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Bioactivity of plant extracts on the larval and pupal stages of *Aedes aegypti* (Diptera, Culicidea)

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ABSTRACT

Introduction: *Aedes aegypti* is responsible for the transmission of the dengue and yellow fever viruses. This study evaluated the effects of extracts from *Cnidosculos phyllacanthus, Ricinus communis*, and *Coutarea hexandra* on the developmental periods of *A.aegypti* larvae and pupae. Crude extracts of *C. phyllacanthus* and *C. hexandra* and oil from *R. communis* and *C. phyllacanthus* were used. **Methods:** Bioassays of the larvicidal and pupicidal effects of these products at different concentrations and times of exposure were evaluated. The lethal and sublethal effects were determined using different concentrations in larvicidal tests. Mortality data were evaluated by Probit analysis to determine the LC_{50} and LC_{90} values. **Results:** The vegetable oils from *C. phyllacanthus* and *R. communis* demonstrated greater efficiency for larval control with an LC_{50}=0.28µl/mL and an LC_{90}=1.48µl/mL and LC_{50}=0.029µl/mL and a LC_{90}=0.26µl/mL, respectively. In pupal tests toxic effects for all insects were verified after exposure to the products at significant LC_{50} and LC_{90} values for 24 and 48h. The effects of sublethal concentrations of *C. phyllacanthus* (oil) were more effective on the insects. **Conclusions:** The vegetables oils from *C. phyllacanthus* and *R. communis* demonstrated greater potential from the control of different developmental periods in the life cycle of this insect.

Keywords: Larvicidal. Control. Vector.

INTRODUCTION

Aedes aegypti, Diptera, Culicidae (Linnaeus, 1762); can be found in human dwellings where it obtains food, copulates and spawns. As ubiquitous insect, this species exhibits a high ability to adapt to artificial habitats allowing for their occupation and expansion and, consequently, emergence of epidemias[1]. The highest epidemiologic importance of this study is that mosquitoes play a role as transmitters of yellow fever and dengue virus[2].

The control of the dengue vector and important insect species related to public health has been managed in the last decades mainly with synthetic chemical insecticides. The intense use of these synthetic compounds has many exposed mosquito populations to intense selective pressure and hence the prevalence of some populations are resistant to products used to control these vectors. Thus, the use of secondary metabolites of plants that have insecticidal potential is being studied to minimize the impact of synthetic compounds on the environment and human health[3].

The close relationship between plants and insects and the possible coevolution of plants led to the development of strategies to attack these invertebrates that, are physical or chemical in nature[4]. Thus, *Cnidoscolus phyllacanthus*, *Ricinus communis* (Euphorbiaceae) and *Coutarea hexandra* (Rubiaceae) are chemical compounds whose biological activities have been described[5-8].

In view of the operational and economic difficulties generated by increased mosquito resistance to synthetic insecticides, alternative methods have gained increasing attention[9]. Several plant species have been investigated and tested with potential larvicides directed against several insect species including *Aedes aegypti*, *Magonia pubescens*[10], *Atlantia monophylla*[11], *Cybistax antisyphilitica*[12], *Anacardium humile*[13] and *Spathelia excelsa*[14]. In this study we report the insecticidal activity (larval and pupal) of *C. phyllacanthus*, *C. hexandra* and *R. communis* on *Aedes aegypti*.

METHODS

The population of *A. aegypti* was collected in the District of Monte Santo, Campina Grande, State of Paraíba, Brazil. The collection and establishment of strains in the laboratory occurred between December 2009 and August 2010. Eggs were collected with 50 ovitraps that were installed inside and outside homes distributed in ten blocks where five traps were installed per block.

Establishment and maintenance of *Aedes aegypti* in the laboratory

The laboratory bioassays were conducted in a temperature-controlled room (26±2°C and 12h light). Vanes eucatex containing *A. aegypti* eggs from the field were dried for 48h and then packed in white plastic trays (40x27x7.5cm) with a

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third of its capacity filled with non chlorinated water. After the outbreak, ornamental fish food (Goldfish growth) was added (100mg/tray). Adults were maintained in cages with a wooden frame coated with organza (40cm x 40cm x 30cm) containing 200 individuals (100 males and 100 females). Adults were fed with a 20% honey solution and the females were fed blood from the quail *Coturnix japonica* three times a week for 30min. After meals were introduced inside each cage, a disposable cup containing 200ml of distilled water with a plastic funnel coated with filter paper served as a substrate for oviposition.

