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EVALUATION OF PHYTOCHEMICAL PROPERTIES AND LARVICIDAL ACTIVITIES OF TARAXEROL EXTRACTED FROM LEAF OF *CODIAEUM VARIEGATUM* AGAINST *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) LARVAE

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ABSTRACT

The larvicidal activity of biologically active compound, taraxerol extracted from *Codiaeum variegatum* (family: Euphorbiaceae) leaf obtained through soxhlet apparatus against larvae of *Culex quinquefasciatus* which is the serious vector of filarial worm, *Wuchereria bancrofti* causing lymphatic filariasis in human beings. The active compound taraxerol extracted through petrol from the leaf of *Codiaeum variegatum* was administered for 24h or 96h to the larvae of *Culex quinquefasciatus*. Mortality produced by taraxerol for the periods ranging from 24 to 96h. The toxicity of petrol extract was time and dose dependent for *Culex quinquefasciatus* larvae. The LC_{50} values of taraxerol were a significant negative correlation between LC values and exposure periods. i.e. LC_{50} values of petrol extract of *Codiaeum variegatum* leaf decreased from 73.03 mg/L (24h) > 66.05 mg/L (48h) > 57.64mg/L (72h) > 50.90mg/L (96h) in case of *Culex quinquefasciatus*. Exposure of larvae over 24h to sub-lethal doses (40% and 80% of LC_{50}) of petrol extract of *Codiaeum variegatum* leaf, significantly ($P<0.05$) altered the level of total protein, total free amino acid, glycogen and enzyme activity i.e. acetyl cholinesterase activity, acid and alkaline phosphates activity in whole body tissue of *Culex quinquefasciatus* larvae. The alterations in all these biochemical parameters were significantly ($P<0.05$) time and dose dependent. *Codiaeum variegatum* (Family: Euphorbiaceae) commonly known as croton in India, which is used as traditional medicine for the treatment of various human ailments such as antiamoebic, antibacterial, anticancer, antifungal, antioxidant, emmenagogue, purgative and sedative. A decoction of the crushed

leaves is used in the treatment of diarrhea. The root is used in the treatment of gastric ulcers and wound healing activity.

KEYWORDS: *Codiaeum variegatum,* Euphorbiaceae, *Culex quinquefasciatus,* biochemical effects, *Wuchereria bancrofti*.

INTRODUCTION

Mosquitoes have a great influence on human well being than any other insects because of their involvement in transmitting dreadful diseases like malaria, filariasis, dengue, chikungunya, Japanese encephalitis, Zika virus, yellow fever etc., besides gorging blood from human beings. The most important thing to reduce the incidence of vector borne diseases is controlling the mosquito population. Vector control is facing a problem that due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either adopting counter measures or development of newer insecticides.[1] Mosquitoes are a potential vector of several tropical diseases, including numerous viral diseases: of 3,000 species existing, 100 are known to be vectors.[2] It has been reported that mosquitoes aremore effective in disease transmission than any other arthropods, and thus are regarded public enemy number one, as reported by the World Health Organization (WHO).[3] Mosquitoes are known to transmit such diseases as malaria, dengue fever, chikungunya, Rift Valley fever, filariasis, West Nile fever and Japanese encephalitis.[4,5] It is estimated that more than 700 million people are infected with mosquito-transmitted diseases annually, which leads to death, poverty, and social and economic disturbances.[6] Synthetic chemical pesticides have been used for a long time in controlling mosquitoes, but their arbitrary use has given rise to known and serious problems, including genetic resistance, increasing cost of application, hazards from handling, and environmental pollution.[7,8] The search for effective and biodegradable pesticides, including mosquito repellents, is of paramount importance. One of the potential sources is plants that are known to be used by communities in the management of insects.

Plants acts as alternative agents for control of vectors because they are rich in bioactive chemicals, are active against specific target insects, and are biodegradable. Phytochemical insecticides considered to be more environmentally safer and biodegradable than synthetic insecticides. Numerous plants have shown insecticidal activities and in India nearly 2400 species of plants possess insecticidal properties.[9] The adulticides may only reduce adult

population temporarily thus most of the mosquito control programmes target the breeding sites with larvicides.[10,11]

In the present study, the larvicidal activity of taraxerol extracted from *Codiaeum variegatum* leaf as well as its biochemical effects on larvae of *Culex quinquefasciatus* were investigated, these extracts cannot be applied to commercial use without a study of these aspects as well.

