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Screening of plants found in the State of Amazonas, Brazil for larvicidal activity against *Aedes aegypti* larvae.

Adrian Martin POHLIT,[a,1] Etienne Louis Jacques QUIGNARD,[a] Sergio Massayoshi NUNOMURA,[a] Wanderli Pedro TADEI,[b] Ari de Freitas HIDALGO,[c] Ana Cristina da Silva PINTO,[d] Elba Vieira Mustafa dos SANTOS,[d] Sabrina Kelly Reis de MORAIS,[d] Rita De Cássia Guedes SARAIVA,[e] Lin Chau MING,[f] Alexandre Mascarenhas ALECRIM,[a] Alfeu de Barros FERRAZ,[a] Andreza Cristiana da Silva PEDROSO,[a] Elisangela Vieira DINIZ,[a] Ellen Kathryn FINNEY,[a] Erika de Oliveira GOMES,[a] Hercules Bezerra DIAS,[a] Katiuscia dos Santos de SOUZA,[a] Laura Cristina Pereira de OLIVEIRA,[a] Luciana de Castro DON,[a] Maria Mireide Andrade QUEIROZ,[a] Marycleuma Campos HENRIQUE,[a] Mirian dos SANTOS,[a] Orivaldo da Silva LACERDA JÚNIOR,[a] Patrícia de Souza PINTO,[a] Suniá Gomes SILVA.[a] Yara Rodrigues GRAÇA.[a]

ABSTRACT

Ethanol, methanol and water extracts representing mostly native plant species found in the Amazon region were prepared, respectively, by maceration, continuous liquid-solid extraction and infusion, followed by evaporation and freeze-drying. The freeze-dried extracts were tested for lethality toward *Aedes aegypti* larvae at test concentrations of 500 µg / mL. In general, methanol extracts exhibited the greatest larvicidal activity. The following 7 methanol extracts of (the parts of) the indicated plant species were the most active, resulting in 100 % mortality in *A. aegypti* larvae: *Tapura amazonica* Poepp. (root), *Piper aduncum* L. (leaf and root), *P. tuberculatum* Jacq. (leaf, fruit and branch). and *Simaba polyphylla* (Cavalcante) W.W. Thomas (branch).

KEY WORDS

larvicide, *Aedes aegypti*, *Tapura*, *Piper*, *Simaba*, Amazonia.

Triagem de plantas encontradas no Estado do Amazonas para atividade larvicida contra Aedes aegypti.

RESUMO

Extratos aquosos, etanólicos e metanólicos, representando principalmente espécies vegetais nativas encontradas na região Amazônica, foram preparados, respectivamente, por infusão, maceração e extração contínua líquido-sólido, seguida de evaporação e liofilização. Os extratos liofilizados foram testados para atividade contra larvas de Aedes aegypti*, na concentração única de 500 µg / mL. Os extratos metanólicos foram, em geral, os que apresentaram maior atividade larvicida. Os seguintes 7 extratos metanólicos das (partes das) espécies vegetais indicadas foram os mais ativos, provocando 100 % de mortalidade em larvas de* A. aegypti*:* Tapura amazonica *Poepp. (raiz),* Piper aduncum *L. (folha e raiz),* P. tuberculatum *Jacq. (folha, fruto e galho) e* Simaba polyphylla *(Cavalcante) W.W. Thomas (galho).*

PALAVRAS-CHAVE

larvicida, Aedes aegypti*,* Tapura*,* Piper*,* Simaba*, Amazônia.*

[1] Author to whom correspondence should be addressed (e-mail: ampohlit@inpa.gov.br).

[a] Coordenação de Pesquisas em Produtos Naturais (CPPN), [b] Coordenação de Pesquisas em Ciências da Saúde (CPCS), Instituto Nacional de Pesquisas da Amazônia (INPA), Av. André Araújo, 2936, 69083-000 Manaus, Amazonas; [c] Faculdade de Ciências Agrárias (FCA), [d] Curso de Pós-graduação em Química de Produtos Naturais, Universidade Federal do Amazonas (UFAM), Av. Gal. Rodrigo O. J. Ramos, 3000, 69077-000 Manaus, Amazonas; [e] Curso de Pós-graduação em Química, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Rio de Janeiro; [f] Universidade do Estado de São Paulo (UNESP), Fazenda Experimental Lageado, 237, 18603-970 Botucatu, São Paulo.

