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Felix G Coe
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs

Dimpi M Parikh
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs

Caley A Johnson
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs

Monika Kucharczyk
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs

Janet A Tovar
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs

Corresponding Author:
Felix G Coe
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs

Bioactivity of 68 Species of Medicinal Plants of Eastern Nicaragua

Felix G Coe, Dimpi M Parikh, Caley A Johnson, Monika Kucharczyk and Janet A Tovar

Abstract
Context: Plant based medicinals form the basis for a significant part of medical treatment in many places Including Nicaragua. Tests that indicate the presence/absence of alkaloids, and general biotoxicity evaluations are critical elements to predicting effectiveness and evaluating safety.
Objective: To determine the bioactivity and cytotoxicity 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: Forty-five of the 68 species (55 genera and 33 plant families) tested positive for alkaloids. The median lethal concentration that kills 50% of the larvae within 24 h of contact with the extract (LC50) was less than 249 µg/mL for 6 (9%) species, 250-499 µg/mL for 7 (10%) species, 500-1000 µg/mL for 27 (40%) species, 1001-2500 µg/mL for 10 (15%) species, 2501-5000 µg/mL for 12 (18%) species, 5001-
7500 µg/mL for 5 (7%) species and between 7501-10000 µg/mL for 1 (1%) species. Of the 46 alkaloid bearing species only 6 (13%) species were considered highly cytotoxic at a concentration of < 249 µg/mL.
Discussion and Conclusion: The overall results from the study of these new species studied are in accord with our previous conclusions: the majority of medicinals used in traditional medicine do contain likely bioactive alkaloids and other secondary metabolites, and most are not cytotoxic. Of course, even medicinals without alkaloids also can be effective in numerous ways.

Keywords: Alkaloids screening, brine shrimp toxicity, eastern Nicaragua, medicinal plants

Introduction
According to Norman Farnsworth (1990) [68] about 64% of the world's population (thus, about 3.2 billion people) have very little access to modern medicine, and thus rely on plants as pharmaceutical sources. The focus of our work is on a part of Nicaragua (eastern region or the Atlantic coast), where most people continue to rely on traditional medicine for primary health care (Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005; Coe *et al*. 2010, 2020, in prep.) [34, 40, 41, 42, 35-39, 43, 45]. Nicaragua is a country where, impressively, about 1500 (25%) of the estimated 6000 species of vascular plants (Sutton 1989; Stevens *et al*. 2001, 2009) [149, 146, 147] are used for medicinal purposes. The eastern region of Nicaragua (according to Stevens *et al*. 2001) [146] has a relatively rich and diverse flora, estimated at 3000-3500 species of vascular plants (Taylor 1959, 1962, 1963; Sutton 1989; Coe 1994; Coe and Anderson 1996a, 1996b, 1997, 1999; Stevens *et al*. 2001; Coe and Anderson 2005; Coe 2008a, 2008b, 2008c; Stevens *et al*. 2009; Coe *et al*. 2010) [153, 154, 155, 149. 34, 40, 41, 42, 35-38, 146, 147, 43]. The medicinal flora includes native and introduced species with documented medicinal use in the eastern region of Nicaragua. The medicinal flora of the eastern region consists of 1189 species in 602 genera in 156 families (compiled in Coe 2020 in press) [45]. Based on studies the ethnopharmacopoeia of the indigenous groups in eastern Nicaragua conducted over the past four decades, consist of 780 species of vascular plants (Coe 1994; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005; Coe 2008a, 2008b; Coe *et al*. 2010, Coe *et al*. 2012, 2020, in press) [34, 40, 41, 42, 35-39, 43-45]. The remaining 409 species not part of the ethnopharmacopoeia of eastern Nicaragua are part of the ethonopharmacopoeia for groups in other regions of Nicaragua and elsewhere (Quisumbing 1951; Asprey and Thornton 1953 1954a, 1954b, 1955; Morton 1981; Williams 1981; García-Barriga 1992; Núñez-Meléndez 1992; Duke 1994, 2008; House *et al*. 1995; Balick and Arvigo 1993, 2015; Mors *et al*. 2000; Grijalva 2006; Hernández Quesada 2008-2010; Coe 2020, in press) [125, 7-10, 111, 92, 117, 60, 58, 89, 13,

[14, 110, 83, 87, 45]. Perhaps the reasons why these 409 medicinals are are excluded from the regional ethonopharmacopoeia are due to differential distribution, and/or the lack of empirical testing by the local healers or cultural idiosyncrasies and acculturation. Natural products continue to play an important role in modern drug development in spite of the advent of robotics, bioinformatics, high throughput screening (HTS), molecular biology-biotechnology, combinatorial chemistry, in silico (molecular modeling) and other methodologies (Newman and Cragg 2012, 2016; Mc Chesney *et al*. 2007) [113, 114, 102]. To date, about 70,000 plant species have been screened for their medicinal use (Veeresham 2012) [157]. Current drug discovery from plants has mainly relied on bioactivity–guided fractionation, that has led to isolation of many important drugs. Today the pharmaceutical industry is highly dependent on plant-based medicines, with over 50% of drug substances derived from nature (Krief *et al*. 2004) [95]. Plants are known to produce phytochemicals, which are potential sources of anticarcinogenic, anticancer, anti- inflammatory, antimicrobial, and antioxidant activity; these compounds include alkaloids, flavonoids, glycosides, phenols, terpenoids, steroids, and tannins (Tavassoli and Djomeh 2011; Bruneton 1995) [152, 24]. Recently much research has focused on the discovery of useful antipathogens drugs from natural products for pharmaceutical uses (Davies 1994; Sajid *et al*. 2012) [57, 133]. Additionally, the increasing interest in traditional ethnomedicine may lead to the discovery of novel therapeutic agents. Plants, especially those with ethnopharmacological uses, have been the primary sources of medicine for early drug discovery. For instance, in a study by Fabricant and Farnsworth (2001) [65] 80% of 122 plant derived drugs were related to their original ethnopharmacological uses.

