University of Brasília
Institute of Biological Sciences
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FITOQUÍMICA DA AYAHUASCA PRODUZIDA COM DIFERENTES ETNOTAXA DE CIPÓS (MALPIGHIACEAE):REVISÃO DE LITERATURA E CROMATOGRAFIA

PHYTOCHEMISTRY OF AYAHUASCA PRODUCED WITH DIFFERENT ETHNOTAXA OF VINES (MALPIGHIACEAE): LITERATURE REVIEW AND CHROMATOGRAPHY

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I dedicate this work to Abby, Noah, and Aaron, who are my deepest inspiration and my constant motivation to become a better person and professional.

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RESUMO

A Ayahuasca é um enteógeno de grande importância cultural e medicinal, consumido por diversas comunidades indígenas da Amazônia e de outras regiões da América Latina. Tradicionalmente, a bebida é preparada a partir de uma mistura complexa de plantas, sendo as mais comuns o cipó *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton (Malpighiaceae) e as folhas de *Psychotria viridis* Ruiz & Pav. (Rubiaceae). O objetivo deste estudo foi revisar a literatura e os herbários para compilar as espécies historicamente utilizadas no preparo da Ayahuasca. A pesquisa foi realizada em várias bases de dados e incluiu uma busca nos herbários COAH, COL, F, GH, IAN, K, L, MICH, MO, NY, UB, UBC e US, utilizando nomes vernaculares relacionados à bebida. Foram identificadas 27 espécies designadas como *Ayahuasca*, das quais 15 já estavam documentadas na literatura e 12 foram reveladas pela primeira vez por meio da análise dos herbários, o que destaca a importância da documentação científica no registro do conhecimento tradicional. Essas espécies pertencem predominantemente à família Malpighiaceae, com ênfase no gênero *Banisteriopsis*, além de 12 espécies conhecidas como *Chacrona* ou *Chacropanga*, com predominância do gênero *Psychotria*. Adicionalmente, o estudo também investigou os compostos químicos presentes em bebidas preparadas com diferentes etnotaxa de *B. caapi*, com o objetivo de verificar se as diferenças nos efeitos fisiológicos relatados pelos usuários estão associadas às variações químicas dessas linhagens. A análise foi realizada utilizando técnicas cromatográficas avançadas, como Cromatografia em Camada Fina (TLC), Cromatografia Líquida de Alta Eficiência com Detector de Arranjo Diodo (HPLC-DAD), Cromatografia Líquida Acoplado a Espectrômetro de Massas (HPLC-MS/MS), Cromatografia Preparativa (Prep-LC), Cromatografia em Coluna de Fase Reversa (RP-CC) e Espectrometria de Ressonância Magnética Nuclear (RMN). Os resultados indicaram que as bebidas apresentaram composições químicas distintas. Esses achados sugerem que a divergência nos efeitos percebidos pelos usuários pode ser explicada pelas diferenças químicas entre os etnotaxa de *B. caapi*, o que reforça a ideia de que o efeito fisiológico da Ayahuasca é fortemente influenciado pela composição química da planta utilizada. Este estudo não apenas contribui para o entendimento das características químicas das linhagens de *B. caapi*, mas também destaca a importância da preservação e documentação do conhecimento tradicional, essencial para futuras aplicações medicinais e culturais da Ayahuasca.

Palavras-chave: Chacrona, Conhecimento tradicional, Malpighiaceae, Rubiaceae e Yagé.

ABSTRACT

Ayahuasca is a highly significant entheogen, both culturally and medicinally, consumed by various indigenous communities in the Amazon and other regions of Latin America. Traditionally, the brew is prepared from a complex mixture of plants, with the most common being the vine *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton (Malpighiaceae) and the leaves of *Psychotria viridis* Ruiz & Pav. (Rubiaceae). The aim of this study was to review literature and herbarium collections to compile the species historically used in the preparation of Ayahuasca. The research was conducted across several databases and included a search of the herbariums COAH, COL, F, GH, IAN, K, L, MICH, MO, NY, UB, UBC, and US, using vernacular names related to the brew. A total of 27 species designated as Ayahuasca were identified, 15 of which were already documented in the literature, while 12 were revealed for the first time through herbarium analysis, highlighting the importance of scientific documentation in recording traditional knowledge. These species predominantly belong to the Malpighiaceae family, with a focus on the *Banisteriopsis* genus, as well as 12 species known as *Chacrona* or *Chacropanga*, with an emphasis on the *Psychotria* genus. Additionally, the study also investigated the chemical compounds present in brews prepared with different *B. caapi* ethnotaxa, aiming to determine whether the differences in physiological effects reported by users are linked to chemical variations among these strains. The analysis was carried out using advanced chromatographic techniques such as Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography with Diode-Array Detector (HPLC-DAD), Liquid Chromatograph Coupled to a Mass Spectrometer (HPLC-MS/MS), Preparative Chromatography (Prep-LC), Reverse-Phase Column Chromatography (RP-CC), and Nuclear Magnetic Resonance (NMR) Spectrometry. The results indicated that the brews exhibited distinct chemical compositions. These findings suggest that the divergence in perceived effects by users may be explained by chemical differences between *B. caapi* ethnotaxa, reinforcing the idea that the physiological effect of Ayahuasca is strongly influenced by the chemical composition of the plant used. This study not only contributes to the understanding of the chemical characteristics of *B. caapi* strains but also emphasizes the importance of preserving and documenting traditional knowledge, which is crucial for future medicinal and cultural applications of Ayahuasca.

Key words: Chacrona, Conhecimento tradicional, Malpighiaceae, Rubiaceae e Yagé.

INTRODUCTION

Medicinal plants have played a central role in human health and cultural practices for millennia, providing not only therapeutic resources but also shaping systems of knowledge and identity. In the Amazon basin, one of the most biologically diverse regions on Earth, these practices are deeply intertwined with ecological dynamics and spiritual worldviews. Among the many plant-based preparations originating in this region, Ayahuasca stands out as a particularly significant example due to its long history of ritual use, its cultural importance for Indigenous and local communities, and its growing visibility in global scientific and medical discussions.

Ayahuasca is an ancestral preparation traditionally used by Indigenous and local communities to address both physical and spiritual conditions. In recent decades, scientific research has suggested its potential therapeutic applications in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's, as well as mental health conditions including anxiety, substance dependence, and depression. Typically, Ayahuasca is prepared using the vine *Banisteriopsis caapi* in combination with the leaves of *Psychotria viridis*, although alternative plant mixtures are also employed depending on cultural practices.

Despite its cultural and potential therapeutic significance, the increasing demand for Ayahuasca has generated ecological concerns. The harvesting of *B. caapi* and associated species often conflicts with their natural growth cycles, and the limited cultivation of these plants has contributed to localized deforestation in the Amazon rainforest. This not only threatens regional biodiversity but also endangers the transmission of traditional knowledge that has sustained the preparation of Ayahuasca for generations.

Within this context, the study and dissemination of scientific knowledge about Ayahuasca may contribute to the dual goals of biodiversity conservation and the preservation of ancestral practices. This dissertation presents a synthesis of findings generated by a team of Latin American researchers, who, through interdisciplinary approaches, seek to advance the understanding of Ayahuasca from ethnobotanical, and phytochemical perspectives. The work is organized into two chapters: *"Unveiling*

Ayahuasca: Comprehensive Study of Botanical and Chemical Diversity" and *"Phytochemical Profiles of Ayahuasca: Insights from Different Ethnotaxa of Vines.*

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CHAPTER 1. Unveiling Ayahuasca: Comprehensive Study of Botanical and Chemical Diversity

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Abstract

Ethnopharmacological relevance: Ayahuasca is an entheogenic beverage used in rituals by traditional communities. Its composition varies regionally, with the vine of *Banisteriopsis caapi* (MAO inhibitor) and the leaves of *Psychotria viridis* (DMT source) as key ingredients. Different *B. caapi* ethnotaxa exhibit distinct morphological and physiological effects.

Aim of the study: To compile *B. caapi* ethnotaxa and species used in Ayahuasca preparation and assess their ethnobotanical and phytochemical records.

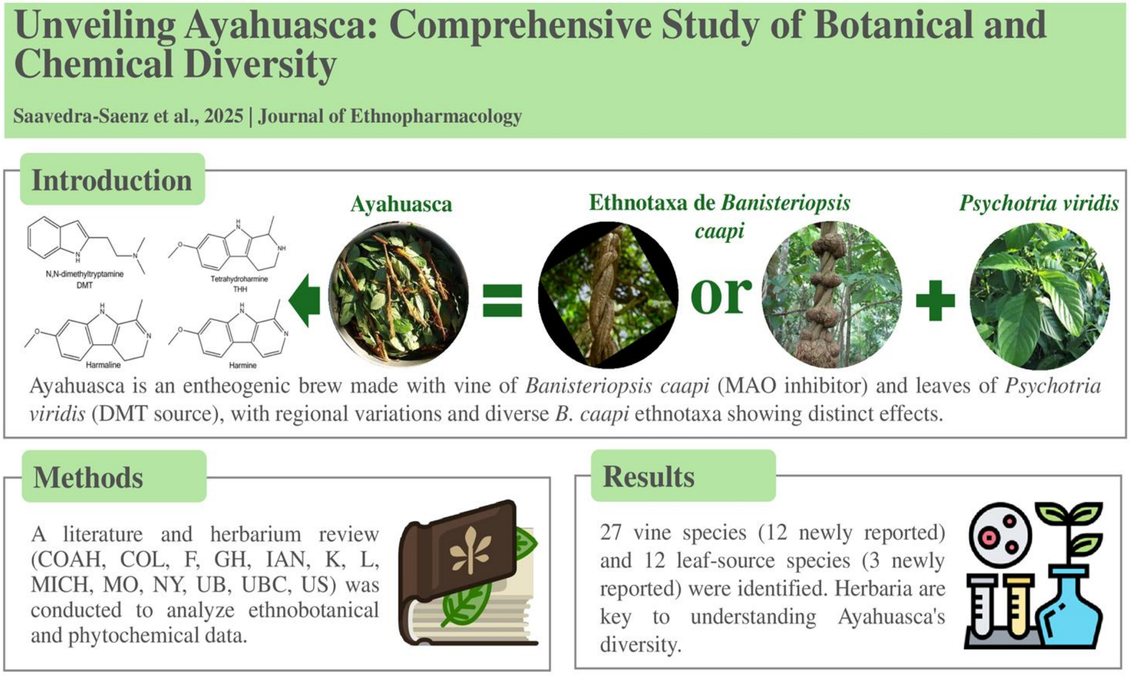
Materials and methods: A literature review and herbarium consultation were conducted, including COAH, COL, F, GH, IAN, K, L, MICH, MO, NY, UB, UBC, and US. Ethnobotanical and phytochemical data were reviewed from these collections.

Results: A total of 27 vine species were identified, 12 newly reported, all from Malpighiaceae, with 119 documented ethnotaxa. Regarding leaf sources, 12 species from *Diplopterys, Palicourea,* and *Psychotria* were recorded, including three new ones. Phytochemical data remain scarce.

Conclusions: Herbaria are crucial for ethnobotanical research. Ayahuasca formulations vary widely, requiring further phytochemical studies to understand their diversity.

Keywords: *Chacrona, herbaria, Malpighiaceae, Rubiaceae, Yagé*

Graphical abstract



Highlights

1. 27 species named *Ayahuasca* or *Yagé*, 12 reported for the first time.
2. Eight species named *Chacrona*, three newly reported in the literature.
3. 119 *B. caapi* etnotaxa reported for the first time, with morphological diferences.
4. Herbaria are key for documenting Ayahuasca's ethnobotanical and phytochemical data.

1. Introduction

The entheogenic beverage known as Ayahuasca originates in South America and is traditionally consumed by Indigenous peoples inhabiting the Amazonian regions of Brazil, Bolivia, Colombia, Ecuador, and Peru, as well as areas along the Orinoco River in Venezuela and the Pacific Coast of Colombia and Ecuador (Schultes and Hofmann, 1980). Its use is primarily associated with rituals of spiritual and medicinal significance.

The first accounts of Ayahuasca shared with the non-Indigenous world were disseminated by Jesuit monks during the colonization of the Amazon in the 17th century. They described it as a "diabolical beverage" made from bitter plants that caused Indigenous people to lose their senses (Chantre y Herrera and Mera, 1901).

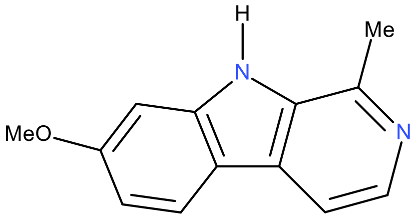
Richard Spruce encountered Ayahuasca during his expeditions in the Amazon and Andes while interacting with the Tukano, Guahibo, and Zaparo peoples in 1851, 1853, and 1857, respectively. He described Ayahuasca as a beverage inducing sensations of alternating cold and heat, fear and boldness. Spruce noted that while the plants used in the Amazon and Andes were the same, the names given to the components varied significantly among Indigenous groups (Spruce, 1908).

Although Spruce first observed Ayahuasca in 1851, the earliest publication about the beverage came from geographer Manuel Villavicencio in 1858. He described it as a "psychoactive beverage that stimulates all senses", made from a vine called *Ayahuasca* (Villavicencio, 1858).

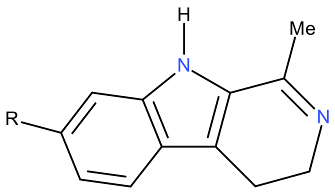
In recent centuries, Ayahuasca has been adopted by rural healers in Colombia and Peru, who practice folk medicine through the use of entheogenic plants and chants (Labate, 2002).

The arrival of João Gabriel Carvalho e Mello, rubber plantation owner, in the Amazon region in 1878 marked the beginning of the First Rubber Boom (Pontes, 2014), which prompted the migration of approximately 50,000 northeastern Brazilians to work in latex production (Pantoja and Silva, 2009). These rubber tappers became acquainted with Ayahuasca, which led to the emergence of religions that placed the beverage at the center of their rituals (ICEFLU, 2024a; Pantoja and Silva, 2009). The first of these religions, Santo Daime, was founded in 1930 by Mestre Raimundo Irineu Serra. Later, Barquinha and União do Vegetal (UDV) emerged in 1946 and 1961, respectively (CEBUDV, 2024; ICEFLU, 2024b). Today, the religious use of Ayahuasca has spread to North America, Europe, and Asia (Labate et al., 2016; Labate and Jungaberle, 2011). This expansion, which includes recreational immersions, has altered the beverage's composition due to the absence or limited access to its original components (Kaasik et al., 2021). It has also fostered the emergence of an online market for psychoactive plant species, readily accessible on the internet.