INTRODUCTION

In response to strong selective pressures of herbivorous insects, toxic secondary metabolites have evolved in plants and often affect insect nerve function and behaviour (Sharma *et al.*, 1998). Plant extracts show potential as sources of natural larvicides for the control of mosquito vectors and an early review has been published in which more than 300 larvicidal plant species were identified (Sukamar *et al.*, 1991). In recent times, studies on the activity of plant extracts towards *Aedes* sp. larvae from different parts of the world, such as, North America (Bergeron *et al.*, 1996), Argentina, Bolivia, Brasil and Peru (Chantraine *et al.*, 1998; Ciccia *et al.*, 2000; Macedo *et al.*, 1997), Trinidade and Tobago (Chariandy *et al.*, 1999), Mali (Diallo *et al.*, 2001), Negev Desert (Sathiyamoorthy *et al.*, 1997) and Africa (Marston *et al.*, 1993), among others, have been published and have revealed numerous examples of active plant extracts representing diverse taxonomic groups. More systematic and directed studies have revealed a number of very active plant extracts, essential oils, and isolated larvicidal phytochemicals (Bandara *et al.*, 2000; Bernard *et al.*, 1995; Hostettmann & Potterat, 1997; Latha & Ammini, 2000; Lee, 2000; Oberlies *et al.*, 1998; Park *et al.*, 2002; Pushpalatha & Muthukrishnan, 1999; Rahuman *et al.*, 2000; Sharma *et al.*, 1998; Thorsell *et al*., 1998). In the present study, we have screened polar extracts of mostly native plants from the Brazilian Amazon for activity against larvae of the hemorrhagic dengue fever vector, *Aedes aegypti* (Diptera: Culicidae), at a single test concentration of 500 μg / mL. Data (species names, plant parts, extraction solvent) for both active and inactive extracts are presented.

MATERIAL AND METHODS

Plant collecting, processing, and extract preparation.

Plants were collected from 1999 to 2002 in the State of Amazonas. Some genera (for example, *Piper* and *Aspidosperma*) and families (Euphorbiaceae, Simaroubaceae, Apocynaceae, Piperaceae, etc.) were better represented among the plants collected due to our specific interest in the bioactivity of these taxonomic groups. The plant materials were generally dried on the ground, in the shade, then stored in plastic bags under the protective warmth and luminosity of incandescent lighting. Where possible, fertile specimens of plant species were collected, used for identification and deposited at the INPA Herbarium. Many samples for study were collected from individual trees in INPA's Ducke Reserve that had been catalogued and identified in recent systematic studies (Ribeiro *et al*., 1999). Ethanol, methanol and water extracts were prepared, respectively, by maceration, continuous liquid-solid extraction, and infusion, followed by rotary evaporation under vacuum with heating in a bath to ca. 40-45 °C and freeze-drying. After this last step, extracts were presumed to be solvent-free. Dry extracts were deposited in our extract bank at the Coordenação de Pesquisas em Produtos Naturais (CPPN / INPA) where they were stored at –19 °C. Many of these extracts were screened previously by some of us against larvae of the brine shrimp species *Artemia franciscana* (Quignard *et al.*, 2003).

Assay for larvicidal activity against *Aedes aegypti*.