Screening of the ethnomedicinal flora is a very important component in the discovery of new drugs (Farnsworth 1990, 1994; Cox 1994; Cox and Balick 1994; King and Tempesta 1994; Cragg *et al*. 1997; Balick and Cox 1999) [68, 70, 50, 51, 93, 52, 15], as evident by the many new anticancer drugs, all plant derived, discovered during the past 25 years (Cragg and Newman 1999; da Rocha *et al*. 2001) [53, 56] such as: taxol, (palclitaxel®), irinotecan (camptosar®), topotecan (hycamtin®), etoposide (toposar®), vinblastine, vincristine (oncovin®), vinorelbine (navalbine®), and teniposide (vumon®). Studies to determine the presence of bioactive compounds and, as, or perhaps more importantly, the toxicity of medicinal plant extracts (Coe *et al*. 2010, 2011) [43]; have been conducted for the medicinals in many countries including Argentina (Mongelli *et al*. 1996) [107]; Brazil (Quignard *et al*. 2004) [124]; Guatemala (Franssen *et al*. 1997; Cáceres *et al*. 1998; Michel *et al*. 2007) [74, 25, 106]; Honduras (Lentz *et al*. 1998) [101]; India (Padmaja *et al*. 2002) [120]; Jamaica (Facey *et al*. 1999) [66]; New Guinea (Rao 1996) [128]; Philippine Islands (Horgen *et al*. 2001) [88]; Spain (Serrano *et al*. 1996) [138]; Tanzania (Moshi *et al*. 2004) [112]; and Turkey (Sener *et al*. 1998). In the case of eastern Nicaragua, the screening of medicinal plants to determine their bioactivity and toxicity began fairly recently (Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005; Coe *et al*. 2010, 2011) [34, 40, 41, 42, 35-39, 43]. In this study, we provide the results from the alkaloid tests and brine shrimp lethal assay (BSLA) of aqueous plant extracts of 68 species of vascular plants used by traditional healers in eastern Nicaragua. This brings the total we have studied to 216 of the 780 medicinal species used in this region – nearly 30% of the medicinal flora of this region.

Materials and Methods

Plant collection

The species chosen for study are those used by traditional healers in eastern Nicaragua. This group consists of species not previously analyzed in Coe's comprehensive work on the eastern Nicaraguan medicinal flora. Plant material was collected during field trips with traditional healers (shamans, midwives, and herbalists) by one of us (F. Coe) during several months over nearly two decades
(1992-2008). Plant parts were collected at different times of the day and consisted of both young and mature plant parts. Further details about the ethnobotanical studies are published elsewhere (Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005) [34, 40, 41, 42, 35-39].

Voucher specimens were deposited at the Missouri Botanical Garden (MO) and the University of Connecticut (CONN). Vouchers were identified by F. Coe with the assistance of specialists listed in the acknowledgements.

Alkaloid screening: Sixty-eight 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 both young and mature individuals, collected, dried and processed according to standard practices. (Lawrence 1951; Bridson and Forman 1992; Soejarto 1993; Soejarto *et al*. 1996) [100, 23, 142, 143]. Alkaloid tests were performed in the same way in the field and in the laboratory, using Dragendorff's reagent (Harborne 1988; Stermitz *et al*. 1989) [85, 145] and Thin-Layer Chromatography (TLC) (Stermitz *et al*. 1989; Stahl 1969) [145, 144]. Alkaloids were determined qualitatively by macerating10-15 mg (dry weight) of plant material in a test tube in 1-2 ml of 1M Na2CO3. Once macerated, 0.5-1.0 mL of 2:1 CHCl3-MeOH was added. The mixture was then mixed with a stirring rod for 3-5 minutes, and afterward allowed to stand and separate into two phases (upper and lower). The lower phase containing the plant extract dissolved in the CHCl3 was drawn off with a disposable pipette into a depression in a spot plate. The CHCl3 was allowed to evaporate to about a drop (0.025 mL). This amount was spotted on an aluminum backed thin layer chromatography (TLC) strip 10 mm x 40 mm in size. The strips were developed in CHCl3, 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 this kind of alkaloid test can sometimes produce false-positive reactions, especially in latex-bearing families, e.g., Apocynaceae, Araceae, Clusiaceae, Convolvulaceae, and Moraceae (Farnsworth 1966) [67]. However, the method (Stermitz *et al*. 1989) [145] we used includes a purification procedure (adding a base-Na2CO3 and extraction with a water immiscible organic solvent-CHCl3) that helps avoid false-positive reactions. But, given the doubt, as an additional measure, we compared our test results for the latex-bearing families with reports in the literature (Rätsch 2008; Vejarano Jara and Guerrero Vejarano 2011; Agrawal *et al*. 2012; Petricevich and Abarca-Vargas 2019) [129, 158, 2, 121].

