DIVERSITY OF METHYLXANTHINE CONTENT IN *ILEX CASSINE* L. AND *ILEX VOMITORIA* AIT.: ASSESSING SOURCES OF THE NORTH AMERICAN STIMULANT CASSINA[1]

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Adam L. Edwards and Bradley C. Bennett *(Center for Ethnobiology and Natural Products, Department of Biological Sciences, Florida International University, Miami, FL 33199; e-mail addresses: adam.edwards@fiu.edu and bennett@fiu.edu).* DIVERSITY OF METHYLXANTHINE CONTENT IN *ILEX CASSINE* L. AND *ILEX VOMITORIA* AIT.: ASSESSING SOURCES OF THE NORTH AMERICAN STIMULANT CASSINA. *Economic Botany 59(3):275–285, 2005. Indigenous people of southeastern North America drank cassina, a stimulant and emetic decoction that the colonial British termed "black drink." Though most authors cite* Ilex vomitoria *Ait. as the botanical source of cassina, confusion persists because some researchers identify the source as* I. cassine *L. To clarify the link between plant and product, the methylxanthine alkaloid contents of* I. vomitoria *and* I. cassine *were compared. Since methylxanthines (i.e., caffeine, theobromine, and theophylline) have pharmacological properties congruent with the recorded effects of cassina consumption, the alkaloids provide a chemical basis for the evaluation of both taxa as sources of the beverage. Methylxanthine levels are higher in* I. vomitoria *than in* I. cassine, *and the principal alkaloid of the former is caffeine. Based on its alkaloid content,* I. vomitoria *is the best-supported candidate source of cassina.*

Key Words: Black drink, caffeine, cassina, *Ilex cassine, Ilex vomitoria,* methylxanthine alkaloids.

Human plant use features an enduring, cross-cultural continuity of experience with stimulants. As part of the development of human stimulant consumption, people of Asia, Africa, and the Americas used several caffeine-containing plant species in the production of stimulant beverages, such as coffee (*Coffea arabica* L. and *C. canephora* Pierre ex Froehner), tea (*Camellia sinensis* Kuntze), cola (*Cola acuminata* Schott & Endl. and *C. nitida* Schott & Endl.), cocoa (*Theobroma cacao* L.), guaraná (*Paullinia cupana* H.B. & Kunth), and yoco (*Paullinia yoco* R. E. Schult. & Killip) (Ashihara and Crozier 1999; Eteng et al. 1997; Schultes 1987; Whitney, Rolfes, and Sizer 1987). Yerba maté, guayusa, and cassina, which are also stimulant beverages, are produced from New World hollies (Alikaridis 1987).

The monotypic holly family Aquifoliaceae, sister to Helwingiaceae in Aquifoliales, comprises around 400 species in the cosmopolitan genus *Ilex* (Manen, Boulter, and Naciri-Graven 2002; Stevens 2003). *Ilex* species are dioecious trees and shrubs with evergreen or deciduous, alternate leaves that have variable but often spiny margins. Axillary, cymose inflorescences bear 4–9-merous hypogynous flowers, and the carpellate flowers mature into red, orange, purple, or black drupes with four to six pyrenes (Stevens 2003). Though *Ilex* occurs on every continent except Antarctica, the centers of extant diversity are East Asia and South America (Cuenod et al. 2000; Manen, Boulter, and Naciri-Graven 2002).

Methylxanthine alkaloids (i.e., caffeine, theobromine, and theophylline) are the secondary constituents responsible for the biological activity of holly stimulants (see Fig. 1) (Eteng et al. 1997). Methylxanthines are water-soluble derivatives of the purine xanthosine 5′-monophosphate. Theobromine (3,7-dimethylxanthine) is the direct biosynthetic precursor of caffeine (1,3,7-trimethylxanthine), but a separate methylation sequence produces theophylline (1,3-dimethylxanthine, Ashihara and Crozier 1999). *Ilex* species as well as other plants with methylxanthines contain the purine alkaloids as

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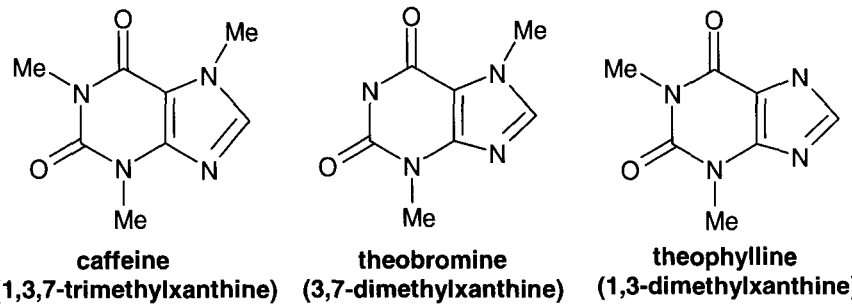