Collection and preparation of solutions

The ethanolic fraction was obtained from cauline tissue from *C. phyllacanthus* and *C. hexandra,* which was placed in an incubator at 40°C for 48h. When dry, the shells were crushed and sieved. The powder was moistened with 90% ethanol and subsequently percolated for cold extraction for 48h. After this period, the ethanolic fractions were collected and concentrated by spin evaporation to yield extracts. Seeds from *C. phyllacanthus* and *R communis* were used for the extraction of vegetable oil. These were first crushed and macerated and then subjected to a hydraulic press in the cold. The fixed oils were stored in glass bottles covered with aluminum foil and kept in the refrigerator.

Larvicidal and pupicidal bioassays

Larvicidal and pupicidal test were performed according to the methodology of the World Health Organization[15] with some adjustments. The biological activity of the extracts of the specimens used in the laboratory was found in late L_3 and/or early L_4 larval and pupal stages of populations of *A. aegypti* to obtain of the lethal concentrations (LC_{50} and LC_{90}) values. We used 30, 60, 125, 250 and 500mg/mL of ethanolic fractions diluted in 1mL of dimethyl sulfoxide (DMSO). For vegetable oils, a 1:1 ratio with tween 20 was made at the following concentrations: 100, 190, 380, 750 and 1.5µl/mL. DMSO and tween and water were used as controls. For each concentration there were four replicates with 25 individuals per replicate, and mortality assessed was after 24 and 48h of exposure. For pupal tests, the occurrence of adults, if they were dead, was counted as pupae.

Effect of sublethal (lethal concentrations of cumulative effect), plant extracts were established via the LC_{50} and LC_{90} values determined with the test larvicide after 48h of exposure to plant products. There were four replicates with each containing 30 third instar *A. aegypti* larvae. Daily readings were made with verification of the larval stage, behavior changes, the presence of exuvia, adult emergence, possible mortality of the larvae, pupae and adults, and the water temperature. The experiment was conducted until the last pupa or adult died or until the last adult completely emerged.

Mortality data for the larvae and pupae of the studied population were subjected to Probit analysis using the Polo-PC program to determine the lethal concentrations (LC_{50} and LC_{90}). There was no need to correct the data with the Abbott formula[16].

RESULTS

With respective to the larvicidal activity of the plant products analyzed, it was observed that after 24 and 48h of exposure, only vegetable oils demonstrated relevant results against L3 *A. aegypti* larvae. Tests including vegetable oils from *R. communis* and *C. phyllacanthus* on L3 *A. aegypti* larvae proved to be the most promising for the control of the mosquito larvae, which was confirmed by the LC_{50}=0.029µl/mL and LC_{90}=0.261µl/mL and LC_{50}=0.288µl/mL and LC_{90}=1.485µl/mL values obtained after 24h of exposure. These tests indicated that lower concentrations are needed for these products to obtain a larval mortality of 50-90% of the population **(Table 1)**. Among the species studied, oil from *R. communis* was the most effective with regard to lethal concentrations (LC_{50} and LC_{90}) and had lower values compared to other species studied. The X^2 values indicated the adequacy of the data for the entire experiment using the Probit model ($p<0.95$).

Seed oil from *C. phyllacanthus* also showed larvicidal potential after 24h of exposure. However, crude extract made from the bark of this vegetable demonstraded activity after 24 and 48h of exposure with larvae of this vector when the values from the LC_{50} and LC_{90} levels were obtained **(Table 1)**.

The results obtained from a crude extract from the stem of *C. hexandra* allowed us to deduce that these dosages treat yourself a non-toxic plant on L3 stage *A. aegypti* because they could not be estimated after 24h of exposure lethal concentrations LC_{50} and LC_{90} **(Table 1)**.

After exposing the larvae to vegetable products for 24 and 48h, it was observed that oil extract from the seeds of *R. communis* had the lowest lethal dose (LC_{50}=0.016µl/mL and LC_{90}=0.082µl/mL). However, for fractions extracted from the stems of *C. phyllacanthus* and *C. hexandra* after 48h of treatment, it was noted that despite a considerable reduction in the LC_{50} and LC_{90} values compared with the test performed after 24h, the levels were still considered high for this mosquito larval control **(Table 1)**.