Aedes aegypti larvae were obtained by incubation of eggs from a laboratory colony at the Coordenação de Pesquisas em Ciências da Saúde (CPCS/INPA) in tap water. Stock solutions or suspensions of extracts in DMSO or de-ionized water (50 mg / mL) were prepared. Each stock solution (100 μL) was then transferred to plastic cups containing 10 third instar (three-day old) larvae in tap water having a final volume of 10 mL and a final extract concentration of 500 μg / mL. Negative controls were prepared analogously, substituting 100 μL of tap water or DMSO for sample solution in each cup, resulting in a final DMSO concentration of 1 %. Each experiment was run in triplicate and compared with a control set after 24h at 26 – 27 °C. Dead larvae were counted and the larvicidal activity expressed as % mortality based on live larvae present initially. In general, no dead larvae were observed in the controls after 24 h.

RESULTS AND DISCUSSION

Table 1 presents ethnobotanical and other information on the species investigated in this study. A number of these plants are traditionally used medicinal plants for the treatment of malaria, fevers, liver problems, and other symptoms caused by malaria infections, whose study is a major focus of our research group. Other plants studied were collected using a random sampling technique in INPA forest reserves in or near Manaus (Reserva Florestal Adolpho Ducke, Reserva da Campina / ZF-2). Still other species, from such genera as *Aspidosperma* (Apocynaceae) and *Piper* (Piperaceae), or from families such as Simaroubaceae, were studied here as part of a systematic approach to the investigation of the chemistry and biological activity of Amazon flora of these, and other taxonomic groups. Thus, the plants studied herein were not selected *a priori* for their known insecticidal, larvicidal or repellent properties. The discovery of larvicidal activity, as described below, serves as a demonstration of the power of bioprospection in the discovery of new larivicidal extracts and compounds based on many plants for which no such activity has been described or is known in the literature.

Tables 2 and 3 present, respectively, data for larvicidal and inactive plant extracts. Comparison of the results presented in Tables 2 and 3 reveals in a general way that extracts prepared from the less polar solvents ethanol and methanol were more active against *A. aegypti* larvae than

Table 1 - Ethnobotanic and other information for the plants studied.

FAMILY *Scientific name*	Common name	Part	Medicinal and other use(s) / property(-ies)	Ref
ANACARDIACEAE				
Spondias mombin L.	taperebá, cajá	leaf, fruit, root, bark	treatment of malaria, fever, diarrhea; vaginal infections	1, 2
APOCYNACEAE				
Aspidosperma araracanga Marcandes-Ferreira		tree		4
A. desmanthum Benth. ex Müll. Arg.	araracanga	leaf	treatment of fever	4, 6
A. marcgravianum Woodson	carapanaúba	wood, bark	treatment of malaria, diabetes, cancer; construction	1 , 4
A. nitidum Benth. ex Müll. Arg.	carapanaúba	wood, bark, latex	treatment of malaria, leprosy, cancer; construction	1, 4
A. sandwithianum Markgr.		tree		4
A. schultesii Woodson		bark	treatment of fever, mycoses; protection against termites	4, 7
A. spruceanum Benth. ex M. Arg.		wood	construction	3, 4
A. vargasii A. DC.		bark	treatment of fever; wounds, insect bites	2, 7
Geissospermum argenteum Woodson	acariquara branca	bark	treatment of fever; malaria	2, 4
G. urceolatum A.H. Gentry	acariquara branca	bark		4
Himatanthus sucuuba (Spruce ex Müll. Arg.) Woodson	sucuúba	latex	treatment of fever, bone fractures, toothaches	1, 4
ASTERACEAE				
Bidens pilosa L.	picão-preto	whole plant	treatment of hepatitis, dropsy, dysentery; diuretic	1,5
Spilanthes acmella (L.) Murray	jambú	flower	treatment of lung diseases, tuberculosis	1
BIGNONIACEAE				
Tabebuia incana A.H. Gentry	pau d'arco	bark	treatment of candidiasis; tumors	1
BIXACEAE				
Bixa orellana L.	urucum	shoot, leaf	treatment of fever, venereal diseases; antiseptic, aphrodisiac	1
BURSERACEAE				
Protium aracouchini (Aubl.) Marchand	breu vermelho	resin	laxative	9
CLUSIACEAE				
Calophyllum brasiliense Cambess.	jacareúba	bark	treatment of diarrhea, diabetes, worms, rheumatism	1
CUCURBITACEAE				
Momordica charantia L.	melão de São Caetano	whole plant	treatment of intestinal cramps, worms; emetic	1

cont. >

Table 1 - Ethnobotanic and other information for the plants studied. (cont.)

