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RESEARCH ARTICLE

The good and the bad: Alkaloid screening and brine shrimp bioassays of aqueous extracts of 31 medicinal plants of eastern Nicaragua

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Abstract

Context: Presence/absence tests for alkaloids of 31 medicinal vascular plant species from 31 genera and 26 families of eastern Nicaragua provided a baseline for bioactivity tests.

Objective: To determine the bioactivity and cytoxicity of aqueous extracts of widely used medicinal species in eastern Nicaragua.

Materials and methods: Ethnomedicinal applications were obtained from interviews of traditional healers. We used Dragendorff's reagent to test alkaloids and brine shrimp for cytotoxicity of aqueous extracts.

Results: Twenty-nine of the 31 species tested positive for alkaloids. The median lethal concentration that kills 50% of the larvae within 24 h of contact with the extract (LC_{50} was less than 1000 μg/mL for 4 (13%) species (the usual cytotoxic category), 1001–5000 μg/mL for 23 (74%) species, and between 5001–7500 μg/mL for the remaining 4 (13%) species.

Discussion and Conclusion: Twenty-five of the ethnomedicines contain alkaloids but are not cytotoxic. In contrast to first suppositions, we suggest that this is a good and desirable, and perhaps expected, outcome. Medicinal plants that are cytotoxic may obviously control or kill bacteria or other pathogens, but may also negatively affect the patient; some high alkaloid levels have been associated with carcinogens. Thus, perhaps the majority of effective medicinals should be expected to be noncytotoxic. We suggest that this is a new paradigm for consideration of the overall value and effectiveness of medicinals. Of course, medicinals also can be effective in numerous ways (e.g., organ stimulation or other physiological functions) other than simply as antimicrobials or antipathogens.

Keywords: Biological screening, cytotoxic activity, ethnomedicinal plants

Introduction

Plants are the oldest source of pharmacologically active compounds and serve as the primary source of medicine for people in developing countries, particularly in the tropics (Farnsworth, 1994; Farnsworth & Soejarto, 1991). In spite of this, the flora of many tropical countries is poorly documented, and more importantly, the pharmaceutical potential of most tropical species remains untested, a situation that exists in eastern Nicaragua.

Nicaragua is a nation of 140,000 km^2, of which 41,700 km^2 (30%) is forested with over 6000 species of vascular plants (Stevens et al., 2001, 2009; Sutton, 1989). In 1977, the Missouri Botanical Garden began the first systematic study of the flora and published the "Flora de Nicaragua" (Stevens et al., 2001, 2009). The Eastern Lowlands, which is the most extensive ecoregion in Nicaragua at about 33% of the country, remained botanically poorly known to modern systematics until recently (Taylor, 1959, 1962, 1963; Seymour, 1980; Dennis, 1988; Boucher, 1990; Boucher et al., 1990, 1994, 1996, 2001; Vandermeer et al., 1990a, 1990b, 1991, 1996, 1997, 1999, 2001; Yih et al., 1991; Barrett, 1994; Coe, 1994, 2008a, 2008b, 2008c; Perfecto et al., 1994; Ferguson et al., 1995; Coe & Anderson, 1996a, 1996b, 1997, 1999, 2005; Urquhart, 1997; Granzow et al., 1998; Stevens et al., 2001, 2009; Vandermeer & Granzow, 2004; Mascaro et al., 2005). The vascular plant flora of eastern Nicaragua is estimated at over 3000 species (Coe & Anderson, 1999; Stevens et al., 2001). Unfortunately, this unexplored

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reservoir of botanical resources and its phytochemical potential are being lost due to rampant deforestation much faster than it is being surveyed (Langley, 1981; Coe & Anderson, 1999).