To evaluate potential overlaps in the larvicide behavior induced by the studied extracts was performed principal component analysis (PCA). The analysis demonstrated that in PC1/PC2, there was a split into three groups (*C. hexandra* with *C. phyllacanthus* (stem), *R. communis* and *C. phyllacanthus* (oil)) which explained 98% of the variance in the data. This result alloweds us to infer that there were differences in the behavior induced by larvicidal oils and extracts from the stem but not from the extracts of *C. phyllancanthus* (stem) and *C. hexandra* (stem) which formed a single group **(Figure 1)**.

For both PCs, the natural products analyzed were completely separate from the toxicity of the plant extracts. When evaluating PC2, which apresented only 5% of the variance the data, the plants had a dimension of differential larvicidal behavior. In agreement with this result the LC_{50} and LC_{90} values were not the same between treatments, they quantitatively differed in the dose-response **(Figure 1)**.

TABLE 1 - Analysis of larvicidal effect of plant extracts *Cnidoscolus phyllacanthus*, *Ricinus communis* and *Coutarea hexandra* with their respective lethal concentrations, confidence intervals (95%), chi-square (χ^2) and (Slope) after an exposure of 24 and 48h.

		Ricinus communis (oil)	*Coutarea hexandra* (crude extract)	*Cnidoscolus phyllacanthus* (crude extract)	*Cnidoscolus phyllacanthus* (oil)
24h	LC_{50}	0.029µl/mL	-	1.103µg/mL	0.288µl/mL
	CI	0.008 - 0.054	-	0.588 - 2.037	0.232 - 0.353
	LC_{90}	0.261µl/mL	-	-	1.485µl/mL
	CI	0.192 - 0.376	-	-	1.067 - 2.427
	X^2	18.428	18.856	15.48	25.990
	Slope	1.35 ± 0.24	0.11 ± 0.41	0.72 ± 0.25	1.802 ± 0.162
48h	LC_{50}	0.016µl/mL	-	0.246µg/mL	0.067µl/mL
	CI	0.009 - 0.036	-	0.106 - 0.378	0.033 - 0.099
	LC_{90}	0.082µl/mL	-	2.145µg/mL	0.390 µl/mL
	CI	0.034 - 0.114	-	1.021-	0.294 - 0.606
	X^2	14.351	26.280	38,597	26.655
	Slope	1.795 ± 0.607	0.235 ± 0.295	0.167 ± 0.325	1.682 ± 0.228

LC_{50}: lethal concentrations; CI: confidence interval.

When assessing the effect of plant extracts on the pupal stage of *A. aegypti*, all results were significant with LC_{50} and LC_{90} values thet were more promising than those obtained by the larvicide test. The larvicide test with vegetable oils *R. communis* and *C. phyllacanthus* demonstrated greater efficiency in controlling the pupae of this vector, which was confirmed by the (LC_{50} and LC_{90} values) obtained after 24h of exposure **(Table 2)**. Oil extracted from the seeds of *C. phyllacanthus* was among the products evaluated, which demonstrated greater pupicidal activity with lower LC_{50} and LC_{90} value after 24 and 48h of exposure.

A greater interaction between the plant products and pupae of *A. aegypti* was observed when evaluating extracts made after 48h of exposure, which resulted in lower lethal doses capable of killing 50 and 90% of the population. Thus, lethality was enhanced with increasing time of exposure to plant products.

With regard to the species evaluated after 48h of treatment *R. communis* extract efficiently killed mosquito pupae, resulting in a mortality rate ranging from 50 to 90% for the lower stage and with small LC_{50} and LC_{90} values **(Table 2)**. During this period of exposure (48h), it was found that adults at all concentrations and treatments were susceptible to the extracts tested verifying mortality in all replicates. This newly emerged adult mortality might be related to the action of contact with plant extracts present in solutions for the adult mosquito. Thus, one can conclude that the solutions tested negatively affected adult emergence.