CYPERACEAE				
Scleria pratensis Lindl. ex Nees	tiririca	leaf, root	treatment of intestinal cramps, gastric problems	6,10
DICHAPETALACEAE				
Tapura amazonica Poepp.	tapura	leaf	toxic	7
EUPHORBIACEAE				
Croton cajucara Benth.	sacaca	bark	treatment of liver inflammation, diabetes; diarrhea	1
C. lanjouwensis Jabl.	dima	bark	treatment of fever	2, 10
Micrandropsis scleroxylon W.A. Rodrigues	piãozinho, aca purí	tree		
GENTIANACEAE				
Tachia grandiflora Maguire & Weaver		small tree		4
LAMIACEAE				
Ocimum micranthum Willd.	alfavaca	leaf	treatment of malaria	2, 5
LAURACEAE				
Aniba rosaeodora Ducke	pau rosa	oil	treatment of allergies, rheumatism; odontologic uses	5
LECYTHIDACEAE				
Corythophora alta R. Knuth	ripeiro (vermelho)	tree		4
LEGUMINOSAE: CAESALPINIOIDEAE				
Cassia fastuosa Willd. ex Vogel	chuva-de-ouro	tree	ornament	11
C. spruceana Benth.	mari-mari-da-terra-firme	root	treatment of fever	2, 4
Senna occidentalis (L.) Link	mata pasto, manjerioba	root, seed	treatment of fever, malaria, asthma, bronchitis; abortive, toxic	1, 2
LEGUMINOSAE: PAPILIONOIDEAE				
Swartzia prouacensis (Aubl.) Amshoff	mututi	tree		8
MENISPERMACEAE				
Abuta grandifolia (Mart.) Sandwith	cipó de bota	root, stem, leaf	treatment of malaria, anemia, sterility	1
OLACACEAE				
Minquartia guianensis Aubl.	acariquara vermelha	wood, bark	posts, civil construction; fish poison	1
PIPERACEAE				
Piper aduncum L.	pimenta longa	leaf, fruit	aromatic, styptic, antimicrobial, antimycotic	7
P. amapaense Yunck.	tapi-ipele	leaf, stem	treatment of dizziness	9
P. baccans (Miq.) C. DC.		shrub		
P. capitarianum Yunck.		shrub		
P. cyrtopodum C. DC.	jacamim	leaf	aromatic baths	10
P. dilatatum Rich.		shrub	antifungal properties	
P. erectipillum Yunck.		shrub		

cont. >

Table 1 - Ethnobotanic and other information for the plants studied. (cont.)

P. hostmannianum (Miq.) C. DC.	cordoncillo	leaf	treatment of warts	7
P. tuberculatum Jacq.	pimenta longa	leaf	fish poison	6,7
Pothomorphe peltata (L.) Miq.	caapeba	leaf	treatment of fevers; diuretic, emetic	1
PORTULACACEAE				
Portulaca pilosa L.	flor de onça	whole plant	treatment of hepatitis, nephritis, worms; diuretic.	1
RHAMNACEAE				
Ampelozizyphus amazonicus Ducke	saracura-mirá	root, bark, leaf	treatment of malaria, insect bites	1, 2
RHIZOPHORACEAE				
Cassipourea guianensis Aubl.	araçá bravo			4
RUBIACEAE				
Ladenbergia undata Klotzsch	quina quina	leaf, bark	treatment of fever, malaria	8
RUTACEAE				
Citrus aurantifolia (Christ.) Swingle	limeira de umbigo	root	treatment of malaria	2
SAPOTACEAE				
Micropholis venulosa (Mart. & Eichler) Pierre	abiurana branca	fruit	food	1
SIMAROUBACEAE				
Picrolemma sprucei Hook. f.	caferana		treatment of malaria, fever, gastritis	2,4
Simaba polyphylla (Cavalcante) W.W.Thomas		tree		4
Simaba sp. nov.		tree		4
Simarouba amara Aubl.	marupá	bark	treatment of fever, anemia, dyspepsia,	1,4
SOLANACEAE				
Physalis angulata L.	bolsa mullaca camapú	leaf, fruit	treatment of malaria, asthma, inflammation; narcotic	1, 2

