Priprioca (*Cyperus articulatus* var. *nodosus*, Cyperaceae) Hydrolate as Larvicidal against *Aedes aegypti*

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Recebido em 30/04/2020 – Aceito em 31/08/2020

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ABSTRACT – Dengue cases have grown significantly in Brazil in recent years. Studies with plant extracts show the insecticidal potential of bioactive substances that become candidates for future commercial products. This work evaluated the larvicidal effect of essential oil, without hydrolysis of *Cyperus articulatus* var. *nodosus* against the third and fourth wide stage of *Aedes aegypti*, which reduces larvae survival by as much as the 3th and 4th instar of *Ae. aegypti*. A chemical characterization of the hydrogen skeletons was performed by the GC-MS, revealing verbenone (%), trans-sabinol (%) and mirtenol (%) as major compounds.

Keywords: Dengue; *Aedes aegypti*; priprioca; *Cyperus articulatus* var. *nodosus*, larvicidal activity.

Hidrolato de Priprioca (*Cyperus articulatus* var. *nodosus*, Cyperaceae) como Agente Larvicida contra *Aedes aegypti*

RESUMO – Os casos de dengue apresentam importante crescimento no Brasil nos últimos anos. Estudos com extratos de plantas mostram o potencial inseticida de substâncias bioativas que as torna candidatas a futuros produtos comerciais. Este trabalho avaliou o efeito larvicida do óleo essencial contido no hidrolato dos rizomas de *Cyperus articulatus* var. *nodosus* contra o terceiro e quarto estágio de larvas de *Aedes aegypti*, reduzindo a sobrevivência de larvas tanto do terceiro quanto do quarto instar do *Ae. aegypti*. A caracterização química dos sesquiterpenos do hidrolato foi realizada por GC-MS, revelando como compostos majoritários a verbenona (%), o trans-sabinol (%) e o mirtenol (%).

Palavras-chave: Dengue; priprioca; *Cyperus articulatus* var. *nodosus*; atividade larvicida.

Hidrolato de Priprioca (*Cyperus articulatus* var. *nodosus*, Cyperaceae) como Agente Larvicida contra *Aedes aegypti*

RESUMEN – Los casos de dengue han crecido significativamente en Brasil en los últimos años. Los estudios con extractos de plantas muestran el potencial insecticida de sustancias bioactivas que las hace candidatas para futuros productos comerciales. Este trabajo evaluó el efecto larvicida del aceite esencial contenido en el hidrolato de los rizomas de *Cyperus articulatus* var. *nodosus* contra larvas del tercer y cuarto estadio de *Aedes aegypti*, lo que reduce la supervivencia larvaria del tercer y cuarto estadio de *Ae. aegypti*. La caracterización química de los sesquiterpenos hidrolatos se realizó mediante GC-MS, revelando verbenona (%), trans-sabinol (%) y mirtenol (%) como compuestos principales.

Palabras clave: Dengue; Priprioca; *Cyperus articulatus* var. *nodosus*; actividad larvicida.

DOI: 10.37002/biobrasil.v10i3.1648

Introduction

Aedes aegypti (*Diptera: Culicidae*) is a cosmopolitan mosquito occurring in tropical and subtropical regions around the world. It is essentially anthropophylic found in urban areas and rural villages (Edman *et al.* 1992, OMS 2011, Tauil 2002, Silva *et al*. 2004). This mosquito has medical importance because it is the dengue and yellow fever vector. Actually, the reappearance of dengue in the last decades transformed this disease in a serious public health issue, mainly in tropical countries (Instituto Virtual da Dengue 2012).

Dengue can infect more than 390 million persons per year (WHO 2014). World Health Organization point out Brazil as one of the most endemical countries with more registrations of the disease. Dengue is arising due not planned urbanization, lack of sanitation, ineffective control of mosquitos, demographic growth and long distance trips. Furthermore, there is no vaccine against the disease. Estimation of 40% of the population is at risk worldwide. Dengue in severe form affects mainly Asian and Latin American countries, being one of the main causes of hospitalization and child mortality in these regions.