MATERIALS AND METHODS

Collection and maintenance of experimental animal

The adult females of Culicines were collected from the different residential areas of Gorakhpur district. Collections were made from human dwellings with the help of an aspirator supplied by W.H.O. and kept in 30x30x30 cm cages with cotton pads soaked in 10% glucose solution and water containing enamel bowl for egg laying. Experimental conditions of water determined by the method of APHA/AWWA/WEF[12] were atmospheric temperature 30.3°±1.7°C, water temperature 27.8°±1.2°C, pH 7.2-7.5, dissolved oxygen 7.5-8.2mg/L, free CO_2 4.2-5.3mg/L, bicarbonate alkalinity 104.5-105.1 mg/L.

Collection of plant material

Plant *Codiaeum variegatum* (family: Euphorbiaceae) was collected locally from Botanical garden of Deen Dayal Upadhyay Gorakhpur University, Gorakhpur and identified by Prof. S.K. Singh, ex-plant taxonomist, Department of Botany, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, Uttar Pradesh, India, where a voucher specimen was deposited.

Extraction of active compounds

Pure taraxerol was isolated from the leaves of *Codiaeum variegatum* by the method of Subramanian et al.[13] The leaf of *Codiaeum variegatum* was dried in an incubator at about 35^0C and powdered with the help of a mechanical device. From 50 g of dried powdered leaf, a concentrated solution of *Codiaeum variegatum* was extracted with petrol, for about 70 hours, in soxhlet apparatus. After evaporation of the solvent by vacuum pump, the isolated compound in dried form was obtained. Confirmation of the compound was also made through IR and Rf values when compared to the authentic sample obtained from Sigma Chemical Company, USA.

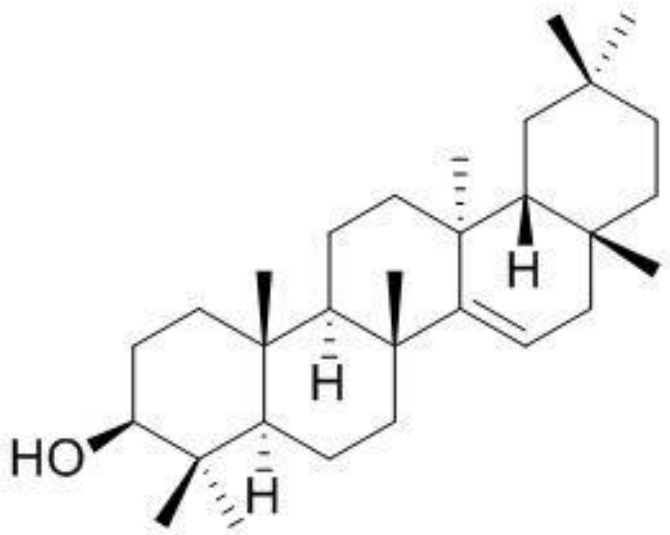

Figure: Chemical Structure of Taraxerol.

Toxicological experiment

Toxicity experiment was performed by using the method of W.H.O.[14] Twenty late third instar larvae of *Culex quinquefasciatus* mosquito were exposed to four different concentrations of taraxerol. Doses were maintained in 500 ml of de-chlorinated tap water in glass beakers (15 cm in diameter and 7.5cm in height) containing twenty mosquito larvae in each test concentration. Six replicates were maintained for each concentration. Control larvae were kept in similar conditions without treatment. *Culex* larvae were exposed for 24 h to 96 h at four different concentrations of taraxerol. Mortality was recorded after every 24 h up to 96 h exposure periods. LC values, upper and lower confidence limits, slope value, t-ratio and heterogeneity were calculated by probit log analysis method using POLO computer programme of Russel et al.[15]