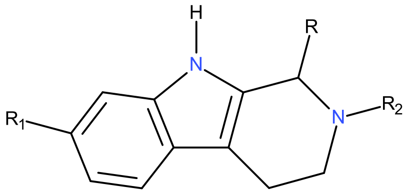
Ayahuasca, also known by various names such as *Cadána, Caapi, Daime, Honi, Iyona, Natema, Nepe, Nixi Pae, Pindé, Vegetal*, and *Yagé*, is prepared using a complex mixture of plants. The most common preparation includes the vine *Banisteriopsis caapi* (Spruce ex Griseb.) C.V.Morton (Malpighiaceae), also referred to as *Ayahuasca* or *Yagé*, which contains the β-carboline alkaloids harmine (***1***), harmaline (***2a***), and tetrahydroharmine (THH, ***3a***). It is combined with the leaves of *Psychotria viridis* Ruiz & Pav. (Rubiaceae Juss.), also known as *Chacrona, Folha,* or *Rainha*, which contain the indole alkaloid *N,N*-dimethyltryptamine (DMT, ***4a***).



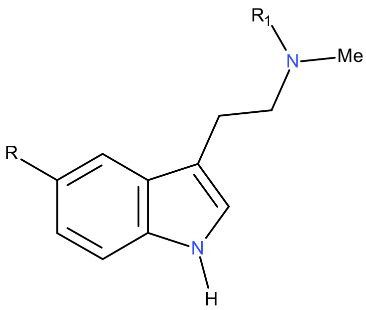
1 harmine



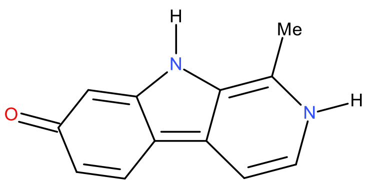
	R
2a harmaline	OMe
2b harmalol	OH



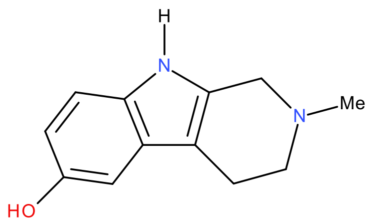
	R	R_1	R_2
3a tetrahydroharmine	Me	OMe	H
3b tetrahydronorharmine	H	OMe	H
3c 2-methyltetrahydro-β-carboline	H	H	Me



	R	R1
4a *N,N*-dimethyltryptamine	H	Me
4b bufotenine	OH	Me
4c *N*-methyltryptamine	H	H
4d 5-methoxy-*N*-methyltryptamine	OMe	H
4e *O*-methylbufotenine	OMe	Me



5 harmol



6 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline

The complexity of Ayahuasca is further highlighted by the inclusion of various other species in the beverage's basic composition, as recorded in literature and herbaria, many of which remain underexplored by science. Additionally, there are uncertainties about whether these lesser-known vine and leaf species contain the same compounds as the more extensively documented species, such as *B. caapi* and *P. viridis*.

The literature also describes "types" of *B. caapi*, here referred to as ethnotaxa (ethnobotanical taxa) (Oliveira et al., 2023), which are recognized and cultivated by Indigenous peoples and members of Ayahuasca-based religions. Communities that interact with Ayahuasca have developed a detailed taxonomy of these ethnotaxa, based on characteristics such as stem morphology, properties of the beverage—color, viscosity, flavor—and its physiological effects on consumers (Luna, 1986; Luz et al., 2022; Monteles, 2020; Oliveira et al., 2023; Schultes, 1986).

The understanding of this ethnoclassification has generated hypotheses, including the idea that within what has been accepted as *B. caapi*, there may be, for example, seven lineages that can be recognized as specific taxa (Luz et al., 2022). In the past, it was identified that certain ethnotaxa corresponded to different species (Langdon, 1986). Most of the herbarium material of *B. caapi* consists of sterile plants from cultivation (Gates, 1982).

It is believed that the discrepancies in purgative and emetic effects experienced by individuals consuming Ayahuasca prepared from different ethnobotanical taxa are correlated with chemical variations. In this way, the ethnobotanical taxa have been considered chemotypes of *B. caapi* (Gates, 1982; Langdon, 1986; Schultes, 1986). Oliveira et al. (2023) interviewed 38 people, usually those responsible for the beverage's production or the leaders of houses, from Brazilian religions that use Ayahuasca in their rituals. There is unanimous agreement among the interviewees that beverages made from an ethnobotanical taxon called *Caupuri* have a stronger physical effect than those from *Tucunacá* (Callaway, 2002; Oliveira et al., 2023).

Ayahuasca induces a range of physical effects beyond visions, which are well discussed in Politi et al. (2021). According to Politi et al. (2021), they highlight the frequency of vomiting and diarrhea events during Ayahuasca use, although these do not occur in all rituals or simultaneously in all individuals. Without clear evidence of the biochemical driver of the reported effects, the authors concluded that vomiting and diarrhea are also cultural effects.

Politi et al. (2021) did not explore the relationship between the physical effects and the taxonomic entity used in the preparation of the beverage. The first to analyze beverages made from ethnobotanical taxa were Rivier and Lindgren (1972), who studied beverages prepared with seven distinct ethnobotanical taxa, although they did not discuss the specific effects of each. On the other hand, Santos et al. (2020) analyzed the β-carboline content in various ethnobotanical taxa without finding statistically significant differences. Both studies focused on quantitative data regarding harmine (***1***), harmaline (***2a***), THH (***3a***), and harmol (***5***); however, we question whether qualitatively different compounds might exist that could explain the varying effects of beverages made from different vines. We are also intrigued by which traditionally used species might be a source of DMT (***4a***) or other variant alkaloids.

Another concern discussed in this review is the importance of Ayahuasca for science, but also for Indigenous peoples, that species and traditional knowledge be clearly recognized and protected. In 1986, an American businessman and scientist patented a plant called *Ayahuasca*, which he considered different, and years later, Ecuadorian Indigenous people requested that the patent be annulled. This request was accepted, based on specimens deposited at the US Botanical Museum (Press, 2022). We aim to organize the records to provide an idea of what is documented and to direct new research lines to safeguard this knowledge. Brazilian legislation provides for benefit-sharing with providers of genetic resources (Presidência da República do Brasil, 2015).

The origin date of Ayahuasca consumption is unknown and intriguing, as there are two main viewpoints: one suggests that Ayahuasca has been used for millennia, while the other defends a more recent use of the beverage, which we will address next.

Ogalde et al. (2010) found evidence of *B. caapi*, the most used component of Ayahuasca, in the hair of two mummies dated to 500-1000 AD from the Azapa Valley, Chile, using gas chromatography and mass spectrometry (GC-MS). Miller et al. (2019), using liquid chromatography and mass spectrometry (LC-MS), discovered the presence of the same species in a bag dated to 905-1,170 AD, found in Bolivia. The chemical composition found in the bag contained only *B. caapi* branches, not the beverage, leading the authors to assume a medicinal use rather than an entheogenic one. Varella (2005), Naranjo (1995) and Zuluaga (2002) also supported the idea of an ancestral use of the beverage, with theoretical grounding. On the other hand, the arguments of Gow (1994), de Mori (2011), Brown (2012), De Assis and Rodrigues (2017) and others authors presented elements that lead to the defense of a relatively recent origin for the beverage, which is believed to have spread by mestizos along the rubber tapper routes in the Amazon.

There are studies that provide literature reviews on Ayahuasca plants (Barriga, 1992; Cuatrecasas, 1958; Schultes and Hofmann, 2000, 1980; Schultes, 1957). However, the data remains scattered, with occasional mentions of species, and herbarium documentation has been underappreciated. Herbaria, as source of ethnobotanical data, could even surpass literary references (Million et al., 2020; Souza and Hawkins, 2017), and they are important for verifying identifications.

Considering the complexity of Ayahuasca's composition and the large number of scattered pieces of information, this article aims to list the species and ethnotaxa of *B. caapi* traditionally used in Ayahuasca production, as documented in the literature and herbarium vouchers. Furthermore, it seeks to associate the names with the chemical compounds present in the involved entities to determine if there is qualitative correspondence between these ethnobotanical taxa.

All plant names in this review were verified using the World Flora Online database (www.worldfloraonline.org) and the Medicinal Plant Names Services (MPNS, http://mpns.kew.org), accessed on April 1st, 2025.

2. Materials and Methods

To identify the species associated with Ayahuasca, research was conducted on vouchers from the COAH, COL, F, GH, IAN, K, L, MICH, MO, NY, UB, UBC, and US herbaria, as well as through platforms such as GBIF, *Nombres comunes de las plantas de Colombia*, and *species*Link. This investigation aimed to document the species linked to vernacular names commonly associated with the beverage, including *Ayahuasca, Yagé, Caapi, Mariri, Chacrona,* and *Cacouna*.

Additionally, a narrative and non-systematic review of the literature was performed, manually selecting texts that contained information about species used as raw materials in the preparation of Ayahuasca. The search was conducted in the BHL and JSTOR databases using keywords such as *Malpighiaceae*, *Rubiaceae*, and *Ayahuasca*. These databases were chosen because they include older articles reporting species beyond the traditional formula of the beverage, composed of *P. viridis* and *B. caapi*. On the JSTOR platform, 51 results were found, of which 9 articles were selected for mentioning species used in the preparation of Ayahuasca. In the BHL database, 79 records were identified, with 8 chosen for their references to species associated with Ayahuasca's vernacular names.

Furthermore, a narrative review of the literature on the chemical compounds presents in plants used for Ayahuasca preparation was carried out. The keywords employed were *Chemical Composition of Ayahuasca*. In the BASE database, 24 records were found, with 5 articles selected. In PubMed, 207 results were identified, and 8 articles were chosen. On SpringerLink, out of 119 results, 2 articles were selected, while in ScienceDirect, 3 articles were prioritized from the 181 records retrieved.

Subsequently, a search was conducted in databases for each species identified through the previous method to determine the chemical composition of each one, using the keyword *Chemical Composition of [Species Name]*. In the BASE database, 1 article out of 10 available for *B. caapi* and 1 out of 6 for *T. mucronata* were found. In PubMed, 3 articles out of 56 for *B. caapi*, 2 out of 4 for *T. mucronata*, 1 out of 3 for *P. carthagenensis*, and 2 out of 40 for *P. viridis* were selected. On Springer, 1 article for *B. caapi* out of 67 available and 1 for *P. viridis* out of 54 were found. In ScienceDirect, 2 articles out of 110 for *B. muricata*, 1 out of 5 for *D. cabrerana*, and 1 out of 114 for *P. viridis* were selected.

Additionally, all previously obtained results were analyzed to identify ethnotaxa related to the *B. caapi* vine.

Searches were conducted in Spanish, English, and Portuguese, considering these are the predominant languages in countries where Ayahuasca is traditionally consumed. The studies including herbarium vouchers were prioritized, and the nomenclature used follows the accepted names according to the Plants of the World Online (POWO) project and the *Flora e Funga do Brazil* initiative.

The chemical structures were built using the software BioviaDraw®, Dassault Sistèmes, version 18.1. NET. (2017).

3. Results

3.1. Species Used as Vine Sources for Ayahuasca or Yagé

The species designated by indigenous peoples as *Ayahuasca*, according to the literature and herbarium reviews, are cataloged in Table 1. This analysis identified 27 species, of which only 15 had been previously documented in written texts. The remaining 12 species were identified through herbarium analysis. Among these species, 25 belong to the family *Malpighiaceae* and are distributed across the following genera: *Alicia* W.R.Anderson, *Banisteriopsis* C.B. Rob., *Bronwenia* W.R.Anderson & C. Davis, *Callaeum* Small, *Christianella* W.R.Anderson, *Dicella* Griseb., *Diplopterys* A.Juss., *Glicophyllum* R.F. Almeida, *Heteropterys* Kunth, *Mascagnia* (Bertero ex DC.) Bertero, *Mezia* Schwacke ex Nied., *Niedenzuella* W.R.Anderson, *Stigmaphyllon* A.Juss., and *Tetrapterys* Cav. Figure 1 illustrates the flowers of some species referred to as *Ayahuasca*.

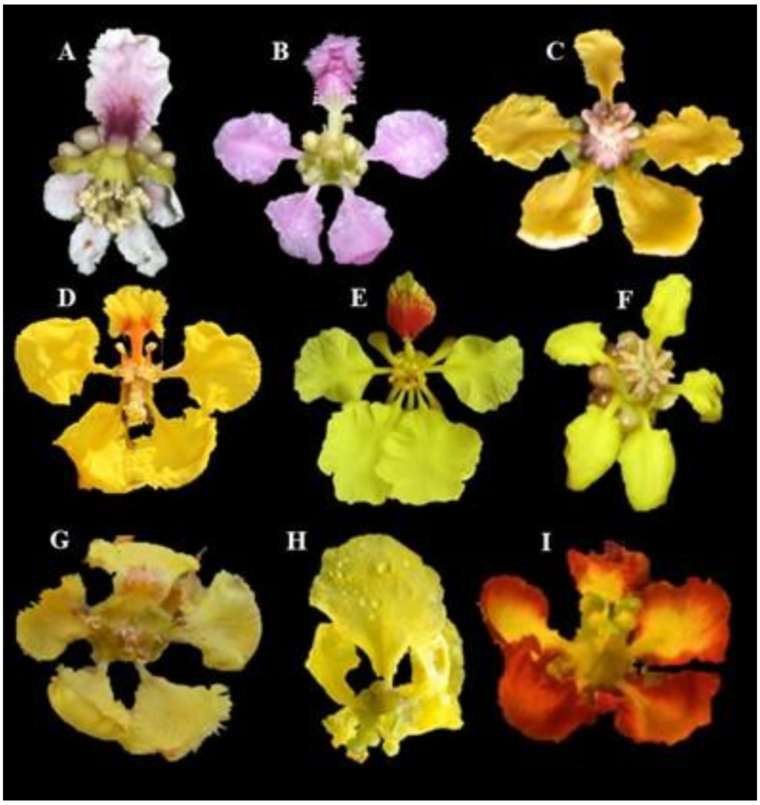


Figure 1. Flowers of various Malpighiaceae species commonly referred as Ayahuasca: *Alicia anisopetala* (A), *Banisteriopsis caapi* (B), *Dicella julianii* (C), *Diplopterys lutea* (D), *Mezia mariposa* (E), *Niedenzuella stannea* (F), *Stigmaphyllon florosum* (G), *Stigmaphyllon maynense* (H) and *Stigmaphyllon sinuatum* (I).