It is well documented that the screening of the ethnomedicinal flora is important in the discovery of new drugs (Farnsworth, 1990; Cox, 1994; Cox & Balick, 1994; King & Tempesta, 1994; Cragg et al., 1997; Balick & Cox, 1999). Of additional value is the standard screening of plants for the presence of bioactive compounds, and the assessment of extract toxicity via bioassays. Toxicity assays have been performed on plant extracts from the ethnomedicinal flora of several countries, including Argentina (Mongelli et al., 1996), Brazil (Quignard et al., 2004), Guatemala (Franssen et al., 1997; Cáceres et al., 1998; Michel et al., 2007), Honduras (Lentz et al., 1998), India (Padmaja et al., 2002), Jamaica (Facey et al., 1999), New Guinea (Rao, 1996), Philippine Islands (Horgen et al., 2001), Spain (Serrano, 1996), Tanzania (Moshi et al., 2004), and Turkey (Sener et al., 1998). A massive new and important project recently announced is the Herbalome Project by the Chinese government to assay for chemical constituents and toxicity the 400,000 medicinal preparations derived from 10,000 medicinal species used in Chinese traditional medicine (Stone, 2008).

Despite the importance of screening plant extracts for the discovery of new drugs, the ethnopharmacopoeia of many Mesoameriandian groups remains untapped; such is the case in eastern Nicaragua. Over the past 15 years, several studies were conducted to screen the medicinal species of eastern Nicaragua for alkaloids (Coe, 1994, 2008a, 2008b, 2008c; Coe & Anderson, 1996a, 1996b, 1997, 1999, 2005). However, bioassays to determine the toxicity of these medicinal species were not conducted until recently (Coe et al., 2010). This study reports on measures of potential effectiveness and cytotoxicity of plant extracts derived from medicinals used by traditional healers in eastern Nicaragua. In what follows, we report the results of alkaloid tests and brine shrimp bioassays of medicinal plant aqueous extracts of 31 species currently used by healers in eastern Nicaragua. We develop the arguments as well that cytotoxicity levels, high *and* low, are of considerable interest. High levels offer insights into effectiveness in treating such things as bacteria or protozoan-based illnesses. But some high cytotoxic levels may be associated with detrimental effects on patients as well. Thus, either way, cytotoxicity tests are of importance.

Materials and methods

Plant collection

Plant material was collected during field studies by one of us (Coe) working with traditional healers (shamans, midwives, and herbalists) during several months over many years (1992–2008). Further details about the ethnobotanical studies are published elsewhere (Coe, 1994, 2008a, 2008b, 2008c; Coe & Anderson, 1996a, 2008b, 1997, 1999, 2005). Voucher specimens were deposited at the Missouri Botanical Garden (MO, USA) and the University of Connecticut (CONN, USA). Vouchers were identified by Coe with the assistance of specialists listed in the acknowledgments.

Alkaloid screening

Thirty-one medicinal species were screened for alkaloids (Table 1). Plant parts tested are the same as those used in the preparation of herbal remedies, and included both aerial and underground parts as appropriate (Table 1). Plant materials were obtained from mature individuals in flower or fruit, and collected and processed according to standard practices (Lawrence, 1951; Bridson & Forman, 1992; Soejarto, 1993; Soejarto et al., 1996). Alkaloid tests were performed using Dragendorff's reagent (Harborne, 1988; Stermitz et al., 1989) and thin-layer chromatography (TLC) (Stahl, 1969; Stermitz et al., 1989). Alkaloids were determined qualitatively by macerating 10–15 mg of plant material in a test tube in 1–2 ml of 1M Na_2CO_3. Once macerated, 0.5–1.0 mL of 2:1 $CHCl_3$-MeOH was added. The mixture was then mixed with a stirring rod for 3–5 min, and afterward allowed to stand and separate into two phases (upper and lower). The lower phase containing the plant extract dissolved in the $CHCl_3$ was drawn off with a disposable pipette into a depression in a spot plate. The $CHCl_3$ was allowed to evaporate to about a drop (0.025 mL). This amount was spotted on an aluminum-backed TLC strip 10 mm × 40 mm in size. The strips were developed in $CHCl_3$ and alkaloids were visualized (color ranges are yellow/orange, red/orange, red/black, pink, and even purple depending on the species or genus) by spraying with Dragendorff's reagent. Alkaloids were considered present when at least two of three replicates gave positive results. We are aware that these kinds of alkaloid tests can sometimes produce false-positive reactions, especially in latex-bearing families, e.g., Apocynaceae, Araceae, Clusiaceae, Convolvulaceae, and Moraceae (Farnsworth, 1966). However, the method (Stermitz et al., 1989) we used includes a purification procedure (adding a base-Na_2CO_3 and extraction with a water immiscible organic solvent-$CHCl_3$) that helps avoid false-positive reactions. But, given the doubt, as an additional measure, we verified our test results, for the five latex-bearing families with reports in the literature.

