SCREENING OF PLANTS FOUND IN AMAZONAS STATE FOR LETHALITY TOWARDS BRINE SHRIMP

Etienne Louis Jacques QUIGNARD, Adrian Martin POHLIT, Sergio Massayoshi NUNOMURA, Ana Cristina da Silva PINTO, Elba Vieira Mustafa dos SANTOS, Sabrina Kelly Reis de MORAIS, Alexandre Mascarenhas ALECRIM, Andreza Cristiana da Silva PEDROSO, Barbara Rachel Brito CYRINO, Christiane Santana de MELO, Ellen Kathryn FINNEY, Erika de Oliveira GOMES, Katiuscia dos Santos de SOUZA, Laura Cristina Pereira de OLIVEIRA, Luciana de Castro DON, Luiz Francisco Rocha e SILVA, Maria Mireide Andrade QUEIROZ, Marycleuma Campos HENRIQUE, Mirian dos SANTOS, Patrícia de Souza PINTO, Suniá Gomes SILVA*

ABSTRACT: 226 methanol and water extracts representing 74 mainly native plant species found in Amazonas State, Brazil, were tested at a standard concentration of 500 µg/mL for lethality towards larvae of the brine shrimp species *Artemia franciscana*. Several cytotoxic plant species were identified in this work: *Aspidosperma marcgravianum, A. nitidum, Croton cajucara, Citrus limetta, Geissospermum argenteum, Minquartia guianensis, Piper aduncum, P. amapense, P. capitarianum, P. tuberculatum* and *Protium aracouchini*. The results were analyzed within the context of the available traditional knowledge and uses for these plants.

KEYWORDS: *Artemia franciscana*, cytotoxicity, amazonian plants.

SCREENING DE PLANTAS ENCONTRADAS NO ESTADO DO AMAZONAS PARA LETALIDADE AO MICROCRUSTÁCEO *Artemia franciscana*

RESUMO: Foram testados 226 extratos metanólicos e aquosos de 74 espécies vegetais (a maioria nativas) encontradas no Estado do Amazonas, Brasil, para letalidade às larvas da espécie microcrustácea *Artemia franciscana* na concentração teste de 500 µg/mL. Várias espécies citotóxicas foram identificadas nesse estudo: *Aspidosperma marcgravianum, A. nitidum, Citrus limetta, Croton cajucara, Geissospermum argenteum, Minquartia guianensis, Piper aduncum, P. amapense, P. capitarianum, P. tuberculatum* e *Protium aracouchini*. Os resultados foram analisados levando em consideração as informações populares, quando disponíveis.

PALAVRAS-CHAVE: *Artemia franciscana*, citotoxicidade, plantas amazônicas.

* Instituto Nacional de Pesquisas da Amazônia, INPA/CPPN. Avenida André Araújo, 2936, Petrópolis, CEP 69083-000, Manaus, AM. Brasil, e-mail: ampohlit@inpa.gov.br.

INTRODUCTION

Amazonian plant species are potential sources of phytotherapeutic agents, nutraceuticals, and leads for the development of new drugs (duke, 1994). Experience has shown that an obvious starting point for research in these areas is the literature on traditional knowledge of plants of the Amazon region (grenand, 1987; lorenzi, 1998; pio Corrêa, 1978; revilla, 2002; schultes, 1990; silva, 1977). These texts identify plants described by their users as having medicinal and other useful properties. However, for many plants there is no relevant literature available and so biological activity must be found using more direct methods such as farmacological testing or screening.