Plant crude extract preparation: We made every attempt here to follow the general preparation procedure that would be utilized by field medicinal practitioners. That means, especially, using the same plant parts as employed in the field, and extracting in water--the most common mode of preparation (see

Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005; Coe *et al*. 2010, 2011). Plant crude extracts (stock solution) were prepared by boiling 1 g of plant material (dry weight) in 100 ml of distilled water as described by Bertani *et al*. (2007) and Coe *et al*. (2010); 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 10000 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*) were purchased from Carolina Biological Supply (Burlington, NC, USA) and were incubated for 48 h in a culture vessel (15 by 15 by 15 cm) containing saltwater (1% NaCl) prepared from nitrate, phosphate, and silicate-free sea salt and deionized water (35 g/L) at 24 to 28 °C under a lamp. The saltwater solution was aerated continuously during incubation with an aquarium air pump (AirTech-2KO). 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, following standard procedures as described in: Meyer *et al*. 1982; McLaughlin *et al*. 1991; Cepleanu *et al*. 1994; Coe *et al*. 2010, 2011. Ten brine shrimp larvae were placed in each of the triplicate vials (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 percent death at each dose and control was determined.

Data analysis: The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the computer program Probit Analysis Version 1.5 developed by the 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 brine shrimp bioassay was recorded as a lethal concentration (LC_{50}) when 50% of the larvae were killed within 24 h of contact with the extract. LC_{50} values of 249 µg/ml or less were considered as highly cytotoxic, 250–499 µg/ml as median cytotoxicity and 500–1000 µg/ml as light cytotoxicity in accordance with standard practice (Meyer *et al*. 1982; McLaughlin *et al*. 1991; Cepleanu *et al*. 1994; Coe *et al*. 2010, 2011.) LC_{50} values greater than 1000 µg/mL for plant extracts were considered non-cytotoxic or inactive.

Sources of Plant Chemical Composition and Bioactivity: Peer reviewed articles were obtained from search engines and databases (MEDLINE/PubMed, EBSCO, PRO-Quest, Google Scholar, SciELO and NAPRALERT). Toxicological information was obtained from the Toxicology Data Network (TOXNET) by entering as keyword the scientific name of each species.

Results

The results from the alkaloid tests and brine shrimp mortality experiments cited (Table 1) are used by healers to treat 23 ailments (Table 2). The 68 species assayed belong to a wide diversity of vascular plants distributed in 55 genera and 33 families. Almost 66% (45/68) of the species screened for alkaloids tested positive (Table 1). However, in the BSLAs, only 40 (59%) were cytotoxic to brine shrimp at 1000 µg/mL or less - the standard level treated as cytotoxic. Of the 68 species, the LC_{50} was 249 µg/mL or less for 6 (9%) species, 250-499 µg/mL for 7 (10%) species, 500-1000 µg/mL for 27 (40%) species, 1001-2500 µg/mL for 10 (15%) species, 2501-5000 µg/mL for 12 (18%) species, 5001-7500 µg/mL for 5 (7%) species and 7501-10000 µg/mL for 1 (1%) species. Only 6 (9%) species were at the level considered highly cytotoxic at LC_{50} of 249 µg/mL or less. All of them bear alkaloids. They include: *Syzygium malaccensis* (Myrtaceae) at 144 µg/mL, *Quassia amara* (Simarubaceae) at 180 µg/mL, *Cissampelos pareira* (Menispermaceae) at 200 µg/mL, *Psychotria elata* (Rubiaceae) at 212 µg/mL, *Aristolochia trilobata* (Aristolochiaceae) at 220 µg/mL, and *Asclepias curassavica* (Asclepiadaceae) at 243 µg/mL. In addition, five nonalkaloid bearing species were cytotoxic at LC_{50} values between 575-863 µg/mL, they are *Fevillea cordifolia* (Menispermaceae) at 575 µg/mL, *Lygodium venustum* (Schizaeaceae) at 580 µg/mL, *Kalanchoe pinnata* (Crassulaceae) at 635 µg/mL, *Smilax spinosa* (Smilacaceae) at 765 µg/mL, and *Hymenaea courbaril* (Fabaceae) at 863 µg/mL. The cytotoxicity of the nonalkaloid bearing species is very low and likely due to other secondary metabolites in their tissues. A review of the literature indicated that the 28 non-cytotoxic species contain fatty acids, flavonoids, glycosides, phenols, polyphenols, saponins, tannins, terpenoids and steroids (Gibbs 1974; Chang *et al*. 2004; Yan and Yang 2005; Chang and Kuo 2007). Obviously, these compounds might play a role in their effectiveness as medicinals and cytotoxicity. The 68 medicinal species are used to treat a wide variety of illnesses, among them seven of the most prevalent pathogenic ailments in eastern Nicaragua (Table 2).

Discussion

Screening of the botanical ethnopharmacopoeia, such as this one, are important steps for at least two reasons (Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005; Coe *et al*. 2010, 2011). First, the screening analyses show whether there are included constituents (such as alkaloids) that are common elements of treatments known to have physiological effects (Farnsworth 1966, 1993). Secondly, and perhaps even more important at least ethnobotanically (Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005; Coe *et al*. 2010, 2011), the bioassays are critical next steps in flagging the potential for toxicity in extracts. The cytotoxicity indications are important ethnobotanically, in particular for providing alpha-level studies to inform ethnobotanical/bush medicine practices. In that regard, Coe is in the last stages of finishing a compendium of over 40 years of study of the ethnomedicine of eastern Nicaragua – a book that will be published in Spanish and fully illustrated (Coe 2020 in press). The hope is that the cytotoxicity data of ethnomedicinal species will be valuable in promoting the cautious use (or avoidance) of toxic species.