Biochemical experiment

The late third instar larvae were treated with 40% and 80% of 24h LC_{50} of taraxerol obtained from *Codiaeum variegatum* leaf for 24 h. Six beakers were set up for each dose and each beaker contained 50 larvae in 1 L de-chlorinated tap water. The LC_{50} value of taraxerol was 75.53 mg/L for 24 h against *Culex quinquefasciatus* larvae. 40% and 80% of 24 h, LC_{50} of petrol extract was selected as sub-lethal dose to analyze its time and dose dependent effects in the present study and at that dose there was no mortality were observed in the treated larvae. After the stipulated time (24 h), the dead larvae were removed from the beaker and washed

with water and the whole body tissue stored in deep freezer, for biochemical analysis. Control larvae were held in the same condition without any treatment. Each experiment was replicated six times and the values are expressed as mean ±SE of six replicates. Student's't' test was applied to locate significant changes with controls.[16,17]

Total protein: Total protein level was estimated by the method of Lowry et al.[18] Homogenates (10 mg/mL) was prepared in 10% tri-chloroacetic acid (TCA). Bovine serum albumin was used as standard.

Total free amino acids: Total free amino acids level was estimated by the method of Spies.[19] Homogenates (10mg/mL) were prepared in 95% ethanol. Glycine was used as standard.

Glycogen: Glycogen level was estimated by the method of Van der Vies.[20] Homogenate (10 mg/mL) was prepared in 5% TCA. Glucose was used as standard.

Acetylcholinesterase activity: Acetylcholinesterase activity was measured by the method of Ellman et al.[21] Homogenate (50 mg/ml, w/v) was prepared in 0.1 M-phosphate buffer, PH 8.0 for 5 min in an ice bath. The change in optical density at 412nm, caused by the enzymatic reaction, was monitor for 3 min at 25°C.

Acid and alkaline phosphatase activity: Acid and alkaline phosphatase activity was determined by the method of Andersch and Szcypinski.[22] Homogenates (2% w/v) were prepared in ice-cold 0.9% NaCl solution and centrifuged at 5000 xg at 0°C for 15 min.

Statistical analysis: Each experiment was replicated at least six times and data has expressed as mean ±SE. Student's t-test as applied for locating significant differences Sokal and Rohlf.[16]

RESULT

Exposure to the taraxerol extracted from *Codiaeum variegatum* leaf caused significant behavioural changes in the larvae of mosquito *Culex quinquefasciatus.* Behavioural changes appear after 4-5 hours of exposure. Larvae were incapable of rising to the surface, show restlessness, loss of equilibrium, lethargic and finally death. No such behavioral symptoms and mortality occurred in the control groups indicating that the plant moieties were actual factors responsible for altered behavior and larval mortality.

Percent mortality produced by taraxerol for the periods ranging from 24h to 96h is shown in Table 1. The toxicity of ethyl alcohol extract was time and dose dependent for *Culex quinquefasciatus* larvae. The LC_{50} values of taraxerol are shown in Table 1. There was a significant negative correlation between LC values and exposure periods. i.e. LC_{50} values of petrol extract of *Codiaeum variegatum* leaf decreased from 73.03mg/L (24h)> 66.05mg/L (48h)> 57.64mg/L (72h)> 50.90mg/L (96h) in case of *Culex quinquefasciatus* larvae (Table 1).

Exposure to sub-lethal doses of 40% and 80% of LC_{50} of taraxerol extracted from *Codiaeum variegatum* leaf for 24h or 96h caused significant ($P<0.05$) alterations in total protein, total free amino acids and glycogen metabolism in whole body tissue of the larvae of *Culex quinquefasciatus* (Table 2). Total protein and glycogen levels were significantly reduced, while free amino acid level was significantly enhanced after the exposure to sub-lethal doses. Total protein levels were reduced to 87% of control after exposure to (24h) of taraxerol extracted from *Codiaeum variegatum* leaf. The maximum decrease in protein level (77% of control) was observed in larvae treated with 80% of LC_{50} (24h). Total free amino acid levels were induced to 113% of controls after treatment with 40% of LC_{50} (24h) and maximum increase in total free amino acids level (121% of control) was observed in larvae treated with 80% of LC_{50} (24h) of petrol extract of *Codiaeum variegatum* leaf and the glycogen level was reduced up to 78% and 51% respectively (Table 2).