The brine shrimp lethality assay consists of exposing larvae to plant extract in saline solution and lethality is evaluated after a day. The commercial availability of inexpensive brine shrimp eggs, the low cost, safety and ease of performing the assay, as well as the lack of a need for special equipment make this a very helpful bench-top tool for the phytochemistry laboratory (mcLaughlin, 1991). First developed by meyer (1982), this assay has wide application in research towards the discovery of cytotoxic and other active principles present in plant extracts (mongelli, 1996). For example, a very positive correlation between the lethality to brine shrimp and antitumoral activity has been established by researchers working on the development of new anti-cancer drugs from plants at the National Cancer Institute (NCI) in the United States (anderson, 1991). This correlation is considered so good that lethality to brine shrimp is recommended by these authors as an effective pre-screen to existing cytotoxicity and antitumor assays. More recently, it has been shown that there is a very good correlation between the median lethal concentrations (LC_{50}) of plant extracts to brine shrimp larvae and the median lethal doses (LD_{50}) of these same extracts, administered orally in mice (Parra, 2001). A number of other studies have demonstrated the use of the brine shrimp assay to screen plants popularly used as pesticides (Fatope, 1993), plants having ethnomedical uses related to cancer (mongelli, 1996), and tropical plants used medicinally (together with screening for larvicidal, fungicidal, and molluscicidal activity in cepleanu (1994)). Lastly, this assay has been used successfully to biomonitor the isolation of cytotoxic (siqueira, 2001), antineoplastic (Badaway, 1997), antimalarial (pérez, 1997), insecticidal (oberlies, 1998) and anti-feedant (labbe, 1993) compounds from plant extracts.

In what follows, the results of a screening of methanol and water extracts of some common medicinal and other plant species (collected in Amazonas State, Brazil) for lethality towards *Artemia franciscana* larvae are presented.

METERIALS AND METHODS

Collection and drying of plants. Plants were collected in or near Benjamin Constant (located near the Brazilian border with Peru and Colombia), Novo Airão (110 km from Manaus) and Manaus (at the INPA Adolfo Ducke Reserve). Identification was performed by employees at the Herbarium at the University of Amazonas (Manaus, Amazonas) or at the Herbarium at the National Institute for Amazon Research (*Instituto Nacional de Pesquisas da Amazônia*, INPA). Plants were air dried in the shade and later separated by part (leaf, stem, bark, *etc.*).

Preparation of water extracts. Extraction was performed by infusion of the ground plant material in boiling water for 15 minutes after which the mixture was filtered and the filtrate evaporated to dryness under vacuum and then freeze-dried. The extracts were stored in a freeezer (-19 °C).

Preparation of methanol extracts. A known mass of each ground plant underwent continuous liquid-solid extraction with methanol in a Soxhlet apparatus for three consecutive 6 h periods (total 18 h). After each 6 h interval, the extract was removed and the Soxhlet apparatus was recharged with fresh methanol to avoid undue exposure of the extracts to heat during extraction. The combined methanol extracts were evaporated and stored as described for the water extracts.

Preparation of stock saline solution. Nitrate, phosphate and silicate free saline stock solution was prepared by dissolving mineral enriched sea salt (*SERA*, Germany*)* in de-ionized water at a concentration of 35 g/L.

Hatching of brine shrimp larvae. Brine shrimp eggs (Brine Shrimp Direct, U.S.A.) were sprinkled into a Petri dish containing saline solution and left for two days at 26 ± 1 ºC under an incandescent lamp. After this period, the eggs had hatched and second instar nauplii (larvae) were observed to be swimming near the light source.

Sample preparation. Methanol extracts were reconstituted in DMSO and water extracts in deionized water at a standard concentration of 50 mg/mL using sonication, shaking and/or mild heating.

Brine shrimp lethality assay. The wells of 24-well microtiter plates were pre-filled with 1.8 mL of saline solution. Next, 10 two-day old nauplii (second instar larvae) in a minimum of saline solution, 20 µL of each reconstituted extract solution and stock saline solution were added to give a final volume of 2.0 mL in each well. This corresponds to a 500 µ/L extract concentration in each experimental well. Each extract was tested in triplicate. Control wells received 20 µL of the corresponding solvent (DMSO or de-ionized water) used for the tested samples, instead of extract, and had the same final volume (2.0 mL) as experimental wells. The plates were allowed to stand in the absence of direct light (to avoid possible false positive results due to the generation of phototoxic compounds) at room temperature and mortality was evaluated after 24 h as the percentage of dead larvae present after 24 h relative to live larvae present originally.