There are many cases of resistance related to the mosquito-vector in the world and particularly for the population of *Ae. aegypti* in Brazil. There are reports of occurrence of resistance to organophosphorated insecticides, at least in eigth cities of São Paulo State with detection of resistance against larvicide temephós (Abate®) (Andrade & Modolo 1991, Macoris *et al*. 1999).

Control of the vector *Ae. aegypti* includes vigilance, reduction of the source (or environmental management), biological control, chemical control by using insecticides and repellents, traps and management of the resistance to insecticides. Chemical control with insecticides of organic or inorganic origin is one of the methodologies adopted as part of integrated management for the control of vectors in Public Health (Rose 2011).

Brazil has a great diversity of plants, more than 56 thousand species, around 19% of the total flora of the world (Giulietti *et al.* 2005). Investigations with vegetal species show the potential to find insecticidal molecules, some of them highly selective, that could be used in the development of new formulations.

Species of Cyperaceae present great importance in Amazonian pharmacopoeia, being used mainly as contraceptive, analgesic and in the treatment of diarrhea. In Pará State, priprioca have aroused growing scientific and economical interest due to the pleasant aroma of essential oil obtained from the rhizomes that are very used to prepare perfumes. Essential oils from these species have mainly sesquiterpenes belonging to the class of cyperene, caryophyllane, eudesmane, patchoulane and rontundane (Zoghbi *et al.* 2006). Volatile terpenes and phenylpropanoids can have, depending of the insect, attractive properties (feeding, polinization) and/or deterrent and insecticide (Kainulainen *et al.* 1998). Larvicide activity of terpenoids were reported on literature.

Hydrolates are aqueous solutions obtained as subproducts from hydrodistillation or steam distillation process of essential oils. These subproducts are normally discarded at the end of the process. The main compounds of hydrolates are the more hydrophilic molecules presented in essential oils. Many studies showed activity of plant extracts against different species of mosquitos (Guimarães *et al.* 2001), including *Ae. aegypti* (Silva *et al.* 2004).

The present study had as aim to evaluate the larvicidal effect of hydrolate obtained from priprioca (*Cyperus articulatus* var. *nodosus*) rhizomes against third and fourth stages of *Ae. aegypti* larvae.

Material and Methods

Hydrolate preparation of priprioca (*C. articulatus* var. *nodosus*)

Priprioca roots (4kg) were collected at Tabocal 1 district, 23km far away of Santarém, Pará, Brazil, between 8 and 10am. Tubers were washed and dried in the oven at 40°C during 24h until constant weight. After grinding in a grinder Trapp 200, the powder was extracted with distilled water in a steam hydrodistillator of 150L. Essential oil was separated and remanescent hydrolate was collected.

Aedes aegypti larvae (Rockfeller strain)

Adult mosquitos of *Ae. aegypti* were procreated in plastic recipients covered with cloth

DOI: 10.37002/biobrasil.v10i3.1648

and maintained at 25°C, 75% relative humidity, 12h photoperiod. An immobilized mouse in a wire mesh was exposed to the mosquitos for blood feeding. Oviposition of female *Ae. aegypti* occurred in filter paper that covered a plastic vase (100mL) filled with 50mL of tap water. Larvae hatching was stimulated by total immersion of filter paper containing the eggs in a recipient with 100mL water, which was treated 24h prior with mice feed. Larvae of 3th or 4th stages were selected to be use in the experiments. A group of larvae was maintained to continue the reproduction of mosquitos. These larvae were treated daily with mice feed until the formation of pupas, when they were transferred to plastic vases and kept in a plastic recipient. Adult mosquitos were fed with saccharose solution (10%) and then the reproductive cycle was restarted.

Plastic vases of 100mL were used in the experiments. Each vase was filled with tap water and the hydrolate (totalizing 50mL of formulation), followed by addition of 10 *Ae. aegypti* larvae. Larvae were daily fed with 0.05% of cat feed. Hydrolate was evaluated in three different concentrations, diluting the hydrolate in water: 1:1, 1:0.5 and 1:0.125. A control was prepared just containing tap water. The experiment was conducted in triplicates.

Larval survival was checked daily 24h after the beginning of the assay, during seven days. Larvae were considered alive when they showed movements or reaction to the contact with a Pasteur pipette. The assay was kept at 27-30°C. Larval survival curves and average survival time (S50) of the larvae were calculated by GraphPad Prism 3.0 Software. Larval survival percentage were done using Excel 2010 Software.