Fig. 1. Chemical structures of the methylxanthine alkaloids caffeine, theobromine, and theophylline (Dewick 2002).

chemical defenses with broad-spectrum activity against detrimental organisms. At concentrations up to 1.0%, caffeine causes dose-dependent growth reduction in *Aspergillus, Penicillium,* and fungi of the *Saprolegniales* order. Caffeine also causes mortality of the larvae of *Manduca sexta* (Lepidoptera: Sphingidae) at a concentration of 0.3%, reduces weight gain of the larvae at a concentration of 1.5%, and results in 100% sterility of *Callosobruchus chinensis* (Coleoptera: Bruchidae) at a concentration of 1.5% (Frischknecht, Ulmer-Dufek, and Baumann 1986). In concentrations of 1% to 2%, methylxanthine kills *Veronicella cubensis* slugs in spray applications. At a concentration of 2%, methylxanthines repel them from soil, and at concentrations as low as 0.01%, methylxanthines reduce their feeding activity. Methylxanthines also kill *Zonitoides arboreus* snails at concentrations of 0.5% and greater (Hollingsworth, Armstrong, and Campbell 2002). In humans methylxanthine alkaloids cause central nervous system stimulation, bronchial smooth muscle relaxation, and diuresis by competitively inhibiting phosphodiesterase activity, which results in the build up of cyclic adenosine monophosphate (cAMP), leading to increased release of adrenaline (Dewick 2002).

Yerba maté is the Spanish name for the beverage that was a pre-Columbian innovation of the Guaraní of southern South America. The caffeine-containing plant from which the beverage was prepared is *I. paraguariensis* St. Hil (Pronczuk, Laborde, and Heuhs 1987). Yerba maté is a hot infusion of the dried, chopped leaves of *I. paraguariensis,* which are a commodity in modern global commerce (Mazzafera 1997; Saldana et al. 1999). Popular status and demand have transformed yerba maté from a characteristic drink of the gaucho into an important agro-forestry crop for consumption and export in the economies of Argentina, Brazil, and Paraguay (Eibl et al. 2000; Gorzalczany et al. 2001; Vilcahuaman et al. 1999).

Use of a second caffeine-containing holly arose among traditional cultures of tropical northwestern South America. While there are several indigenous names for the beverage, such as waís from the Shuar of modern-day Ecuador, Spanish speakers use the Quichua name guayusa (Bennett, Baker, and Andrade 2002). Guayusa is a hot decoction of the roasted leaves of *I. guayusa* Loes. that, in contrast to yerba maté, has not received global attention (Patino 1968; Schultes 1979). Both yerba maté and guayusa function as stimulants in the human diet, but consumption of guayusa may be accompanied by emesis (Lewis et al. 1991).

Indigenous groups of North America consumed cassina, a third holly stimulant (Alston and Schultes 1951; Hudson 1979). Despite an independent origin with respect to its New World counterparts, cassina featured notable similarities with the South American stimulant guayusa, including the use of toasted leaves and associated emesis. Nearly all knowledge of the North American holly stimulant is based on notes and descriptions found in the chronicles of early contacts between native societies and Europeans (e.g., Narváez in Alston and Schultes 1951; Cabeza de Vaca 1993; Ribault in Hale 1891; Laudonniere 1975; Le Moyne in Alston and Schultes 1951; Gourges in Connor 1968; Dickinson in Blosser 1996; Lawson 1966; Bossu 1962; Catesby in Vogel 1970; Adair 1974; Romans 1962; and Bartram 1998). Synthesis of the cassina accounts reveals a stimulant leaf decoction that historically occupied a position of pro-

found importance in the cultures of the Creek, Catawba, Timicua, Alabama, Cherokee, Natchez, and Seminole as a ceremonial medicine and psychological aid (reviewed in Hudson 1979; Moerman 1998).

Shortly after arriving in North America, Europeans adopted the use of cassina from their indigenous neighbors (Lawson 1966; Vogel 1970). British colonists eventually incorporated the North American holly stimulant into their economy and vocabulary as black drink (Hudson 1979). In 1730, Francis Veale of North Carolina recorded that the leaves used to make the drink were "sold Cur'd att 2£ Barrell Sterling or 48 Bills" (Berkeley and Berkeley 1964). However, by the time the U.S. Government relocated the southeastern Native American populations to the Midwest, cassina use among North Americans of European descent had subsided in the face of open access to the popular stimulants coffee and tea (e.g., Wright 1986). In spite of a brief spike in its use among people of the southeastern United States during the Civil War, when access to coffee and tea was restricted by a trade blockade, the only North American holly stimulant had declined in popularity by the end of the nineteenth century (Hale 1891). Seven decades later, leaf tea remained a customary drink in the Carolinas (Morton 1974).