Table 1. Vines referred to as *Ayahuasca, Caapi, Cabi, Jagubo,* or *Yagé*, Family, Species, Vernacular Names, Herbarium Voucher Data (Collector, Collection Number, and Herbaria Acronym), and Sources of Information. **Indicates species with unconfirmed use.*

Family	Species	Vernacular Name	Voucher	Reference
Apocynaceae	*Prestonia amazonica* (Benth. ex Müll.Arg.) J.F.Macbr.	*Yagé, Caapi-pinima*	-	(Hochstein and Paradies, 1957; Perrot and Raymond-Hamet, 1927; Rivier and Lindgren, 1972)
Malpighiaceae	*Alicia anisopetala* (A.Juss.) W.R.Anderson*	*Mirac Huasca*	*Schunke, J. 12534* (US, L!, MO)	
Malpighiaceae	*Alicia macrodisca* (Triana & Planch.) W.R.Anderson	*Yage de pescado, Jagubo*	*Pabón, M. 691* (COL!, COAH!)	
Malpighiaceae	*Banisteriopsis caapi* (Spruce ex Griseb.) C.V.Morton	*Caapi, Yagé, Pindé, Natema, Vegetal*	*Garcia-Barriga, H. 15721* (COL!)	(Barriga, 1992; Cuatrecasas, 1958; Gates, 1982; Schultes, 1957)
Malpighiaceae	*Banisteriopsis elegans* (Triana & Planch.) Sandwith	*Yagé*	-	(Duke and Vasquez, 1994)

Malpighiaceae	*Banisteriopsis martiniana* (A.Juss.) Cuatrec.	*Yagé, ñucña-wasca.*	*Garcia-Barriga, H. 13706* (COL!, US)	(Barriga, 1992; Schultes, 1978)
Malpighiaceae	*Banisteriopsis muricata* (Cav.) Cuatrec.	*Ayahuasca negra*	*Schunke, J. 2037* (COL!, US)	(Barriga, 1992; Bristol, 1966; Davis and Yost, 1983; Gates, 1982; Schultes, 1957)
Malpighiaceae	*Bronwenia megaptera* (B.Gates) W.R.Anderson & C.Davis	Does not cite	-	(Fontella-Pereira et al., 2010)
Malpighiaceae	*Callaeum antifebrile* (Ruiz ex Griseb.) D.M.Johnson	*Cabi*	*Ducke, A. 819* (US!, IAN!, MO!)	(Albuquerque, 2012; Johnson, 1986)
Malpighiaceae	*Christianella glandulifera* (Cuatrec.)	Does not cite		(Schultes, 1978)
Malpighiaceae	*Dicella julianii* (J.F.Macbr.) W.R.Anderson	*Chunchullo Ayahuasca*	*Schunke, J. 9985* (F!, L!, MO, MICH)	

Malpighiaceae	*Diplopterys cabrerana* (Cuatrec.) B.Gates	*Yagé, Yaji Chaliponga, Chagropona, Chacruna, OcoYagé*	*Irvine, D. 699* (F!)	(Barriga, 1992; Duke and Vasquez, 1994; Gates, 1982; Schultes, 1978)
Malpighiaceae	*Diplopterys lutea* (Ruiz ex Griseb.) W.R.Anderson & C.Davis	*Yagé*	-	(Barriga, 1992)
Malpighiaceae	*Diplopterys schunkei* (B.Gates) W.R.Anderson & C.Davis	*Auca Ayahusca*	*Schunke, J. 4635* (K!, MO!, F!, NY!, US, GH, IAN)	(Gates, 1982)
Malpighiaceae	*Glicophyllum stylopterum* (A.Juss.) R.F.Almeida	*Caapi*	-	(Cuatrecasas, 1958; Schultes, 1969, 1978, 1957; Schultes and Smith, 1976)
Malpighiaceae	*Heteropterys aureosericea* Cuatrec.	*Ayahuasca Amarilla*	*Schunke, J. 1714* (COL!, US!)	

Malpighiaceae	*Mascagnia divaricata* (Kunth) Niedenzu	*Yacu Ayahuasca, Ayahuasca Negra, Ayahuasca Rosada*	*Schunke, J. 12536* (NL!, MICH, F)	
Malpighiaceae	*Mascagnia ovatifolia* (Kunth) Griseb.	*Yacu Ayahuasca*	*Schunke, J. 1621* (US!)	
Malpighiaceae	*Mezia mariposa* W.R. Anderson	*Ayahuasca Máman*	*Schunke, J. 2062* (F!, US)	
Malpighiaceae	*Mezia includens* (Benth.) Cuatrec.	*Ayahuasca Negro*	-	(Schultes, 1983)
Malpighiaceae	*Niedenzuella stannea* (Griseb.) W.R.Anderson	*Ayahuasca Sacha, Ayahuasca Amarilla*	*Schunke, J. 4071* (F!, US, MO)	
Malpighiaceae	*Stigmaphyllon florosum* C.E.Anderson*	*Añushe huasca*	*Graham, J.G. 503* (MICH, US, F!)	
Malpighiaceae	*Stigmaphyllon maynense* Huber	*Ayahuasca Blanco*	*Schunke, J. 4549* (COL!, MO, US)	

Malpighiaceae	*Stigmaphyllon sinuatum* (DC.) A.Juss.*	*Canoa huasca panga*	*Gudiño, E. 19* (COL!)	
Malpighiaceae	*Tetrapterys crispa* A. Juss	*Ayahuasca Amarilla, Sacha Ayahuasca*	*Schunke, J. 3726* (COL!, US)	
Malpighiaceae	*Tetrapterys mucronata* Cav.	*Yajé*	*Schultes, R.E. 12107* (US)	(Schultes, 1975, 1990, 1972a, 1978)
Solanaceae	*Hawkesiophyton ochraceum* (Cuatrec.) A.Orejuela & C.I.Orozco	*Ayahuasca*	*Plowman, T.C. 2176* (COL!, US)	(Schultes, 1978)

Barriga (1992) suggested that, in addition to the species reported by him—*B. caapi*, *B. martiniana*, *B. muricata*, *D. cabrerana*, and *D. lutea* (see Table 1)—the following could also be considered alternatives to Ayahuasca due to morphological similarities: *Banisteriopsis pubescens* (Nied.) Cuatrec., *Bronwenia cornifolia* (Kunth) W.R.Anderson & C.Davis, and *Diplopterys heterostyla* (A.Juss.) W.R.Anderson & C.Davis. This assertion supports the argument presented by Cuatrecasas (1958), who suggests that other species of the genus, as well as species from other genera within the family Malpighiaceae, may possess psychoactive properties identical or similar to those of *B. caapi*.

Banisteriopsis inebrians C.V.Morton, although considered synonymous with *B. caapi* by Gates (1982), is recognized as a valid species by some authors, such as Cuatrecasas (1958), Barriga (1992) and Schultes and Hofmann (2000). According to Cuatrecasas (1958), when studying the Malpighiaceae family, the two species can be differentiated based on the morphology of their leaves and fruits. He noted that the leaves of *B. inebrians* are thicker, ovate, and briefly attenuate, while the samaras represent the main distinguishing characteristic of the species. These samaras have a semi-obovate wing, significantly expanded at the apex, with the lower margin strongly constricted at the base. He also stated that the size of the stipules, described as longer in *B. inebrians*, may be variable (see Figure 2).

Figure 2. A: Isotype of Banisteriopsis caapi (Spruce 2712 (NY!, K!, G, GH, MO)). B: Isotype of B. inebrians (Klug 1964 (K!, MO!, NY!, L!, U!, MICH, US)). It can be observed that the samaroids of B. inebrians are visually distinct, narrowing toward the proximal portion.

Schultes (1982) reinforced that the two types of specimens exhibit morphological differences and agreed with Cuatrecasas's (1958) observations. He added, however, that a larger number of specimens must be evaluated to confirm whether the two species are indeed synonymous.

The use of *Alicia anisopetala* (*Mirac huasca*), *Stigmaphyllon florosum* (*Añushe huasca*), and *Stigmaphyllon sinuatum* (*Canoa huasca panga*) as raw materials for the preparation of Ayahuasca, as recorded in the COL herbarium (see Table 1), was included in the list of species used in the beverage solely because they contain the term "huasca" in their designation. This inclusion is questionable, as "huasca" is a generic term used for all types of vines or lianas, with dozens of plants being referred to as "huasca" or "huaska" without any direct connection to Ayahuasca.

The term "huasca" or “hoasca” is directly related to Ayahuasca only when used to name the beverage, meaning "vine" or the act of "drinking the vine," as commonly referred to by rubber tappers in Acre in the early 20th century (Berlanda and Viegas, 2012; Labate and MacRae, 2016). However, *Alicia anisopetala* has been marketed on various shamanism websites as *Black Ayahuasca*, a name attributed to the species in Peru according to these sources. Personal observations indicate the circulation of this species in Brazil, particularly among non-traditional Ayahuasca groups.

The use of *Callaeum antifebrile* as a vine in Ayahuasca preparation presents contradictory information. On one hand, Ducke (1943) reported that this plant is used as a hallucinogen in the vicinity of Belém, in a manner similar to *B. caapi*, which would justify the generic name *Cabi*. On the other hand, Johnson (1986) observed that in Peru, the plant appears to be used exclusively for medicinal purposes, such as an antipyretic and in the treatment of internal and external wounds. Furthermore, Schultes (1982) demonstrated that the plant contains the alkaloid harmine, also found in *B. caapi*, which reinforces the possibility of its relationship with the traditional use of Ayahuasca.

Although the initial research focus was on species from the families Malpighiaceae and Rubiaceae, this review of literature revealed the presence of the plants *Prestonia amazonica* and *Hawkesiophyton ochraceum*, belonging to the families Apocynaceae and Solanaceae, respectively, as species with the vernacular names *Ayahuasca* or *Yagé* (Hochstein and Paradies, 1957; Perrot and Raymond-Hamet, 1927;

Rivier and Lindgren, 1972; Schultes, 1978). However, an analysis conducted by Schultes and Raffauf (1960), based on a bibliographic review and herbarium material, concluded that *P. amazonica* was reported as Ayahuasca due to a misidentification. Regarding the use of *H. ochraceum* as one of the main components of Ayahuasca, Schultes (1978) mentions uncertainty as to whether the plant is indeed an essential ingredient of the beverage or merely an additive.

It is important to recognize herbaria as valuable sources of ethnobotanical information, as the following species had not been previously reported in the literature as Ayahuasca: *Alicia anisopetala*, *Alicia macrodisca*, *Dicella julianii*, *Heteropterys aureosericea*, *Mascagnia divaricata*, *Mascagnia ovatifolia*, *Mezia mariposa*, *Niedenzuella stannea*, *Stigmaphyllon florosum*, *Stigmaphyllon maynense*, *Stigmaphyllon sinuatum*, and *Tetrapterys crispa*.

3.2. Ethnotaxa of Ayahuasca

Table 2 presents 119 ethnotaxa names recorded among 24 ethnic groups from Brazil, Colombia, Ecuador, Peru, and Venezuela, as well as Brazilian Ayahuasca religions. Brazil is the country with the highest number of reported ethnotaxa, totaling 63 records, followed by Colombia, Peru, Ecuador, and Venezuela, with 51, 25, 18, and 13 records, respectively.

The most comprehensive list related to Brazilian Ayahuasca religions was published by Oliveira et al. (2023), who identified 18 distinct ethnotaxa denominations. Based on stem anatomical analysis, the authors determined that one of the collected ethnotaxa, referred to by participants as *Tucunacá*, corresponds to *Diplopterys cf. pubipetala*, while the remaining specimens were classified as *B. caapi*.

In addition, the ethnotaxa were organized into a Venn diagram and linked to Berlin's (1973) taxonomic system. The analysis revealed two "generic" groups, which were also confirmed by multivariate analyses based on stem anatomy. The two major groups identified were vines with swollen nodes, vernacularly known as *Caupuris*, and those with non-swollen nodes, referred to as *Tucunacás*.

The ethnotaxa exhibits morphological differences in the color of the inner bark, the coloration of the prepared beverages, and the vine structure, including cross-sectional geometry and the presence of swollen nodes. These differences are illustrated in Figures

3 and 4 for the ethnotaxa reported by Oliveira et al. (2023): *Arara, Caupuri, Ourinho, Pingo de Ouro* and *Tucunacá*.