Assessment of the activity pupicidal of plant extracts via analysis of PC1 versus PC2 with 95% of variance shows that there were no differences in the mortality response between treatments after 24h of exposure. In **Figure 2**, all samples were blended. The toxicity of the products was the same for the pupal

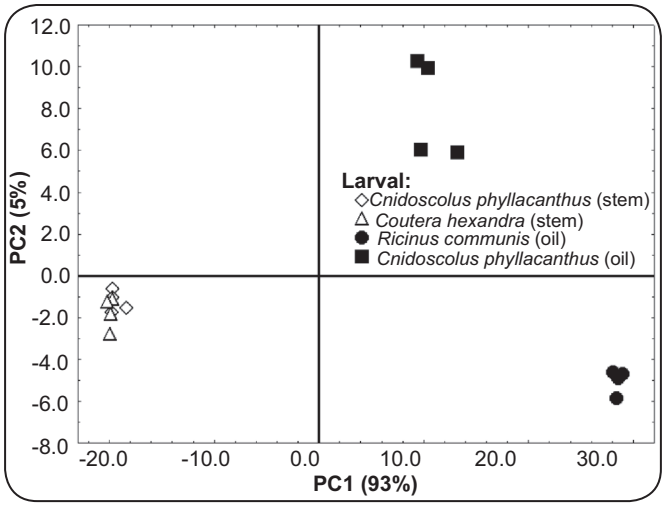

FIGURE 1 - Graph scores for PC1 versus PC2 for the five tested concentrations of plant extracts after 24h of exposure to *Aedes aegypti*. PC: principal component.

stage of this vector. Therefore, at this stage of the life cycle of *A. aegypti,* both of the crude vegetable oils demonstrated efficiency in controlling this vector.

The sublethal effects of *C. phyllacanthus* (oil) LC_{50}=0.067µl/mL lasted 28 days with a larvicide cumulative mortality of ± 84.1% **(Table 3)**. During the experiment it was observed that the larvae were able to develop and reach the adult stage. In addition, the temperature of the water throughout the experiment was verified to be approximately 22ºC.

The concentration that significantly reduced the rate of adult emergence of *A. aegypti* was the LC_{90} (0.39µl/mL) of *C. phyllacanthus* (oil). For this extract, the duration of the experiment was 14 days, and for the 120 larvae exposed to

TABLE 2 - Analysis of the effect of the plant extracts pupicidal *Cnidoscolus phyllacanthus*, *Ricinus communis* and *Coutarea hexandra* with their respective lethal concentrations (LC), confidence intervals (95%), chi-square (χ^2) values and slope (Slope) after an exposure of 24 and 48h.

		Ricinus communis (oil)	*Coutarea hexandra* (crude extract)	*Cnidoscolus phyllacanthus* (crude extract)	*Cnidoscolus phyllacanthus* (oil)
24h	LC_{50}	1932.31µl/mL	90725.0µg/mL	715.8µg/mL	0.027µl/mL
	CI	146.8 - 2965.1	513.0-	12.02 - 976.1	0.007 - 0.049
	LC_{90}	1.271µl/mL	2.068µg/mL	1.253µg/mL	0.187µl/mL
	CI	0.678 - 1.986	0.986 - 3.560	0.496 - 1.967	0.251 - 0.138
	X^2	40,620	39,808	40.614	6.635
	Slope	0.403 ± 0.379	0.276 ± 0.369	0.465 ± 0.392	1.545 ± 0.305
48h	LC_{50}	199.43µl/mL	2101.32µg/mL	0.314µg/mL	0.011µl/mL
	CI	-	-	-	-
	LC_{90}	2.959µl/mL	12.8µg/mL	913.84µg/mL	0.060µl/mL
	CI	-	-	-	-
	X^2	29,044	24,913	20.855	20.331
	Slope	0.701 ± 0.560	0.579 ± 0.692	0.283 ± 0.703	1.730 ± 0.733

LC: lethal concentrations; CI: confidence interval.

TABLE 3 - Duration of the experiments for sublethal effects of the vegetable oils *Cnidoscolus phyllacanthus* and *Ricinus communis* at the lethal LC_{50} and LC_{90} concentrations for *Aedes aegypti*. Total of was 120 L_3 larvae of this vector were in each treatment.

Plant extracts		Duration (days)	Death larvae (%)	Death pupal (%)	Emergence adults (%)	Death adults (%)
Cnidoscolus phyllacanthus	LC_{50}	28	84.1	5.0	10.9	3.1
	LC_{90}	14	88.3	5.8	5.9	5.0
Ricinus communis	LC_{50}	33	87.5	0.8	11.7	1.6
	LC_{90}	24	93.3	0.8	5.9	2.3
Control	Water	35	4.1	-	95.9	-

LC: lethal concentrations.