Plant crude extract preparation

We made every attempt here to follow the general preparation procedure that would be utilized by field medicinal practitioners. This included, especially, using the same plant parts as employed in the field, and extracting in water– the most common mode of preparation of medicinals (Coe, 1994, 2008a, 2008b, 2008c; Coe & Anderson, 1996a, 2008b, 1997, 1999, 2005). Plant crude extracts (stock solution) were prepared by boiling 1 g of

Table 1. Bioactivity and bioassay results of medicinal plants used in eastern Nicaragua.

Scientific Name[a]	Common Names[b]	Medicinal[c]	Part[d]	Alka.[e]	LC_{50}[f]	95% CI[g] (μg/mL)
PTERIDOPHYTA						
POLYPODIOPSIDA (FERNS)						
PTERIDACEAE						
1. *Pityrogramma calomelanos* (L.) Link (Coe-4058)	waha pihni (m)	F,G,H,K,L,U	L,R	+	1843	1531–2196
SCHIZAEACEAE						
2. *Lygodium heterodoxum* Kuntze (Coe-2770)	nata kyuca (m)	A,B,S	L	+	717	523–909
MAGNOLIOPHYTA						
MAGNOLIOPSIDA (DICOTS)						
BIGNONIACEAE						
3. *Crescentia cujete* L. (Coe-3447)	saabang (r)	D,F,L	L	0	4382	2741–5864
BOMBACACEAE						
4. *Pachira aquatica* L. (Coe-2881)	plingkiing (r)	D,I,J,S,T	B,E	+	2684	1304–3998
BORAGINACEAE						
5. *Cordia curassavica* (Jacq.) Roem. & Schult. (Coe-3981)	riskupata (m)	A,C,D,F,H	L	+	2280	1914–2648
CELASTRACEAE						
6. *Salacia belizensis* Standl. (Coe-3365)	lasap (m)	A,T	P	+	6650	None
CLUSIACEAE						
7. *Symphonia globulifera* L.f. (Coe-2363)	samu (m)	A,M,S	B,P,S	+	3114	2612–3613
CONVOLVULACEAE						
8. *Ipomoea batatas* (L.) Lam. (Coe-3637)	paiik (r)	B,I,N,Q,R, S,U,X,Y	L	+	3231	None
EUPHORBIACEAE						
9. *Jatropha hastata* L. (Coe-3518)	pisik (m)	F,S,X	L	+	6211	None
FLACOURTIACEAE						
10. *Casearia aculeata* Jacq. (Coe-3852)	pyuta piaia (m)	I,S	L	+	4478	None
GESNERIACEAE						
11. *Chrysothemis pulchella* (Donn ex Sims) Decne. (Coe-3938)	katuruk saala (r)	A,S	L,P	+	1668	825–3112
MALPIGHIACEAE						
12. *Heteropterys leona* (Cav.) Exell (Coe-3481)	twisa târa (m)	L,Q	L	+	5103	None
13. *Stigmaphyllon puberum* (Rich.) A. Juss. (Coe-3789)	pyuta wâkia (m)	B,Q,V,Z	L,M,P	+	2183	1828–2559
MALVACEAE						
14. *Sida acuta* Burm. f. (Coe-3977)	aras kauka (m)	A,C,F,K, L,V,W	L,P	+	2626	615–4391
MELASTOMATACEAE						
15. *Acisanthera quadrata* Pers. (Coe-3244)	asdura pata (m)	F	L,M	+	1371	None
MORACEAE						
16. *Artocarpus altilis* (Parkinson) Fosberg (Coe-3426)	yiraa (r)	A,H	L,S	+	2809	None
MYRTACEAE						
17. *Eugenia venezuelensis* O. Berg (Coe-3988)	tablira (m)	D,T	L	+	793	600–992
PHYTOLACCACEAE						
18. *Petiveria alliacea* L. (Coe-3567)	prauk (r)	A,D,E,L, O,P,R	L,P,R	+	1332	1761–5093
RUBIACEAE						
19. *Borreria assurgens* (Ruiz & Pav.) (Griseb. (Coe-3898)	kalila (m)	B,I,L,S,U,W	L	+	2508	2104–2916
20. *Guettarda elliptica* Sw. (Coe-4342)	lasat (m)	T	L	+	2726	2274–3187
21. *Hamelia barbata* Standl. (Coe-2589)	silbyara (m)	B,I,S,Y	L,P	+	2662	None
SAPINDACEAE						
22. *Melicoccus bijugatus* Jacq. (Coe-3437)	suksuk (m)	D,Q	E,L	0	3653	3189–4122
SAPOTACEAE						
23. *Chrysophyllum cainito* L. (Coe-3533)	apil (m)	D,F,I,Q,V	F,L	+	3435	None
TILIACEAE						