Beginning with the earliest scientific investigations of cassina, researchers have cited *Ilex vomitoria* Ait., *I. cassine* L., or both Latin binomials, often without reference to authority or voucher specimens, as sources of the beverage. *Ilex cassine* (dahoon holly, Christmas berry) is a shrub or small tree that attains heights up to 12 meters (m). Its leaves are elliptic or obovate, evergreen, and usually bear a few spinulose-serrate teeth on their margins. *Ilex cassine* grows in flatwood depressions and along swamp or pond edges from Virginia to Florida and west to southeastern Texas. Populations also are found in the Bahamas and in Cuba. *Ilex vomitoria* (yaupon holly) is a shrub or small tree that rarely grows taller than 8 m. Leaves of *I. vomitoria* are oval or elliptic and have crenate margins (see Fig. 2). *Ilex vomitoria* grows in and on the edges of upland, sandy, maritime woods on the coastal plain stretching from Virginia to Florida and westward to Arkansas and Texas. *Ilex vomitoria* also grows in Bermuda and the Mexican states of Chiapas and Veracruz (Wunderlin and Poppleton 1977).

In 1872, Henry M. Smith published articles in *Scientific American* and the *American Journal of Pharmacy* that identified *I. cassine* as the source of cassina (cited in Hale 1891). Hale (1891) described *I. cassine* as the aboriginal North American tea or "the famous black drink of the Southern Indians." More recently, Alikaridis (1987), Lewis and Elvin-Lewis (2003), and Weinberg and Bealer (2001) allude to *I. cassine* as a source of cassina. However, most researchers consider *I. vomitoria* to be the botanical source of the North American black drink (Alston and Schultes 1951; Blosser 1996; Hudson 1979; Merrill 1979).

One cause of the confusion may be the long-standing history of taxonomic confusion in the genus, especially with respect to *I. cassine* and *I. vomitoria.* Since Linnaeus published the generic name *Ilex* in *Species Plantarum* in 1753, botanists have proposed 18 additional generic names for all or parts of the group. The result is a lengthy synonymy for most *Ilex* species (e.g., Wunderlin and Poppleton 1977). It follows that nomenclatural confusion surrounds the purported botanical sources of cassina despite recognizable differences between them (Hu 1979).

Before publication of *Species Plantarum,* Caspar Bauhin (who provided no specimen or description of the plant) used the phrase *Cassena herba e cuius succo* in 1623; Leonord Plukenet (who provided the earliest botanical description) used the name *Cassine vera floridanourum* in 1700 for the taxon now recognized as *I. vomitoria* (Alston and Schultes 1951). During the preparation of *Species Plantarum,* Linnaeus had two American holly specimens. Borrowing from the Plukenet name, Linnaeus named one *I. cassine* (the dahoon holly) and the other *I. cassine* var. β, which would later become *I. vomitoria.* While Linnaeus's recognition of both specimens as varieties of *I. cassine* might be the starting point of confusion between the two modern species, the post-Linnean nomenclatural history of *I. vomitoria* further complicates the story (Hu 1979).

Authors have applied 16 different binomial names to the yaupon holly since Linnaeus first described the species as *I. cassine* var. β in 1753 (Table 1). Thomas Walter named the taxon in his posthumous 1788 publication *Flora Caroliniana. Ilex cassine* Walt. is thus a later homonym of *I. cassine* L. and has no botanical legitimacy. *Ilex vomitoria* Ait., the current ac-

