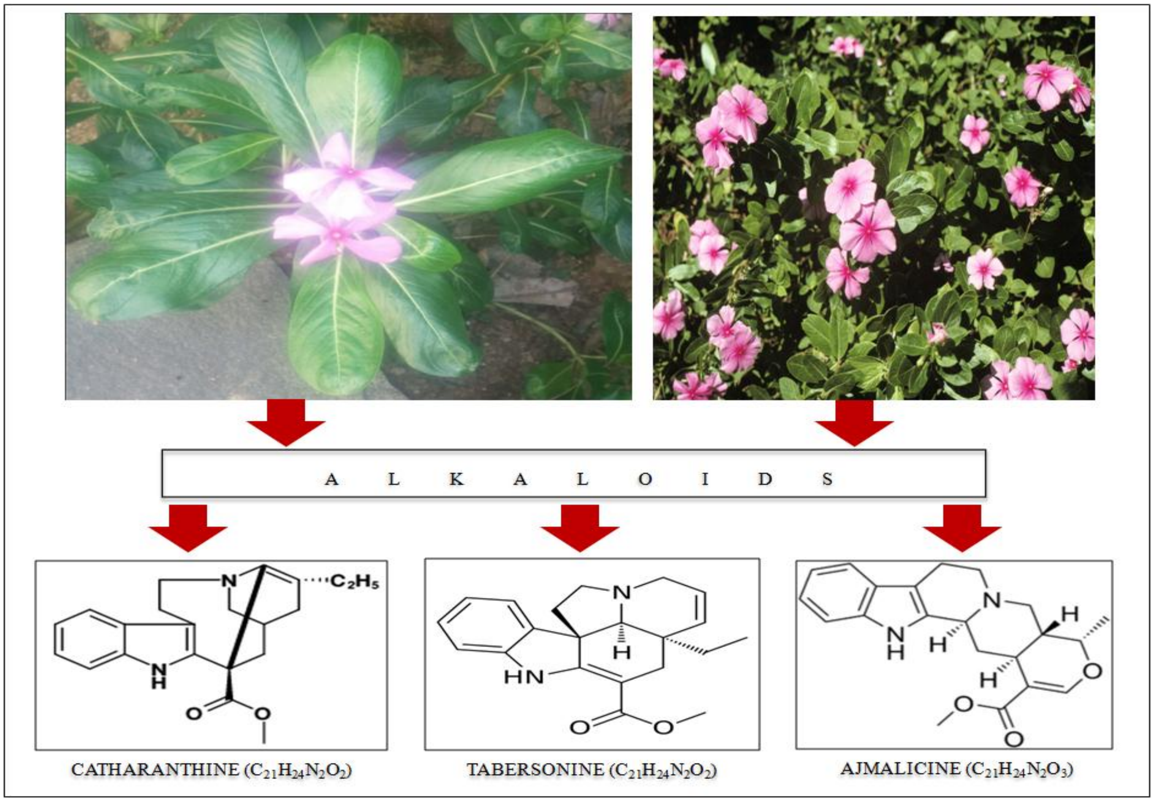

Figure-2: *Catharanthus roseus.*

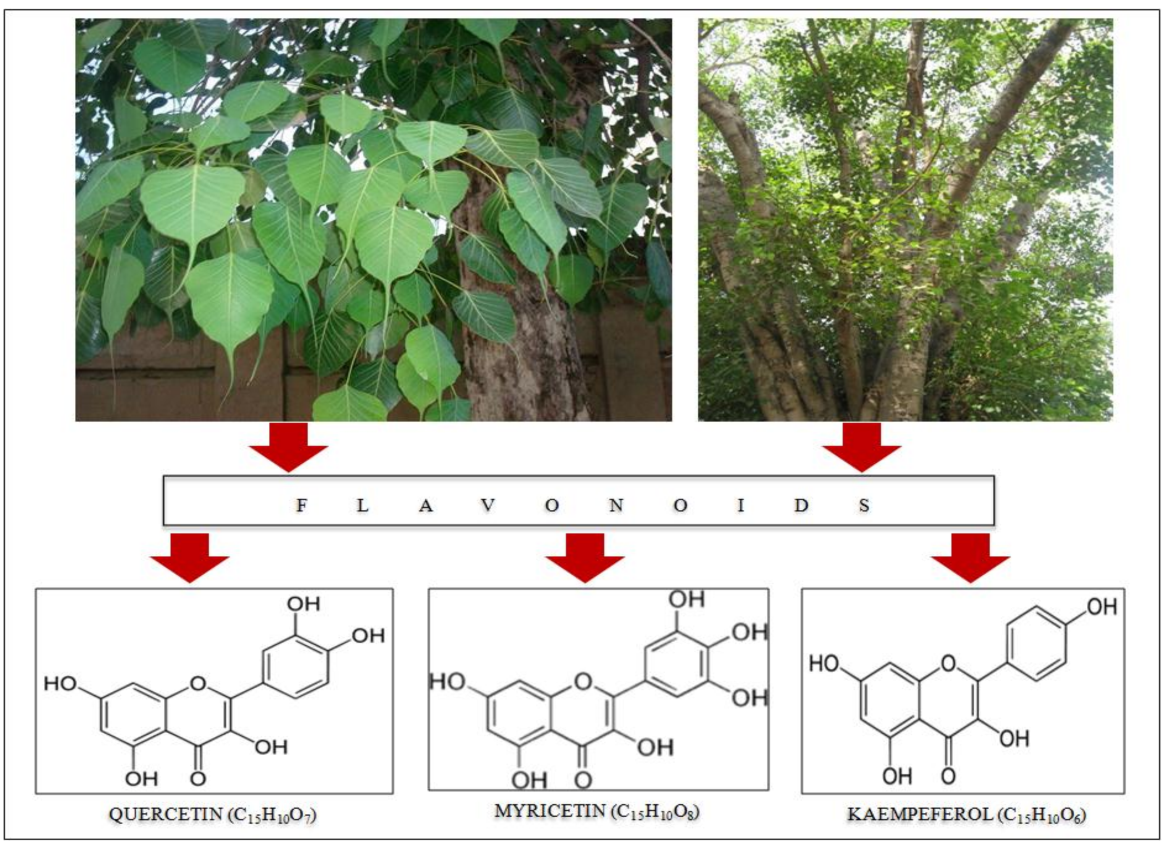