Extraction of hydrolate with methylene chloride

A 50mL of hydrolate was transferred to a separation funnel and 25mL methylene chloride was added. After 3-fold extraction, organic phase was separated, dried with anhydrous Na_2SO_4 and evaporated under vacuum to dryness.

GC-MS analysis

Volatile constituents were analysed by GC Agilent, HP-6890, with a selective mass detector, Agilent HP-5975, using capilar column HP-5MS (30m x 0,25mm x 0,25μm). The following conditions were adopted: injector temperature = 220°C, column = 60°C, heating rate from 3°C/min to 240°C/min, and detecto r = 250°C. Helium was the carrier gas in an outflow of 1mL/min. Selective mass detector operated at 70 eV, *m/z* = 30 a 500amu.

Essential oil was dissolved in ethyl acetate in the concentration of 20mg/mL and identification was carried out by retention index of the compounds, using a coinjection of a mixture of hydrocarbon standards (C8 to C22), comparing with the electronic library of the GC system (NIST-11) and literature data (Adams 2007).

Results

Third stage larvae exposed to the highest concentration of hydrolate (1:1 dilution) showed the less survival rate (36.6%) compared to the others treatments, after seven days evaluation (Table 1). The untreated control had 83.3% live larvae at the end point of the test. Dilution 1:1 of the hydrolate was the only one to present S_{50} (3.5 days).

Table 1 – Surviving rate (%) ± SE and average time of surviving (S_{50}) of *Ae. aegypti* 3th stage larvae exposed to hydrolate.

Hydrolate dilutions	1:1	1:0.5	1:0.25	Control
Surviving of the larvae (%)	36.6 ± 2.36	60.0 ± 1.60	80.0 ± 0.69	83.3 ± 0.48
S_{50}	3.5	ND	ND	ND

ND = not determined data.

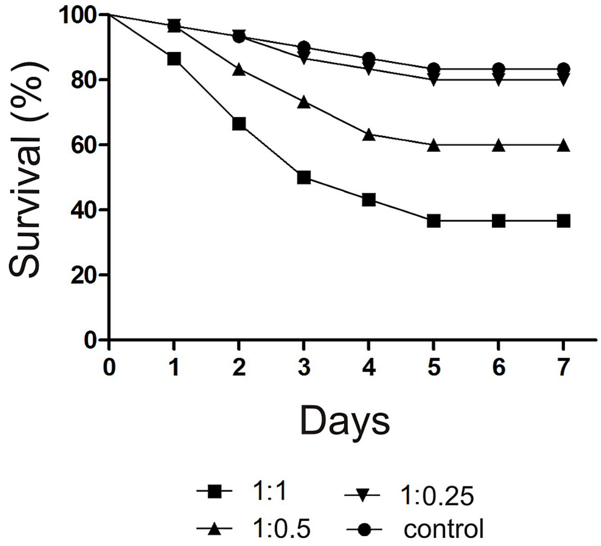

Figure 1 – Daily survival curves of 3th instar larvae exposed to three dilutions of the hydrolate. Untreated control was prepared with water only.

All fourth stage larvae died (0%) when treated with 1:1 dilution of the hydrolate, compared to the other treatments, after seven days (Table 2). The untreated control showed 80% live larvae at the end of the experiment. 1:1 dilution caused less S_{50} value (3 days), while 1:0.5 dilution caused S_{50} value of four days to the larvae. Figure 2 shows daily survival curves of larvae exposed to three dilutions of the hydrolate. Treatment control was done with water only.

Table 2 – Surviving rate (%) ± SE and average time of surviving (S_{50}) of *Ae. aegypti* 4th stage larvae exposed to hydrolate.

Hydrolate dilutions	1:1	1:0.5	1:0.25	Control
Surviving of larvae (%)	0.0 ± 3.90	26.6 ± 3.43	76.6 ± 0.81	80.0 ± 0.69
S_{50}	3.0	4.0	ND	ND

ND = not determined data.