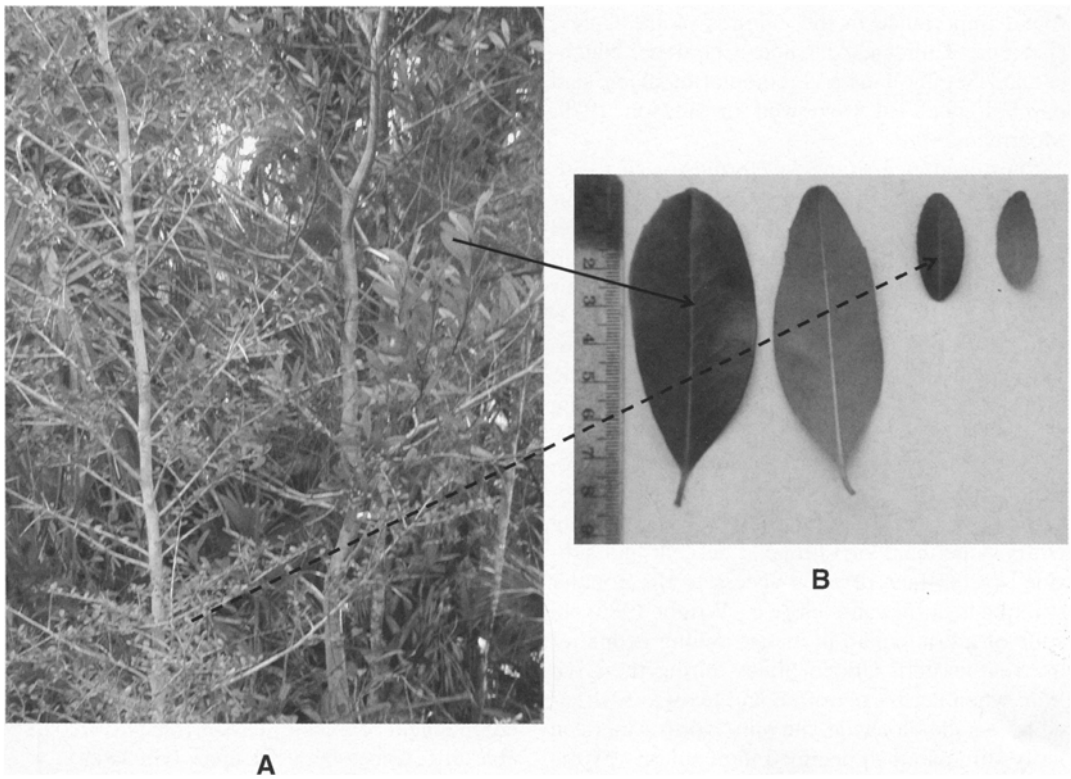

Fig. 2. **A.** *Ilex vomitoria* (on the left) and *I. cassine* (on the right) growing side-by-side in Crystal River Ecological Preserve, Citrus County, FL. **B.** *Ilex cassine* and *I. vomitoria* leaves (adaxial and abaxial view for each species). Scale to the left is in millimeters.

cepted binomial, did not appear until 1789 in *Hortus Kewensis.* During the next century, Lamarck, Michaux, and other botanists added another nine scientific names to the growing list of *I. vomitoria* synonyms. Furthermore, Asa Gray and other authors of major floras of the eastern United States misapplied *I. cassine* L. to *I. vomitoria,* a mistake that others perpetuated until the early twentieth century. Researchers often exacerbate the problem by excluding authors from Latin binomials in discussions of botanical sources of cassina, thereby making it unclear whether they intend *I. cassine* L. or *I. cassine* Walt., the former being a valid name for dahoon holly and the latter an invalid name for yaupon holly (Dudley 1985; Hu 1979).

Though careful examination of the literature provides a means by which to sort through the taxonomic confusion associated with *Ilex cassine* and *Ilex vomitoria,* the published literature supplies little information that distinguishes the species with respect to their secondary chemistry. The methylxanthine alkaloid content of *I. vomitoria* was the subject of only two studies in the twentieth century. Power and Chesnut (1919) reported that the caffeine content of *I. vomitoria* leaves ranges from 0.12% to 1.67% dry mass. Lewis et al. (1991) analyzed the leaves from one plant in Mississippi and reported its caffeine and theobromine contents as 0.09% and 0.04% dry mass, respectively. While the few studies of the methylxanthine content of *I. vomitoria* at least agree that the foliage contains caffeine, the methylxanthine alkaloid content of *I. cassine* is a point of disagreement in the literature. Lewis and Elvin-Lewis (2003) write that *I. cassine* contains caffeine and theobromine, without reference to any specific organ, even though Power and Chesnut (1919) failed to detect caffeine in this species. Alikaridis (1987) also wrote that *I. cassine* contains theobromine. Small sample sizes, incomparable sampling methods, and the frequent lack of vouchers hamper the synthesis of previous work

TABLE 1. CHRONOLOGICAL SYNONYMY IN *ILEX CASSINE* L. AND *ILEX VOMITORIA* AIT. SINCE LINNAEUS (HU 1979, WUNDERLIN AND POPPLETON 1977).

Ilex cassine L.		*Ilex vomitoria* Ait.	
Synonym, author, and source	Year	Synonym, author, and source	Year
I. cassine L., Sp. Pl. 125.; *non* Walt.	1753	*I. cassine* var. β L., Sp. Pl. 125.	1753
I. caroliniana Mill., Gard. Dict. Ed. 8.	1768	*Cassine peragua* L.	1753
I. dahoon Walt., Fl. Carol. 241.	1788	*Prinos glaber* L.	1762
I. cassine var. *augustifolia* Ait., Hort. Kew. 1:170.	1789	*Cassine paragua* Mill., Gard. Dict. Ed. 8.	1768
I. cassine var. *latifolia* Ait., Hort. Kew. 1: 170.	1789	*Cassine caroliniana* Lamarck	1782
I. cassinoides Link, Enum. Pl. Berol. 1:148.; *non* Du Mont de Courl	1811	*I. cassine* Walt., Fl. Carol. 241.; *non* L.	1788
I. laurifolia Nutt., Amer. J. Sci. 5:289.	1822	*I. vomitoria* Ait., Hort. Kew. 1:170.	1789
I. dahoon var. *laurifolia* (Nutt.) DC., Prodr. 2:14.	1825	*I. floridana* Lamarck	1791
Ageria palustris Raf., Sylva Tellur. 48.	1838	*Cassine yapon* Bartram	1791
Ageria germinata Raf., Sylva Tellur. 48.	1838	*I. cassena* Michx., Fl. Bor.-Amer. 2:229.	1803
Ageria heterophylla Raf., Sylva Tellur. 48.	1838	*I. religiosa* Barton	1812
Ageria obovata Raf., Sylva Tellur. 48.	1838	*Hierophyllus cassine* Raf.	1830
I. dahoon var. *augustifolia* (Ait.) Torr. & Gray, Bibl. Index N. Amer. Bot. 158.	1878	*Ageria cassena* Raf.	1838
		I. peragua (L.) Trelease	1889
		I. caroliniana (Lam.) Loesener	1891
		Cassine vomitoria Swanton	1946