In this study, the majority of alkaloid bearing species belongs to a small group of angiosperm families reputed to have alkaloid-rich taxa, they are: Apocynaceae (indole alkaloids), Asclepiadaceae (indole, pyridine alkaloids), Asteraceae (indole, pyrrolizidine alkaloids), Boraginaceae (pyrrolizidine alkaloids), Fabaceae (indole, pyridine, pyrrolizidine, quinolizidine alkaloids), Liliaceae (pyrrolizidine alkaloids), Rubiaceae (indole,

isoquinoline purine, quinoline alkaloids), and Solanaceae (indole, pyrrolizidine, tropane alkaloids). The species in these families contain a wide variety of alkaloids (e.g., imidazole, indole, isoquinoline, piperidine, purine, pyridine, pyrrolizidine, quinoline, tropane) and other secondary metabolites (e.g., glycosides, phenols, saponins, steroids, terpenoids). These chemical compounds have the potential to confer various pharmaceutical activity. The majority of extracts contained alkaloids, and only some of them were cytotoxic. In fact, only six (9%) of the 68 species tested showed high levels of cytotoxicity at LC_{50} values of 249 µg/mL or less. Of the 68 species essayed, four non-alkaloid bearing species were cytotoxic, and even these species produced light levels with LC_{50} values between 575-863 µg/mL. The responses of the brine shrimp to the extracts from these plants suggests that in addition to alkaloids, other types of secondary metabolites such as glycosides, monoterpenes, polyphenols, sapogenins, saponins, sesquiterpenes and steroids may play a role in their cytotoxicity (Gibbs 1974; Chang *et al*. 2004; Yan and Yang 2005; Chang and Kuo 2007). For example, *Syzygium malaccensis*, which in addition to alkaloids, has many other bioactive compounds such as polyphenols in the form of phenolic acids (p-coumaric acid, benzoic acid), carotenoids, saponins, tannins (e.g., tannic acid), flavonoids (e.g., isoquercitrin, isorhamnetin, kaempferol, myricitrin, quercetin, rutin), anthocyanins (e.g., cyanidin 3-*O*-glucoside, peonidin-3-*O*-glucoside), 2- phenylethanol and its esters (2- phenylethyl acetate, 2- phenylethyl isopentanoate, 2- phenylethyl benzoate and 2- phenylethyl phenylacetate, monoterpenoids, sesquiterpenoids) (Pino *et al*. 2004; Nunes *et al*. 2016; Batista *et al*. 2017). Clearly, *Syzygium malaccensis* contains many potentially toxic compounds and had the highest cytotoxicity in this study (LC_{50} value of 144 µg/mL). The high toxicity level of this species is probably due to the alkaloids casuarine 6-O-α-glucoside (Talaviya *et al*. 2014) and myrciaine, a nicotinic ester (Wagner and Bladt 1996; Rodrigues 2009; Oyinlade 2014) or synergism of alkaloids with other secondary metabolites. The fruit pulp is rich of soluble fibers, flavonoids, phenols, and reducing sugars; the peel has insoluble fibers, lipids and anthocyanins; the seeds are rich in lipids; and the leaves contain large amounts of catechins, quercetin, carotenoids, ellagitannins, flavanones, flavonol glycosides, proanthocyanidins, anthocyanidins, triterpenoids, chalcones, volatile terpenoids and tannins (Khandaker and Boyce 2016; Batista *et al*. 2017). In other studies, the edible part of the fruits of *Syzygium malaccensis* showed antioxidant properties (Lako *et al*. 2007) and antitumoral activity (Nile and Park 2014; Paredes-López *et al*. 2010), the leaves extracts had antiinflammatory, antioxidant effects (Arumugam *et al*. 2014), antihyperglycaemic effect (Arumugama *et al*. 2016) and cytotoxicity (Savitha, Padmavathy and Sundhararajan 2011), the trunk bark extract had glycemia/cholesterolemia-lowering effects (Bairy, Sharma and Shalini 2005). It seems that each tissue type of the plant has different chemical characteristics, perhaps to ward off predation by herbivores and pathogenic organisms. Second in toxicity was *Quassia amara* with a LC_{50} value of 180 µg/mL. In our screening, the wood tested positive for alkaloids (Coe and Anderson 1996b). *Quassia amara* contains a variety of secondary metabolites with high bioactivity that include the alkaloids amarastelline A, cantine, and ß-carboline alkaloids, isolated from the bark (Bajaj 2012). The plant contains ß-sitosterol, gallic acid, malic acid, potassium acetate, and quassol (Duke 1992). Other compounds present include triterpenoids (quassin, quassinol, 18-hydroquassin, neoquassin, a bitter principle that is 50 times more bitter than quinine (Duke 2018), quassinoids, quassinacin, similakolactone D, isoquassin), steroid (sterol), glaucarubin, and volatile oil (Duke 2008). Cantine is a cardiac tonic that increases the strength and rate of heart contractions. Cantine alkaloids have glucoregulatory properties because they are antihyperglycemic, that is, they reduce blood glucose (Sasaki *et al*. 2015). The extract of *Quassia amara* had a high antimalarial activity both in vitro and in vivo (Bertani *et al*. 2006). The quassinoid simalikalactone D and E (SkD, SkE) is the active compound, with an IC50 value of 10 nM against the chloroquine resistant strain of *Plasmodium falciparum* FcB1 in vitro (Bertani *et al*. 2006). Lastly, it inhibits 50% of *Plasmodium yoelii yoelii* rodent malaria parasite at 3.7 mg/kg/day in vivo by oral route (Bertani *et al*. 2006). These findings confirm the traditional use of this plant (Bertani *et al*., 2006). The plant extract contains quassimarine that showed promising activity against lymphocytic leukemia in rats and nasopharyngeal carcinoma cells in humans (Kupchan and Streelman 1976).