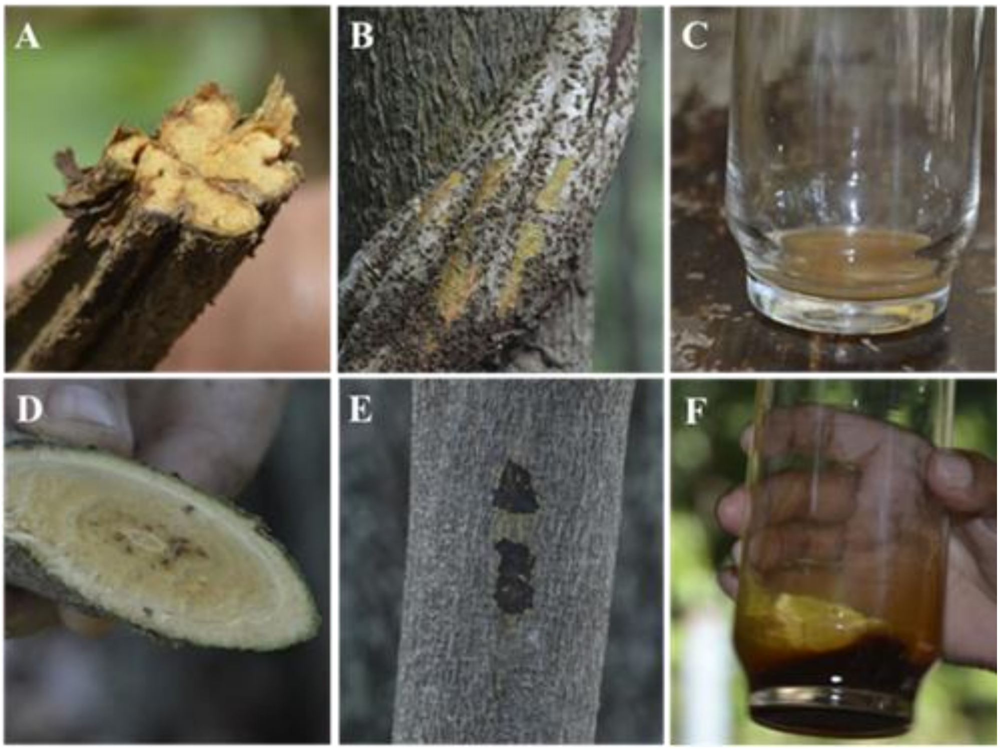


Figure 3. Ethnotaxa of *Banisteriopsis caapi*. A, B, and C: *Ourinho*: Cross-section of the stem, inner bark, and coloration of Ayahuasca prepared with *Ourinho*. D, E, and F: *Arara*: Cross-section of the stem, inner bark, and coloration of Ayahuasca prepared with the *Arara* vine, respectively.

Figure 4. Ethnotaxa of *Banisteriopsis caapi*. A, B, and C: Vines without swollen nodes, forming braids: A. *Tucunacá*, B. *Ourinho*, and C. *Pingo de Ouro*. D: *Caupuri*, characterized by the presence of swollen nodes, indicated by an arrow.

Langdon (1986) reported the existence of 18 Ayahuasca ethnotaxa within the Siona community, representing the largest record of ethnotaxa for a single community in Colombia. However, due to the indigenous belief that these vines harbor spirits, only six could be collected. Among the analyzed ethnotaxa, the one referred to as *Yajé agua de pajarito* was identified as belonging to the genus *Diplopterys*, rather than *Banisteriopsis*, as initially expected.

Table 2. Ethnotaxa and Vernacular Names of Vines Referred to as Ayahuasca Recognized by Different Indigenous Ethnicities and Religious Communities, and References.

Community Type	**Name of Community**	**Country**	**Ethnotaxa**	**Reference**
Indigenous peoples	Barasana	Colombia and Brazil	*Yajé de cabeça, Yajé de cobra, Yajé de guamo, Yajé de jaguar vermelho, Yajé de sangue, Yajé dos animais da selva*	(Reichel-Dolmatoff, 1990)
Religion	Centro Espírita Beneficente União do Vegetal	Brazil	*Amarelinho, Caupuri sem-nós, Mariri-caupuri, Mariri-nativo, Mariri-tucunacá, Pajezinho, Tucunacá-com-nós*	(Luz et al., 2022)

Indigenous peoples	Cofan	Colombia	*Oofa, Yageuco, Yagê*	(Friedberg, 1965)
Indigenous peoples	Cubeo	Brazil and Colombia	*Mihí*	(Reichel-Dolmatoff, 1990)
Indigenous peoples	Cuiba	Colombia and Venezuela	*Buntaijuipa, Mapqjuipa: Capi de matapalo, Newaüjuipa, Ukuyorijuipa, Wiripanejuipa: Capi de guamo, Jomowabijuipa: Capi de güío, Urarijuipa: Capi de cuare*	(Gomez, 1989)
Indigenous peoples	Culina	Peru	*Ramiwetsem: Ayahuasca amarela, Tsipu-makuni: Ayahuasca branca, Tsipu-tsueni: Ayahuasca Negra, Tsipu-wetseni: Ayahuasca vermelha*	(Rivier and Lindgren, 1972; Schultes, 1986)
Indigenous peoples	Desana	Colombia	*Yajé de dhutú-puu-sereda, Yajé de guamo: Merepida, Yajé de nós: Korepida, Yajé de tooka*	(Reichel-Dolmatoff, 1990)
Indigenous peoples	Embera	Colombia	*Pildé*	(Reichel-Dolmatoff, 1990)
Indigenous peoples	Huni kuin	Brazil and Peru	*Bakã-huni: Cipó peixe, Keya-huni: Cipó transformação, Nii-huni: Cipó floresta, Nixi-huni: Cipó homem, Shane-huni: Cipó*	(Martini, 2014)

			periquito azul, Shawã-huni: Cipó arara vermelha, Xawan-huni, Xani-huni.	
Indigenous peoples	Indigenous peoples living between the Napo and Japurá Rivers	Brazil and Colombia	*Kaxpi e Kulikaxpiro*	(Koch-Grünberg, 1910)
Indigenous peoples	Kaxinawá	Brazil and Peru	*Bakã-huni, Xane-huni, Xawan-huni*	(Keifenheim, 2009)
Indigenous peoples	Marinahua	Peru	*Tukondi*	(Rivier and Lindgren, 1972)
Mixed heritage peoples	Mixed heritage people near Iquitos	Peru	*Cielo-Ayahuasca: Ayahuasca blanco, Piturijacu*	(Rivier and Lindgren, 1972)

Mixed heritage peoples	Mixed heritage people from Peru	Peru	*Cielo, Lucero, Pucahuasca, Rumi*	(McKenna et al., 1984)
Indigenous peoples	Noanamá	Colombia	*Dápa*	(Reichel-Dolmatoff, 1990)
Indigenous peoples	Piaroa	Venezuela	*Duhui-huoika, Kunahua, Kohö, Mäe, Ubaku-ukhuä, Yurina*	(Rodd, 2008)
Indigenous peoples	Pira-Tapuya	Colombia	*Gahpí-da-vaí: Yajé-rama-peixe*	(Reichel-Dolmatoff, 1990)
Indigenous peoples	Piro	Peru	*Kamalampi*	(Rivier and Lindgren, 1972)
Religions	Santo Daime	Brazil	*Arará, Cabôco, Caupuri, Doce, Juaruá, Ourinho, Pajezinho, Peixe, Quebrador, São Francisco, Tucunacá.*	(Monteles, 2020)
Religions	Santo Daime, Barquinha e	Brazil	*Arara, Arara-vermelha, Ararinha, Caboquinho, Caboquim-do-pará, Cabi-rosário, Caupuri, Caupuri de nós longos, Caupuri*	(Oliveira et al., 2023)

	grupos neo-ayahuasqueiros		*em touceira, Caupuri-sem-nó, Gema-de-ovo, Jagube-de-árvore, Juruá, Mariri, Mariri-de-cuzco, Nó-de-cachorro, Ourinho, Ourinho-branco, Peixe, Pingo de ouro, Quebrador, Rei, Roxinho, Tucunacá, Tucunacá-mariri-boliviano*	
Indigenous peoples	Sharanahua	Peru	*Shuri-fisopa: Ayahuasca negra, Shuri-oshinipa: Ayahuasca branca, Shuri-oshinipa: Ayahuasca vermelha*	(Rivier and Lindgren, 1972)
Indigenous peoples	Siona	Colombia and Ecuador	*Armadillo, Collar de pecarí, Espíritu, Fresco, Guacamayo, Indios wititi, Jaguar, Jaguar de río, Mono negro lanoso, Pájaro, Pájaro canastero, Pecarí de labelo blanco, Pez gato negro, Pléyades, Selva, Tapir, Yagé agua de pajarito, Yagé datura*	(Langdon, 1986)
Indigenous peoples	Tucano	Brazil and Colombia	*Ajúwri-kahi-má, Kahi de la cabeza de mono, Kahi del jaguar rojo*	(Schultes and Hofmann, 2000)
Indigenous peoples	Tucano	Colombia	*Yagé de cabeça, Yagé de guamo, Yagé de mamífero, Yagé de peixe*	(Reichel-Dolmatoff, 1990)

3.3. Species Used as Leaf Sources in Ayahuasca or Yagé

The leaves traditionally known as *Chacrona*, *Chacuruna*, *Folha*, *Rainha*, or *Chacropanga* are key components in the preparation of the Ayahuasca beverage. Through a literature review and herbarium analysis, 12 species associated with these vernacular names were identified, belonging to the genera *Diplopterys* A.Juss. (Malpighiaceae), *Palicourea* Aubl., and *Psychotria* L. (Rubiaceae) (see Table 3). Of the 12 species listed in Table 3, three had not been previously recorded in the literature: *P. borjensis*, *P. remota*, and *P. trivialis*. This fact highlights the importance of herbarium documentation as a key tool for recording traditional knowledge.

Table 3. Species Used as Leaf Sources in Ayahuasca Preparation: Family Name, Species, Vernacular Names, Herbarium Voucher with Collector's Name, Herbaria Number and Acronym, and Reference Sources.

Family	Species	Vernacular Name	Voucher	Reference
Malpighiaceae	*Diplopterys cabrerana* (Cuatrec.) B.Gates	*Yageuco, Ocoyage, Yage-oco, Chacropanga & Chacuruna*	*Cuatrecasas, J. 10597* (COL!, US, F!)	(Schultes and Hofmann, 2000)
Malpighiaceae	*Diplopterys longialata* (Nied.) W.R.Anderson & C.Davis	*Chacropanga, Chacrona huambisa*	*Naranjo, C. 5* (COL!)	(Barriga, 1992; Cuatrecasas, 1958; Schultes, 1969, 1972b; Schultes and Hofmann, 1980)
Rubiaceae	*Palicourea stenostachya* (Standl.) C.M.Taylor	Does not cite	-	(Duke and Vasquez, 1994)
Rubiaceae	*Psychotria alba* Ruiz & Pav.	Does not cite	-	(Duke and Vasquez, 1994)

Rubiaceae	*Psychotria borjensis* Kunth	*Chacrona macho*	*Lozano, A. 22* (COAH!)	
Rubiaceae	*Psychotria carthagenensis* Jacq.	*Chacuruna*	*Schunke, J. 2199* (US!)	(Schultes and Hofmann, 1980; Schultes, 1972b)
Rubiaceae	*Psychotria nervosa* Sw.	*Tupumaqui*	-	(Duke and Vasquez, 1994)
Rubiaceae	*Psychotria marginata* Sw.	Does not cite	-	(Duke and Vasquez, 1994)
Rubiaceae	*Psychotria psychotriifolia* (Seem.) Standl.	Does not cite	-	(Pinkley, 1969)
Rubiaceae	*Psychotria remota* Benth.	*Chacuruna*	*Schunke, J. 5407* (MO, L!)	
Rubiaceae	*Psychotria trivialis* Rusby	*Chacuruna Amarillo*	*Graham, J. 354* (F!)	
Rubiaceae	*Psychotria viridis* Ruiz & Pav.	*Chacuruna*	*Schunke, J. 4795* (US, COL!, MO!)	(Schultes and Hofmann, 1980; Schultes, 1972b)

3.4. Other Plants Added to Ayahuasca

In addition to the species listed in the previous tables, there are records of occasional additions to the beverage from plant families other than *Malpighiaceae* and *Rubiaceae* (see Table 4) to provide specific healing powers (Schultes and Hofmann, 2000; Schultes, 1957). The diversity of species used in the preparation of Ayahuasca is reflected in 35 families and 58 genera. Nonetheless, the beverage can be made exclusively with the vine known as Ayahuasca (Barriga, 1992; Schultes, 1987).

The UDV, an Amazonian-origin religion that combines elements of Catholicism, Afro-Brazilian cults, Kardecist Spiritism, and European esotericism, incorporates other plants known vernacularly as *Breuzinho, Apuí, Samaúma, Castanheira, Pau-d'Arco, Imburana de Cheiro, Mulateiro, Maçaranduba* and *Carapanaúba*, to prepare the beverage called "9 Vegetais." According to practitioners, the beverage has healing properties for the spirit. However, since 2001, the "9 Vegetais" has not been consumed in the UDV. When the previously described mixture is supplemented with the *João Brandinho* plant, the beverage is named after the plant (Labate and Jungaberle, 2011).

Table 4. Families and Species Occasionally Added to the Ayahuasca Beverage (Labate and Jungaberle, 2011; Ott, 1993; Rivier and Lindgren, 1972).

Family	Species
Amaranthaceae	*Alternanthera lanceolata* (Benth.) Schinz, *Iresine* P.Browne spp., *Pfaffia iresinoides* (Kunth) Spreng.
Acanthaceae	*Lepidagathis alopecuroidea* (Vahl) R.Br. ex Griseb.

Apocynaceae	*Aspidosperma carapanauba* Pichon, *Aspidosperma excelsum* Benth., *Himatanthus articulatus* (Vahl) Woodson, *Malouetia tamaquarina* (Aubl.) A.DC., *Mandevilla scabra* (Hoffmanns. ex Roem. & Schult.) K.Schum., *Tabernaemontana sananho* Ruiz & Pav., *Thevetia* L. spp.
Aquifoliaceae	*Ilex guayusa* Loes.
Araceae	*Montrichardia arborescens* (L.) Schott, *Spathiphyllum* Schott spp.
Bignoniaceae	*Handroanthus impetiginosus* (Mart. ex DC.) Mattos, *Handroanthus incanus* (A.H.Gentry) S.O.Grose, *Handroanthus ochraceus* (Cham.) Mattos, *Handroanthus serratifolius* (Vahl) S.O.Grose, *Mansoa alliacea* (Lam.) A.H.Gentry, *Tynanthus panurensis* (Bureau) Sandwith.
Burseraceae	*Protium heptaphyllum* (Aubl.), *Protium opacum* Swart*,* Marchand
Cabombaceae	*Cabomba aquatica* Aubl.
Cactaceae	*Epiphylium* Haw. spp.*, Opuntia* Mill spp.
Caryocaraceae	*Anthodiscus pilosus Ducke*
Clusiaceae	*Clusia* L. spp., *Clusia insignis* Mart., *Clusia grandiflora* Splitg.
Convolvulaceae	*Ipomoea carnea* Jacq.