RESULTS AND DISCUSSION

Information on the native and exotic species studied is presented in Table 1. Many of these plants were collected as part of an ongoing research program on the biological activity and chemistry of Amazonian antimalarial plants used in traditional medicine and include the barks of *carapanaúba*, *acariquara branca, taperebá* (also frequently called *cajá*), *abiu*, *castanha do Pará* (Brazil nut) trees and other plant species. Other species, such as *Aspidosperma* spp., *Piper* spp. and *Cassia* spp., were collected following a chemosystematic approach, *i.e.* they belong to genera from which cytotoxic, antimalarial and other interesting medicinal substances have been isolated. When possible, several parts of the plant were collected, not restricting the parts collected to those used traditionally. This allows for a more complete analysis and understanding of the cytotoxicity of the plants and the part(s) of the plants where cytotoxic substances are concentrated.

Table 2 shows the results of the screening of methanol and water extracts of plants using the brine shrimp assay. In all, 226 extracts representing 74 species were screened for lethality to brine shrimp nauplii and 24.3 % (55 extracts) had lethalities > 20 %, 14.6 % (33 extracts) had lethalities > 50 %, while only 8.8 % (20 extracts) were considered highly active, having lethalities > 90 % to brine shrimp.

Table 1. Ethnobotanic information for the plants studied.

FAMILY *Scientific name*	Common name	Part(s) used	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
ANNONACEAE				
Bocageopsis multiflora (Mart.) R.E.Fries	envira surucucu	wood	construction, tools, ornament, reforestation	3, 4
APIACEAE				
Eryngium foetidum L.	chicória	leaf, root, stem, fruit	perfumary, treatment of diarrhea, fever, headaches	2, 5
APOCYNACEAE				
Aspidosperma araracanga Marcondes-Ferreira				4
Aspidosperma desmanthum Benth. ex Müll. Arg.	araracanga	leaf	febrifuge	4, 6
Aspidosperma marcgravianum Woodson	carapanaúba	wood, bark	construction; treatment malaria, diabetes, cancer	1 , 4
Aspidosperma nitidum Benth. ex Müll. Arg.	carapanaúba	wood, bark, latex	construction; treatment leprosy, malaria, cancer	1, 4
Aspidosperma sandwithianum Markgr.				4
Aspidosperma schultesii Woodson		bark	termite protector; antimycotic; febrifuge	4, 7
Aspidosperma spruceanum Benth. ex Müll. Arg.		wood	construction	3, 4
Aspidosperma vargasii A. DC.			fever; wound treatment, insect bites	2, 7
Aspidosperma sp.				4
Geissospermum argenteum Woodson	acariquara branca	bark	fever; malaria	2, 4
Himatanthus sucuuba (Spruce ex Müll. Arg.) Woodson	sucuuba	latex	fever, bone fractures, tooth aches	1, 4
ARECACEAE				
Euterpe oleracea Mart.	açaizeiro	root, fruit, seed	beverage; treatment jaundice; fever	1
Mauritia flexuosa L.f.	buriti	fruit	food, beverage	1, 4
ASTERACEAE				
Bidens bipinnatus L.	carrapicho de agulha			8
Spilanthes acmella (L.) Murray	jambú	flower	lung diseases, tuberculosis	1
BIGNONIACEAE				
Arrabidaea chica (Humb. & Bonpl.) B. Verl.	crajirú	leaf	dye, astringent; treatment infections, inflammation	1, 5
Tabebuia incana A.H. Gentry	pau d'arco	bark	candidiasis; tumors	1
Tabebuia serratifolia (Vahl) G. Nichol.	pau d'arco	wood, bark	candidiasis; tumors; fever; leishmaniosis	1
BURSERACEAE				
Protium aracouchini (Aubl.) Marchand	breu vermelho	resin	laxative	9
DICHAPETALACEAE				
Tapura amazonica Poepp.	tapura	leaf	toxic	7
EUPHORBIACEAE				
Croton cajucara benth.	sacaca	bark	treatment of diabetes; diarrhea; liver inflammation	1
Croton lanjouwensis jabl.	dima	bark	fever	2,10
Mabea subsessilis Pax & K. Hoffim.	taquari	wood		4
Micrandra siphonioides Benth.	seringarana	wood		4

Table 1. Ethnobotanic information for the plants studied (Continued).

