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Metabolite Profiling of Antiaddictive Alkaloids from Four Mexican *Tabernaemontana* Species and the Entheogenic African Shrub *Tabernanthe iboga* (Apocynaceae).

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Ibogaine and other ibogan type alkaloids present antiaddictive effects against several drugs of abuse and occur in different species of the Apocynaceae family. In this work, we used chromatography-mass spectrometry (GC-MS) and principal component analysis (PCA) in order to compare the alkaloid profiles of the root and stem barks of four Mexican *Tabernaemontana* species with the root bark of the entheogenic African shrub *Tabernanthe iboga*. PCA demonstrated that separation between species could be attributed to quantitative differences of the majority alkaloids coronaridine, ibogamine, voacangine, and ibogaine. While *T. iboga* mainly presented high concentrations of ibogaine, *Tabernaemontana* samples either showed a predominance of voacangine and ibogaine, or coronaridine and ibogamine, respectively. The results illustrate the phytochemical proximity between both genera, and confirm previous suggestions that Mexican *Tabernaemontana* species are viable sources of antiaddictive compounds.

Keywords: Alkaloids • Phytochemistry • *Tabernaemontana* (Apocynaceae) • *Tabernanthe iboga* (Apocynaceae) • Ibogaine

Introduction

Ibogaine and addiction treatment

Ibogaine is a monoterpenoid indole alkaloid (MIA) belonging to the ibogan type subclass. The substance has aroused interest among the scientific community since its first isolation in 1901 due to its strong central nervous system (CNS) effects. During the last decades, the substance has found growing use as an aid to drug detoxification and alternative psychotherapy through a widespread network of medical practitioners, lay therapists, activists, and some practitioners adapting forms of traditional practice [1]. Despite the fact that supportive evidence of ibogaine's antiaddictive effects is often drawn from a wide sample of anthropological anecdotes, the compound has been successfully tested in animal models of drug abuse [2]. Other ibogan type alkaloids like coronaridine and ibogamine may present an ever higher efficacy while being less toxic than ibogaine, possibly due to the absence of a methoxy group [3]. Notwithstanding, the development of the medical use of ibogaine is limited by legislation in several countries. In the USA it was added to Schedule 1 of the US Controlled Substances Act in 1970 because of its psychoactive effects [4]. It is similarly restricted in a number of other countries, while New Zealand, South Africa, and the city of Sao Paolo, Brazil, all have versions of policies that allow for its use [5]. However, the majority of ibogaine treatment occurs outside of prescription programs, through private providers, some of which are licensed treatment centers [6]. Although the total synthesis of ibogaine and structurally related compounds was achieved in the mid-1960s and more efficient chemical procedures were presented a few years ago [7,8], virtually all the ibogaine currently in existence originates from natural sources [6].

Tabernanthe iboga *and the* Bwiti *practice*

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Tabernanthe iboga BAILL. (Apocynaceae) (Fig. 1) is a shrub endemic to Central African rainforests where it is commonly known as *iboga*, although the term may apply as well to any of the other eight species of the genus [9]. The plant has a long history of cultural use as a ritual entheogen and medicine which is predominantly rooted and concentrated in Gabon, especially in the context of *Bwiti*, a spiritual practice of great national importance [6,10]. Although the whole plant of *T. iboga* contains up to 17 MIAs of the ibogan type [11–15], the ritually most valued material is the bitter inner layer of the root bark which tends to accumulate high concentrations of ibogaine and cause strong stimulant and oneirogenic effects [16]. Being the best known ibogaine-containing plant species, natural populations of *T. iboga* have been exposed to unregulated exploitation in order to meet the expanding demand for this substance from mostly western therapeutic communities. In consequence, *iboga*'s market price has increased at least tenfold in Gabon since the mid-1990s, creating potential challenges and barriers to access for local traditional practitioners, as well as leading to concerns about sustainability, since traditionally, there is no significant agricultural *iboga* production [6,9,17–19].