Cyperaceae	*Cyperus* L.
Euphorbiaceae	*Alchornea castaneifolia* (Humb. & Bonpl. ex Willd.) A. Juss., *Euphorbia* L. spp., *Hura crepitans* L.
Fabaceae	*Amburana acreana* (Ducke) A.C.Sm., *Amburana cearensis* (Allemão) A.C.Sm., *Erythrina* L. spp., *Jupunba laeta* (Benth.) M.V.B.Soares, M.P.Morim & Iganci.
Lamiaceae	*Cornutia odorata* (Poepp.) Schauer, *Ocimum campechianum* Mill., *Vitex triflora* Vahl.
Lecythidaceae	*Bertholletia excelsa* Bonpl., *Couroupita guianensis* Aubl.
Lomariopsidaceae	*Lomariopsis japurensis* (Mart.) J. Sm.
Loranthaceae	*Passovia pyrifolia* (Kunth) Tiegh., *Tripodanthus acutifolius* (Ruiz & Pav.) Tiegh.
Malvaceae	*Ceiba insignis* (Kunth) P.E.Gibbs & Semir, *Ceiba pentandra* (L.) Gaertn., *Ceiba samauma* (Mart.) K.Schum., *Ceiba speciosa* (A.St.-Hil., A.Juss. & Cambess.) Ravenna, *Cavanillesia hylogeiton* Ulbr., *Cavanillesia umbellata* Ruiz & Pav.
Marantaceae	*Goeppertia veitchiana* (Veitch ex Hook.f.) Borchs. & S.Suárez
Myristicaceae	*Virola* Aubl. spp, *Virola surinamensis* (Rol. ex Rottb.) Warb.

Moraceae	*Ficus citrifolia* Mill., *Ficus insipida* Willd., *Ficus trigona* L.f.
Petiveriaceae	*Petiveria alliacea* L.
Piperaceae	*Piper* L. spp.
Plantaginaceae	*Scoparia dulcis* L.
Polygonaceae	*Triplaris weigeltiana* (Rchb.) Kuntze
Pontederiaceae	*Pontederia cordata* L.
Rubiaceae	*Calycophyllum spruceanum* (Benth.) Hook.f. ex K.Schum., *Capirona macrophylla* (Poepp.) Delprete, *Guettarda ferox* Standl., *Rudgea guianensis* (A.Rich. ex DC.) Sandwith, *Sabicea amazonensis* Wernham, *Uncaria guianensis* (Aubl.) J.F.Gmel., *Uncaria tomentosa* (Willd. ex Schult.) DC.
Sapindaceae	*Paullinia yoco* R.E.Schult. & Killip
Sapotaceae	*Manilkara bidentata* (A.DC.) A.Chev., *Manilkara cavalcantei* Pires & Rodr. ex T.D.Penn., *Manilkara huberi* (Ducke) Standl.
Schizaeaceae	*Lygodium venustum* Sw.

Solanaceae	*Brugmansia insignis* (Barb.Rodr.) Lockwood ex R.E.Schult., *Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Sweet, *Brunfelsia chiricaspi* Plowman, *Brunfelsia grandiflora* D.Don, *Brunfelsia* Plum. ex L. spp., *Capsicum annuum* L., *Capsicum frutescens* L., *Iochroma fuchsioides* (Bonpl.) Miers, *Nicotiana rustica* L., *Nicotiana tabacum* L.
Urticaceae	*Coussapoa tessmannii* Mildbr.
Violaceae	*Rinorea viridiflora* (Tul.) Baill.

3.5. Phytochemistry of the Ayahuasca Beverage

The most commonly identified compounds in Ayahuasca are the β-carbolines harmine (***1***), harmaline (***2a***), and THH (***3a***), alkaloids derived from the vine, and the indole alkaloid DMT (***4a***), derived from the leaf (Callaway, 2005; Gambelunghe et al., 2008; McKenna et al., 1984; Pires et al., 2009; Rivier and Lindgren, 1972; Rodríguez et al., 2022; Santos et al., 2020; Schultes and Hofmann, 1980).

It is believed that the β-carbolines in the beverage are chemically stable over time, as Arbouche et al. (2023) analyzed samples of the beverage stored for over 100 years, detecting concentrations of 130 to 1500 mg/L of harmine (***1***), 53 to 80 mg/L of harmaline (***2a***), and 49 to 142 mg/L of THH (***3a***). However, DMT (***4a***) was not detected in the beverage; the authors suggested this might be due to the absence of *Psychotria* leaves in the preparation. Nevertheless, Verotta et al. (1999) reported that some species of this genus do not contain detectable levels of DMT (***4a***).

The synergy between the previously mentioned alkaloids generates an entheogenic effect in users, as the β-carboline alkaloids inhibit the degradation of monoamine oxidase (MAO), thereby allowing the activation of DMT (***4a***) (McKenna, 2004; Ott, 1999; Riba et al., 2012).

Zerda-Bayon (1906) and Fischer-Cardenas (1923) identified a substance in the beverage which they called telepathina, due to its supposed ability to facilitate telepathic communication between participants in Ayahuasca consumption rituals (Schultes and Hofmann, 2000).

Barriga-Villalba (1925) isolated yageine and yagenine from Ayahuasca. Later, Lewin (1928) isolated banisterine, believing it to be the main alkaloid of the beverage. Elger (1928), Wolfe and Rumpff (1928), Bruckl and Mussgnug (1929), Dalmer (1929), and Chen and Chen (1939) determined that telepathina, yageine, and banisterine were compounds identical to harmine (***1***), a substance previously isolated from *Peganum harmala* L..

McKenna et al. (1984) analyzed 8 Ayahuasca samples from Peru and identified the presence of harmine (***1***), harmaline (***2a***), THH (***3a***), harmol (***5***), and, exclusively, harmalol (***2b***) in all of them. DMT (***4a***) was found in all samples except one, which was prepared with *P. carthagenensis* instead of *P. viridis*, the most used species.

Callaway (2005), in his analysis of 29 Ayahuasca samples from different Brazilian Ayahuasca traditions (Barquinha, Santo Daime, and UDV), identified varied alkaloid profiles, corroborating the results of Byrska et al. (2022). In some of these samples, DMT (***4a***) was not detected, as reported by McKenna et al. (1984). According to Berger et al. (2022), among the *Psychotria* species, only *P. viridis* seems to contain DMT (***4a***). The fact that beverages are produced without DMT (***4a***) leads to the hypothesis that the β-carbolines alone may exert a psychoactive effect or that other phytochemicals may act as an alternative to DMT (***4a***).

Callaway (2005) also reported that the beverage's alkaloid content can remain stable at room temperature in a dark room for up to 80 days. However, the beverage's acidic characteristic gradually shifted to neutral as it began to ferment over time. The results indicated that the practice of churches storing the samples does not alter the beverage's alkaloid profile.

Rodriguez et al. (2022) identified fructose as the most abundant compound in 8 Ayahuasca samples donated by Ayahuasca communities in Uruguay. They also found glucose, ethanol, lactate, acetate, besides harmine (***1***), harmaline (***2a***), harmalol (***2b***), THH ***(3a***), tetrahydronorharmine (***3b***), DMT (***4a***), bufotenine (***4b***), *N*-methyltryptamine (***4c***), and harmol (***5***).

Understanding the chemical composition of Ayahuasca is essential, as its consumption has increased in recent years, along with added modifications. Gonçalves et al. (2021) reported that the β-carboline alkaloids harmine (***1***), harmaline (***2a***), harmalol (***2b***), THH ***(3a***), harmol (***5***), and the indole alkaloid (DMT, ***4a***) present in Ayahuasca are bioavailable and non-cytotoxic. Furthermore, Guimarães et al. (2021) determined that the concentrations of Li, Al, Mn, Fe, Co, Cu, Zn, As, Cd, Hg, Pb, Ca, Mg, K and P, in Ayahuasca samples were below the recommended or tolerable limits, thus Ayahuasca presents safe administration levels concerning total elemental content. Due to the expansion of Ayahuasca use for recreational, medicinal, and religious purposes in European and North American countries, the chemical composition of the beverage has been modified with the addition of substances like medications (moclobemide: MAO inhibitor) and other psychoactive alkaloids (psilocybin: alkaloid derived from psilocybin mushrooms, or yuremamine: indole alkaloid from *Mimosa tenuiflora* (Willd). Poir.) (Kaasik et al., 2021).

Therefore, it is important to highlight that the safety of the addition of other drugs to the original composition of Ayahuasca remains unknown, as well as the occurrence of interactions among the compounds.

3.6. Phytochemistry of the Vines Used in the Preparation of Ayahuasca

Phytochemical information was found for three of the 27 species designated as Ayahuasca, previously reported in Table 1: *B. caapi*, *B. muricata* and *T. mucronata*. Information was also found for *B. inebrians*, considered by Gates (1982) as a synonym of *B. caapi*. Regarding the genus *Mascagnia*, which includes two species designated as Ayahuasca, *M. divaricata* and *M. ovatifolia*, Schultes (1975) reported that their chemical characteristic is the presence of saponins. Table 5 lists the alkaloids present in the vines used in the preparation of Ayahuasca.

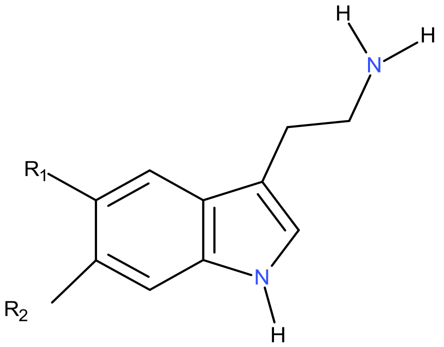
Table 5. Some nitrogenated compounds, species in which they are found, and bibliographic references of vines used in the preparation of Ayahuasca. NI: Not information

Alkaloid	Species	Sample Origin	References
harmine (telepathine, yageine, banisterine) (*1*)	*Banisteriopsis caapi* (100 years old); *B. caapi* ethnotaxa *Tsipi makuni*, *Tsipi wetseni*, *Shuri fisopa*, *Shuri oshinipa*, *Pituricaju*, *Cielo*, *Tukondi Pucahuasca, Kamalampi, Rumi, Tucunacá, Amarelinho, Ourinho* and *Caupuri; B. inebrians; B. muricata* (leaves)	Peru, Brazil, NI	(Ghosal et al., 1971; Hochstein and Paradies, 1957; McKenna et al., 1984; O'Connell and Lynn, 1953; Rivier and Lindgren, 1972; Santos et al., 2020; Schultes et al., 1969)

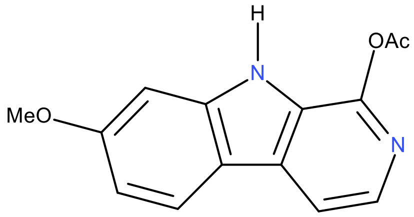
h**armaline (*2a*)**	*Banisteriopsis caapi, B. caapi* ethnotaxa *Cielo*, *Tsipu wetseni*, *Shuri fisopa*, *Shuri oshinipa*, *Piturijacu, Pucahuasca, Kamalampi, Rumi, Tucunacá, Amarelinho, Ourinho* and *Caupuri; B. muricata* (leaves)	Peru, Brazil, NI	(Ghosal et al., 1971; Hochstein and Paradies, 1957; McKenna et al., 1984; Santos et al., 2020; Schultes et al., 1969)
harmalol (*2b*)	*Banisteriopsis caapi* ethnotaxon *Pucahuasca*.	Peru	(McKenna et al., 1984)
tetrahydroharmine (*3a*)	*Banisteriopsis caapi, B. caapi* from ethnotaxa: *Tsipi makuni*, *Tsipi wetseni*, *Shuri fisopa*, *Shuri oshinipa*, *Pituricaju*, *Kamalampi*, *Cielo*, *Pucahuasca, Tukondi, Rumi, Tucunacá, Amarelinho, Ourinho* and *Caupuri*; *B. muricata* (leaves)	Peru, Brazil, NI	(Ghosal et al., 1971; Hochstein and Paradies, 1957; McKenna et al., 1984; Rivier and Lindgren, 1972; Santos et al., 2020; Schultes et al., 1969)
tetrahydronorharmine (*3b*)	*Banisteriopsis caapi*	United States	(Samoylenko et al., 2010)

***N,N*-dimethyltryptamine (*4a*)**	*Banisteriopsis muricata* (leaves)	NI	(Ghosal et al., 1971)
bufotenine (*4b*)	*Tetrapterys mucronata*	Brazil	(Queiroz et al., 2014)
5-methoxy-*N*-methyltryptamine (*4d*)	*Tetrapterys mucronata*	Brazil	(Queiroz et al., 2014)
harmol (*5*)	*Banisteriopsis caapi* from ethnotaxa: *Shuri oshinipa, Cielo*, *Piturijacu Pucahuasca* and *Rumi*	Peru	(McKenna et al., 1984; Rivier and Lindgren, 1972)
2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (*6*)	*Tetrapterys mucronata*	Brazil	(Queiroz et al., 2014)
6-methoxy-*N*-tryptamine (*7a*)	Cultivated *Banisteriopsis caapi*; *B. caapi* ethnotaxa: *Piturijacu*	Peru	(Rivier and Lindgren, 1972)
acetylnorharmin (*8*)	*Banisteriopsis caapi*	NI	(Hashimoto and Kawanishi, 1976)
banistenoside A (*9*)	*Banisteriopsis caapi*	United States	(Samoylenko et al., 2010)
banistenoside B (*10*)	*Banisteriopsis caapi*	United States	(Samoylenko et al., 2010)
caffeine (*11*)	*Banisteriopsis inebrians*	NI	(O’Connell, 1969)