Table 2 clearly shows that sub-lethal exposure of taraxerol at (40% and 80%) of LC_{50} the AChE activity decreases 77% , 63% at 24h with respect to control but at 96h exposure the AChE activity also decreases as 56%, 41% at (40% and 80%) of LC_{50} respectively with respect to control.

According to Table 2 at sub-lethal treatment of taraxerol of 40% and 80% of LC_{50} (24h), Acid phosphatase activity decreases by 84% to 74% respectively with respect to control. At longer duration (96h) exposure, 40% and 80% of LC_{50} of taraxerol also decreases the activity of acid phosphatase by 65%, 60% respectively with respect to control. In the case of enzyme alkaline phosphatase, exposure of 40%, 80% LC_{50} of taraxerol also decreases the enzyme activity by 83% to 71% and 68% to 55% at 24h or 96h respectively with respect to control (Table 2).

Statistical analysis of the data on toxicity brings out several important points. The X^2 test for goodness of fit (heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep values. The steepness of the slope line indicates that there is a large increase in the mortality of the larvae of *Culex quinquefasciatus* with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the target animal to the plant origin pesticides used.

Table 1: Toxicity (LC values) of different concentrations of Taraxerol extracted from petrol of *Codiaeum variegatum* leaf against *Culex quinquefasciatus* larvae at 24h to 96h exposure period.

Exposure Period (hours)	Effective dose (mg/L)	Limits (mg/L)		Slope value	't' ratio	Heteroge-neity
		LCL	UCL			
24	LC_{10}=47.43	22.21	55.77	6.84±4.39	2.84	0.02
	LC_{50}=73.03	65.12	99.11			
	LC_{90}=112.44	88.76	378.83			
48	LC_{10}=44.59	24.13	52.53	7.51±4.26	3.19	0.10
	LC_{50}=66.05	58.62	76.39			
	LC_{90}=97.84	81.88	193.24			
72	LC_{10}=38.94	17.63	47.58	7.52±4.28	3.17	0.02
	LC_{50}=57.64	46.74	64.07			
	LC_{90}=85.33	73.77	144.91			
96	LC_{10}=33.71	8.73	43.72	7.16±4.52	2.84	0.16
	LC_{50}=50.90	32.10	57.60			
	LC_{90}=76.85	67.26	133.21			

- Batches of twenty mosquito larvae were exposed to four different concentrations of the extract.
- Concentrations given are the final concentration (w/v) in the glass beaker containing de-chlorinated tap water. Each set of experiment was replicated six times.
- Mortality was recorded after every 24h.
- Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
- LCL: Lower confidence limit; UCL: Upper confidence limit.
- There was no mortality recorded in the control group.

Table 2: Changes in total protein, glycogen, total free amino acid, acetylcholinesterase activity, acid and alkaline phosphatase activity in whole body tissue of *Culex quinquefasciatus* larvae after 24h or 96h exposure to sub-lethal doses (40% and 80% of LC_{50} of 24h) of taraxerol compound extracted through petrol from leaf of *Codiaeum variegatum*.

Parameters	**Control**	**40% of LC_{50} (+, ±£)** (29.21 mg/L, 24h LC_{50})	**80% of LC_{50} (+, ±£)** (58.42 mg/L, 24h LC_{50})
Protein	**24h**		
	2.30±0.004 (100)	2.00±0.003 (87)	1.77±0.003 (77)
Glycogen	**24h**		
	1.70±0.004 (100)	1.32±0.006 (78)	1.14±0.005 (51)
Amino acid	**24h**		
	0.48±0.004 (100)	0.54±0.003 (113)	0.58±0.003 (121)
AChE	**AChE activity (µm SH hydrolyzed/min/mg protein**		
	24h		
	0.088±0.004 (100)	0.068±0.006 (77)	0.055±0.004 (63)
	96h		
	0.088±0.004 (100)	0.049±0.004 (56)	0.036±0.004 (41)
Acid phosphatase	**µm *p*-nitrophenol formed/30 min/mg protein**		
	24h		
	0.190±0.003 (100)	0.160±0.003 (84)	0.140±0.003 (74)
	96h		
	0.200±0.003 (100)	0.129±0.003 (65)	0.120±0.003 (60)
Alkaline phosphatase	**µm *p*-nitrophenol formed/30 min/mg protein**		
	24h		
	0.420±0.005 (100)	0.350±0.003 (83)	0.300±0.003 (71)
	96h		
	0.440±0.005 (100)	0.300±0.003 (68)	0.240±0.003 (55)