Mexican Tabernaemontana *species*

Apart from *T. iboga*, ibogaine has been found in a variety of species of the Apocynaceae family, particularly within the *Tabernaemontana* and *Voacanga* genera [20]. Actually, a significant share of the commercial ibogaine preparations available on the market are now produced via semisynthesis from voacangine isolated from the stem bark of *Voacanga africana* STAPF EX SCOTT-ELLIOT [6]. Being phytochemically more diverse than *Tabernanthe* and *Voacanga*, the pantropical *Tabernaemontana* genus produces 11 classes of MIAs and can be characterized by the presence of compounds belonging to the aspidospermatan, corynanthean, plumeran, and above all ibogan types [21]. Mexico is a diversity hotspot for the genus, with so far 16 identified species, six of which are endemic to the country [22–24]. *Tabernaemontana alba* MILL., *Tabernaemontana amygdalifolia* JACQ., *Tabernaemontana arborea* ROSE EX J.D.SM., and *Tabernaemontana donnell-smithii* ROSE EX J.D.SM. (Fig. 2 and 3) are known to produce ibogan type alkaloids as majority compounds [21,25–33]. These species frequently thrive in disturbed areas associated with tropical deciduous, semi-deciduous, and evergreen forest [34–36]. In the Los Tuxtlas region in the south of the State of Veracruz, *T. alba*, *T. arborea*, and *T. donnell-smithii* are dominant species of the secondary vegetation surrounding the human-induced pasturelands, partially due to the presence of a secondary metabolite-rich latex which acts as a cattle feeding deterrent [37]. The first and the third species are considerably more abundant than the second [38]. The peculiar morphology of *Tabernaemontana* fruits has earned many species local names alluding to the testicles of mammals, and in the case of the Los Tuxtlas region, the expressions *cojón de gato* (cat balls; *T. alba*), *cojón de venado* (dear balls; *T. arborea*), and *cojón de toro* (bull balls; *T. donnell-smithii*) are common, taking into account the increasing size of the fruits of the three above-mentioned species, respectively (Fig. 3).



Figure 1. *Tabernanthe iboga.* Whole plant, fruit, and flowers.



Figure 2. *Tabernaemontana amygdalifolia.* Whole plant and flowers.

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Figure 3. Fruits of *Tabernaemontana donnell-smithii*, *T. arborea*, and *T. alba* (from left to right).

We have previously reported that *T. alba* and *T. arborea* are potentially viable sources of ibogaine and voacangine, which accumulated particularly in the root and stem barks [26]. However, there is a need for quantitative studies that take into account the whole range of alkaloids present in these plants, as some compounds may show antiaddictive effects similar to ibogaine, while others may be toxic. Additionally, these studies should include information regarding the intraspecific variability of alkaloid contents, and the results should be compared with a reference species for these substances. The same procedure could then be applied to other species of the genus. For these reasons, we followed a metabolite profiling approach based on a microextraction protocol, chromatography-mass spectrometry (GC-MS), and principal component analysis (PCA), that allowed for a more comprehensive quantitative analysis of the alkaloid profiles of the barks of four Mexican *Tabernaemontana* species and *T. iboga* than any previous study. The results confirm both the potential of the former as sources of antiaddictive ibogan type alkaloids, as well as the phytochemical proximity between the five species.

Results and Discussion

Interspecific comparison of the MIA contents by PCA

Only quantifiable Total Ion Chromatogram (TIC) peaks that could be identified as MIAs were considered relevant to statistical analysis. Fig. 4 illustrates that generally speaking, the alkaloid profiles of *T. arborea* were more similar to *T. donnell-smithii* than to *T. amygdalifolia*. *T. alba* root bark samples were divided into two groups, one (2r, 3r, 6r) resembling the mostly ibogamine and coronaridine-containing *T. amygdalifolia* whole root bark, and the other (4r, 5r) being more similar to *T. arborea* root bark which showed a strong association with voacangine and, to a lesser extent, ibogaine. Regarding *T. alba* stem bark, only 3s was associated with *T. amygdalifolia* root bark. The other samples presented greatest similarity to *T. arborea* root bark and/or both types of *T. donnell-smithii* bark, with the latter composing a reasonably homogeneous group defined by its main compound voacangine. *T. amygdalifolia* stem bark formed a quite heterogeneous group, but coronaridine was the majority MIA in 12s and 13s. The quantitative predominance of vobasine over voacangine was a unique feature of *T. arborea* stem bark. The *T. iboga* samples 14r and 15r could be distinguished from the different *Tabernaemontana* clusters by their high concentrations of ibogaine.