FAMILY *Scientific name*	Common name	Part(s) used	Medicinal and other use(s) / property(-ies)	Ref
EUPHORBIACEAE				
Micrandropsis scleroxylon W.A. Rodrigues	piãozinho	wood		
Phyllanthus niruri L.	quebra-pedra	whole plant	treatment of malaria, kidney stones, liver disease	1
Piranhea trifoliata Baill.	piranheira	wood	construction, carpentry	11
GENTIANACEAE				
Tachia grandiflora Maguire & Weaver				4
LAMIACEAE				
Ocimum micranthum Willd.	alfavaca	leaf	malaria	2, 5
LAURACEAE				
Aniba canelilla (Kunth.) Mez.	preciosa	bark, seed, leaf	perfumary, tonic; treatment malaria, syphilis, dysentery	5
LECYTHIDACEAE				
Bertholletia excelsa Bonpl.	castanha do Pará	seed, bark	food; cosmetics; treatmento liver ailments, anemia	1
Corythophora alta R. Kunth.	ripeiro (vermelho)	wood		4
Eschweilera bracteosa (Poepp. ex O. Berg) Miers	matamatá amarelo	wood		4
Gustavia elliptica S.A. Mori	mucurão	wood	construction, ornament	4
LEGUMINOSAE: MIMOSOIDEAE				
Abarema floribunda (Spruce ex Benth.) Barneby & J.W. Grimes				4
LEGUMINOSAE: CAESALPINIOIDEAE				
Cassia fastuosa Willd. ex Vogel	chuva-de-ouro		ornament	
Cassia siamea Lam.	acácia de Siam			
Cassia spruceana Benth.	mari-mari-da-terra-firme	root	fever	2, 4
Senna occidentalis (L.) Link	mata-pasto	root, seed	treatment fever, malaria, asthma; abortive, toxic	1, 2
Senna reticulata (Willd.) H.S. Irwin & Barneby	mata-pasto	leaf, flower	liver diseases; stomach aches; kidney inflammation	1, 2
LEGUMINOSAE: PAPILIONOIDEAE				
Bowdichia nitida Spruce ex Benth.	sucupira	bark	treatment of syphilis, skin diseases	7, 4
Swartizia pronacencis Amsh.	mututi			8
MENISPERMACEAE				
Abuta grandifolia (Mart.) Sandwith.	cipó-de-bota	root, stem, leaf	treatment of sterility, malaria, anemia	1
OLACACEAE				
Minquartia guianensis Aubl.	acariquara vermelha	wood, bark	posts, civil construction; fish poison	1
PHYTOLACCACEAE				
Petiveria alliacea L.	mucuracá	whole plant	abortive; antispasmodic; antirheumatic; antipyretic; diuretic	1
Agonandra brasiliensis Miers ex Benth. & Hook. f.	pau marfim	wood	tools, carpentry	10
PIPERACEAE				
Piper aduncum L.	pimenta longa	leaf, fruit	aromatic, styptic, antimicrobial, antimycotic	7
Piper amapense Yunck.	tapi-ipele	leaf, stem	treatment of dizziness	9
Piper capitarianum Yunck.				

Table 1. Ethnobotanic information for the plants studied (Continued).