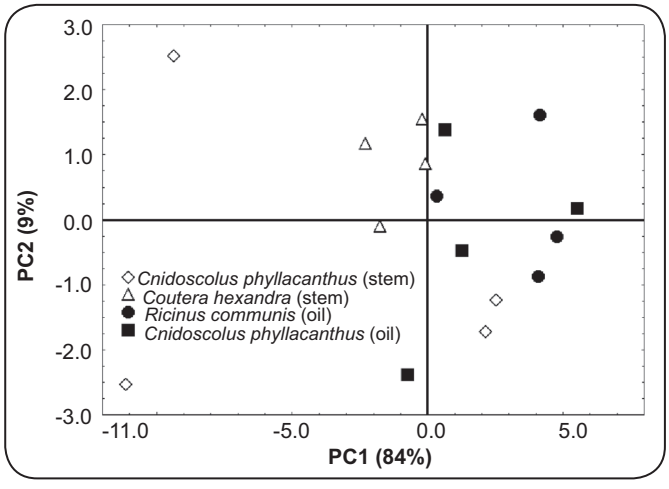

FIGURE 2 - Graph scores for PC1 versus PC2 for the five tested concentrations of plant extracts after 24h of exposure for *Aedes aegypti* larvae. PC: principal component.

this concentration, there was a mortality rate of ± 88.3%. The efficiency in reducing adult emergence helped to confirm that at this dose ± 11.7% of the larvae developed to the pupal stage and only 0.9% became viable adults **(Table 3)**. With regard to a treatment with an LC_{50} (0.067µl/mL) of *C. phyllacanthus,* of the larvae that did could develop, 19 turned into pupae, but there was a 5% mortality rate even at this stage, and only ± 10.9% reached adulthood (± 3.1% died in this stage).

The sublethal effects of *R. communis* at an LC_{50} of 0.016µl/mL lasted approximately 33 days, and the LC_{90} concentration lasted 24 days. At the end of the 33 day exposure to 120 larvae, the mortality rate was ± 87.5% LC_{50} *R. communis*. Between those that managed to develop, 10 became pupae with only one death. The highest number of emerged adults (11.7%) and ± 1.6% mortality was recorded of the (LC_{50}) of the *R. communis* extract. This was the most effective treatment for the larvae of *A. aegypti* with a mortality rate of 87.5%. The highest larval

mortality observed for the larvicide test was obtained with a LC_{90} of 0.082µl/mL, where at the end of 24 days, the larval mortality was ± 93.3%. The remaining ± 6.7% developed into pupae, only one death was recorded, and 5.9% emerged into adults and ± 2.3% died at this stage.

The control experiment lasted 35 days with only five larval mortalities (± 4.1%). If the mortality of the control group was between 5 and 20%, it was be adjusted using the Abbott formula or the bioassay would have been repeated according to recommendations of the World Health Organization[17,18] **(Table 3)**. Moreover, there was no mortality for pupae and adults, verifying the emergence of 95.9% viable adults.

DISCUSSION

The larvicidal activity of the *R. communis* oil can be attributed to its chemical components which include the a derivative of ricin ricinoleic acid, a toxic substance that is present in abundance in the seed oil of this plant[7]. This constituent had a negative effect on the larval of this vector. No studies have reported on the effects of *R. communis* on *A. aegypti*; however, the insecticidal effect of this vegetable was evaluated in other species of insects. Hebling[19], assessed the bioactivity of *R. communis* on ants and demonstrated its efficacy in combating Hymenopteran, Burg and Mayer[20] tested the effect of *R. communis* seed oil on aphids and different species of lice, describing how promising this oil was for the control of these insects. In a study of the effects of aqueous extract from the green fruits of castor on the larvae and pupae of *Spodoptera frugiperda,* Santiago[21] observed a reduction in the lifetime of these stages, and Rother[22] identified toxic effects of leaf extracts of *R. communis* on *Apis mellifera* worker larvae.

The plant extracts studied here showed greater efficiency in the control *A. aegypti* pupae. The promising effects of these products on the pupal stage of *A. aegypti* are related to the morphological differences between the pupae and larvae of this insect, indicating that the mode of action of these products is by contact or choking and not swallowing, because the pupal stage does not involve ingestion. A curious observation was made with crude extracts from *C. hexandra* and *C. phyllacanthus* the concentrations studied were nontoxic to the L3 larvae of this vector, but were lethal to the pupae. Furthermore, there was similarity between the pupal mortality of products *C. phyllacanthus* (stems and seeds) after 24h. According to Santos[23] despite being a source of extracts from different parts of the plant chemicals biosynthesis is not restricted to one part of the plant; thus, the products are accumulated in the whole plant or different organs because of an intercellular system.