- Values are mean ±SE of six replicates.
- Values in brackets indicate percent biochemical activity with control taken as 100%.
- Doses are 40% and 80% of LC_{50} for period for which animals were exposed.
- +, significant ($P<0.05$) when two way analysis of variance was applied to see whether enzyme inhibition was time and dose.

- £, significant (P<0.05) when Student 't' test was applied between control and treated groups.

DISCUSSION

Here, in the current study, we demonstrated that taraxerol extracted from *Codiaeum variegatum* leaf, though quite in chemical composition, provided pronounced larvicidal activities against *Culex quinquefasciatus* larvae. Mosquito larval control using larvicidal agents is a major component in the control of vector borne diseases. Plant as potential larvicides is considered as viable and preferred alternative in the control of the mosquito species at the community level. A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control.[23,24,1]

The detrimental effects on mosquitoes and their larvae by the plant extracts was mainly due to the phytochemicals present in it.[25] The Phytochemicals present in the plants were grouped into two categories, primary metabolites such as amino acids, chlorophyll etc. and secondary metabolites which are alkaloids, flavonoids, tannins, saponins etc.[26] Phytochemicals present in the plant extracts have been found to have insecticidal, antimicrobial, anticonstipative, antispasmodial and antioxidant activities.[27, 28]

We are noticed that the LC_{50} values recorded in different studies were various comparing with each other. It's clear if we focus on these studies; Komalamisra et al [29] tested the *Nerium oleander* larvicidal activity against *Aedes aegypti* with the LC_{50} value of 197.97mg/L. A study deal with the larvicidal activity of different plants extracts against *Culex quinquefasciatus* and recorded the LC_{50} values. Of these, *Gleoonis coronarium* flowers extracts LC_{50} value of 53.0 ppm, *Sonchus arvensis* stem extracts LC_{50} value of 68.0 ppm, *Matricaria maritima* flowers extracts LC_{50} value of 72.0 ppm.[30] A study has tested the effects of some plants extracts against the larvae of *Culex quinquefasciatus* included *Tagetes erectes* leaf extract LC_{50} value of 100.0 ppm, *Achilea millefolium* stem extract LC_{50} value of 120.0 ppm, *Tanacetum vulgare* flower extract LC_{50} value of 178.0 ppm and *Otanthus maritimus* stem extract LC_{50} 195.0 ppm.[31] The mechanism of extracts effect on mosquito larvae due to digestive system effectiveness through the entrance of phytochemicals and bounded with lipids or cell metabolic effectiveness through moulting or skin effectiveness or cuticle hardness through Tyrosinase enzyme effectiveness or respiratory bores closing.[32] A

big number of plant chemicals have larvicidal activity. Mosquito different responses to these extracts due to many causes such as plant species, phytochemical type, plant part, extraction solvent, and extraction method Ghosh et al and Shaalan et al.[3,33]

In the present study the taraxerol extracted with petrol from *Codiaeum variegatum* leaf has potent larvicidal activity of *Culex quinquefasciatus* mosquitoes. Exposure to sub-lethal doses of compound taraxerol of *Codiaeum variegatum* leaf against larvae of *Culex quinquefasciatus* significantly altered the level of total protein, total free amino acid, glycogen and enzyme activity of acetylcholinesterase, acid and alkaline phosphatase activity. Significant exceptional changes as given in result section of *Culex quinquefasciatus* larvae like ecdysial failure, abnormalities during intermediate stages, prolongation of the life span of treated instars, emergence of adultoids after treatment with petrol extract of *Codiaeum variegatum* leaf may be due to the effect of active moiety present in the plant extract. The effect of compound depends on the synthesis or release of ecdysone and in absence of it, the insect lapses into a state of developmental stand still.[34] It resulted into ecdysial failure. The male and female emerged from treated groups were unable to feed on sugar solution as well as on mammal blood ultimately they died sooner. Laboratory observations revealed that, their mouth parts were undeveloped, legs were paralyzed and the females were incapable of egg lying after treatment, eventually they died sooner.

