

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

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**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*, *Minuartia 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*, *Minuartia 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.

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## 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 pharmacological 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.

## MATERIALS 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 freezer (-19 °C).

*Preparation of methanol extracts.* A known mass of each ground plant underwent con-

tinuous 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  $26 \pm 1$  °C 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 µL 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*, *acariguara 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.

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

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

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

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

<b>FAMILY</b> <i>Scientific name</i>	Common name	Part(s) used	Medicinal and other use(s) / property(-ies)	Ref
<b>PIPERACEAE</b>				
<i>Piper cyrtopodum</i> C. DC.	jacamim	leaf	aromatic baths	10
<i>Piper dilatatum</i> Rich.				
<i>Piper erectipilum</i> Yunck.				
<i>Piper hostmannianum</i> (Miq.) C. DC.	cordoncillo	leaf	treatment of warts	7
<i>Piper tuberculatum</i> Jacq.	pimenta longa	leaf	fish poison	7, 6
<i>Pothomorphe peltata</i> (L.) Miq.	caapeba	leaf	diuretic, antipyretic and emetic; sudorific	1
<b>POACEAE</b>				
<i>Cymbopogon citratus</i> (DC.) Stapf.	capim -santo	leaf, root	beverage; digestive; antipyretic	1
<b>RHAMNACEAE</b>				
<i>Ampelozizyphus amazonicus</i> Ducke	saracura-mirá	root, bark, leaf	treatment of insect bites; depurative; antimalarial	1, 2
<b>RHIZOPHORACEAE</b>				
<i>Cassipourea guianensis</i> Aubl.	araçá bravo			4
<b>RUBIACEAE</b>				
<i>Ladenbergia undata</i> Klotzsch	quina-quina			
<i>Palicourea carymbifera</i> (Müll. Arg.) Standl.		bark	treatment of persistent cough, chest ailments	7, 4
<i>Palicourea guianensis</i> Aubl.		root, leaf	styptic and hemostatic; vermifuge	7
<i>Palicourea virens</i> (Poepp. & Endl.) Standl.				4
<b>RUTACEAE</b>				
<i>Citrus limetta</i> Risso	limeira de umbigo			8
<b>SAPOTACEAE</b>				
<i>Micropholis venulosa</i> (Mart. & Eichler) Pierre	abiurana branca	fruit	food	1
<i>Pouteria caimito</i> (Ruiz & Pav.) Radlk.	abieiro	fruit, wood	food, construction	3
<i>Pouteria guianensis</i> Aubl.	abiurana, abieiro	fruit	food	1
<b>SIMAROUBACEAE</b>				
<i>Picramnia Spruceana</i> Engl.	sam panga	fruit	dye; treatment of skin irritation	1
<i>Simaba cedron</i> Planch.			pungent	4
<b>SOLANACEAE</b>				
<i>Physalis angulata</i> L.	camapú	leaf, fruit, root	narcotic; diuretic; anti-inflammatory;	1
<b>VIOLACEAE</b>				
<i>Rinorea guianensis</i> Aubl.	falsa cupiuba	wood	construction	4
<i>Rinorea racemosa</i> (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 Coite (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 potential 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	Part	Brine Shrimp Lethality (%)		BOTANICAL FAMILY	Part	Brine Shrimp Lethality (%)	
		MeOH	Water			MeOH	Water
Scientific name				Scientific name			
<b>ANACARDIACEAE</b>				<i>Tapura amazonica</i>			
<i>Spondias Mombin</i>	bark	42	14		leaf	81	ND
					bark	7	ND
<b>ANNONACEAE</b>				<b>EUPHORBIACEAE</b>			
<i>Bocageopsis multiflora</i>	root	0	ND	<i>Croton cajucara</i>	leaf	98	5
<b>APIACEAE</b>				<i>Croton lanjouwensis</i>			
<i>Eryngium foetidum</i>		0	0		branch	97	72
					leaf	44	0
					fruit	7	0
					bark		
<b>APOCYNACEAE</b>							
<i>Aspidosperma araracanga</i>	bark	ND	3		branch	0	7
<i>Aspidosperma desmanthum</i>	bark	6	0		seed	91	17
<i>Aspidosperma marcgravianum</i>	bark	100	0	<i>Mabea subsessilis</i>	branch	0	0
<i>Aspidosperma nitidum</i>	bark	70	25	<i>micrandra siphonioides</i>	leaf	ND	2
<i>Aspidosperma sandwithianum</i>	bark	0	0		root	5	0
<i>Aspidosperma shultesii</i>	bark	0	0	<i>Micrandropsis scleroxylon</i>	root	95	0
<i>Aspidosperma spruceanum</i>	bark	65	0	<i>Phyllanthus niruri</i>	whole plant	0	0
<i>Aspidosperma vargasii</i>	bark	94	3	<i>Piranhea tripholiata</i>	bark	ND	3
<i>Aspidosperma sp.</i>	bark	17	6	<b>GENTIANACEAE</b>			
	bark	11	0	<i>Tachia grandiflora</i>	leaf	0	0
<i>Geissospermum argenteum</i>	wood	100	3		root	13	0
	bark	0	21	<b>LAMIACEAE</b>			
<i>Himatanthus sucuuba</i>	bark	8	0	<i>Ocimum micranthum</i>	fruit, flower	11	0
<b>ARECACEAE</b>							
<i>Euterpe oleracea</i>	root	0	14		stem	0	0
<i>Mauritia flexuosa</i>	root	2	0		root	0	ND
<b>ASTERACEAE</b>				<b>LAURACEAE</b>			
			0	<i>Aniba canelilla</i>	bark	ND	0
<i>Bidens bipinnatus</i>	stem	0	0	<b>LECYTHIDACEAE</b>			
	leaf	0	0	<i>Bertholletia excelsa</i>	bark	5	0
<i>Spilanthes acmella</i>	leaf	0	0	<i>Corytophora alta</i>	root	20	ND
<b>BIGNONIACEAE</b>				<i>Eschweilera bracteosa</i>			
<i>Arrabidaea chica</i>	leaf	0	2		root	9	0
<i>Tabebuia incana</i>	bark	49	3	<i>Gustavia elliptica</i>	bark	ND	0
<i>Tabebuia serratifolia</i>	bark	82	0		root	12	ND
<b>BURSERACEAE</b>				<b>LEGUMINOSAE: MIMOSOIDEAE</b>			
<i>Protium aracouchini</i>	root	100	46	<i>Abarema floribunda</i>	flower	0	0
	bark	81	ND		leaf	0	11
	leaf	22	ND		branch	ND	0
	branch	52	ND				
<i>Tapura amazonica</i>	bark	18	0				