There are several studies of the activity of plant extracts on *A. aegypti*, mainly examining its larvicidal action. However, there are few studies of the effect of natural products on the pupal stage. Thus, we found no studies in the literature involving these species and their effects on the pupal stage of *A. aegypti*. However, studies of the pupicidal activity of other plant species have been reported by Macchioni et al.[24] with *Condonopsis javanica* on the dipteran *Aedes albopictus*, demonstrating 75% mortality of pupae at a concentration of 60.000ppm. Using the extracts of leaves and seeds from *Melia azedarach* on *Anopheles stephensi*, Nathan[25] found a 92.3 and 90.9% mortality rate, respectively, at 20,000ppm. Nathan[26] also tested *Eucalyptus tereticornis* on *Anopheles stephensi* and found a 160ppm 88% mortality for pupae. Although different material was used in this study, the results discussed above only corroborate the need for development studies using plant extracts to evaluate their effects on different stages of *A. aegypti*.

Regarding the effects of the sublethal plant extracts studied, we observed that although there was a small immediate response for some of the products analyzed, when evaluating larval development, these products interfered with the in timing and number of hatched adult insects. The sublethal effects of chemical compounds on natural specimens of *A. aegypti* were investigated by Shaalan[27] who evaluated the effect of synthetic insecticides and *Callitris glaucophyllam* extracts and various chemical insecticides Adanan et al.[28] and Kamaraj[29] showed that, while not fatal, these sublethal concentrations lead to a disorder in insect biology, both morphologically and physiologically, interfering with the duration of the larval period and the pupae exposed to these concentrations, reflected in the emergence of adults.

These results need corroboration further, in addition to the more detailed study on the insecticidal properties with phytochemicals present in *R. communis* and *C. hexandra* and *C. phyllacanthus*. The next challenge consists identify chemical compounds that were responsible for the larval and pupal mortality and control programs in the future serve as a tool to control of the actions to *A. aegypti*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Silva HHG, Silva IG, Santos SRMG, Rodrigues FE, Elias CN. Atividade larvicida de taninos isolados de *Magoniua pubescens* St. Hil. (Sapindaceae) sobre *Aedes aegypti* (Diptera, Culicidae). Rev Soc Bras Med Trop 2004; 37:396-399.
2. Agrelo RS. *Aedes aegypti*, *Aedes albopictus* (Díptera, Culicidae) y su papel como vectores en lãs américas. La situación de Uruguay. 1996. [Cited 2012 November 11]. Available at: http://www.cepes.ops-oms.org/brsoir/repimdex/repi78/ref.pdf.
3. Barreto CF. *Aedes aegypti* resistência aos inseticidas químicos e as novas alternativas de controle. Rev Eletr Fac Montes Belos, Goiás 2005; 1:62-73.
4. Freitas AC, Lima MP, Ferreira AG, Tadei WP, Pinto ACS. Constituintes Químicos do caule de *Spathelia excelsa* (RUTACEAE) e atividade frente a *Aedes aegypti.* Quim Nova 2009; 32:2068-2072.