References (ref) cited: 1 Duke & Vasquez (1994), 2 Milliken (1997), 3 Lorenzi (1998), 4 Ribeiro *et al.* (1999), 5 Revilla (2002), 6 Mors *et al.* (2000), 7 Schultes & Raffauf (1990), 8 Pio Corrêa (1978), 9 Grenand *et al.* (1987), 10 Silva *et al.* (1977), 11 Le Cointe (1947).

Table 2 - Data for Plant Extracts Exhibiting Lethality to *Aedes aegypti* Larvae.

FAMILY *Scientific name*	Part[1]	Extract[2]	Mortality (%)
APOCYNACEAE			
Aspidosperma araracanga Marcandes-Ferreira	Bk	M	4
A. marcgravianum Woodson	Bk	W	4
A. schultesii Woodson	Bk	M	33
ASTERACEAE			
Bidens pilosa L.	Rt	M	10
Spilanthes acmella (L.) Murray	Wp	M	37
BIGNOGNACEAE			
Tabebuia incana A. H. Gentry	Bk	E	4
CLUSIACEAE			
Calophyllum brasiliense Cambess.	Bk	W	20
CUCURBITACEAE			
Mormodica charantia L.	St	M	7
DICHAPETALACEAE			
Tapura amazonica Poepp.	Br	M	7
	Rt	M	100
EUPHORBIACEAE			
Croton cajucara Benth.	Lf	M	4
	Br	M	83
C. lanjouwensis Jabl.	Fr, Bk	M	90
	Sd	M	30
Micrandropsis scleroxylon W.A. Rodrigues	Rt	M	7
GENTIANACEAE			
Tachia grandiflora Maguire & Weaver	St	M	7
	Lf	M	10
	Br	M	30
	Rt	E	64
LAURACEAE			
Aniba rosaeodora Ducke	Lf	M	43
	Br	W	33
	Wd	M	23
PIPERACEAE			
Piper aduncum L.	Lf	M	100
	Rt	M	100
P. amapense Yunck.	Lf	M	17
	Br,St	W	4
P. capitarianum Yunck.	Lf	M	4
P. dilatatum Rich.	Br,St	M	7
P. hostmanianum (Miq.) C. DC.	Lf	M	17
	Br,St	M	7
P. tuberculatum Jacq.	Lf	M	100
	Fr	M	100
	Fr	W	53
	Br,St	M	100
Pothomorphe peltata (L.) Miq.	Lf	E	7
	Rt	E	7

cont. >

Table 2 - Data for Plant Extracts Exhibiting Lethality to *Aedes aegypti* Larvae. (cont.)

SIMAROUBACEAE			
Picrolemma sprucei Hook.	St	M	57
	Rt	M	74
		W	44
Simaba polyphylla (Cavalcante) W.W. Thomas	Br	M	100
Simaba sp. nov.	Br	M	70
Simarouba amara Aubl.	Br	M	37

1 - Bk – bark; Br – branch; Fl – flower; Fr – fruit; Lf – leaf; Rt – root; Sd – seed; St – stem; Wd – wood; Wp – whole plant.
2 - E – ethanol; M – methanol; W – water.

Table 3 - Inactive Plant Extracts.