Table 1. continued on next page

Table 1. Continued.

Scientific Name[a]	Common Names[b]	Medicinal[c]	Part[d]	Alka.[e]	LC_{50}[f]	95% CI[g] (μg/mL)
24. *Apeiba membranacea* Spruce ex Benth. (Coe-2371)	urus bamba (m)	L,Q,S	B,L	+	4989	4266–5594
TURNERACEAE						
25. *Turnera ulmifolia* L. (Coe-3887)	klua tangni (m)	A,F,L,X	L	+	6650	None
VERBENACEAE						
26. *Lippia alba* (Mill.) N.E. Br. ex Britton & Wilson (Coe-3496)	sîka siahka (m)	C,F,G,I,L,S,W	L	+	3086	2539–11109
VOCHYSIACEAE						
27. *Vochysia ferruginea* Mart. (Coe-2755)	yamari (m)	A,S	L,S	+	966	820–1154
LILIOPSIDA (MONOCOTS)						
COMMELINACEAE						
28. *Commelina erecta* L. (Coe-3596)	butku sirpi (m)	I,S	L,M	+	4314	None
POACEAE						
29. *Cymbopogon citratus* (DC.) Stapf (Coe-3686)	twî rih (m)	A,F,G,L	L,R	+	2252	1878–2651
30. *Mesosetum blakei* Swallen (Coe-4323)	wâlang (s)	I,S	L,R	+	1985	1638–2370
31. *Olyra latifolia* L. (Coe-2495)	sagádi (g)	I,S	L,R	+	958	795–1156

[a]Scientific name of the families follow Stevens et al. (2001, 2009), the order within families, genera, and species is alphabetical; F.G. Coe voucher number is given in parenthesis () after the scientific name.
[b]Common Names: g, Garífuna; m, Miskitu; r, Rama; s, Sumu; spelling follows CIDCA (1986, 1987, 1989), Heath (1913, 1927, 1950), Heath & Marx (1961), Smutko (1985).
[c]Medicinal Applications: A, Aches and Pains; B, Bites and Stings (snake, scorpion, insects); C, Childbirth and Pregnancy; D, Diarrhea; E, Emetic, F, Fever; G, Digestive; (stomach ache, ulcers, etc.); H, Hypertension; I, Infections; J, Diabetes; K, Diuretic; L, Respiratory & Pulmonary Disorders (cold, coughs, etc.); M, Malaria; N, Burns; O, Abortifacient; P, Worms and Intestinal Parasites; Q, Astringent; R, Aphrodisiac; S, Skin Rashes and Sores; T, Tonic and Anemia (blood fortifier); U, Cuts and Hemorrhage; V, Venereal Diseases; W, Female Disorders (Menstruation, Hemorrhage); X, Purgative and Laxative; Y, Constipation; Z, Tooth Extraction.
[d]Material Used (the same plant parts were used for medicinal preparations and assays extracts): B, Bark; C, Flower; E, Seed; F, Fruit; L, Leaf; M, Stem; P, Whole Plant; R, Root; S, Sap.
[e]Alkaloid Tests: + (present) or 0 (absent) in Coe tests (see Methods and Materials).
[f]Toxicity: Concentration Lethal to 50% of the Brine Shrimp (LC_{50}).
[g]Confidence interval (CI) using the Probit method to determine the toxicity of the extract; see methods.