on the methylxanthine alkaloids of *I. vomitoria* and *I. cassine.*

Examination of the methylxanthine contents of *Ilex cassine* and *Ilex vomitoria* would clarify their status as candidate botanical sources of cassina by allowing us to consider whether or not each species has an alkaloid profile consistent with, on a pharmacological basis, the reported stimulant effects of cassina consumption. To clarify the plant-product link between cassina and the North American *Ilex* species, we asked the following questions: 1) What are the methylxanthine alkaloid contents of *I. vomitoria* and *I. cassine*? 2) Are the methylxanthine alkaloid contents of the two species different? 3) Based on its methylxanthine alkaloid content, could *Ilex cassine* be a botanical source of cassina? With the observation that the majority of references to botanical sources of cassina in the literature point to *Ilex vomitoria,* we hypothesized that the methylxanthine content of *I. vomitoria* leaves would be sufficiently different from that of *I. cassine* foliage to suggest the former species as the principal source of cassina or black drink.

MATERIALS AND METHODS

Foliage samples from *Ilex cassine* and *I. vomitoria* plants were collected in natural populations from Florida. We gathered leaves from 10 *Ilex vomitoria* individual plants in the maritime-hammock and the marsh-edge habitats of Anastasia State Park in Florida's St. Johns County on May 8, 2003. On May 12, 2003, we collected leaves from 10 separate plants of the same species in the hammock habitat of Faver-Dykes State Park, which is also in St. Johns County, Florida. Samples from 12 *Ilex cassine* individual plants were collected from a hammock-marsh ecotone in Broward County, Florida on May 15, 2003; samples from eight other individual plants were gathered from tree island-marsh ecotones within the Florida International University (FIU) Singletary Preserve (Dade County, FL) on May 23, 2003. In total, we amassed foliage samples from 20 individuals of both *Ilex* species. Voucher specimens are deposited at Fairchild Tropical Garden in Miami, Florida.

All foliage samples were dried at 55 degrees Celsius (C) in a Thelco Model 6 (Precision Scientific Co., Chicago, IL) oven to a constant mass. Dried leaves were powdered with an Oster Osterizer (Oster, McMinnville, TN) blender and stored at room temperature until extraction. We placed 1 gram (g) of each powdered foliage sample in 20-milliliter (ml) scintillation vials along with 10 ml of a 1:1 water-and-methanol mixture to perform 10% w/v extracts for six

hours. Vials were maintained under constant mixing conditions on a Junior Orbit Shaker (Lab-Line Instruments, Inc., Melrose Park, IL). In preliminary tests, the extraction procedure produced ≥90% methylxanthine recovery after four hours. After six hours, 1-ml aliquots of the 20 crude extracts were filtered with Gelman 0.2-μm nylon syringe filters (Ann Arbor, MI) into 2-ml glass injection vials for analysis of their methylxanthine contents.

We analyzed the 1-ml crude extract aliquots by high-pressure liquid chromatography (HPLC) on an HP 1090LC (Hewlett-Packard, Palo Alto, CA) in line with a PC running HP ChemStation Rev A.06.03. The chromatographic solid phase was a reverse-phase Phenomenex Luna C18(2) column (150 × 2 mm, 5 μm), and the mobile phase consisted of water and methanol. The method comprised a linear methanol gradient from 5% to 100% over a period of 0 to 5 minutes (min) and 100% back to 5% methanol over a period of 5 to 10 min at a constant flow rate (0.5 ml/min), resulting in a total run time of 10 min. Each extract was monitored at 271 nm with a diode array detector. The chromatographic method used in this study was a modification of that of Baumann, Schulthess, and Hanni (1995).