Figure-3: *Ficus religiosa.*

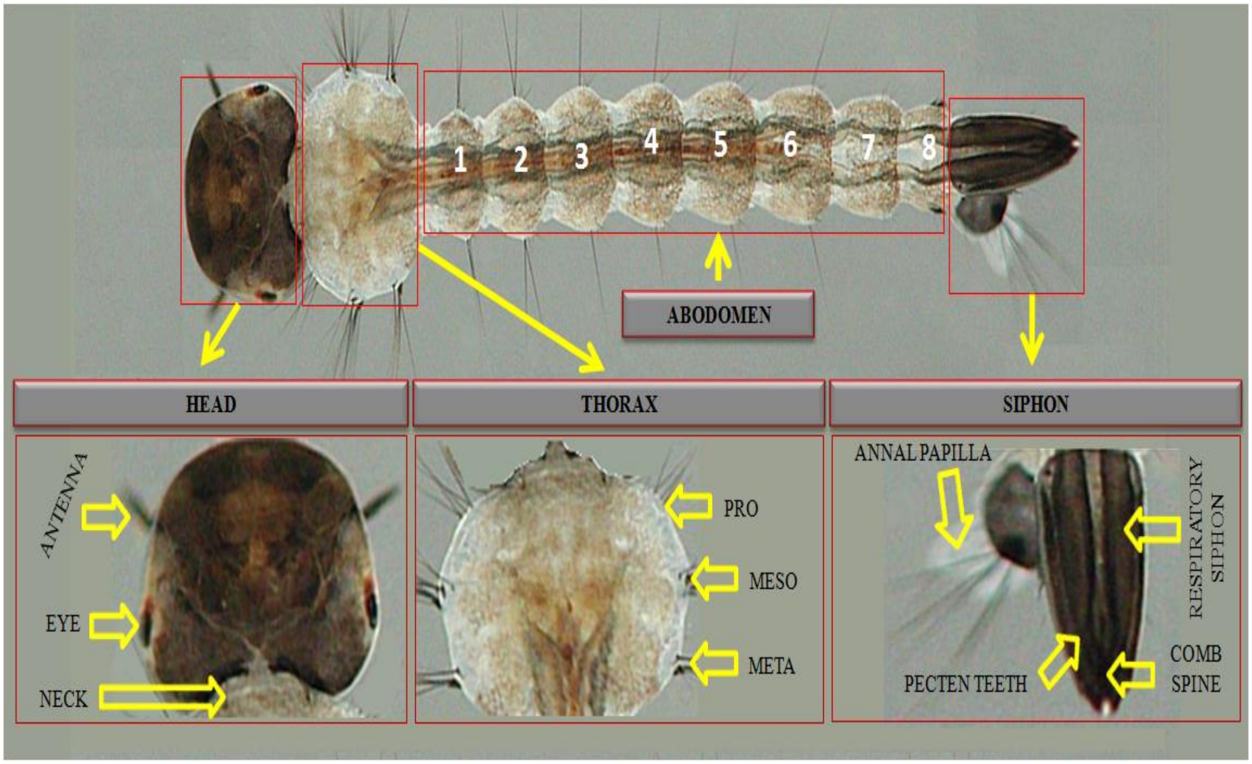

Figure-4: Morphology and morphometry of *Aedes aegypti* Larvae.