Nome Científico	Part(s)[1] [Extract(s)] [2]
Abuta grandiflora (Mart.) Sandwith	V [M, W]
Ampelozizyphus amazonicus Ducke	V [M, W]
Aniba rosaeodora Ducke	Br [M], Lf [W], Wd [W]
Aspidosperma araracanga Marcandes-Ferreira	Bk [W]
A. desmanthum Benth. ex Müll. Arg.	Bk [M, W]
A. marcgravianum Woodson	Bk [M]
A. nitidum Benth. ex Müll. Arg.	Bk [M, W]
A. sandwithianum Markgr.	Bk [M, W]
A. schultesii Woodson	Bk [W]
A. spruceanum Benth. ex M. Arg.	Bk [M]
A. vargasii A. DC.	Bk [M]
Bidens pilosa L.	Rt [W]
Bixa orellana L.	Lf [M, W]
Calophyllum brasiliense Cambess.	Bk [W]
Cassia fastuosa Willd. ex Vogel	Lf [M], Rt [W]
C. spruceana Benth.	Rt [E]
Cassipourea guianensis Aubl.	Rt [M]
Citrus aurantifolia (Christ.) Swingle	RT [W]
Corythophora alta R. Knuth	Rt [M]
Croton cajucara Benth.	Br [W], Lf [M, W]
C. lanjouwensis Jabl.	Br [M, W], Fr [W], Lf [M, W], Sd [W]
Geissospermum argenteum Woodson	Bk [E, M]
G. urceolatum A.H. Gentry	Bk [E, M]
Himatanthus sucuuba (Spruce ex Müll. Arg.) Woodson	Bk [M, W]
Ladenbergia undata Klotzsch	Bk [M, W]
Micropholis venulosa (Mart. & Eichler) Pierre	Rt [M]
Minquartia guianensis Aubl.	Bk [M]
Momordica charantia L.	St/Lf [W]
Ocimum micranthum Willd.	Fl/Lf [M]
Physalis angulata L.	Rt [M, W], St [M, W], St/Lf [M, W]
Picrolemma sprucei Hook	Lf [M, W], St [W]
Piper aduncum L.	Br [M, W], Lf [W], Rt [W], St [M, W]
P. amapaense Yunck.	Br [M], Lf [M, W], Rt [M]
P. baccans (Miq.) C. DC.	Br [M, W], Lf [M, W], Rt [W]

cont. >

Table 3 - Inactive Plant Extracts. (cont.)

P. cyrtopodum C. DC.	Br [M, W], Lf [M, W], Rt [W]
P. dilatatum Rich.	Br [W], Lf [M, W], Rt [M, W]
P. erectipilum Yunck.	Br [M, W], Lf [M, W], Rt [M, W]
P. hostmannianum (Miq.) C. DC.	Br [W], Lf [W]
P. tuberculatum Jacq.	Br [W], Fr [M, W], Lf [W]
Portulaca pilosa L.	St/Lf [M, W]
Pothomorphe peltata (L.) Miq.	Rt [M, W]
Protium aracouchini (Aubl.) Marchand	Rt [M, W]
Scleria pratensis Lindl. ex Nees	Rt [M, W]
Senna occidentalis (L.) Link	Rt [W], Br [W], Lf [W]
Simaba polyphylla (Cavalcante) W.W.Thomas	Br [E, W], Lf [M, W], Br [E, W]
Simaba sp. nov.	Br [W], Lf [M, W]
Simarouba amara Aubl.	Br [W], Lf [M, W]
Spilanthes acmella (L.) Murray	Wp [W]
Spondias mombin L.	Bk [M]
Swartzia prouacesis (Aubl.) Amshoff	Br [W], Lf [W]
Tabebuia incana A.H. Gentry	Bk [M]
Tachia grandiflora Maguire & Weaver	Br [E, W], Lf [E, M,W], Rt [M, W], St [E]
Tapura amazônica Poepp.	Lf [M], Bk [M], Rt [W]

1 - Bk – bark; Br – branch; Fl – flower; Fr – fruit; Lf – leaf; Rt – root; Sd – seed; St – stem; Wd – wood; Wp – whole plant.
2 - E – ethanol; M – methanol; W – water.

the extracts prepared by extraction with the more polar water as solvent, which is probably an indication that toxicity to these larvae should be associated in general with substances of medium or even low polarity.