Individual external calibration curves for caffeine, theobromine, and theophylline were constructed by relating peak areas to a range of concentrations (0.01, 0.10, 0.50, 1.00, 5.00, 10.00, 100.00, and 1,000.00 μg alkaloid/ml 80% aqueous methanol) of each alkaloid with simple linear regression [PROC REG in SAS (2002)]. Caffeine and theophylline showed linear peak responses from 0.50 μg/ml to 1,000.00 μg/ml; theobromine did so from 1.00 μg/ml to 1,000.00 μg/ml. The limits of detection for 5-μl injections were 0.1 μg/ml for caffeine and theophylline and 0.5 μg/ml for theobromine. Randomized, duplicate 5-μl injections for all 20 *I. cassine* and *I. vomitoria* foliage extracts were then analyzed. Average percentages of dry mass values from the duplicate samples for each extract were used in statistical analyses.

Nonparametric confidence interval estimates for the median caffeine, theobromine, and total methylxanthine contents (i.e., caffeine + theobromine) of *Ilex cassine* and *I. vomitoria* foliage samples were calculated. In addition, we evaluated the presence of a species effect in the distribution of the total methylxanthine contents data from all foliage extracts (N = 40) with the Wilcoxon two-sample rank sum test against the null statistical model of no species effect [PROC NPAR1WAY in SAS (2002)].

Results

Of the 40 foliage samples, quantifiable levels of caffeine were detected in 36, theobromine in 34, and both alkaloids in 31. No sample contained measurable levels of theophylline. For the purpose of statistical analysis, we calculated the contents of alkaloids present below the limit detection as one half of the lowest detected concentration for each alkaloid in all samples separately for each species. Table 2 shows summary statistics and nonparametric 95.86% confidence interval estimates for the caffeine, theobromine, and total methylxanthine contents of *I. cassine* and *I. vomitoria* foliage samples along with published values for the methylxanthine contents of *Camellia sinensis* and *Coffea arabica* for comparison.

The PROC NPAR1WAY procedure yielded a Wilcoxon two-sample test statistic (S) = 581 ($P < 0.001$) under the null model of equal total methylxanthine contents in *I. cassine* and *I. vomitoria.* The data provide sufficient evidence to reject the null model that total methylxanthine content is the same in *I. cassine* and *I. vomitoria* (see Fig. 3). When each sample was plotted as a point in the plane formed by caffeine and theobromine vectors, samples from the two species formed two discrete groups (Fig. 4).

Discussion

The caffeine, theobromine, and total methylxanthine alkaloid contents of comparable *Ilex cassine* and *I. vomitoria* leaf samples from Florida populations of both species were quantified. The caffeine and total methylxanthine contents of *I. vomitoria* foliage are five times and two times, respectively, that of *I. cassine.* On the other hand, the theobromine content of *I. cassine* leaves is twice that of *I. vomitoria* foliage. There is evidence of a detectably clear difference in the methylxanthine profile of the two North American *Ilex* species.

In comparison to the raw materials for tea and coffee, the methylxanthine contents of *I. cassine* and *I. vomitoria* leaves are an order of magnitude lower. For example, the median caffeine contents of foliage samples from both *Ilex* species in our analysis were less than 1% while the caffeine contents of tea leaves and coffee py-

TABLE 2. METHYLXANTHINE CONTENTS OF *ILEX CASSINE* L. AND *ILEX VOMITORIA* AIT. FOLIAGE SAMPLES (N = 20 PER SPECIES) WITH VALUES FOR *CAMELLIA SINENSIS* (L.) KUNTZE LEAVES AND *COFFEA ARABICA* L. PYRENES

Species	Alkaloid	Median	Range	CV[a]	95.86% CIE[b]
Ilex vomitoria Ait.	Caffeine	0.56[c]	0.34–0.94	30.6	0.46–0.69
	Theobromine	0.11	0.03–0.31	57.6	0.08–0.13
	Σ Methylxanthines	0.66	0.41–1.10	29.3	0.51–0.86
Ilex cassine L.	Caffeine	0.12	0.05–0.19	37.4	0.09–0.14
	Theobromine	0.22	0.05–0.67	62.6	0.17–0.28
	Σ Methylxanthines	0.33	0.09–0.83	46.4	0.26–0.41
Camellia sinensis (L.) Kuntze	Caffeine	3.40[d]	2.70–4.10		
	Theobromine	1.10	0.11–2.00		
	Σ Methylxanthines	4.50	2.81–6.10		
Coffea arabica L.	Caffeine	1.40[e]	0.80–2.00		
	Theobromine	<0.01	—		
	Σ Methylxanthines	1.40	0.80–2.00		

[a] CV = coefficient of variation.
[b] CIE = confidence interval estimate.
[c] All values are in units of % dry mass.
[d] Median and range values for *C. sinensis* based on data from Duke (1992) and Graham (1978).
[e] Median and range values for *C. arabica* based on data from Duke (1992), Eteng et al. (1997), Graham (1978), and Nathanson (1984).

renes are reported in the literature as 3.4% and 1.4%, respectively (see Table 2).