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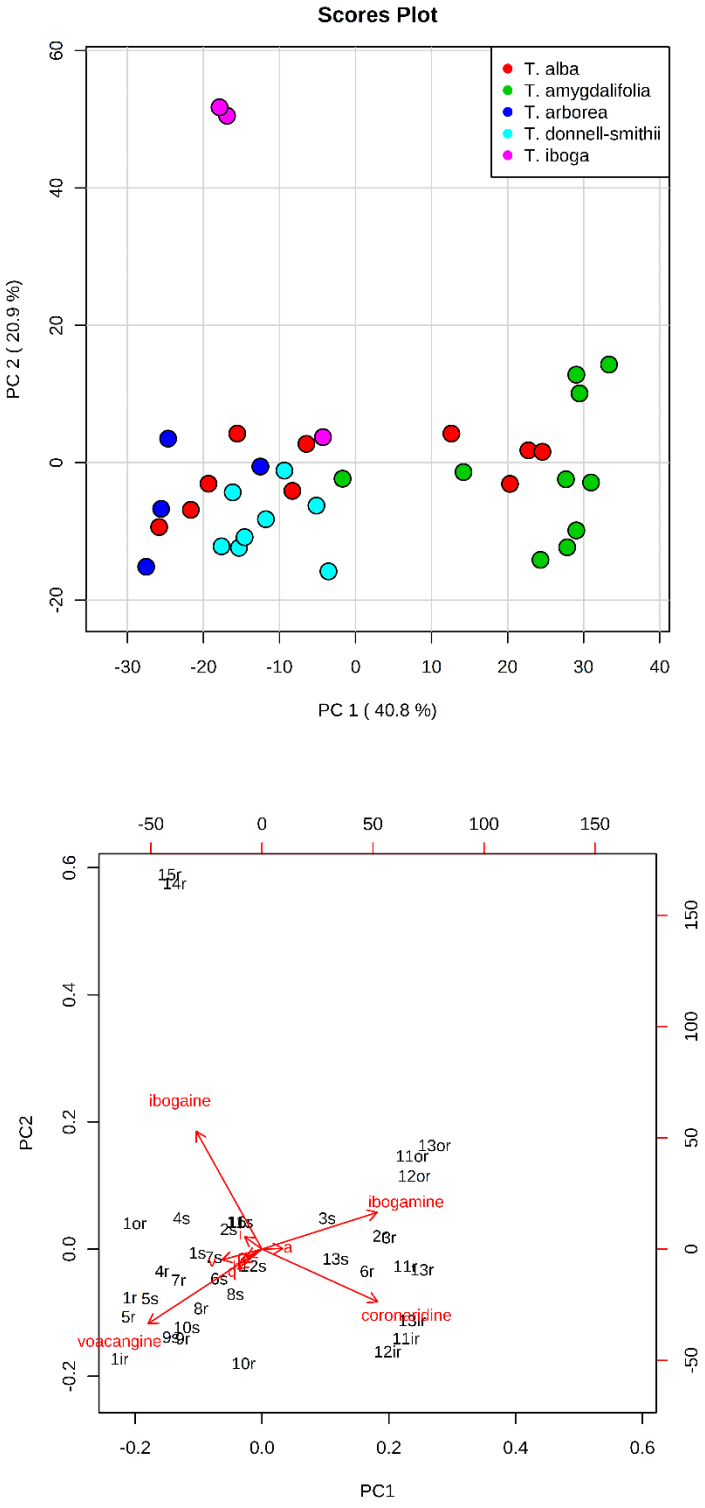


Figure 4. Scores (above) and biplot (below) derived from PCA of the MIA concentrations detected in root and stem bark extracts.

1 = *Tabernaemontana arborea*; 2-6 = *Tabernaemontana alba*; 7-10 = *Tabernaemontana donnell-smithii*; 11-13 = *Tabernaemontana amygdalifolia*; 14-16 = *Tabernanthe iboga* (each number represents an individual *Tabernaemontana* spp. plant or *T. iboga* sample)

ir = inner root bark; or = outer root bark; r = (whole) root bark; s = stem bark

ß = ß-hydroxyquebrachamine; a = appericine; h = 10-hydroxycoronaridine; i = ibogaline; q = quebrachamine; v = vobasine

On an individual scale, the alkaloid profiles of root and stem bark from the same *T. alba* plant could be either similar or differ considerably: specimens 3, 4, and 5 showed good coincidence between both types of bark, whereas the opposite was true for specimens 2 and 6. As a general rule for all four *Tabernaemontana* species, the majority alkaloid concentrations were always significantly higher in root than in stem bark (Table S1).