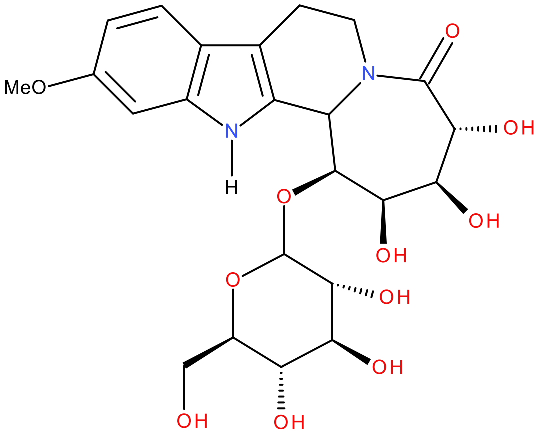
dihydroshihunine (*12*)	*Banisteriopsis caapi*	NI	(Kawanishi et al., 1982)
harmalinic acid (*13a*)	*Banisteriopsis caapi*	NI	(Hashimoto and Kawanishi, 1975)
harmic acid methyl ester (*13b*)	*Banisteriopsis caapi*	NI	(Hashimoto and Kawanishi, 1975)
harmic amide (*13c*)	*Banisteriopsis caapi*	NI	(Hashimoto and Kawanishi, 1976)
harmine-*N*-oxide (*14*)	*Banisteriopsis caapi*	NI	(Hashimoto and Kawanishi, 1975)
ketotetrahydronorharmine (harmalacidine) (*15*)	*Banisteriopsis caapi*	NI	(Hashimoto and Kawanishi, 1976)
shihunine (*16*)	*Banisteriopsis caapi*	Brazil	(Kawanishi et al., 1982)



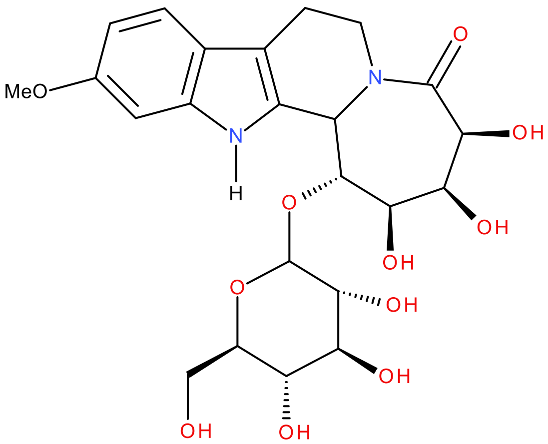
	R_1	R_2
7a 6-methoxy-*N*-tryptamine	H	OMe
7b 5-methoxy-*N*-tryptamine	OMe	H



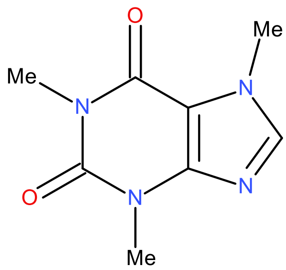
8 acetylnorharmin



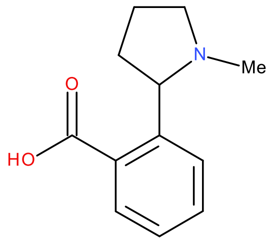
9 banistenoside A



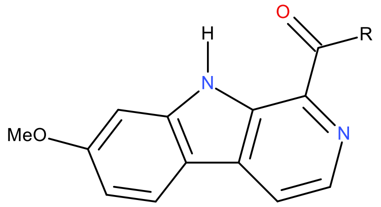
10 banistenoside B



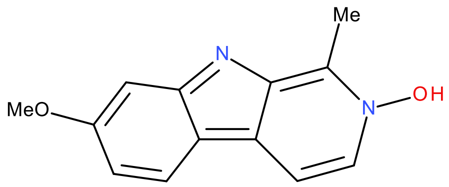
11 caffeine



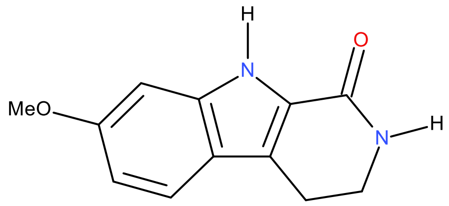
12 dihydroshihunine



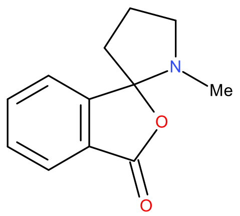
	R
13a harmalinic acid	OH
13b harmic acid methyl ester	OMe
13c harmic amide	NH_2



14 harmine-*N*-oxide



15 ketotetrahydronorharmine



16 shihunine

3.6.1. Phytochemistry of *Banisteriopsis caapi*

In addition to collecting the type specimens of *B. caapi* (*Spruce, R. 2712* (K!, NY!, GH, MO)), Richard Spruce also deposited branch fragments in the Economic Botany Museum at Kew Gardens. These fragments were subjected to chemical analysis nearly a century later, in 1968 (Schultes, 1968), providing a unique opportunity to investigate the phytochemical properties of a historical material from this species.

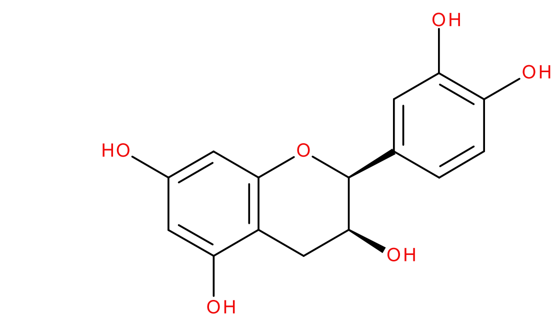
Using GC-MS to analyze the *B. caapi* specimens (*Spruce, R. 2712*) and a fresh stem fragment of the same species, Schultes et al. (1969) concluded that both samples contained a similar alkaloid content (0.4% and 0.5%, respectively). However, the sample collected by Spruce contained only harmine (***1***), whereas the fresh sample contained harmine (***1***), harmaline (***2a***), and THH (***3a***). This finding suggests that alkaloid content remains stable over time but undergoes oxidation to harmine (***1***).

Seil and Putt (1924) analyzed *B. caapi* stems, quantifying the presence of tannins (0.86%), sugars (1.30%), fiber (47.25%), oil (0.0042%), non-phenolic alkaloids (1.88%), and phenolic alkaloids (0.03%). They also isolated three unidentified alkaloids. Although no herbarium voucher information is available, it is known that the analyzed specimen was collected by Henry Hurd Rusby and Orland E. White during the "Mulford Exploration of the Amazon Basin"(Seil and Putt, 1924). It is presumed that the material studied corresponds to the specimen *White, O.E. 970* (US!, MICH), as this was the only one found in the collection associated with the aforementioned expedition.

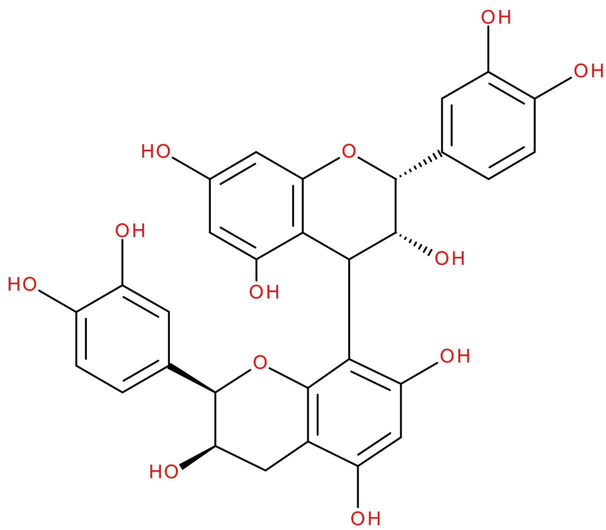
Hochstein and Paradies (1957) isolated harmine (***1***), harmaline (***2a***), and THH (***3a***) from *B. caapi* vines collected by H. Allen in the Napo River region of Peru, identified by Ramon Ferreyra of the University of San Marcos, Peru. To verify the identity of these compounds, paper chromatography results were compared with synthetic standards of the same substances.

Hashimoto and Kawanishi (1976, 1975) identified six additional β-carboline compounds: acetylnorharmin (***8***), harmalinic acid (***13a***), harmic acid methyl ester (***13b***), harmic amide (***13c***), harmine-*N*-oxide (***14***), and ketotetrahydronorharmine (***15***). Later, Kawanishi et al. (1982) reported the occurrence of the pyrrolidine alkaloids dihydroshihunine (***12***), and shihunine (***16***). However, none of these studies provided herbarium specimens to confirm the botanical identification of the analyzed materials.

Samoylenko et al. (2010), while analyzing two *B. caapi* specimens — one collected by *Kenneth M.N. 2789* (HLA) and another purchased online — identified the alkaloids harmine (***1***), harmaline (***2a***), tetrahydroharmine (***3a***), tetrahydronorharmine (THNH, ***3b***), harmol (***5***), banistenoside A (***9***) and B (***10***), besides the non=alkaaloidic compounds epicatechin (***17***) and procyanidin B2 (***18***), and the carbohydrates β-D-fructofuranosyl-(2→5)-fructopyranose, sucrose and glucose. Furthermore, they observed that while both the collected and commercially purchased samples had similar chemical compositions, the commercial sample contained lower concentrations of harmine (***1***), banistenoside A (***9***), banistenoside B (***10***), and procyanidin B2 (***18***), resulting in reduced monoamine oxidase (MAO) inhibition and lower antioxidant activity. Subsequently, Wang et al. (2010) analyzed the same samples and found that compounds harmine (***1***), THH (***3a***), banistenoside A (***9***), banistenoside B (***10***), epicatechin (***17***), and procyanidin B2 (***19***) were more concentrated in the bark of mature vines compared to other plant parts.



17 epicatechin



18 procyanidin B2

Rivier and Lindgren (1972) were the first to analyze *B. caapi* vines from different ethnobotanical taxa: *Tsipi makuni, Tsipi wetseni, Shuri fisopa, Shuri oshinipa, Pituricaju*, and Cultivado, corresponding to vouchers *Rivier, L. 1, Rivier, L. 2, Rivier, L. 3, Rivier, L. 4, Holmstedt, B. s.n., Martin, R.T. 1805* (US, INPA), and *Pinkley, H.V. 445*. Using GC and GC-MS, they reported that alkaloid content varied between 0.11% and 0.83% in the vine, 0.28% and 0.70% in the leaves, and 0.64% and 1.95% in the roots. They noted that all analyzed taxa contained primarily harmine (***1***) and THH (***3a***), though their chemical composition varied among ethnotaxa. Additionally, *Shuri oshinipa* and *Pituricaju* were the only taxa to exhibit harmol (***5***).

McKenna et al. (1984), using HPLC, analyzed the ethnotaxa *Cielo, Pucahuasca,* and *Rumi,* corresponding to vouchers *McKenna, D.J. 110* (UBC!), *McKenna, D.J. 124* (UBC!), *McKenna, D.J. 125* (UBC!), *McKenna, D.J. 129* (UBC), and *Plowman, T. 6041* (MICH), all identified as *B. caapi*. All samples contained harmine (***1***), harmaline (***2a***), THH (***3a***), and harmol (***5***), though in varying amounts. Additionally, harmalol (***2b***) was uniquely identified in the Pucahuasca ethnotaxon.

Callaway (2002) analyzed 35 *B. caapi* samples from the ethnobotanical taxa *Caupuri* and *Tucunacá*, concluding that *Caupuri* exhibited higher levels of β-carboline alkaloids, potentially explaining the stronger physical effects reported by consumers, such as tremors, vomiting, and diarrhea. Although herbarium vouchers were not created, the material was sourced from the Brazilian União do Vegetal religious tradition and referenced by Oliveira et al. (2023), though statistical analyses were not performed to substantiate their conclusions.

In another study, Santos et al. (2020) analyzed 159 *B. caapi* vine samples, including 11 *Amarelinhos*, 18 *Caupuris*, 7 hybrids, 10 *Ourinhos*, and 113 *Tucunacás*, using LC-MS/MS. They determined that β-carboline content was higher in *Tucunacás* compared to *Caupuris*, although this difference was not statistically significant, raising questions about whether the lack of significance was due to the sample size imbalance, with six times more *Tucunacás* vines than *Caupuris*. Additionally, the researchers observed that wild vines had higher harmine (***1***) content than cultivated ones. The plant specimens analyzed are stored in the UB herbarium and the UBw wood collection.

Subsequently, Santos et al. (2022) evaluated the bioactivity of the samples previously studied by Santos et al. (2020) and determined that harmine (***1***), while modulating inflammatory responses, may also exhibit cytotoxicity. Moreover, harmaline (***2a***) and THH (***3a***) demonstrated anti-inflammatory potential by reducing pro-inflammatory cytokines associated with detrimental processes in the central nervous system.

From another perspective, Nagamine-Pinheiro et al. (2021) analyzed two *Tucunacá* samples—one fresh and one mature—corresponding to the collection numbers *Sonsin-Oliveira, J. 414* (UB) and *Sonsin-Oliveira, J. 239* (UBw), respectively. Through histochemical analysis, they identified alkaloids, tannins, phenols, essential oil terpenoids, pectins, and saponins in the parenchymal cells of the mature vine. In the fresh vine, the same compounds were present, except for phenols and tannins.

3.6.2. Phytochemistry of *Banisteriopsis inebrians*

O'Connell and Linn (1953) isolated the alkaloid harmine (***1***) from the vine *Banisteriopsis inebrians*, with a reported content of 0.15%. Using Amelink's acetaldehyde tests and observing green fluorescence under ultraviolet light, they confirmed the absence of harmaline (***2a***) and harmalol (***2b***) —compounds present in the synonymous species *B. caapi*. However, no details were provided regarding the reference material used in their analysis.

Similarly, O'Connell (1969) identified the presence of caffeine (***11***) in *B. inebrians* and verified its identity through infrared (IR) spectroscopy. Nonetheless, no information about the reference material employed in this study was disclosed.

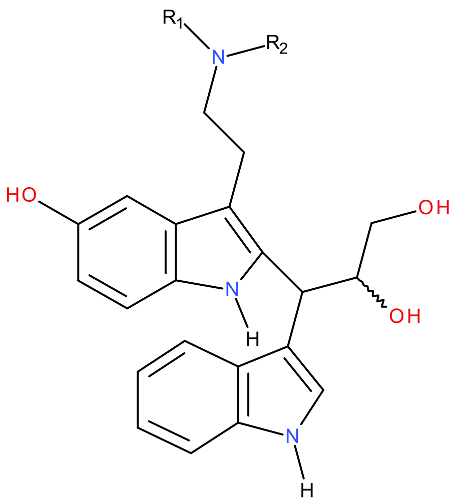
3.6.3. Phytochemistry of *Banisteriopsis muricata*

Ghosal et al. (1971) identified harmine (***1***), harmaline (***2a***), THH (***3a***), and DMT (***4a***) in the leaves of *B. muricata*. However, they did not report the herbarium voucher used in their study. In contrast, Nagamine-Pinheiro et al. (2021) analyzed a sample of *B. muricata* corresponding to the voucher *Oliveira, R.C. 3392* (UB). Through histochemical analysis, they observed an abundant presence of saponins in the parenchymal cells of the vine. Additionally, alkaloids, tannins, phenols, and essential oil terpenoids were identified.