FAMILY *Scientific name*	Common name	Part(s) used	Medicinal and other use(s) / property(-ies)	Ref
PIPERACEAE				
Piper cyrtopodum C. DC.	jacamim	leaf	aromatic baths	10
Piper dilatatum Rich.				
Piper erectipilum Yunck.				
Piper hostmannianum (Miq.) C. DC.	cordoncillo	leaf	treatment of warts	7
Piper tuberculatum Jacq.	pimenta longa	leaf	fish poison	7, 6
Pothomorphe peltata (L.) Miq.	caapeba	leaf	diuretic, antipyretic and emetic; sudorific	1
POACEAE				
Cymbopogon citratus (DC.) Stapf.	capim -santo	leaf, root	beverage; digestive; antipyretic	1
RHAMNACEAE				
Ampelozizyphus amazonicus Ducke	saracura-mirá	root, bark, leaf	treatment of insect bites; depurative; antimalarial	1, 2
RHIZOPHORACEAE				
Cassipourea guianensis Aubl.	araçá bravo			4
RUBIACEAE				
Ladenbergia undata Klotzsch	quina-quina			
Palicourea carymbifera (Müll. Arg.) Standl.		bark	treatment of persistent cough, chest ailments	7, 4
Palicourea guianensis Aubl.		root, leaf	styptic and hemostatic; vermifuge	7
Palicourea virens (Poepp. & Endl.) Standl.				4
RUTACEAE				
Citrus limetta Risso	limeira de umbigo			8
SAPOTACEAE				
Micropholis venulosa (Mart. & Eichler) Pierre	abiurana branca	fruit	food	1
Pouteria caimito (Ruiz & Pav.) Radlk.	abieiro	fruit, wood	food, construction	3
Pouteria guianensis Aubl.	abiurana, abieiro	fruit	food	1
SIMAROUBACEAE				
Picramnia Spruceana Engl.	sam panga	fruit	dye; treatment of skin irritation	1
Simaba cedron Planch.			pungent	4
SOLANACEAE				
Physalis angulata L.	camapú	leaf, fruit, root	narcotic; diuretic; anti-inflamatory;	1
VIOLACEAE				
Rinorea guianensis Aubl.	falsa cupiuba	wood	construction	4
Rinorea racemosa (Mart.) Kuntze	branquinha	wood	construction	1

References (ref) cited: 1 duke (1994), 2 milliken (1997), 3 lorenzi (1998), 4 ribeiro (1999), 5 revilla (2002), 6 mors (2000), 7 schultes (1990), 8 pio Corrêa (1978), 9 grenand (1987), 10 silva (1977), 11 le Cointe (1947).

The most active species were *Aspidosperma marcgravianum, A. nitidum, Citrus limetta, Croton cajucara, Geissospermum argenteum, Minquartia guianensis, Piper tuberculatum, P. aduncum, P. amapense, P. capitarianum and Protium aracouchini.* It is interesting that species such as *Piper tuberculatum* and *Minquartia guianensis*, which are traditionally used for their toxic properties, and *Tapura amazonica*, which is reportedly toxic, show significant lethality to brine shrimp. This result is similar in kind to that of Parra (2001) who found a correlation between the toxicity of orally administered plant extracts in mice and the lethal concentration of the extracts to brine shrimp.

Our systematic approach to screening of the genus *Aspidosperma* revealed that only the barks of a few species were active in the brine shrimp assay. This would seem to indicate that cytotoxic compounds are not generally distributed in the barks of the *Aspidosperma* species studied. The two species *A. nitidum* and *A. marcgravianum*, both popularly called *carapanaúba*, are traditionally used for the treatment of a number of diseases, including cancer. Since it is known that *Aspidosperma* species produce antitumor alkaloids such as ellipticine (Ohashi, 1996), the activity of these two species in the brine shrimp assay make them interesting as potencial sources of anticancer agents. Our results show that several *Piper* species are also active in the brine shrimp assay which is consistent with existing phytochemical knowledge of this genus as a source of cytotoxic and antitumor compounds (Parmar, 1997).

CONCLUSION

A number of cytotoxic plants were identified during this work. Several of them are now under biomonitored phytochemical analysis and should yield isolated cytotoxic components in due time. These results represent an important first step in a planned long term collaboration between labs at CPPN and those at the College of Medicine at the University of Ceará (Fortaleza, Ceará State, Brazil) for the discovery of anticancer compounds present in Amazonian plant species.

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Table 2. Lethality of Plant Extracts in the Brine Shrimp Assay.