Carbohydrates are the primary and immediate source while the protein acts as the next alternative source of energy to meet the increase energy demand. The depletion of the protein fraction in treated mosquito larvae of *Culex quinquefasciatus* may have been due to their degradation and the possible utilization for metabolic purposes. The protein content is depends on the rate of protein synthesis and its depletion might have been due to their degradation and possible utilization for metabolic purposes. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains.[35] The decreased protein content attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery.[36] The total free amino acids content showed a significant increase in whole body tissue of mosquito larvae exposed to sub-lethal doses of ethyl alcohol extract of *Codiaeum variegatum* leaf. The augmentation in total free amino acids level in the whole body tissue suggests high proteolytic activity. The accumulation of free amino acids can also be attributed to lesser use of amino acids[37] and their involvement in the maintenance of an acid base balance.[38] Another possibility for enhancement of free

amino acid level might be due to transamination and amination to keto acids. Stress conditions induce elevation in the transamination pathway.[39] The transamination reaction is probably the most important pathway in the metabolism of many amino acids.[40] In stress condition, carbohydrate reserve depleted to meet energy demand. In the present study, the diminished glycogen content in body tissues of *Culex* larvae indicates its rapid utilization for energy generation; a demand caused by rutin extracted from *Codiaeum variegatum* leaf as a consequence toxic stress during the experiment.

Finally, glycogenolysis seems to be the result of increased secretion of catecholamine due to stress of plant extracts treatment.[41] Larvae also secrete catecholamine in excess amount, during stress, which depletes glycogen reserves.[42] Anaerobic and aerobic segments are two important components of carbohydrate metabolism. In first case, breakdown of glucose or glycogen through Embden- Meyerhof pathway (glycolysis) takes place while the next one consists oxidation of pyruvate to acetyl co-A to be utilized through citric acid cycle.[43]

The AChE is the most important enzymes that confer resistance capacity to mosquitoes. From our study high activity of AChE was observed in control *Aedes aegypti*. Similarly, Lima et al., (2011) investigated the resistance capacity of *A. aegypti* and found that the AChE values ranged from (65-131) nM/min/mg protein and (51-116) µM/min/mg tissue protein, respectively. Larvae tissue homogenates when pre-incubated with plant extracts, both the enzyme showed reduced activity.

Effect of toxicants on enzymatic activity is one of the most important biochemical parameters, which Affect physiology of body. When an organ is diseased due to the effect of a toxicant, enzyme activity appears to be increased or it may be inhibit due to the active site being either denature or destroyed. Acetylcholinesterase, or acetyl-hydrolase, is a serine protease that hydrolyses the neurotransmitter acetylcholine. AChE found mainly at neuromuscular junctions and brain synapse, where its activity serves to terminate synaptic transmission. It belongs to carboxyl esterase family of enzymes.

Enzyme alkaline phosphatase plays an important role in animal metabolism. Vorbrodt [44] has reported that the role of this enzyme is in the transport of metabolites across the membrane. The enzyme has been shown to be intimately associated with protein synthesis and is thus involved in the synthesis of certain enzymes.[45] Acid phosphatase is the lysosomal enzyme

and plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis.[46]

CONCLUSION

In conclusion, the larvicidal activity of the taraxerol extracted through petrol from *Codiaeum variegatum* leaf is highly toxic to larvae of *Culex quinquefasciatus* mosquito. This extract significantly suppresses the population build up of the mosquito by morphogenetic action on insect. Sub-lethal doses of ethyl alcohol extract significantly alter the protein, amino acids, glycogen, enzyme activity like acetylecholinesterase, acid and alkaline phosphatase activity of *Culex* larvae. We therefore believe that the plant extracts may eventually be of great value for the control of *Culex quinquefasciatus* mosquitoes in aquatic stage.

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