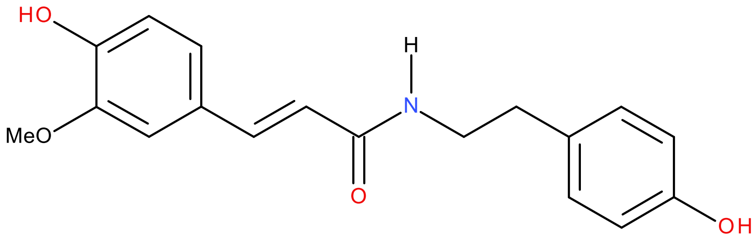
3.6.4. Phytochemistry of *Tetrapterys mucronata*

Queiroz et al. (2015, 2014), using high-performance liquid chromatography coupled with ultraviolet spectroscopy (HPLC-UV) and tandem mass spectrometry with electrospray ionization (HPLC-ESI/MS/MS), identified various compounds in the vine of *T. mucronata* (barcode SP146620). These compounds included bufotenine (***4b***), 5-methoxy-*N*-methyltryptamine (***4d***), O-methylbufotenine (***4e***), 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (***6***), mucronatin A (***19a***) and B (***19b***), trans-*N*-feruloyltyramine (***20***), grossamide (***21***), and cannabisin F (***22***). Moreover, the following non-nitrogenated compounds were detected: gentisic acid (***23a***), gentisic acid 5-O-β-xyloside (***23b***), salicylic acid (***23c***), (+)-catechin (***24***), vanillic acid (***25***), (*Z*)-3-methoxy-4,5-(methylenedioxy)cinnamic acid (***26***), (*E*)-3-methoxy-4,5-(methylenedioxy)cinnamic acid (***27***), lyoniside (***28***), 2,6-phenanthrenediol (***29a***), 7-methyl-2,6-phenanthrenediol

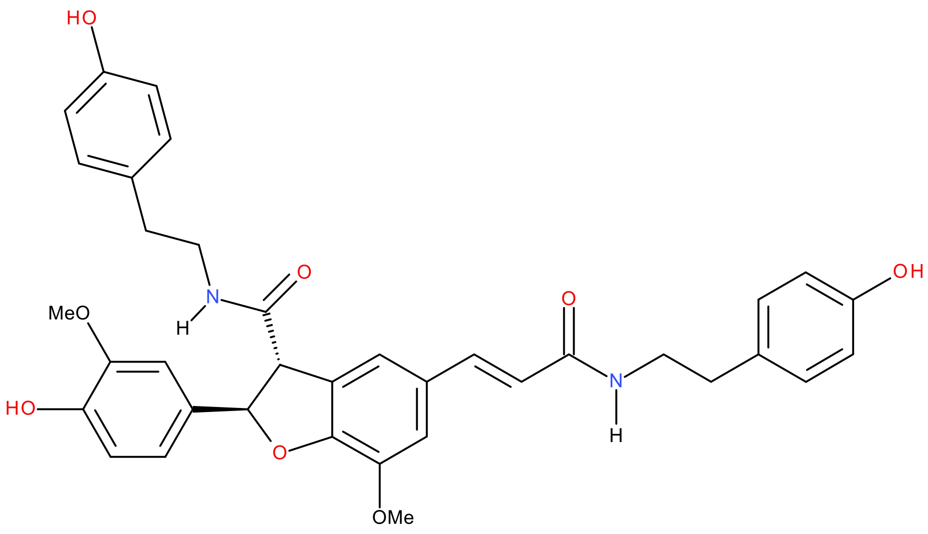
(***29b***), 2,6-dihydroxy-9,10-dihydrophenanthrene (***30***), nudiposide-9′-dihydroxybenzoic acid (***31***), and smilaside L (***32***). The alkaloids bufotenine (***4b***) and *O*-methylbufotenine (***4e***) were particularly significant in their findings.



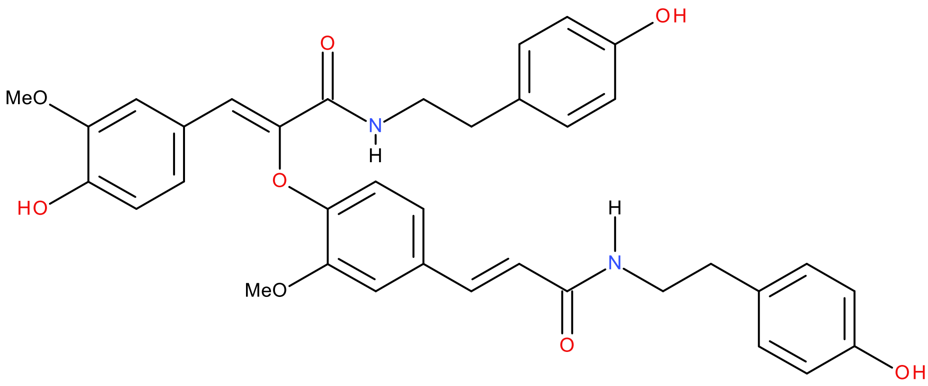
	R_1	R_2
19a mucronatin A	Me	Me
19b mucronatin B	H	H



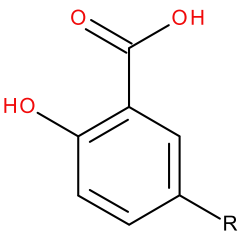
20 trans-*N*-feruloyltyramine



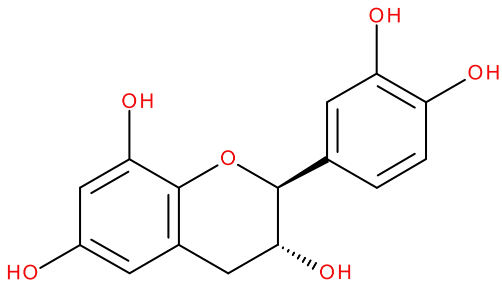
21 grossamide



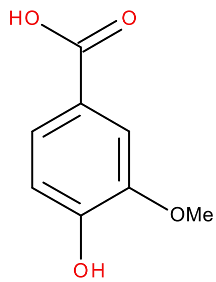
22 cannabisin F



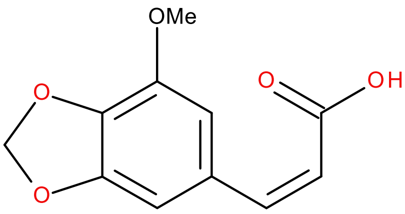
	R
23a gentisic acid	OH
23b gentisic acid 5-*O*-β-xyloside	O-xyl
23c salicilic acid	H



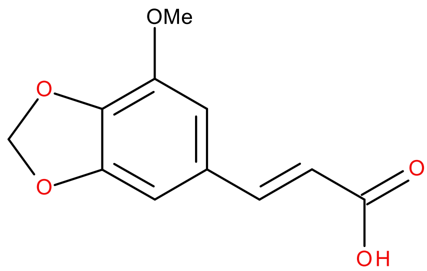
24 catechin



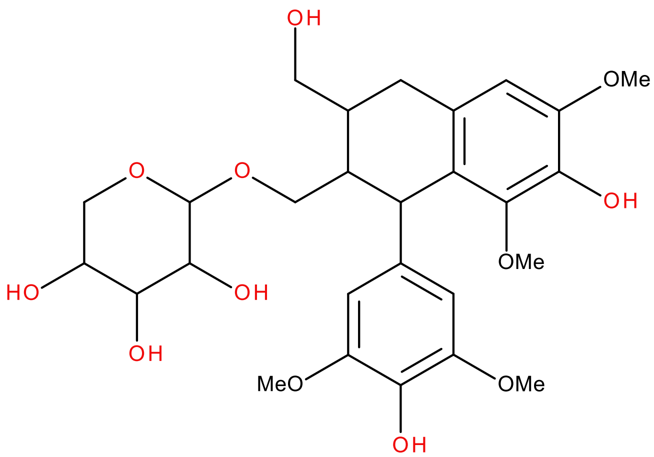
25 vanillic acid



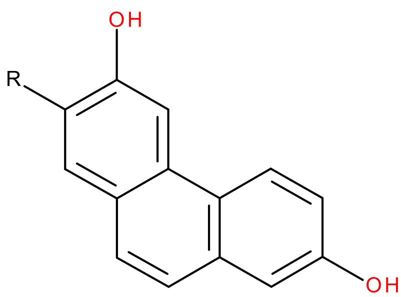
26 (Z)-3-methoxy-4,5-(methylenedioxy)cinnamic acid



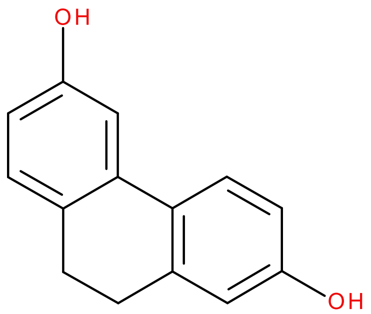
27 (E)-3-methoxy-4,5-(methylenedioxy)cinnamic acid



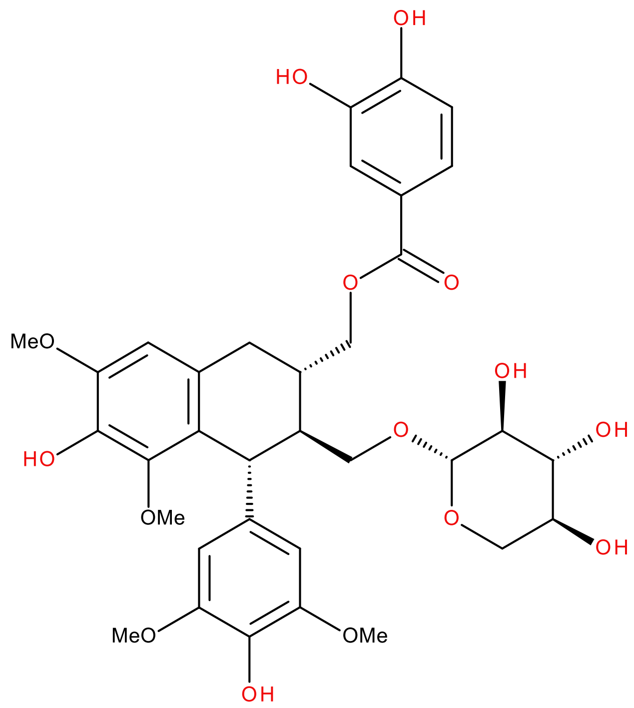
28 lyoniside



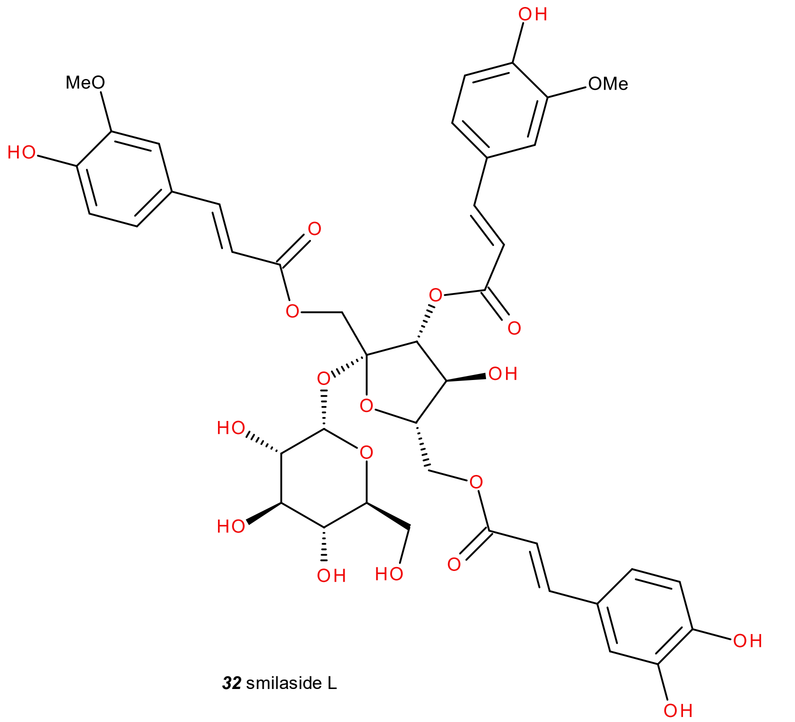
	R
29a 2,6-phenanthrenediol	H
29b 7-methyl-2,6-phenanthrenediol	Me



30 2,6-dihydroxy-9,10-dihydrophenantrene



31 nudiposide-9'-dihydroxybenzoic acid



32 smilaside L

3.7. Chemical Composition of Leaf Sources Used in Ayahuasca Preparation

Chemical composition data are available for four of the 12 species listed in Table 3 as sources of leaves used in the preparation of Ayahuasca: *Diplopterys cabrerana*, *D. longialata*, *P. carthagenensis*, and *P. viridis*. For the remaining eight species, no publications addressing their chemical compositions were found. The results of this investigation are presented in Table 6.

Additionally, it was discovered that, like *B. caapi*, communities also classify *P. carthagenensis* and *P. viridis* into different ethnobotanical taxa. However, the criteria used to define these taxa remain uncertain. Table 7 presents the ethnobotanical taxa.

Table 6. Alkaloids Present in the Species Used as Leaf Sources in Ayahuasca Preparation and References.