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Two outliers were identified in the scores plot: One *T. amygdalifolia* stem bark sample (11s) that contained only trace amounts of coronaridine, ibogamine, and voacangine, as well as one *T. iboga* root bark sample (16r) with a unique alkaloid profile which was devoid of any of the MIAs detected in the other samples (Table S1), but the only one that presented ajmalicine, reserpiline, and yohimbine (data not shown). These three substances were eliminated from the raw data before subjecting it to PCA. Other alkaloids were of minor importance with regard to the PCA outcomes, and are discussed in the following paragraphs.

Predominance of the CIVI-complex reflects phytochemical and taxonomic proximity between Tabernaemontana *and* Tabernanthe

Ibogamine, coronaridine, ibogaine, and voacangine contributed the most to the separation between samples determined by PCA (Fig. 4). Interestingly, these alkaloids form a group of structurally related compounds pertaining to the ibogan type of MIAs which hereinafter we will refer to as the "coronaridine-ibogamine-voacangine-ibogaine (CIVI)-complex:" Ibogamine represents the basic molecular skeleton, coronaridine its esterified, ibogaine its methoxylated, and voacangine its esterified and methoxylated forms, respectively. However, as the presumed precursor of the ibogan type alkaloids, stemmadenine, presents an ester but not a methoxy group [39], it seems plausible that from a biosynthetic point of view, ibogamine is the deesterified-decarboxylated and voacangine the hydroxylated-methoxylated derivative of coronaridine. Ibogaine could then originate either from the hydroxilation-methoxylation of ibogamine or the deesterification-decarboxylation of voacangine (Fig. 5).

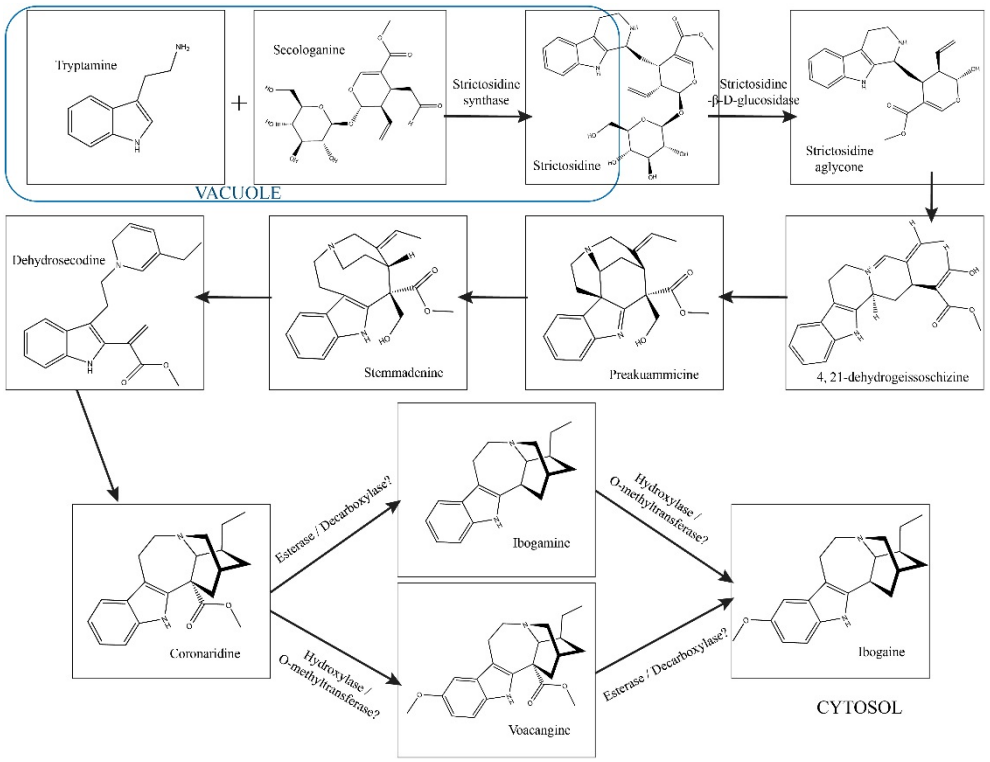


Figure 5. Hypothetical biosynthetic pathway of the CIVI-complex (modified from [39]).
Coronaridine and voacangine are the methyl esters of the 16-carboxylic acids of ibogamine and ibogaine, respectively.