5. Endo Y, Ohta T, Nozoe S. Favelines novel tricyclic benzocychoheptans with citotoxic activities from the Brazilian plant, *Cnidoscolus phyllacanthus*. Tetrahedron Lett 1991a; 32:3083-3086.
6. Endo Y, Ohta T, Nozoe S. Favelanone. a novel tetracyclic cyclopropane derivative from de Brazilian plant, *Cnidoscolus phyllacanthus*. Tetrahedron Lett 1991b; 32:5555-5558.
7. Leite AC, Cabral EC, Santos DAP, Fernandes JB, Vieira PC, Silva MFGF. Isolamento do alcalóide ricinina das folhas de *Ricinus communis* (Euphorbiaceae) através de cromatografias contracorrentes. Quim Nova 2005; 28:983-985.
8. Lucena JEX. Efeito antinociceptivo e antiinflamatório do extrato aquoso da entrecasca de *Coutarea hexandra* Schum (Rubiaceae). Rev Bras Farmacogn 2006; 16:67-72.
9. World Health Organization (WHO). Dengue hemorrhagic fever: Diagnosis, treatment, prevention and control. Geneva: WHO; 1997.
10. Silva HHG, Silva IG, Santos SRMG, Rodrigues FE, Elias CN. Atividade larvicida de taninos isolados de *Magoniua pubescens* St. Hil. (Sapindaceae) sobre *Aedes aegypti* (Diptera, Culicidae). Rev Soc Bras Med Trop 2004; 37:396-399.
11. Sivagname N, Kalyanasundaram MM. Laboratory avaluation of methanolic extract of *Atlantia ophylla* (family: Rutaceae) against immature stages of mosquitoes and non-target organisms. Mem Inst Oswaldo Cruz 2004; 99:115-118.
12. Rodrigues MAS, Paula JE, Roblot F, Fournet A, Espindola LS. Larvicida activity of *Cybistrax antisyphilitica* against *Aedes aegypti* larvae. Fitoterapia 2005; 76:755-757.
13. Porto KRA, Roel AR, Silva MM, Coelho RM, Scheleder JD, Jeller AH. Atividade Larvicida de óleo de Anacardium humile Saint Hill sobre *Aedes aegypti* (Linnaeus, 1762) (Diptera, Culicidade). Rev Soc Bras Med Trop 2008: 41:586-589.
14. Freitas AC, Lima MP, Ferreira AG, Tadei WP, Pinto ACS. Constituintes Químicos do caule de *Spathelia excelsa* (RUTACEAE) e atividade frente a *Aedes aegypti.* Quim Nova 2009; 32:2068-2072.
15. World Heatlth Organization (WHO)**.** Guildelines for laboratory and Field testing of mosquito larvicides. Geneva: WHO; 2005.
16. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol 1925; 18:265-267.
17. World Health Organization (WHO). Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Geneva: WHO; 1981.
18. World Health Organization (WHO). Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides. Geneva: WHO; 1981.
19. Hebling MJA. Toxic effect of *Ricinus communis* (Euphorbeaceae) to laboratory nests of *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Bull Entomol Research 1996; 86:253-256.
20. Burg IC, Mayer PH. Alternativas ecológicas para prevenção e controle de pragas e doenças. 7th ed. Francisco Beltrão (PR): Grafit Gráfica e Editora; 1999.
21. Santiago GP. Efeitos de extratos de plantas na biologia de *Spodoptera frugiperda* (I.E. Smith, 1797), (Lepidoptera: Noctuidae) mantida em dieta artificial. Cienc Agrotec 2008; 32:792-796.
22. Rother DC, Souza TF, Malaspina O, Bueno OC, Silva M, Vieira PC, et al. Suscetibilidade de operárias e larvas de abelhas sociais em relação a ricinina. Serie Zoologia 2009; 99:61-65.
23. Santos RI. Metabolismo Básico e origem dos metabólitos secundários *In*: Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR. Farmacognosia da planta ao medicamento, 5th ed. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2004. p. 1102.
24. Macchioni F, Carugini S, Cecchi F, Siciliano T, Braca A, Cioni P, et al. Aqueous extracts of *Codonopsis Javanica* against larval and pupal stages of *Aedes albopictus*, Analle della Facolta**,** 2004. [Cited 2012 September 10]. Available at: http://eprints.adm.unipi.it/50/1/215.pdf/.
25. Nathan SS. Efficacy of *Melia azedarach* L. extract on the malarial Vector *Anopheles stephensi* Liston (Diptera: Culicidae). Bioresour Technol 2006; 97:1316-1323.
26. Nathan SS. The use of *Eucalyptus Tereticornis* Sm. (Myrtaceae) oil (leof extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Bioresour Technol 2007; 98: 1856-1860.
27. Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab HE, Mansour AH. Effects of sub-lethal concentrations of synthetic insecticides and *Callitris glaucophylla* extracts on the development of *Aedes aegypti*. J Vector Ecol 2005; 30:295-298.
28. Adanan CR, Zaire J, NG KH. Efficacy sublethal effects of mosquito mats on *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Proceedings of the fifth International Conference on Urban Pests. 2005. p. 265-269.
29. Kamaraj C, Rahuman AA, Bagavan A. Antifeedant and larvicidal effects of plant extracts against Spodoptera litura F. *Aedes aegypti* L. and *Culex quinquefasciatus* Say. J Parasitol Res 2008; 103:325-331.