BOTANICAL FAMILY *Scientific name*	Part	Brine Shrimp Lethality (%) MeOH	 Water
ANACARDIACEAE			
Spondias Mombin	bark	42	14
ANNONACEAE			
Bocageopsis multiflora	root	0	ND
APIACEAE			
Eryngium foetidum		0	0
APOCYNACEAE			
Aspidosperma araracanga	bark	ND	3
Aspidosperma desmanthum	bark	6	0
Aspidosperma marcgravianum	bark	100	0
Aspidosperma nitidum	bark	70	25
Aspidosperma sandwithianum	bark	0	0
Aspidosperma shultesii	bark	0	0
Aspidosperma spruceanum	bark	65	0
Aspidosperma vargasii	bark	94	3
Aspidosperma sp.	bark	17	6
	bark	11	0
Geissospermum argenteum	wood	100	3
	bark	0	21
Himatanthus sucuuba	bark	8	0
ARECACEAE			
Euterpe oleracea	root	0	14
Mauritia flexuosa	root	2	0
ASTERACEAE			0
Bidens bipinnatus	stem	0	0
	leaf	0	0
Spilanthes acmella	leaf	0	0
BIGNONIACEAE			0
Arrabidaea chica	leaf	0	2
Tabebuia incana	bark	49	3
Tabebuia serratifolia	bark	82	0
BURSERACEAE			
Protium aracouchini	root	100	46
	bark	81	ND
	leaf	22	ND
	branch	52	ND
Tapura amazonica	bark	18	0
Tapura amazonica	leaf	81	ND
	bark	7	ND
EUPHORBIACEAE			
Croton cajucara	leaf	98	5
	branch	97	72
Croton lanjouwensis	leaf	44	0
	fruit	7	0
	bark		
	branch	0	7
	seed	91	17
Mabea subsessilis	branch	0	0
micrandra siphonioides	leaf	ND	2
	root	5	0
Micrandropsis scleroxylon	root	95	0
Phyllanthus niruri	whole plant	0	0
Piranhea tripholiata	bark	ND	3
GENTIANACEAE			
Tachia grandiflora	leaf	0	0
	root	13	0
LAMIACEAE		11	
Ocimum micranthum	fruit, flower	31	0
	stem	0	0
	root	0	ND
LAURACEAE			
Aniba canelilla	bark	ND	0
LECYTHIDACEAE			
Bertholletia excelsa	bark	5	0
Corytophora alta	root	20	ND
Eschweilera bracteosa	root	9	0
Gustavia elliptica	bark	ND	0
	root	12	ND
LEGUMINOSAE: MIMOSOIDEAE			
Abarema floribunda	flower	0	0
	leaf	0	11
	branch	ND	0

Table 2. Lethality of Plant Extracts in the Brine Shrimp Assay (continued).

BOTANICAL FAMILY *Scientific name*	Part	Brine Shrimp Lethality (%) MeOH	 Water
ANACARDIACEAE			
Spondias Mombin	bark	42	14
ANNONACEAE			
Bocageopsis multiflora	root	0	ND
APIACEAE			
Eryngium foetidum		0	3
APOCYNACEAE			
Aspidosperma araracanga	bark	ND	0
Aspidosperma desmanthum	bark	6	0
Aspidosperma marcgravianum	bark	100	25
Aspidosperma nitidum	bark	70	0
Aspidosperma sandwithianum	bark	0	0
Aspidosperma shultesii	bark	0	0
Aspidosperma spruceanum	bark	65	3
Aspidosperma vargasii	bark	94	6
Aspidosperma sp.	bark	17	6
	bark	11	0
Geissospermum argenteum	wood	100	3
	bark	0	21
Himatanthus sucuuba	bark	8	0
ARECACEAE			
Euterpe oleracea	root	0	14
Mauritia flexuosa	root	2	0
ASTERACEAE			
Bidens bipinnatus	stem	0	0
	leaf	0	0
Spilanthes acmella	leaf	0	0
BIGNONIACEAE			
Arrabidaea chica	leaf	0	2
Tabebuia incana	bark	49	3
Tabebuia serratifolia	bark	82	0
BURSERACEAE			
Protium aracouchini	root	100	46
	bark	81	ND
	leaf	22	ND
Tapura amazonica	branch	52	ND
Tapura amazonica	leaf	81	ND
	bark	7	ND
EUPHORBIACEAE			
Croton cajucara	leaf	98	5
	branch	97	72
Croton lanjouwensis	leaf	44	0
	fruit	7	0
	barch		
	branch	0	7
	seed	91	17
Mabea subsessilis	branch	0	0
micrandra siphonioides	leaf	ND	2
	root	5	0
Micrandropsis scleroxylon	root	95	0
Phyllanthus niruri	whole plant	0	0
Piranhea tripholiata	bark	ND	3
GENTIANACEAE			
Tachia grandiflora	leaf	0	0
	root	13	0
LAMIACEAE		11	
Ocimum micranthum	fruit	31	0
	flower		
	stem	0	0
	root	0	ND
LAURACEAE			
Aniba canelilla	bark	ND	0
LECYTHIDACEAE			
Bertholletia excelsa	bark	5	0
Corytophora alta	root	20	ND
Eschweilera bracteosa	root	9	0
Gustavia elliptica	bark	ND	0
	root	12	ND
LEGUMINOSAE: MIMOSOIDEAE			
Abarema floribunda	flower	0	0
	leaf	0	11
	branch	ND	0