The pharmacological function of cassina as a stimulant beverage results from the ingestion of methylxanthine alkaloids contained in the botanical source. Yet, the effects of the individual methylxanthine alkaloids in the human body are different. Caffeine is the methylxanthine alkaloid with the strongest central nervous system stimulant activity, while theobromine is only one-tenth as potent as a stimulant (Dewick 2002). The higher caffeine and total methylxanthine concentrations in *I. vomitoria* foliage suggest that this species is characterized by an alkaloid profile indicative of a stimulant source. While *I. cassine* leaves contain twice the theobromine that *I. vomitoria* foliage does, the comparably small caffeine content and dominance of the weak stimulant theobromine in the alkaloid profile of *I. cassine* render it a less likely candidate for the botanical source of cassina or black drink. The unraveling of the tangled no-

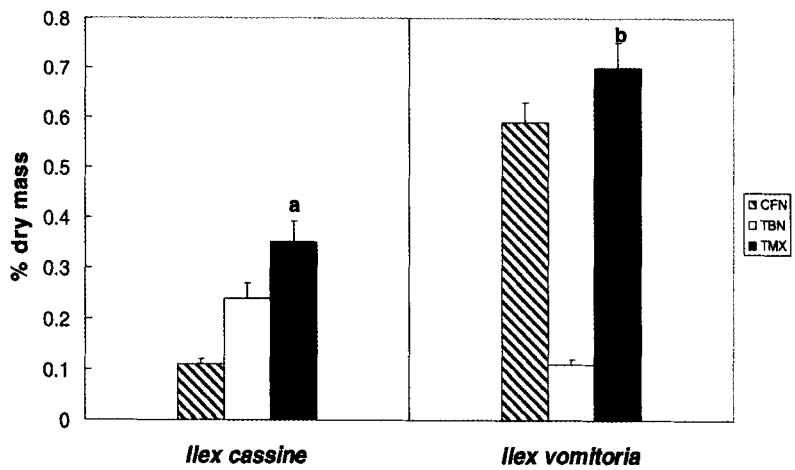

Fig. 3. Caffeine (CFN), theobromine (TBN), and total methylxanthine (TMX) contents for *Ilex cassine* and *Ilex vomitoria* foliage samples (N = 20 per species). Columns represent the mean ± SEM. Different lowercase letters above the TMX columns indicate statistical difference ($P < 0.001$) between species.