Piper auritum a very popular medicinal in eastern Nicaragua and elsewhere (Joly 1981; Morton 1981; Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1996b, 1997, 1999, 2005) contains diosgenin, pyridine alkaloids, terpenoids, benzoic acid derivatives, chromenoic acid, chromanoic acid, sesquiterpenoids (caryophyllene, caryophyllene epoxide, muurolene), cadinene hydrocarbons, β-sitosterol, safrole, trans-phytol (Morton 1981; Gupta *et al*. 1985; Duke and Atchley 1986; Ampofo *et al*. 1987; Monzote *et al*. 2010). The secondary metabolites in *Piper auritum* have many medicinal properties such as anti-inflammatory, antioxidant, antitumor activities, febrifuge, sudorific, diuretic, stimulant, local anesthetic, antibacterial, and antifungal (Gupta *et al*. 1985; Hernández *et al*. 2003; García *et al*. 2007). *Piper auritum* had a median toxicity level with a LC_{50} at a 420 µg/mL. However, in high concentration and prolonged use, it can be highly toxic. For example, safrole, a ubiquitous compound found in *Piper auritum* is a potent hepatoxin and hepatocarcinogen (Monzote *et al*. 2010). Perhaps the use of *Piper auritum* in herbal remedies (and in its use as a condiment) should be guarded or avoided due to high concentration of the carcinogen safrole (Wislocki *et al*. 1977; Tan *et al*. 1994).

Twenty-three of the species tested negative for alkaloids (Table 1). However, they too could also be effective non-cytotoxic medicinals due to the presence of other secondary metabolites such as anthocyanins, bufadienolides, carotenoids, coumarins, flavonoids, glycosides, lectins, malic acid, phenols, quinines, sapogenins, saponins, sitosterol, tannins, terpenoids, and tocopherol (Gibbs 1974; Afzal *et al*. 2012). For example, the widely used medicinal *Kalanchoe pinnata* (Crassulaceae) tested negative for alkaloids, but was cytotoxic with a LC_{50} at 635 µg/mL (Table 1). *Kalanchoe pinnata* is rich in other secondary metabolites such as caffeic acids, citric acid, ferrulic acid, glycosides, lactic acid, malic acid, p-coumaric, tannins, phenols, p-hydroxybenzoic acid, succinic acid, syringic acid, diterpenoidal lactones, steroids, aliphatic compounds, rutin, kaempferol, quercetin, quercetin-3L-rhamonsido-L-arabino, furanoside, quercetin-3-O-diarabinoside, kaempferol-3-glucoside, bryophollenone, bryophollone, cholestane-3,6,14-triol, 3,3',4',5,5',7-hexahydroxyflavan, 3-hydroxy-12,20-ursadien-11-one, 2-(9-decenyl) phenanthrene, bryophyllin-A,4bryophyllin B,5 bryotoxin B, bryotoxin C and 3,5,11,14-tetrahydroxy-12, 19-dioxobufa-20, 22-die-nolide, phenolic glucosides, syringic acid -D-glucopyranosyl ester (1) and 4'-O-D-glucopyranosyl-cis-p-coumaric acid, as well as flavonoids including kaempferol, quercetin, myricetin, acacetin, and