This hypothesis has recently been supported by experimental evidence in a transcriptomic study of *T. iboga* that led to the discovery of two enzymes: Ibogamine 10-hydroxylase (I10H) is capable of converting ibogamine to noribogaine and coronaridine to 10-hydroxycoronaridine, while noribogaine-10-O-methyltransferase (N10OMT) catalyzes the transformation of noribogaine to ibogaine and 10-hydroxycoronaridine to voacangine [40]. Our results seem to further, albeit indirectly, indicate the validity of this pathway in *Tabernaemontana* species, as the outer root barks of *T. amygdalifolia* (11or, 12or, 13or) and *T. arborea* (1or) accumulated higher concentrations of the non-esterified compounds (ibogamine and ibogaine, respectively) than the inner root barks (11ir, 12ir, 13ir, and 1ir) whose majority alkaloids were always present in its esterified form (coronaridine and voacangine, respectively; Fig. 4; Table S1). Taking into consideration that outer root bark consists of more mature and supposedly more differentiated tissue than inner root bark, it would make sense that the former accumulated more alkaloids associated with later biosynthetic stages than the latter. In the case of *T. alba* and *T. donnell-smithii*, the root bark was not thick enough to allow separation into inner and outer layers.

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From a taxonomic perspective, the predominance of the CIVI-complex in all five species reflects the close relationship between the *Tabernaemontana* and *Tabernanthe* genera. According to *Sennblad* and *Bremer* [41], the former is a sister group to a clade formed by the latter plus two other African genera (*Carvalhoa* and *Schizozygia*). The fact that we detected only trace amounts of coronaridine in the *iboga* root bark samples (Table S1) may be due to its occurrence being restricted to the seeds of the species [13]. The minority alkaloids vobasine (corynanthean type; *T. alba* and *T. arborea*), apparicine (aspidospermatan type; *T. alba* and *T. amygdalifolia*), 10-hydroxycoronaridine (ibogan type; *T. donnell-smithii*), ß-hydroxyquebrachamine, quebrachamine (aspidospermatan type; *T. donnell-smithii*), and ibogaline (ibogan type; *T. iboga*) were of minor importance to the PCA outcomes, but could possibly serve as species-specific chemical markers, either on a whole-plant or organ-specific level: For instance, both quebrachamine-related compounds seem to be exclusive to *T. donnell-smithii*, whereas in the case of 10-hydroxycoronaridine, this may only be true for the barks, since the alkaloid has also been found in the leaves and fruits of *T. alba* [26]. Finally, it should be noted that the alkaloid profiles of *T. amygdalifolia* were quite different to previous reports from other researchers, given that we did not detect any compounds of the plumeran type such as cylindrocarpidine and related substances in the barks [27–29].

Potential of Mexican Tabernaemontana *species as alternative sources of antiaddictive alkaloids*

This study basically confirms the proposal of *Krengel et al.* [26] that *T. alba* and *T. arborea* could be used to produce ibogaine, especially when taking into account the combined ibogaine and voacangine contents, as the latter can be semisynthetically converted into the former [42]. The highest yields of these alkaloids detected in the *Tabernaemontana* samples corresponded to 0.95% of voacangine, as well as 0.22% and 0.27% of ibogaine of root bark dry weight of *T. alba* and *T. arborea*, respectively, hence giving combined concentrations of the two MIAs between 1.17 and 1.22% (Table S1). These values are on par with the 1.17% of ibogaine determined in the *T. iboga* root bark sample 14r which originated from a Gabonese farm with commercial goals, and considerably superior (at least 5 times) to what *Krengel et al.* [26] reported for the same species and MIAs. The considerable quantitative differences between the latter and the current study may be explained by the fact that in the first publication, plant material belonging to the same organ and species was pooled together prior to extraction (thus giving average values of high and low yielding individuals), whereas in the present work, samples obtained from individual plants were treated separately. Additionally, the significant variability of ibogaine and voacangine contents between *T. alba* samples suggests that yields could be substantially improved by selecting appropriate chemo- and genotypes, as well as by growing plants in controlled conditions that favor alkaloid production. While in the case of *T. iboga* root bark the inner layer contains most of the ibogaine [6], in *Tabernaemontana* species there seems to be a tendency to basically restrict the occurrence of non-esterified MIAs to the outer root bark. Consequently, separating inner from outer bark prior to extraction could facilitate isolation of the individual alkaloids. Thorough processing of *Tabernaemontana* bark might also provide a higher-yielding produce and make comparisons with the usually refined *iboga* products more exact.