plant material in 100 mL of distilled water as described by Bertani et al. (2007); this was the stock solution. An appropriate amount of 1% NaCl solution was added to the stock solution to give concentrations of 500, 1000, 2500, 5000, 7500, and 10,000 μg/mL. Three replicates were prepared for each dose level. These relatively high doses were used to replicate the concentrations at which herbal remedies are prepared and administered by healers in eastern Nicaragua. The "Control Solution" we used consisted of only 1% NaCl solution.

Hatching of brine shrimp

Eggs of brine shrimp (*Artemia salina* L., Artemiidae) were purchased from Carolina Biological Supply (Burlington, NC, USA) and were incubated for 48 h in a culture vessel (15 × 15 × 15 cm) containing 1% NaCl, the latter, prepared from nitrate, phosphate, and silicate-free sea salt and deionized water (35 g/L) at 24°C to 28°C under a lamp. The saltwater solution was aerated continuously during incubation with an aquarium air pump. After 48 h the nauplii (larvae) were collected from the culture vessel.

Brine shrimp lethality assay

The brine shrimp lethality assay (BSLA) was used to determine if the plant extracts of medicinal species were cytotoxic (Meyer et al., 1982; McLaughlin et al., 1991; Cepleanu et al., 1994; Coe et al., 2010). Ten brine shrimp larvae were placed in each of the triplicate vials (thus, 30 shrimp per concentration) using a plastic pipette with a 2 mm diameter tip. The larvae were released under the surface of the solution to avoid killing them by trapping air under their carapaces. Survivors were counted under the stereomicroscope after 24 h, and the percentage death at each dose and control was determined.

Data analysis

The mean results of brine shrimp mortality were plotted against the logarithms of concentrations using the computer program Probit Analysis Version 1.5 developed by U.S. Environmental Protection Agency, Cincinnati, Ohio, from which median lethal concentrations (LC_{50}) at 95% confidence intervals (CI) were calculated, according to the method of Finney (1971). Biological activity using the BSLA was recorded as a lethal concentration (LC_{50}) when 50% of the larvae were killed within 24 h of contact with the extract. As is standard practice (Meyer et al., 1982; Zhao et al., 1999; Morrow et al., 2001; Holetz et al., 2002; Pimentel Montanher et al., 2002; Silva et al., 2007) LC_{50} values lower than or equal to 1000 μg/mL were considered cytotoxic and greater than 1000 μg/mL for plant extracts were considered noncytotoxic.

Results

The 31 species assayed constitute a diversity of plants as illustrated by their distribution among 31 genera and

26 families, including two fern and 29 angiosperm species. The ethnomedicinal uses, bioactivity, and bioassay information are presented in Tables 1 and 2. Almost 95% (29/31) of the species screened for alkaloids tested positive (Table 1). However, in the BSLAs, only four species (13%) were cytotoxic to brine shrimp at 1000 μg/mL (Table 1) or less – a level that is generally considered cytotoxic (Meyer et al., 1982; Zhao et al., 1999; Morrow et al., 2001; Holetz et al., 2002; Pimentel Montanher et al., 2002; Silva et al., 2007). All four of these species tested positive for alkaloids. Almost 75% of the species (23) had an LC_{50} at between 1000 and 5000 μg/mL and the remaining four required a concentration greater than 5000 μg/mL to be cytotoxic. The two species that did not test positive for alkaloids were in the 1000–5000 μg/mL group. Given that 27 of the species were cytotoxic at levels above the 1000 μg/mL threshold, the vast majority of the species tested with the bioassays should be considered "noncytotoxic."