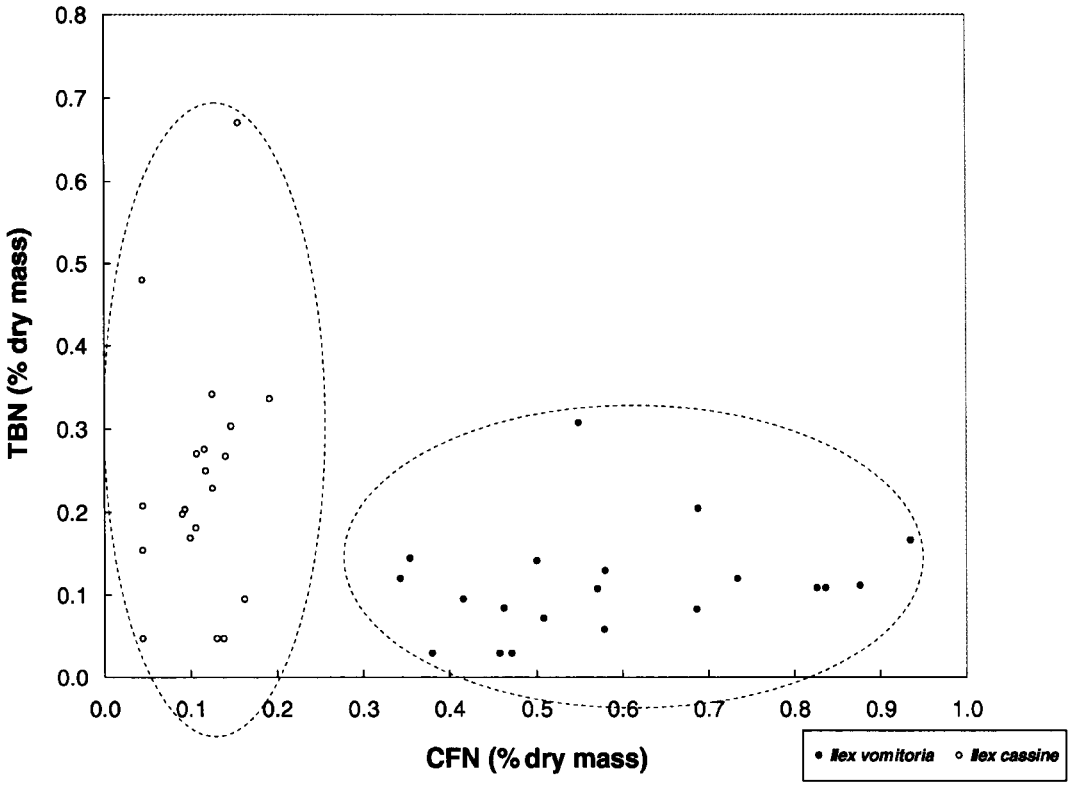

Fig. 4. Caffeine (CFN) and theobromine (TBN) contents of *Ilex cassine* and *Ilex vomitoria* foliage samples (N = 20 per species). Dashed ovals are for visual convenience only and are of no statistical significance.

menclatural history of *I. cassine* and *I. vomitoria* in the technical literature combined with the line of phytochemical evidence presented here provides strong support for distinguishing *I. vomitoria* as the best-supported candidate botanical source of cassina. However, a double-blind, placebo-controlled trial of preparations of *I. cassine* and *I. vomitoria* on human or other animal subjects in future research should also be performed for consideration along with the phytochemical findings.

As beverage products, the stimulant activities of cassina, coffee, and tea cannot be ranked according to the alkaloid contents of the respective plant materials used in preparation. The potency of a stimulant beverage is dependent on the type and amount of plant material used and the method and time of preparation (Bunker and McWilliams 1979; Graham 1978). Even though tea leaves contain higher levels of caffeine than coffee pyrenes, brewed coffee can contain 85 to 148 milligrams (mg) caffeine per cup compared to 7 to 50 mg per cup for tea (Bunker and McWilliams 1979; Conte, Barry, and Rubinstein 1996; Eteng et al. 1997; Graham 1978; Lelo et al. 1986; Pena, Lina, and Silveira 2005). We did not assess directly the potency of cassina as a prepared decoction. While it may be tempting to consider cassina a weak stimulant based on the lower levels of methylxanthines in *I. vomitoria* leaves, the potency of the black drink could approach that of a cup of tea depending on the number of leaves, the volume of water, and the length of time employed in its preparation. Based on an average *I. vomitoria* leaf mass of 25 mg from our samples, it would require at least 70 *I. vomitoria* leaves to provide the caffeine in a cup of tea or 650 leaves to provide the caffeine in a cup of coffee.

If *Ilex cassine* were a botanical source of the stimulant cassina among indigenous groups in North America, one would expect to find that people continued its preparation while they occupied territory within the range of *I. cassine* but outside the range of *I. vomitoria.* However, this predictive hypothesis fails upon examination of at least one example. The Seminole and Miccosukee of south Florida are descendants of

the Creek Nation, a group that once spanned large parts of southern North America (Bennett 1997). Groups ancestral to the modern Seminole and Miccosukee were among those who prepared cassina, and observations of cassina preparation among such groups are frequent in literature from periods of North American exploration and colonization by the Europeans. During the time of contact between the European explorers and colonists, indigenous groups that consumed cassina ranged across the natural distribution of *I. vomitoria*. But, as a result of warfare and migration away from the encroaching pressure of European colonization, ancestors of the Seminole and Miccosukee people settled permanently in southern Florida where *I. vomitoria* does not occur. They substituted *Eryngium yuccifolium* Michx. and other herbs as the source of their black drink, even though *I. cassine* was available in the area. This example adds further geographical and historical evidence to support the conclusion that *I. vomitoria* is the best-supported botanical source of cassina.

While the existence of intraspecific chemical heterogeneity may be assumed for methylxanthines across the ranges of both *Ilex* species, our samples from Florida represent data from the southernmost extent of the ranges of both species. Based on the ecogeographic hypothesis that latitude and alkaloid production are negatively correlated in plants (Levin 1976), our phytochemical findings from foliage samples of *I. cassine* and *I. vomitoria* may represent the upper bound of the methylxanthine contents of both species across their geographic distribution.

Conclusion

Perusal of taxonomic and ethnobotanical literature reveals a complicated history shared by *Ilex cassine* and *I. vomitoria* as touted botanical sources of cassina or black drink. This study of the methylxanthine alkaloid profile of *I. cassine* and *I. vomitoria* represents the largest sample of both species to be analyzed for methylxanthine alkaloids and the only study based on comparable samples of each. Quantifying the caffeine, theobromine, and total methylxanthine alkaloid contents of the dahoon and yaupon hollies enabled phytochemical means of discriminating between the two as candidate botanical sources of cassina. Nomenclatural, ethnobotanical, and phytochemical evidence all strongly support *I. vomitoria* as the principal botanical source of cassina. Future research with samples from across the species ranges to accommodate geographically correlated chemical heterogeneity and human trials of preparations from both *Ilex* species would provide more evidence for the evaluation of the botanical identity of cassina.

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