diosmetin glycosides (Gaind and Gupta 1971, 1973; Gibbs 1974; Morton 1981; Yamagishi *et al*. 1988, 1989; Duke 1994; Quazi Majaz *et al*. 2011; Fürer *et al*. 2013). *Kalanchoe pinnata* has proven to be cytotoxic in pharmacological studies against 9KB cancer cells (Yamagishi *et al*. 1989); anti-bacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Boakye-Yiadom 1977; Akinpelu 2000); antifungal properties on *Trichophyton mentagrophytes* (Rai and Upadhyay 1988), *Ustilago maydis* and *Ustilago nuda* (Singh and Pathak 1984); anti-inflammatory activity (Sidhartha *et al*. 1990; Ojewole, 2005); antinociceptive, antidiabetic effects (Ojewole 2005); and anti-tumor promoting activity (Supratman *et al*. 2001). In our bioassay, the aqueous extracts derived from dried parts of *Kalanchoe pinnata* had light cytotoxicity at a LC_{50} of 635 µg/mL). However, in two earlier studies based on aqueous extracts from fresh material, Biswas *et al*. (2011) and Vijay and Garge (2018) reported extremely higher cytotoxicity at a LC_{50} of 100 µg/mL. The physical state of the plant material (dry vs. wet), like a number of other variables (e.g., time of day, stage of development) also may alter chemical composition (Robinson 1974; Waller and Nowacki 1978; Salemink 1980; Crankshaw and Langenheim 1981; Coley 1983; Croom 1983; Langenheim *et al*. 1986; Coley and Aide 1991; Coe and Anderson 1996a; Coley and Barone 1996; Kursar *et al*. 1999). Similarly, *Lygodium venustum* tested negative for alkaloids but contains a wide variety of secondary metabolites such as flobabenic tannins, flavones, xanthones, chalcones, flavonoids (quercetin, rutin and kaempferol), phenolics acids (gallic, chlorogenic and caffeic acids), phenols and flavononones that accounts for its medicinal properties, particularly as a snakebite remedy. The presence of these secondary metabolites is responsible for its antiparasitary activity against *Leishmania* and *Trypanosoma* (Bravo *et al*. 2001; Batista *et al*. 2007; Fournet *et al*. 2007). In this study, the aqueous extracts derived from dried parts of *Lygodium venustum* were cytotoxic to brine shrimp (*Artemia salina*) at a LC_{50} of 580 µg/mL. In a study by Morais-Braga *et al*. (2013) the methanol fraction showed activity with inhibition percentage of 63% and 68% for promastigotes and epimastigotes, respectively, at a concentration of 500 µg/mL. Even though, *Kalanchoe pinnata*, *Lygodium venustum*, *Fevillea cordifolia*, *Smilax spinosa* and *Hymenaea courbaril* tested negative for alkaloids, their extracts were cytotoxic presumably because they contained other secondary metabolites such as caffeic acids, kaempferol, phenols, quercetin, quercetin-3L- flavonoids, rutin, and tannins. For example, caffeic acid, a type of polyphenol, is cytotoxic to leukemia cells and has very good antiviral activity (Etzenhouser *et al*. 2001); kaempferol is a potent promoter of apotosis (Ramos 2007); phenols are well known for their antiseptic, biocidal and corrosive properties, phenol also denatures proteins and generally acts as a protoplasmic poison with a LD_{50} in animals from 250-500 mg/kg (Michałowicz and Duda 2007); quercetin has potentially toxic effects, including its mutagenicity, prooxidant activity, mitochondrial toxicity, and inhibition of key enzymes involved in hormone metabolism (Dunnick and Halley 1992); rutin, as is most flavonoids, has very good antiseptic activity against many human pathogenic bacteria (e.g., *Escherichia coli*, *Proteus vulgaris*, *Shigella sonnei*, *Klebsiella sp.*, *Pseudomonas auruginosssa*, *Bacillus subtilis*) and fungi (e.g., *Candida albicans*, *C. gattii*) (Ganeshpurkar and Saluja 2017); and tannins (tannic acid) are water-soluble polyphenols with well documented antimicrobial activities (Chung *et al*. 1998). The growth of many bacteria, fungi, and viruses is inhibited by tannins (Chung *et al*. 1998). In addition, tannic acid is toxic to higher organisms such as mice, rats and other mammals (Robinson and Graessle 1943). The overall toxicity of tannins and other polyphenols is due to hydrogen bonding with vital proteins such as enzymes (Field and Lettinga 1992).

The results of this study are similar to earlier findings by Coe *et al*. (2010, 2011) that suggest alkaloid presence in an extract does not necessarily imply cytotoxicity. In the present study, 66% (45/68) of the species tested positive for alkaloids. However, of the 45 alkaloid bearing species, 27% (12/45) were non-cytotoxic. Two of the 12 alkaloid bearing non-cytotoxic species (*Passiflora quadrangularis* and *Pavonia schiedeana*) are both widely used medicinals in eastern Nicaragua and elsewhere (Morton 1981; Coe 1994, 2008a, 2008b, 2008c; Coe and Anderson 1996a, 1997). This broader use also implies that, from an ethnomedicinal perspective, these species are likely safe to use. Another possibility is that the therapeutic efficacy of the non-cytotoxic species could be the result of organ stimulation/destimulation or other physiological functions (Koffi *et al*. 2009).

In this study, the majority of plant extracts tested positive for alkaloids, but with a range of cytotoxicity. Perhaps this wide variation in toxicity is due to: (1) the type of alkaloid (e.g., imidazole, indole, isoquinoline, purine, pyridine, pyrimidine, pyrrolizidine, quinoline, tropane) present, (2) the synergism among alkaloids, or (3) the synergism of alkaloids with other secondary metabolites (e.g., glycosides, polyphenols, steroids, terpenoids). Among plants secondary metabolites, polyphenols are some of the most ubiquitous and bioactive compounds. So, perhaps polyphenols alone, or combined with other secondary metabolites, play an important role in the cytotoxicity of those nonalkaloid bearing species in this study. The results of the present study reaffirm the findings of our earlier studies, in that the presence of alkaloid does not necessarily mean that a plant extract is toxic. Most of the species in this study and our prior studies had very similar chemical constituents and those that differed in chemistry had compounds with similar bioactivity and therapeutic properties (e.g., quinine from *Cinchona* sp. and quassinoid simalikalactone D and E from *Quassia amara*, both antiplasmodial/antimalarial).

In summary, our studies indicated that most of the illnesses treated with the 780 ethnomedicinal species in eastern Nicaragua are of pathogenic origins (Coe and Anderson 1996a, 1997, 1999; Coe, 2008a, 2008b, 2008, 2020 in prep.). Moreover, our studies provide some insight into the use, chemistry, bioactivity and cytotoxicity of the medicinal plants used in eastern of Nicaragua. Over 70% of the medicinal plants presently in use in this area remain untested to determine their bioactivity, cytotoxicity and pharmacological properties. The hope is that the data herein and in our other studies will be valuable in promoting further research of the ethno pharmacology of eastern Nicaragua.