The four species with cytotoxic extracts were distributed across a wide range of plants – and included a fern (see Table 1). In addition to alkaloids, the four cytotoxic species have been tested in other contexts, and apparently contain other secondary metabolites such as fatty acids, flavanoids, glycosides, phenols, saponins, tannins, and terpenoids (Gibbs, 1974; Cambie & Ash, 1994; Hess et al., 1995; Calderon et al., 2000, 2001; Zucaro et al., 2000). Obviously, these other compounds might have been the source of the cytotoxicity as well as, or instead, of the alkaloids.

Discussion

People in eastern Nicaragua use a wide range of plants/plant extracts for treatment of an array of ailments (Coe, 1994, 2008a, 2008b, 2008c; Coe & Anderson, 1996a, 1996b, 1997, 1999, 2005). One of the authors (Coe) has studied the plants in the field and there are several papers citing medicinal applications (Coe, 1994, 2008a, 2008b, 2008c; Coe & Anderson, 1996a, 1996b, 1997, 1999, 2005). In spite of the fact that the 31 species tested in this study are widely used by healers in eastern Nicaragua to treat over 25 ailments (see Tables 1 and 2), there is virtually nothing known of the cytotoxicity. The recent discovery of the carcinogenic effects of the extracts of *Aristolochia fangchi* Y.C.Wu ex L.D.Chow & S.M.Hwang (Aristolochiaceae), a medicinal species widely used in Asia, highlights the importance of toxicity screening (Greensfelder, 2000; Nortier et al., 2000) of medicinals. Toxicity can be good–potentially killing bacteria and parasites. And/or it can be bad, as with *A. fangchi*, negatively impacting the patient–perhaps in ways much worse than the ailments treated. Many species of the genus *Aristolochia* are commonly used in eastern Nicaragua and elsewhere to treat a variety of ailments, from skin rashes to snakebites (Morton, 1981; Coe, 1994, 2008a, 2008b, 2008c; Coe & Anderson, 1996a, 1996b, 1997, 1999, 2005). Thus, *Aristolochia*-bearing medicinals in Nicaragua may be carcinogenic as well. We did not have the *Aristolochia* (that has been a part of our other studies – Coe & Anderson, 1996a, 1996b, 1997, 1999, 2005; Coe, 2008a, 2008b, 2008c) to test, but the same idea applies to the other medicinals used: any of the eastern Nicaraguan medicinals may be cytotoxic.

In our studies, less than 10% (4/31) was considered cytotoxic in spite of the fact that over 90% (29/31) of the species contained alkaloids (Table 1). It is possible that the type of alkaloid in the extract is not toxic to the test organism, or that the solvent used in the alkaloid extraction (which was distilled water – to match the most commonly used method of preparation in eastern Nicaragua) was not effective in removing all the alkaloids from the plant tissue. Thus, we can conclude that our results clearly suggest that the presence of the water-extracted alkaloids do not necessarily mean that the plant extract will be cytotoxic. The lack of cytotoxicity, of course, does not mean the medicinal is not effective (see more on that below).

Similarly, the two species (*Crescentia cujete* L., Bignoniaceae; *Melicoccus bijugatus* Jacq., Sapindaceae) that tested negative for alkaloids are fairly widely used medicinals (Table 1). The effectiveness of these two species as herbal remedies may be due to the presence of other types of secondary metabolites such as flavanoids, glycosides, phenols, sapogenins, saponins, and tannins (Heltzel et al., 1993a, 1993b; Binutu & Lajubutu, 1994; Kaneko et al., 1997, 1998; Betancur-Galvis et al., 1999; Bystrom et al., 2008; Ogbuagu, 2008). For example, the fruit pulp of *C. cujete* contains flavanoids, saponins, cyanogenic glycosides (as HCN), phenols, and tannins (Ogbuagu, 2008). Similarly, the fruit pulp of *M. bijugatus* has phenolic acids, flavonoids, procyanidins, and catechins (Bystrom et al., 2008). The therapeutic values and toxicity of these other compounds are well known.