Our results confirmed the toxicity of several plant species which have been described as being toxic or are used traditionally for their toxicity. For example, methanol root extract of *Tapura amazonica* Poepp. was highly lethal to *A. aegypti* larvae which is interesting in light of the fact that it has been described by Colombian native indians as being a toxic species (Table 1; Schultes & Raffauf, 1990). In similar fashion, *P. tuberculatum* Jacq. presented highly lethal extracts in the assay and is, according to traditional use, an effective fish poison (Table 1). Lastly, *Picrolemma sprucei* Hook (caferana), which exhibited larvicidal root and stem extracts in the present study, is known for its toxic effects towards the human fetus, being used widely in the State of Amazonas and Manaus as an abortifacient tea (Pohlit *et al.*, unpublished data).

Another *Piper* species, *P. aduncum* L., exhibited good activity against *Aedes aegypti* (Table 2) and is known to produce the larvicidal phytochemical compound, dillapiole, which has been shown by Bernard *et al*. (1995) to be active against another *Aedes* sp.. Given that *P. aduncum* is a fast-growing, invasive species, it is potentially useful as an industrial source of dillapiole and other active phytochemicals.

Another interesting observation is that larvicidal activity is definitely specific to certain parts of plants, such as *P. aduncum*, which exhibited very active methanol leaf and root extracts (Table 2), however, extracts of other parts of this plant were inactive (Table 3). Other examples of this specificity of insecticidal activity to certain plant parts is evidenced by comparing the data presented in Tables 2 and 3.

Finally, it is important to note that even the low mortality exhibited by some extracts during screening can be significant for the discovery of bioactive phytochemicals. An example of this is the weak activity of the ethanol root and leaf extracts of *Pothomorphe peltata* (L.) Miq. (each exhibiting only 7 % mortality at 500 µg / mL, Table 2). In further work, bioassay-guided fractionation of this ethanol root extract in our labs yielded very active intermediate fractions and isolation of the phytochemical larvicide, 4-nerolidylcatechol, which exhibited very significant lethality towards *Aedes aegypti* larvae and was shown by chromatographic analytical methods to be a major component of both root and leaf extracts (Pinto, 2002).

CONCLUSION

A number of larvicidal plants were identified during this work. Several of these are now under biomonitored phytochemical analysis and should yield further examples of isolated larvicidal phytochemicals in the future.

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LITERATURE CITED

Bandara, K.A.N.P. *et al*. 2000. Insecticidal piperidine alkaloid from *Microcos paniculata* stem bark. *Phytochemistry*, 54(1):29-32.

Bergeron, C. *et al*. 1996. Screening of plants used by North American indians for antifungal, bactericidal, larvicidal, and molluscicidal activities. *Intl. J. Pharmacog.*, 34(4):233-242.

Bernard, C.B. *et al*. 1995. Insecticidal defenses of Piperaceae from the neotropics. *J. Chem. Ecol.*, 21(6):801-814.

Chantraine, J.M. *et al*. 1998. Insecticidal activity of essential oils on *Aedes aegypti* larvae. *Phytother. Res.*, 12(5):350-354.

Chariandy, C.M. *et al*. 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J. Ethnopharmacol.*, 64(3):265-270.

Ciccia, G., Coussio, J., Mongelli, E. 2000. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J. Ethnopharmacol.*, 72(1-2): 185-189.

Diallo, D. *et al*. 2001. Screening of Malian medicinal plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. *Phytother. Res.*, 15(5):401-406.

Duke, J.A.; Vasquez, R. 1994. *Amazonian Ethnobotanical Dictionary*. CRC Press, Florida, United States. 215 pp.

Grenand, P. Moretti, C., Jacquemin, H. 1987. *Pharmacopées traditionnelles en Guyane: Créoles, Palikur, Wayãpi*. Éditions de l'ORSTOM, Paris. 569pp.