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

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

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

<b>BOTANICAL FAMILY</b>	Part	Brine Shrimp Lethality (%)		<b>BOTANICAL FAMILY</b>	Part	Brine Shrimp Lethality (%)	
<i>Scientific name</i>		MeOH	Water	<i>Scientific name</i>		MeOH	Water
<b>LEGUMINOSAE: CAESALPINIOIDEAE</b>				<i>Piper amapense</i>	leaf	7	ND
<i>Cassia fastuosa</i>	bark	14	0		branch	21	0
	leaf	71	0		root	100	ND
	branch	0	7	<i>Piper capitarianum</i>	leaf	36	ND
	root	0	42		branch	97	ND
<i>Cassia siamea</i>	flower	ND	0		root	97	ND
	leaf	2	2	<i>Piper cyrtopodum</i>	branch	4	ND
	branch	0	0	<i>Piper erectipilum</i>	leaf	0	ND
<i>Cassia spruceana</i>	bark	0	17	<i>Piper hostmannianum</i>	leaf	95	6
	root	0	5		branch	72	0
<i>Senna occidentalis</i>	leaf	9	70	<i>Piper tuberculatum</i>	leaf	95	3
<i>Senna reticulata</i>	flower	16	0		fruit	100	58
	leaf	0	0		branch	100	68
	stem	65	3	<i>Pothomorphe peltata</i>	leaf	14	0
	pod	0	0		fruit	75	0
	branch	0	0		branch	34	0
	root	0	0		root	96	0
<b>LEGUMINOSAE: PAPILIONOIDEAE</b>				<b>POACEAE</b>			
<i>Bowdichia nitida</i>	root	ND	0	<i>Cymbopogon citratus</i>	leaf	50	0
<i>Swartzia pronacensis</i>	root	2	0	<b>RHAMNACEAE</b>			
<b>MENISPERMACEAE</b>		2	0	<i>Ampelozizyphus amazonicus</i>		31	11
<i>Abuta grandifolia</i>							ND
	vine	22	11	<b>RHIZOPHORACEAE</b>			
	whole plant	27	0	<i>Cassiopourea guianensis</i>	pod	25	ND
<b>OLACACEAE</b>				<b>RUBIACEAE</b>			
<i>Minquartia guianensis</i>	bark	98	3	<i>Ladenbergia undata</i>	bark	22	8
<b>PHYTOLACCACEAE</b>				<i>Palicourea carymbifera</i>	leaf	0	ND
<i>Agonandra brasiliensis</i>	branch				stem	ND	0
<i>Petiveria alliacea</i>	root	5	ND	<i>Palicourea guianensis</i>	leaf	0	0
	leaf	0	0	<i>Palicourea virens</i>	leaf	0	0
	root, stem	0	0		stem	ND	0
	stem	ND	0	<b>RUTACEAE</b>			
<b>PIPERACEAE</b>				<i>Citrus limetta</i>	root	100	0
<i>Piper aduncum</i>	stem	7	0	<b>SAPOTACEAE</b>			
	leaf	100	0	<i>Micropholis venulosa</i>	root	8	27
	branch	0	0		bark	ND	0
	root	100	0		root	8	ND

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

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

ND= Not determined. Lethalities should be considered accurate to within  $\pm 3\%$ .

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