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

	Scientific Namea	Common Namesb	Medicinalc	Partd	Alka.e	LC f 50
	Ptridophyta, Filicopsida					
	SCHIZAEACEAE					
1.	*Lygodium venustum* Sw. (Coe-4337)	wire fern (c)	B,S	L,M	O	580
	Magnoliophyta, Magnoliopsida (DICOTS)					
	Apocynaceae					
2	*Allamanda cathartica* L. (Coe-2522)	dumari rauwa (g)	E,X	F,L,S	+	780
3	*Echites umbellata* Jacq. (Coe-3487)	vean withes (c)	B	R	+	2181
4	*Mandevilla villosa* (Miers) Woodson (Coe-2208)	unta kyuka (m)	B	L	O	1243
5	*Odontadenia puncticulosa* (Rich.) Pull. (Coe-2139)	amali (g)	B	L	+	1221
6	*Tabernaemontana chrysocarpa* Blake (Coe-4193)	cachito (h)	A,I	L	+	981
	Aristolochiaceae					
7	*Aristolochia trilobata* L. (Coe-3923)	cuntribo (g)	B,G,H,L,T	L,P	+	220
	Asclepiadaceae					
8	*Asclepias curassavica* L. (Coe-3235)	lamuruhewe (g)	D,P	B,F,P,S	+	243
	Asteraceae					
9	*Clibadium eggersii* Hieron. (Coe-2528)	inma saura (m)	S,T	L,P	O	5571
10	*Mikania cordifolia* (L.f.) Willd. (Coe-3246)	guagu (g)	A,B,S	L,M,P	+	465
11	*Neurolaena lobata* (L.) R. Br. (Coe-2552)	guye arani (g)	F,H,M,P,S	L	+	275
	Boraginaceae					
12	*Cordia inermis* (Mill.) I.M. Johnst. (Coe-4340)	kiasaika (m)	A,F	L	O	5561
	Crassulaceae					
13	*Kalanchoe pinnata* (Lam.) Pers. (Coe-3429)	bradutki (m)	A,L	L	O	635
	Cucurbitaceae					
14	*Fevillea cordifolia* L. (Coe-4432) antidote bush (c)	A,B,E,G	E	O	575	
15	*Momordica charantia* L. (Coe-3633) tasplira, twasplira (m)	A,C,H,l,J,L	L,M	+	560	
	Dilleniaceae					
16	16.Davilla kunthii A. St. Hil. (Coe-2702)	yahal (m)	D,Q	B,L,M	+	8761
	Euphorbiaceae					
17	*Euphorbia hyssopifolia* L. (Coe-4040)	bla saika (m)	A,C,I	L,P	O	4561
	Fabaceae					
18	*Cajanus cajan* (L.) Millsp. (Coe-3361)	pigeon pea (c)	L,S	F,L	+	891
19	*Cassia grandis* L.f. (Coe-3438)	bisbaira mina (m)	P,S,T,X	F,L	+	1451
20	*Cassia occidentalis* L. (Coe-3625)	ganibisi (g)	A,C,F,G,I	L,P,R	+	475
21	*Cassia undulata* Benth. (Coe-3287)	cuscus (m)	F,X	L,R	O	1341
22	*Crotalaria longirostrata* Hook. et Arn. (Coe-3335)	lamuruhewe (g)	E,X	L	+	978
23	*Dalbergia brownei* (Jacq.) Schinz (Coe-4082)	rusul (m)	D,Q,S	B,L,M	O	5671
24	*Desmodium barbatum* (L.) Benth. & Oerst. (Coe-3310)	latawira (m)	A,I,S,V	L,R	O	6732
25	*Dioclea megacarpa* Rolfe. (Coe-3238)	kuakua (g)	A,S	L	+	751
26	*Enterolobium cyclocarpum* (Jacq.) Griseb. (Coe-4374)	tuburus (m)	xx	xx	O	2541
27	*Hymenaea courbaril* L. (Coe-3417)	laka, lawa (m)	A,D,F,L	BS	O	863
28	*Lonchocarpus heptaphyllus* (Poir.) DC. (Coe-2567)	lîl tât (m)	S	F,R	O	1931
29	*Pentaclethra macroloba* (willd.) Kuntz (Coe-2441)	pigeon bush (c)	B,E,F,L,S	B	+	781
	Loganiaceae					
30	*Spigelia anthemia* L. (Coe-2830)	worm bush (c)	P	P	+	654
	Malpighiaceae					
31	*Banisteriopsis cornifolia* C.B. Rob. Ex Small	withes (c)	B,S	B,L,M	+	785
	(Coe-3305)					
32	*Heteropteris multiflora* (DC.) Hochr. (Coe-3476)	inenei (g)	L,Q	L	+	678
	Malvaceae					
33	*Pavonia schiedeana* Steud. (Coe-4150)	mozote (h)	F,I,J,K,V,W	R	+	3415
	Menispermaceae					
34	34.*Cissampelos pareira* L. (Coe-2532)	tamasa, alcotan (h)	B,F,S,V	L,R	+	200
	Moraceae					
35	35.*Ficus insipida* Willd. (Coe-3483)	higo (h)	A,G	S	+	675
	Myrtaceae					
36	*Eugenia axillaris* (Sw.) Willd. (Coe-3988)	tablira (m)	D,T	L	O	2341
37	*Syzygium malaccense* (L.) Merr. & Perry (Coe-3452)	manzana (g)	A,S	B,L,S	+	144
38	Ochnaceae					
	Sauvagesia erecta L. (Coe-4036)	Lilia sara (m)	A,B,F,G,L	P	O	3415
	Onagraceae					
39	*Ludwigia octovalvis* (Jacq.) Raven (Coe-3223)	slilma sirpi (m)	F,G,L	F	+	835
	Passifloraceae					
40	*Passiflora quadrangularis* L. (Coe-3513)	drap (m)	F,I,J,M,P,S	L	+	1876
	Piperaceae					