Cymbopogon citratus Stapf. (Poaceae) is also interesting. This species tested positive for alkaloids, but is also reputed to contain a wide array of other secondary metabolites (Cambie & Ash, 1994). In spite of the alkaloids and secondary metabolites, the test for this species put it into the category of "noncytotoxic." Interestingly, ethanol extracts of *C. citratus* also did not demonstrate any significant biological activity – in this case, antibacterial effect against *Staphyloccocus aureus*, *S. epidermidis* or to methicillin-resistant *S. aureus* (MRSA) (Chomnawang et al., 2009). Thus, the extracts from this species are probably not effective *cytotoxic* medicinals, but rather have an effect on other physiological functions.

In our study, four extracts widely used by many Amerindians to treat a variety of ailments such as inflammation, mouth and skin sores, fever, muscular pain, rheumatism, respiratory disorders, and pulmonary congestion showed the greatest cytotoxicity. The fern species, *Lygodium heterodoxum* Kunze (Schizaeaceae) is particularly interesting because ferns in general are not known for their bioactive compounds (Berti & Bottari, 1968). However, in our study, this fern had the highest cytotoxicity, causing brine shrimp death at slightly above 700 μg/mL (Table 1). The effectiveness of *L. heterodoxum* at this relatively low

concentration may be due to the presence of the alkaloids, or other secondary metabolites such as flavanoids, glycosides, phenols, saponins, and tannins (Gibbs, 1974) that it contains. The additional three species that were cytotoxic also contained alkaloids (Table 1), but, like the *L. heterodoxum*, they posses other secondary metabolites as well (Gibbs, 1974; Hess et al., 1995; Calderon et al., 2000). For instance, *Eugenia venezuelensis* O. Berg. (Myrtaceae) contain coumarins (such as 3,4- benzocoumarin), glycosides, phenols (such as D-quercitol), eugenol, eugenol-acetyl-salicylic acid ester, methyl salicylate, monoterpenoids, triterpenoids (such as oleanolic acid), saponins, and tannins (Gibbs, 1974; Cambie & Ash, 1994). *Olyra latifolia* L. (Poaceae) contains carbohydrates such as fructosans, phenolic acids such as caffeic, ferulic acid, gentisic acid, *p*-OH-benzoic acid, flavonoids such as anthocyanins, leucoanthocyanins, flavones, and flavonols, *p*-coumaric acid (Gibbs, 1974). *Vochysia ferruginea* Mart. (Vochysiaceae) contains fatty acids such as lauric acid, myristic acid, tannins, triterpene such as 6β-hydroxymaslinic acid, polyphenols such as β-sitosterol-glucoside, uvaol, erythrodiol, urosolic acid, oleanolic acid, crategolic acid, bellericagenin A, bellericaside A, betulinic acid (Gibbs, 1974; Zucaro et al., 2000). It is clear, in particular, that *V. ferruginea* contains a number of cytotoxic compounds, and, thus, not surprisingly, showed bioactivity (e.g., anaesthetic, analgesic, antifungal, antiseptic, bactericide, and anti-inflammatory activities) in many other assays (Gibbs, 1974; Cambie & Ash, 1994; Hess et al., 1995; Calderon et al., 2000, 2001; Zucaro et al., 2000). However, in general, it is obvious that all four of the species we found to be most lethal are loaded with compounds that may have caused the cytotoxicity.