T. amygdalifolia and *T. donnell-smithii* barks produced only small amounts of ibogaine, but reasonable concentrations of voacangine. Notably, the first species presented considerable concentrations of ibogamine and coronaridine ranging from 0.76 to 0.95%, and from 1.09 to 1.38% of (whole) root bark dry weight, respectively, and even reaching the very high value of 2.05% of ibogamine in outer root bark (Table S1).

Considering that *T. alba* presented two clusters of chemotypes, one biosynthesizing mainly the non-methoxylated and the other the methoxylated forms of the (both esterified and non-esterified) ibogamine skeleton in the root bark, this species may actually be a good source of the whole CIVI-complex.

Quantification of ibogaine in Tabernanthe iboga *root bark*

Two *T. iboga* samples (14r and 15r) showed alkaloid profiles characteristic of the species, ibogaine being by far the most abundant compound [20,43]. With the exception of *Jenks* [42] who isolated 3.666 g of ibogaine hydrochloride from 1000 g of *T. iboga* root bark, no published quantitative data on the species' MIA content could be found (the total alkaloid content of *iboga* root bark can be as high as 6% [43]). Our results suggest a significant intraspecific variability in ibogaine concentration with one sample (15r) showing a 4 times higher value than the other (14r; Table S1), although it is worth noting that we do not know whether the *iboga* samples were each obtained from only one plant or rather from a pool of several plants. The very high concentration of 4.75% of ibogaine per dry weight of plant material was actually determined in the root bark produced by a Gabonese Pygmy community. This ethnic group has probably the longest history of ethnobotanical use of *T.*

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iboga [6] which could imply that the high alkaloid content in 15r is the result of sophisticated plant selection or specific local growing conditions. In contrast, the 14r sample originated from Otong Mekok, a farm in Northern Gabon that aims to produce *iboga* sustainably and economically for both the local and international market. In any case, our results underline the difficulty of correct dosing when using *T. iboga* root bark directly or in the form of extracts (instead of pure ibogaine) in an ethnobotanical or therapeutic context, as depending on the administered dose, ibogaine can act as a stimulant or entheogen, but can also have cardiotoxic effects, particularly in combination with risk factors involving preexisting cardiovascular diseases or adverse drug interactions [44]. Even in traditional settings with a long history of usage and knowledge of *iboga* such as *Bwiti* rituals, incidental fatalities related to medical complications do occur [20,43]. Actually, there is a controversy concerning the degree to which these fatalities are caused by the consumption of "false *iboga*". In fact, the plant's long harvest cycle, it's rapid rise in value, and its unregulated trade [6] have created conditions that incentivize the adulteration or counterfeit of *T. iboga* root bark with other plant materials, sometimes dangerous. Reports of "false *iboga*" leading to adverse physical reactions and even to death have been heard within user communities, and previously confirmed by *Guignon* [19] in the context of Gabonese traditional medicine, although it is important to point out that the phenomenon is not limited to a local or regional scale, but has also been associated with international mail order and internet sales [45]. It consequently fits the picture that the sample purchased on a local market in Cameroon (16r) did not contain any ibogan type alkaloids, but reserpiline, yohimbine, and ajmalicine (all corynanthean type). The sample was probably obtained from another Apocynaceae species, most likely belonging to the *Rauvolfia* genus [46], and mislabeled as *T. iboga* root bark.