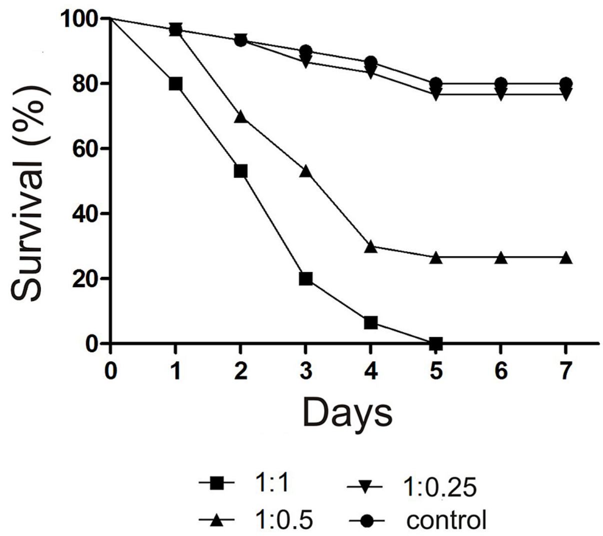

Figure 2 – Daily survival curves of 4th instar larvae exposed to three dilutions of the hydrolate. Untreated control was prepared with water only.

Chromatographic analysis by GC-MS (Figure 3) revealed that major compounds presented on hydrolate are verbenone (31.7%), followed by *trans*-sabinol (13.9%) and myrtenol (13.2%) (Table 3).

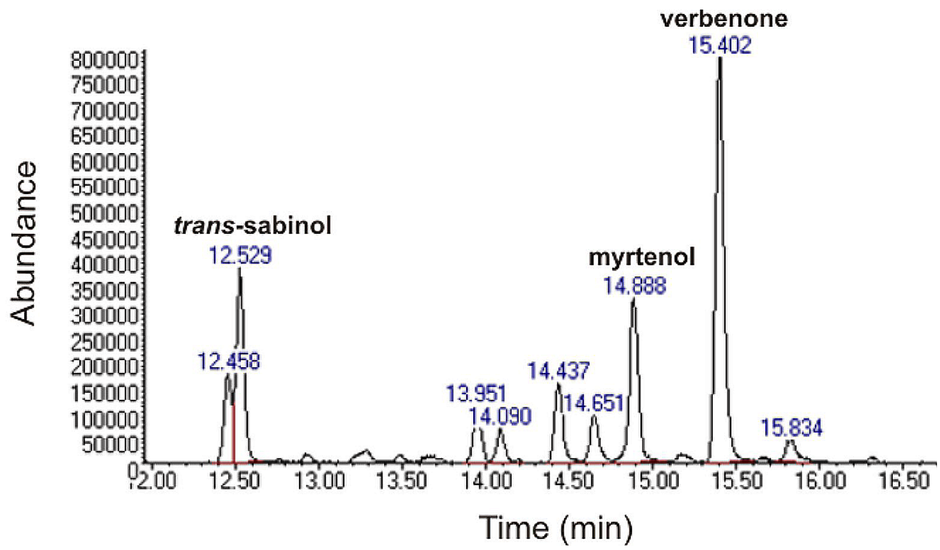

Figure 3 – Chromatographic analysis by GC-MS of *C. articulatus* var. *nodosus* hydrolate.

Table 3 – Volatile compounds identified by GC-MS in sample of the hydrolate from priprioca.

t_R (min)	IR	Compound	% rel.
12,46	1137	Nopinone	5,69
12,53	1139	*trans*-sabinol	13,89
13,95	1174	*cis*-pinocamphone	4,05
14,09	1177	terpin-4-ol	2,59
14,44	1186	M= 150	6,02
14,65	1191	*alfa*-terpineol	3,86
14,89	1197	Myrtenol	13,20
15,40	1209	Verbenone	31,74
15,83	1220	*trans*-carveol	2,19
34,13	1677	Mustacone	2,38
34,28	1682	M= 218	1,80
36,07	1732	M= 236	1,90
40,21	1852	M= 252	1,30
40,82	1871	M= 238	2,55
41,27	1884	M= 234	3,73
43,28	1946	M= 236	3,12

M = molar mass of not identified compounds.

Discussion

Terpenoids, especially limonoids, are compounds known for their insecticide potential. Although, simple monoterpenes also possess this potential, protecting plants from insect attacks (Vieira *et al.* 2011).