Among the 26 ailments/aliment categories that are included in Table 2, there are three that are obviously associated with external bacterial, protozoan, etc., vectors: "Infections" (10 species), "Malaria" (1 species), and "Worms and intestinal parasites" (1 species). We scored the species that are used to treat these vector-borne ailments, and compared their distribution among the three LC_{50} categories (Table 3). The patterns revealed are interesting. Among the four "effective" species (those that had an effect in the bioassay at less than 1000 μg/mL), three are *not* used to treat the vector-borne ailments. That result argues against the concept that a strong bioassay effect ("cytotoxicity" as here used) is a key to application in treating pathogen-borne ailments. Slightly more than half of the 23 species (13) in the next category, 1000–5000 μg/mL, also were not used to treat pathogens, but the difference between those "used" and "not used" is not significant (chi-square goodness of fit, $P = 0.53 = \text{NS}$). Thus, this result is thus "neutral" in arguing for or against medicinal application to vector-borne ailments. The only two medicinal species that lacked alkaloids were in the "not used" category. Finally, none of the four species in the "weakest" category (i.e. more than 5000 μg/mL required for an effect in the bioassays) were used to treat vector-borne ailments, a result one might expect if cytotoxicity bioassays were a good measure of application to vector-borne diseases. Overall, the variable and somewhat contradictory results from this analysis reinforce significant conclusions from our study: that the major application of the native medicinals (alkaloid-bearing *or not*) is (a) not necessarily in the treatment of infectious pathogens, and (b) that cytotoxicity is not a predictor of such an application.

Cytotoxicity could be considered desirable – in that bacteria and other pathogens or parasites might respond like the brine shrimp did. On the other hand, cytotoxicity might be considered undesirable, because, as for *Aristolochia fangchi*, perhaps the alkaloids and cocktail of other compounds might be so strong, so concentrated, that they may kill "host" cells or may be carcinogenic; the treatment might be more detrimental than the illness.

Table 2. Medicinal applications and the number of species used of the 31 medicinal assayed.

Medicinal application	Number of species
Abortifacient (O)	1
Aches and pains (A)	11
Aphrodisiac (R)	2
Astringent (Q)	6
Anemia (T)	4
Bites and stings (B)	5
Burns (N)	1
Childbirth and pregnancy (C)	3
Constipation (Y)	2
Cuts and hemorrhage (U)	3
Diabetes (J)	1
Diarrhea (D)	7
Digestive (G)	3
Diuretic (K)	2
Emetic (E)	1
Female disorders (W)	3
Fever (F)	10
Hypertension (H)	3
Infections (I)	10
Malaria (M)	1
Purgative and laxative (X)	3
Respiratory & pulmonary (L)	10
Skin rashes and sores (S)	15
Tooth extraction (Z)	1
Venereal diseases (V)	3
Worms and intestinal parasites (P)	1

Table 3. Bioactivity and use of medicinal in treatment of "Infections" (I), "Malaria" (M) and "Worms and intestinal parasites" (P).

LC50 μg/mL	Used to treat I, M, P	Not used to treat I, M, P
1 000 μg mL or less	1	3
1 001–5 000 μg mL[b]	10	13[a]
5 001 μg/mL or more	0	4

Notes: [a]The only two species that did not bear alkaloids are in this category;
[b]A chi-square goodness of fit test showed that the distribution of species between "used" and "not used" was not significant.

Accordingly, the question is: are these cytotoxic species sufficiently valuable medicinally to be used anyway, or should they be considered dangerous– as in the case with *A. fangchi*?

This leads to a perhaps unexpected conclusion: the 25 alkaloid bearing, but noncytotoxic species might be, in fact, the safer and more generally applicable medicinals. These 25 noncytotoxic species are also widely used medicinals. We presume that they have some physiological and therapeutical effects. In spite of, or because of, their lack of cytotoxicity, they may or can play a role in other ways that have salutary medicinal applications (e.g., organ stimulation, increase flow of gastric juices, etc.). Perhaps, then the better approach might be to encourage the use of plant extracts that are proven noncytotoxic in tests like the ones we performed, and encourage avoidance, or limited use of the plant extracts that are cytotoxic. These results and this conceptual approach may lead us to reconsider what constitutes a desirable native medicinal.

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