Conclusions

Our research offers rare quantitative data on the MIA profiles of the Mexican *Tabernaemontana* species *T. alba*, *T. amygdalifolia*, *T. arborea*, and *T. donnell-smithii*, as well as the African shrub *Tabernanthe iboga*. The predominance of a complex of four structurally related ibogan type alkaloids – coronaridine, ibogamine, voacangine, and ibogaine – in root and/or stem bark proved to be a characteristic chemical feature of the five species. This illustrates the phytochemical and taxonomic proximity between the two genera, and strongly suggests that *Tabernaemontana* species are promising candidates for the production of antiaddictive alkaloids from alternative plant sources. Furthermore, we hope to contribute to the risk reduction regarding both the traditional and therapeutic use of ibogan type alkaloid-containing plant material, particularly with reference to *T. iboga*, the sacred plant that is so essential to Central African spirituality and innovative addiction treatment.

Experimental Section

Plant material

Root and stem bark was taken from young and mature *Tabernaemontana alba* Mill. (*cojón de gato*), *Tabernaemontana arborea* Rose ex J.D.Sm. (*cojón de venado*), and *Tabernaemontana donnell-smithii* Rose ex J.D.Sm. (*cojón de toro*) plants in the vicinity of the Estación de Biología Los Tuxtlas (UNAM) in Veracruz, Mexico (18°33´ to 18°36´N 95°04´ to 95°09´W), in February 2017. *Tabernaemontana amygdalifolia* Jacq. (*jasmín de perro*) bark was collected in San Antonio Mulix (20°40'6"N 89°45'24"W) and Valladolid (20°41'39.872"N 88°13'38.964"W), Yucatán, Mexico, in April 2017. Taxonomic identification of the species was carried out by Delfino Álvaro Campos Villanueva (Estación de Biología Los Tuxtlas, UNAM) and Leonardo Osvaldo Alvarado Cárdenas (Facultad de Ciencias, UNAM), voucher specimens were deposited at the herbarium of the Facultad de Ciencias (FCME), UNAM (voucher numbers 161424 to 161427), and plant names were checked with http://www.theplantlist.org. Root bark samples of *Tabernanthe iboga* Baill. (*iboga*) were acquired from a distributor in Western Cameroon, a traditional Gabonese Pygmy village, and from Otong Mekok, a commercial farm in Northern Gabon.

Extraction

The dried bark was pulverized using a cutting mill. The extraction procedure was loosely based on the protocol published by *Kim et al*. [47]: 100 mg samples, each associated with an individual plant (trunk bark as well as inner, outer, and whole root bark from five *Tabernaemontana alba*, three *T. amygdalifolia*, one *T. arborea*, and four *T. donnell-smithii* plants, plus the already processed *Tabernanthe iboga* samples), were placed in 2 ml Eppendorf tubes to which a small piece of degreased cotton wool (approximately 10 mg) and a mixture of dichloromethane (1.5 ml) and ammonium hydroxide (50 µl) was added.

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The tubes were vortexed and put into an ultrasonic bath for 30 min. After centrifuging at 14500 rpm for 2 min, the supernatant was sucked up into a Pasteur pipette through the cotton wool and evaporated to dryness at room temperature in darkness. This procedure was repeated four more times for each sample. The alkaloid extracts were stored at -20°C.

Analysis of alkaloid extracts by GC-MS

The extracts were dissolved in 2 ml of methanol and analyzed using an Agilent 7890B/5977A GC/MSD with a HP-5ms (30 m) capillary column following the method described by *Krengel et al.* [26], with minor modifications: EI-mass spectra recording at 70 eV after 8 min of solvent delay; injector in split mode (1:5) at 300°C with automatic injection of 1µl aliquots; ramp temperature program from 150 to 300°C at 4°C/min; Helium carrier gas at 1 ml/min. Peak identification was carried out by the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library (Version 2.2, build Jun 10, 2014) and by comparison of the respective mass spectra with published data or experimentally obtained spectra of standard compounds (representative chromatograms and mass spectra can be found in Fig. S1 and S2 in the Supporting Information). These compounds, ibogaine and voacangine, were kindly donated by Phytostan Enterprises, Inc. (Montreal, Quebec) and used as external standards to construct calibration curves consisting of six points (25, 50, 100, 200, 300, and 400 µg/ml) with three repetitions each. The ibogaine and voacangine contents of the samples were calculated by the respective formula, while other MIAs were quantified based on the calibration curve of the structurally more similar alkaloid.
Ibogaine (apparicine, ß-hydroxyquebrachamine, ibogamine, ibogaline, quebrachamine):
$y = 212807.90171x + 2760970.43093$ ($r^2 = 0.99766$)
Voacangine (coronaridine, 10-hydroxycoronaridine, vobasine):
$y = 194161.98834x + 2381117.06883$ ($r^2 = 0.99789$)

Statistical analyses

Statistical analyses of the MIA concentrations were performed with the free R-based online software MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/#). Data was normalized by sum and Pareto-scaled before subjecting it to PCA.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbdv.2018xxxxx.