41	*Piper auritum* HBK. (Coe-2719)	ugudi bagasu (g)	A,C,F,G,N	L	+	420
42	*Piper hispidum* Sw. (Coe-2457)	Spanish ela (c)	A,F,G	L	+	675
43	*Piper jacquemontianum* (Kunth) DC. (Coe-3956)	lulubakbak (m)	A,F,G	L	+	917
44	*Piper peltatum* L. (Coe-3210)	ugudi bagasu (g)	A,C,F,G,N	L	+	715
	Polygonaceae					
45	*Polygonum punctatum* Elliott (Coe-3419)	pyâwira inma (m)	S	P	O	1912
	Primulaceae					
46	*Stylogyne turbacensis* (Kunth) Mez (Coe-2583)	butku plun (m)	A	L	+	3264
	Rubiaceae					
47	*Posoqueria latifolia* (Rudge) Roem. Et Schult.	uragu (g)	D,Q	B,L	+	962
		(Coe-4313)				
48	*Psychotria elata* (Sw.) Hammel (Coe-2472)	red scholars (c)	B,F,I,J,	F,L,M,R	+	212
			N,O,S			
	Sapindaceae					
49	*Cupania rufescens* Triana & Planch. (Coe-4275)	bila bila (m)	A,D,S	L	O	3305
50	*Cupania scrobiculata* Rich. (Coe-2488)	kalitara (m)	A,D,S	L	O	4151
	Scrophulariaceae					
51	*Lindernia difusa* (L.) Wettst. Ex Dugand et Jacks.	bird bush (c)	X	P	+	3241
		(Coe-2709)				
52	*Scoparia dulcis* L. (Coe-2236)	ri harachan (g)	B,CI,M,N,	L,P,R	+	450
			T,V,W			
53	*Waltheria glomerata* Presley (Coe-2605)	alwani saika (m)	D,O,T,U	L	O	4523
	Simarubaceae					
54	*Quassia amara* L. (Coe- 3540)	wewe gifi (g)	A,B,FM,	M	+	180
			P,Q,T			
	Solanaceae					
55	*Physalis angulata* L. (Coe-2259)	dumadu harachan (g)	F,S	L,P	+	850
56	*Physalis cordata* Mill. (Coe-3695)	turtle egg (c)	K	L,P	+	941
57	*Solanum asperum* Rich. (Coe-3255)	susul (m)	S	L	+	650
58	*Solanum mammosum* L. (Coe-3664)	gene gadaru (g)	A,L,S	E,F,L	+	400
59	*Solanum torvum* Sw. (Coe-4361)	miramara furuda (g)	A,B,F,S	L,R	+	550
	Tiliaceae					
60	*Apeiba aspera* Aubl. (Coe-2369)	urus bamba (m)	L,Q,S	B,L	O	6541
	Verbenaceae					
61	*Clerodendrum thoursomiae* Balf. (Coe-2296)	rice and beans (c)	S	L	O	3514
62	*Stachytarpheta cayennensis* (Rich.) Vahl. (Coe-3551)	vorvine (c)	C,F,G,L,P,V	L	+	620
63	*Stachytarpheta jamaicensis* (L.) Vahl. (Coe-3628)	vorvine (c)	C,F,LP,V,X	L	+	510
	Vitaceae					
64	*Cissus sicyoides* L. (Coe-3861)	karas wihta (m)	A	L,M,R	O	4430
	Liliopsida (Monocots)					
	ARACEAE					
65	*Montrichardia arborescens* (L.) Schott (Coe-3538)	chinchin banana (c) F	F+3452			
	POACEAE					
66	*Coix lacryma*-jobi L. (Coe-2646) sagadi, agusa (g)	I,S	E,R+2315			
67	*Eleusine indica* (L.) Gaertn. (Coe-2273) sagadi (g)	C,F,I,W	R +	850		
	SMILACACEAE					
68	*Smilax spinosa* Mill. (Coe-3820) chiny, ta wakia (m)	B,S,T	R	O	765	

Table 2: Number of the 68 species assayed used for each medicinal application.

	Number of Species
Abortifacient (O)	2
Aches and Pains (A)	25
Astringent (Q)	6
Anemia (T)	8
Bites and Stings (B)	16
Burns (N)	4
Childbirth and Pregnancy (C)	9
Cuts and Hemorrhage (U)	1
Diabetes (J)	4
Diarrhea* (D)	8
Digestive (G)	11
Diuretic (K)	2
Emetic (E)	4
Female Disorders (W)	3
Fever* (F)	21

Hypertension (H)	3
Infections* (I)	10
Malaria* (M)	4
Purgative and Laxative (X)	6
Respiratory & Pulmonary* (L)	13
Skin Rashes and Sores* (S)	24
Venereal Diseases* (V)	6
Worms and Intestinal Parasites (P)	8
*Ailments of pathogenic origins	

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