Hostettmann, K., Potterat, O. 1997. Strategy for the isolation and analysis of antifungal, molluscicidal and larvicidal agents from tropical plants. In: *Phytochemicals for Pest Control*. ACS Sym. Ser.. v. 658. American Chemical Society, Washington. p. 14-26.

Latha, C., Ammini, J. 2000. *Curcuma raktakanda* is a potential larvicide for mosquito control. *Pharm. Biol.*, 38(3):167-170.

Le Cointe, P. 1947. *Árvores e Plantas Úteis: indígenas e aclimadas*. 2ª ed.. Companhia Editora Nacional, São Paulo, 506 pp.

Lee, S.E. 2000. Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, *Piper longum*. *J. Amer. Mosq. Ctrl. Assoc.*, 16(3):245-247.

Lorenzi, H. 1998. *Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil*. Editora Plantarum, Nova Odessa (SP), Brazil. 352 pp.

Macedo, M. E. *et al*. 1997. Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). *Mem. Inst. Osw. Cruz*, 92(4):565-570.

Marston, A. *et al*. 1993. Search for antifungal, molluscicidal and larvicidal compounds from African medicinal plants. *J. Ethnopharmacol.*, 38(2-3):215-233.

Milliken, W. 1977. *Plants for Malaria. Plantas for Fever: Medicinal Species in Latin*

AMERICA – a bibliographic survey. Whitstable Litho, United Kingdom. 116pp.

Mors, W. B., Rizzini, C. T., Pereira, N. A. 2000. *Medicinal Plants of Brazil*. DeFilipps, R.A. Ed.. Reference Publication, Algonac (U.S.A.). 501 p.

Oberlies, N.H. *et al*. 1998. Cytotoxic and Insecticidal Constituents of the Unripe Fruit of *Persea americana*. *J. Nat. Prod.*, 61(6): 781-785.

Park *et al*. 2002. Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. *J. Agric. Food Chem.*, 50(7):1866-1870.

Pinto, A.C.S., 2002. *Estudo fitoquímico e biológico de* Pothomorphe peltata *(L.) Miquel (Piperaceae)*, Master's Dissertation-Natural Products Chemistry, UFAM, Manaus.

Pio Corrêa, M. 1978. *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas,* v. 1-6, Ministério da Agricultura-Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro.

Pushpalatha E., Muthukrishnan, J. 1999. Efficacy of two tropical plant extracts for the control of mosquitoes. *J. Appl. Entom.,* 123(6): 369-373.

Quignard, E.L.J. *et al*. 2003. Screening of plants found in Amazonas state for lethality towards brine shrimp. *Acta Amazonica*, 33(1):93-104.

Rahuman, A. A. *et al*. 2000. Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia*, 71:553-557.

Revilla, J. 2002. *Apontamentos para a Cosmética Amazônica*. SEBRAE-AM / INPA, Manaus. 532pp.

Ribeiro, J.E.L. da S. *et al*. 1999. *Flora da Reserva Ducke - Guia de identificação das plantas vasculares de uma floresta de terra firme na Amazônia Central*. INPA / DFID, Manaus. 800pp.

Sathiyamoorthy, P. *et al*. 1997. Larvicidal activity in desert plants of the Negev and Bedouin market plant products. *Intl. J. Pharmacog.*, 35(4):265-273.

Schultes, R.E., RAFFAUF, R.F. 1990. *The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia*. Dioscorides Press, Oregon, United States. 484pp.

Sharma, N. *et al*. 1998. Larvicidal activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephansi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Pharm. Biol.*, 36(1):3-7.

Silva, M.F., Lisbôa, P.L.B., Lisbôa, R.C.L. 1977. *Nomes vulgares de Plantas Amazônicas*. INPA, Manaus. 222pp.

Sukamar, K., Perich, M.J., Boobar, L.R. 1991. Botanical derivatives in mosquito control: a review. *J. Am. Mosq. Contr. Ass.*, 7:210-237.

Thorsell, W.A. *et al*. 1998. Efficacy of plant extracts and oils as mosquito repellents. *Phytomedicine*, 5(4):311-323.

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