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Author Contribution Statement

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F.K., Q.C., and R.R.C. collected the *Tabernaemontana* plant material. J.D. provided the *iboga* samples. F.K. and Q.C. extracted the samples and conducted the GC-MS and statistical analyses. F.K., J.D., and J.H.S. wrote the draft. Based on the comments of all authors, F.K. and R.R.C. wrote the final version of the manuscript.

Declarations of interest

None to declare.

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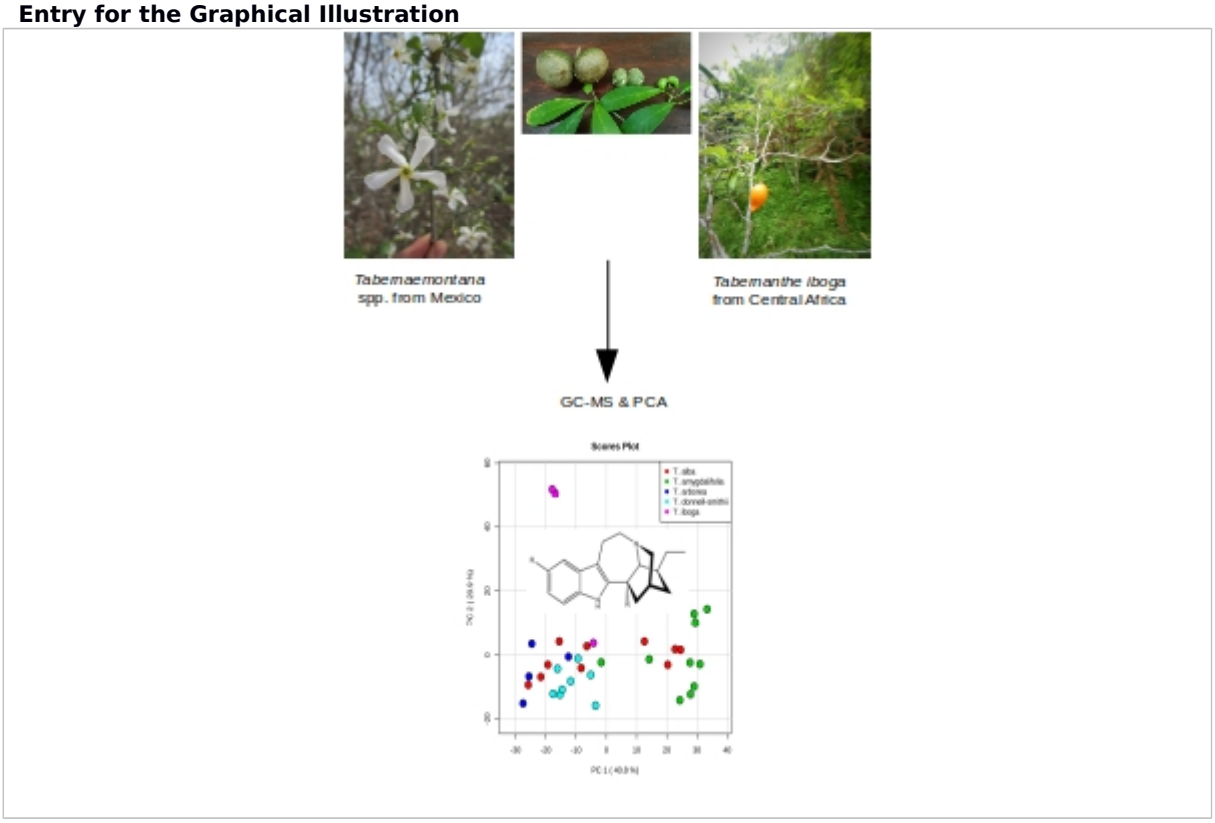
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Similar to *Tabernanthe iboga*, Mexican *Tabernaemontana* species are viable sources of antiaddictive ibogan type alkaloids.

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