Regarding hydrolate of *C. articulatus* var. *nodosus*, 3th stage larvae exposed to 1:1 dilution of the hydrolate presented in 7th day of evaluation, the minor survival rate (36.6%) compared to other treatments (Table 1). By another hand, 4th stage larvae exposed to the same dilution, showed 0% of survival.

According to literature, the most active compounds of essential oil against *Ae. aegypti* are phenylpropanoids and sesquiterpenoids (Simas *et al.* 2004). Larvicide activity of terpenoids have been reported on literature, highlighting monoterpene β-thujaplicina (Jang *et al.* 2005) and sesquiterpenes 6-E-nerolidol, desidrocostos lactona and mansona C (Simas *et al.* 2004, Neves *et al.* 1999, Tiew *et al.* 2003). Monoterpene β-thujaplicina isolated from *Chamaecyparis obtusa*, also known as hinokitiol, has antimicrobial and phytoalexinic properties and larvicide activity against *Ae. aegypti* (Jang *et al.* 2005).

Larvicidal activity of essential oils from Brazilian Northeast plants showed good results, with LC_{50} = 67ppm for *Ocimum americanum* and LC_{50} = 60ppm for *O. gratissimum*. The main components of the essential oils were *E*-methyleugenol and cinnamate both derived from phenylpropanoids (Cavalcanti *et al.* 2004).

Carvalho *et al.* (2003) showed larvicidal activity against *Ae. aegypti* using the pure hydrolate of *Lippia sidoides* (known as pepper rosemary in Brazil) and its dilutions. Further, the major component of essential oil from leaves of this species – timol – was also evaluated. Hydrolate induced mortality almost instantaneously for all larvae, while timol caused 100% mortality with 0.017% concentration after 1.5h.

Larvicidal activity of essencial oil from two Cyperaceae species, *Cyperus giganteus* and *C. rotundus* against *A. albopictus* larvae was assayed (Kempraj & Bhat 2008). The results showed that *C. giganteus* has highest biocidal effect compared to *C. rotundus*. One of the major compounds of essential oil from *C. rotundus* is α-cyperone (Kilani *et al.* 2005, Tam *et al.* 2007) and it present insecticidal potential against clothes moth and beetles (Dadang *et al.* 1996, Adedire *et al.* 1999). Nevertheless, the presence of α-cyperone has not been reported in tested essential oil of *C. rotundus*. Therefore it is presumable that other compounds could act as larvicidal in this essential oil.

Substances as verbenone are known to present insecticidal, antioxidant and antimicrobial properties (Angioni *et al.* 2004, Kabouche *et al.* 2005, Sacchetti *et al.* 2005, Santoyo *et al.* 2005, Prins *et al.* 2006, Celiktas *et al.* 2007, Gachkar *et al.* 2007). The hydrolate of *Cyperus articulatus* var. *nodosus* has verbenone as major compound (31.74%), followed by trans-sabinol and myrtenol, 13.89% and 13.20%, respectively. Although the insecticidal potential of verbenone is already described in literature, it is possible that these three major substances could be acting synergistically or separately causing mortality for 3th and 4th stage larvae of *Ae. aegypti*.

Furthermore, hydrolate of *C. articulatus* var. *nodosus* can presente more plar compounds as flavonoids and glycosides, although GC-MS analysis cannot detect such substances.

Conclusion

Third and fourth stage larvae exposed to 1:1 dilution of priprioca hydrolate presented minor survival rate compared to other treatments (1:0.5 and 1:0.025) and untreated control. The hydrolate decreased the survival of these larvae and for that reason it could be investigated as a control agent against this vector. More studies need to be done aiming to isolate insecticidal substances presented in *C. articulatus* var. *nodosus* hydrolate.

Acknowledgements

We are thankful to Universidade Estadual no Norte Fluminense (UENF), Prof. Richard Ian Samuels and Adriano Rodrigues de Paula for technical support and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) (grants to LESB, IMR and MRA) as well Coordenação Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant to AS).

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Biodiversidade Brasileira – BioBrasil.
Fluxo Contínuo
n. 3, 2020

http://www.icmbio.gov.br/revistaeletronica/index.php/BioBR

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ISSN: 2236-2886