Table 2. Lethality of Plant Extracts in the Brine Shrimp Assay (continued).

BOTANICAL FAMILY	Part	Brine Shrimp Lethality (%)	
Scientific name		MeOH	Water
LEGUMINOSAE: CAESALPINIODEAE			
Cassia fastuosa	bark	14	0
	leaf	71	0
	branch	0	7
	root	0	42
Cassia siamea	flower	ND	0
	leaf	2	2
	branch	0	0
Cassia spruceana	bark	0	17
	root	0	5
Senna occidentalis	leaf	9	70
Senna reticulata	flower	16	0
	leaf	0	0
	stem	65	3
	pod	0	0
	branch	0	0
	root	0	0
LEGUMINOSAE: PAPILIONOIDEAE			
Bowdichia nitida	root	ND	0
Swartizia pronacencis	root	2	0
MENISPERMACEAE		2	0
Abuta grandifolia	vine	22	11
	whole plant	27	0
OLACACEAE			
Minquartia guianensis	bark	98	3
PHYTOLACCACEAE	branch		
Agonandra brasiliensis	root	5	ND
Petiveria alliacea	leaf	0	0
	root, stem	0	0
	stem	ND	0
PIPERACEAE			
Piper aduncum	stem	7	0
	leaf	100	0
	branch	0	0
	root	100	0

BOTANICAL FAMILY	Part	Brine Shrimp Lethality (%)	
Scientific name		MeOH	Water
Piper amapense	leaf	7	ND
	branch	21	0
	root	100	ND
Piper capitarianum	leaf	36	ND
	branch	97	ND
	root	97	ND
Piper cyrtopodum	branch	4	ND
Piper erectipilum	leaf	0	ND
Piper hostmannianum	leaf	95	6
	branch	72	0
Piper tuberculatum	leaf	95	3
	fruit	100	58
	branch	100	68
Pothomorphe peltata	leaf	14	0
	fruit	75	0
	branch	34	0
	root	96	0
POACEAE			
Cymbopogon citratus	leaf	50	0
RHAMNACEAE			
Ampelozizyphus amazonicus		31	11
			ND
RHIZOPHORACEAE			
Cassiopourea guianensis	pod	25	ND
RUBIACEAE			0
Ladenbergia undata	bark	22	8
Palicourea carymbifera	leaf	0	ND
	stem	ND	0
Palicourea guianensis	leaf	0	0
Palicourea virens	leaf	0	0
	stem	ND	0
RUTACEAE			
Citrus limetta	root	100	0
SAPOTACEAE			
Micropholis venulosa	root	8	27
	bark	ND	0
	root	8	ND

Table 2. Lethality of Plant Extracts in the Brine Shrimp Assay (Continued).

BOTANICAL FAMILY / *Scientific name*	Part	Brine Shrimp Lethality (%) MeOH	Water	BOTANICAL FAMILY / *Scientific name*	Part	Brine Shrimp Lethality (%) MeOH	Water
Pouteira caimito	bark	5	5	*Simaba cedron*	stem	42	58
Pouteria guianensis	root	13	5		leaf	0	0
SIMAROUBACEAE				**SOLANACEAE**			
Picramnia spruceana	leaf	6	7	*Physalis angulada*	root	0	0
	minor branch	0	19	**VIOLACEAE**			
	trunk wood	0	0	*Rinorea guanensis*	bark	ND	0
	petiole	2	24		leaf	ND	3
	seed	0	0		root	3	ND
				Rinorea racemosa	root	0	0

ND= Not determined. Lethalities should be considered accurate to within ± 3 %.

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