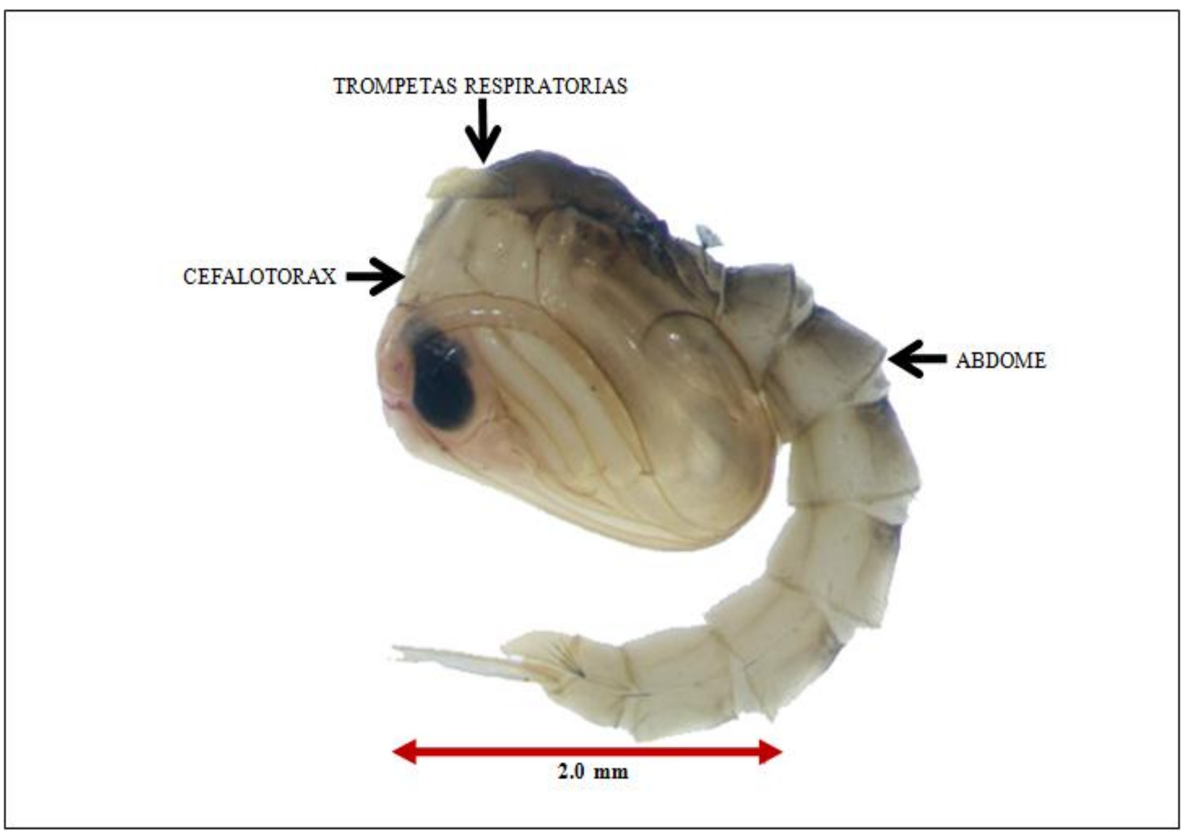

Figure-5: Morphology and morphometry of *Aedes aegypti* Pupae.

Plate 1a: Normal larvae; Plate 1b: Dechitinized larvae with damaged digestive tract; Plate 1c: Exuvia of the proceeding instar attached to the dead larvae; Plate 1d: Death occurred during the larval stage with no initiation of population. Plate 2a: Normal pupae; Plate 2b: Demelanized pupae except eye pigment with straight abdomen; Plate 2c: Brown pupae with some melanization; Plate 2d: Exhibits dwarf pupae with retared abdomen.

Figure-6: Conspicuous morphological deformities was occurred among the larvae and pupae of *Aedes aegypti* exposed to lethal concentrations of aqueous leaf extract of *Lantana camara, Catharanthus roseus* and *Ficus religiosa* using acetone solvents.

Conclusion

This study found that all the three medicinal plant species were evaluated had possessed a different range of larvicidal and pupicidal property; which may use as a Zika and dengue vector control agent. Natural products of these medicinal plants with a less toxic effect on non-target organisms and an intrinsic biodegradability may well be used in Zika and dengue vector control interventions. Key findings of this study have shown that, of these plant species, the aqueous leaf extract of *C. roseus* with acetone solvent is showing a high-level of toxicity even at a low dose; which was targeting as a repellent or anti-feedant, larvicidal and pupicidal approach to control dengue and zika vector, *Aedes aegypti*. The findings of the current study that lead to a reduction in using synthetic insecticides, which enhances the opportunity to promote work aimed at the statistical development of new mosquito control agents based on plant sources as botanical pesticides. Further investigation needed for the isolation of active concepts, synthetic analogs and optimum dosage, necessary for larvicide, pupicide and adult inhabitation behavior in *Aedes aegypti*. Advanced monitoring of the mode of extraction of phytoconstituents, effects on non-target organisms and field-based assessment are important in the preparation of strategies to minimize the spread of infections and prevent future epidemics.

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