Species	Chemical compound	Reference
Diplopterys cabrerana	*N,N*- dimethyltryptamine (DMT, ***4a***)	(McKenna et al., 1984)
	bufotenine (5-hidroxy-*N,N*-dimethyltryptamine, ***4b***)	(McKenna et al., 1984)
Diplopterys longialata	2-methyltetrahydro-β-carboline (2-methyl-1,2,3,4-tetrahydro-β-carboline, MTHBC, ***3c***)	(Agurell et al., 1968)
	bufotenine (***4b***)	(Agurell et al., 1968)
	N-dimethyltryptamine (***4c***)	(Agurell et al., 1968)
	O-methylbufotenine (5-methoxy-*N,N*- dimethyltryptamine, ***4e***)	(Agurell et al., 1968)
	N,N- dimethyltryptamine (***4a***)	(Agurell et al., 1968; Poisson, 1965; Schultes, 1968)
Psychotria carthagenensis	2-methyltetrahydro-β-carboline (MTHBC, ***3c***)	(Rivier and Lindgren, 1972)

	N,N-dimethyltryptamine (DMT, ***4a***)	(Rivier and Lindgren, 1972)
	6-methoxytryptamine (***7a***)	(Rivier and Lindgren, 1972)
Psychotria viridis	2-methyltetrahydro-β-carboline (MTHBC, ***3c***)	(McKenna et al., 1984; Rivier and Lindgren, 1972)
	N,N-dimethyltryptamine (DMT, ***4a***)	Callaway et al., 2005; McKenna et al., 1984; Rivier and Lindgren, 1972)
	N-methyltryptamine (***4c***)	(Rivier and Lindgren, 1972)

Table 7. Reported Ethnotaxa for *Psychotria viridis* and *Psychotria carthagenensis*, Voucher, and Reference.

Ethnotaxa	Specie	Voucher	Reference
Kawa Kui	*Psychotria viridis*	*Rivier, L. 9*	(Rivier and Lindgren, 1972)
Rami appani	*Psychotria carthagenensis, Psychotria viridis*	*Rivier, L. 8, Rivier, L. 7*	(Rivier and Lindgren, 1972)

3.7.1. Phytochemistry of *Diplopterys cabrerana*

McKenna et al. (1984), through the analysis of the sample *Plowman, T. 6040* (US) using HPLC and GC/MS, identified the presence of DMT (***4a***) and the derivative bufotenine(***4b***). This finding was confirmed using the Ehrlich reagent test.

3.7.2. Phytochemistry of *Diplopterys longialata*

Poisson (1965) analyzed the leaves of *D. longialata* and found them to contain 0.64% DMT (***4a***), although the specimen used was not reported. Schultes (1968), meanwhile, analyzed the leaves of the specimen *Pinkley, H.V. 449* (MO), also known as *Oco-yajé* by the Cofán indigenous group, and identified the presence of β-carboline alkaloids and DMT (***4a***).

Agurell et al. (1968), using GC-MS, analyzed the leaves of *D. longialata* from the specimens *Pinkley, H.V. 449* (MO) - also analyzed by Schultes (1968) - and *Pinkley, H.V. 310*. They identified *N*β-methyltetrahydro-β-carboline ***(3c***), DMT (***4a***), 5-hydroxy-DMT (***4b***), *N*-methyltryptamine (***4c***), and 5-methoxy-DMT (***4e***) in the leaves. In the vine material from these same specimens, they detected Nβ-methyltetrahydro-β-carboline ***(3c***), DMT (***4a***), and 5-methoxy-DMT (***4e***).

3.7.3. Phytochemistry of *Psychotria carthagenensis*

Rivier and Lindgren (1972) reported that the leaves of *P. carthagenensis* contain DMT ***(4a***) at concentrations higher than those of *P. viridis*, with amounts doubled or even sixfold greater. They also identified 2-methyl-1,2,3,4-tetrahydro-β-carboline (MTHC) (***3c***) and methoxytryptamine (***7a***) in the sample of the ethnotaxon *Rami appani*. Interestingly, the vernacular name of *P. carthagenensis*, *Rami appani*, coincides with that of an ethnotaxon of *P. viridis* used by the Culina indigenous community.

Despite reports of *P. carthagenensis* being used as a leaf source in Ayahuasca preparation, McKenna et al. (1984) concluded that the species does not contain the alkaloid DMT ***(4a***), after analyzing the sample *McKenna, D.J.* 109 (UBC!) using GC/MS. Similarly, Leal and Elisabetsky (1996) performed pharmacological tests on rats using an ethanolic extract from the specimen identified with barcode ICN 98863 and concluded that *P. carthagenensis* lacks alkaloids.

Formagio et al. (2024) analyzed the specimen corresponding to the voucher *Volobuff, C.R.F.* 214 (DDMS) and determined that *P. carthagenensis* exhibits high antioxidant activity. Additionally, they identified phenols, tannins, flavonoids, and flavonols in the sample.

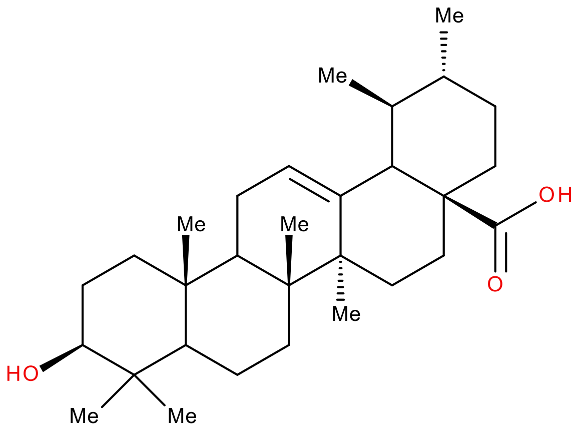
3.7.4. Phytochemistry of *Psychotria viridis*

Rivier and Lindgren (1972), through GC and GC-MS analysis of *P. viridi*s leaves from the ethnotaxa listed in Table 7, identified MTHBC (***3c***), DMT ***(4a***), and *N*-methyltryptamine (MMT, ***4c***) in the ethnotaxon *Rami appani*, while only MTHBC (***3c***) and MMT (***4c***) were detected in *Kawa Kui.*

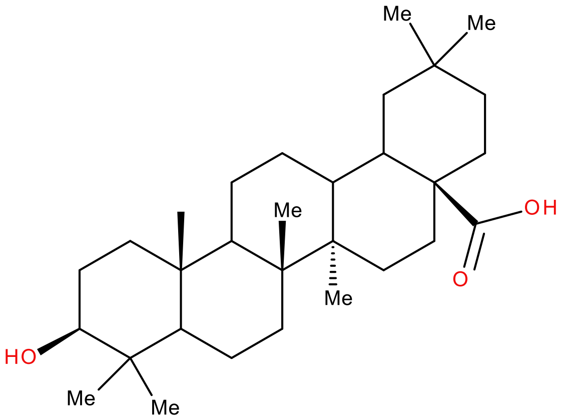
McKenna et al. (1984) reported the presence of DMT ***(4a***) in *P. viridis* leaves from the vouchers *McKenna, D.J. 21* (UBC!), *McKenna, D.J. 108* (UBC!), and *McKenna, D.J.* 139 (UBC!) using HPLC, GC/MS, and Ehrlich reagent staining. The alkaloid content ranged from 1.0 to 1.6 mg/g of dry leaf. Furthermore, the β-carboline alkaloid 2-methyl-tetrahydro-β-carboline (MTHBC, ***3c***) was identified in the sample *McKenna, D.J. 139* (UBC!). No alkaloids were detected in the fruits or stems of these samples.

Callaway et al. (2005) analyzed 37 samples of *P. viridis* and observed a wide range of DMT ***(4a***) concentrations in all of them, except one. For eight of the samples, they detected less than 0.60 mg/g of DMT (***4a***). The authors did not report the specimens used.

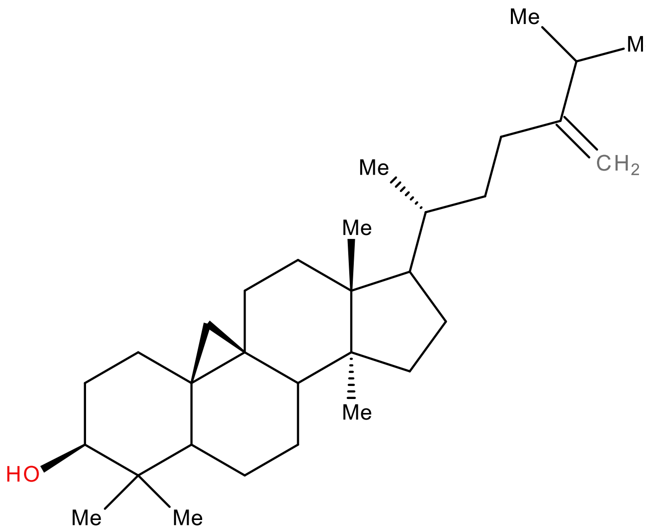
Soares et al. (2017) analyzed leaves from the UDV religion, although they did not specify the voucher. Their analysis identified the pentacyclic triterpenes ursolic acid (***33***) and oleanolic acid (***34***); the steroids 24-methylene-cycloartenol (***35***), stigmasterol (***36a***), and β-sitosterol (***37a***); the glycosylated steroids 3-O-β-D-glucosyl-stigmasterol (***36b***) and 3-O-β-D-glucosyl-β-sitosterol (***37b***); the polyunsaturated triterpene squalene; the glycerol esters 1-palmitoylglycerol and triacylglycerol; a mixture of long-chain hydrocarbons; the aldehyde nonacosanal; the long-chain fatty acids hentriacontanoic, hexadecanoic, and heptadecanoic acids; methyl heptadecanoate ester; 4-methyl-epi-quinate (***38***); and two indole alkaloids, DMT (***4a***) and MMT (***4c***).



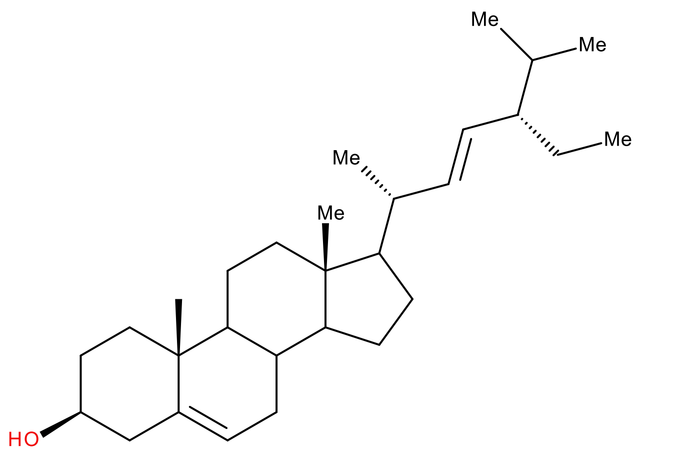
33 ursolic acid



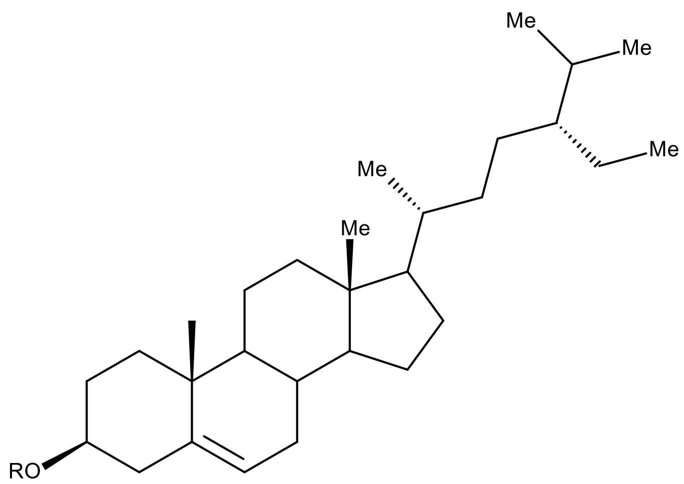
34 oleanolic acid

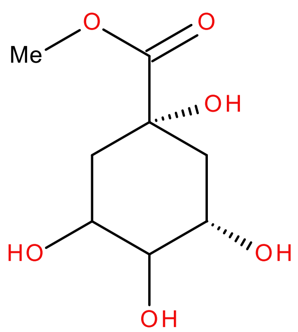


35 24-methylenecycloartanol



	R
36a stigmasterol	H
36b stigmasterol-3-O-β-glucoside	glu





	R
37a β-sitosterol	H
37b β-sitosterol-3-O-β-glucoside	glu

38 4-methyl-4-epiquinate

4. Discussion

Understanding the broad diversity of plants involved in Ayahuasca preparation is essential for preserving ancestral and traditional knowledge while contributing to the protection of the Amazon rainforest. According to Shipibo shaman Walter Ramiro López, the forest is experiencing a form of cultural extractivism, in which "people consume Ayahuasca plants but do not replant them," placing these species at risk of extinction (PDA, 2024).

Additionally, Ayahuasca tourism does not always adhere to sustainable practices. Healing centers, often managed by foreigners, charge exorbitant fees for tourists—up to

$15,000 per person—while Indigenous shamans receive minimal compensation, often around $25 per patient. This disparity not only reflects economic exploitation but also threatens the cultural and ecological sustainability associated with Ayahuasca (PDA, 2024).

Spruce (1908), Cooper (1986), Luz (1996), and Tukano (2022) reported that Indigenous peoples from the following linguistic families have a traditional consumption of Ayahuasca: Arawak, Auishiri, Carib, Cofan, Guahibo, Jivaro, Maku, Munichi, Pano, Quechua, Sabela, Sukuani, Tucano, Tukano, Tupi, Wambisa, Yagua, Záparo, among others. These groups inhabit regions of Brazil, Colombia, Ecuador, Peru, and Venezuela.

All studied groups assign names to the ethnotaxa used in Ayahuasca preparation. Indigenous classification methods are often complex: some names relate to the plants' age, others to different parts of the plant, or to ecological conditions such as soil type, shade levels, and humidity. Many names also reflect entities from nature, which are honored through these denominations. Indigenous peoples believe that different ethnotaxa have distinct physiological effects (Schultes and Hofmann, 2000).

Reports in Brazilian Ayahuasca religions suggest that the spiritual and physiological effects of Ayahuasca vary depending on the ethnotaxon used in the brew's preparation (Oliveira et al., 2023). There is a consensus among users that brews made with *Caupuris* produce more intense physical effects than those prepared with *Tucunacá*s. These effects are so pronounced that they surpass the visible characteristic of conspicuous nodes on the *Caupuris* stems. Thus, there are *Caupuris* "without nodes," referring to vines classified as having swollen nodes due to the similarity of their physiological effects.

The rich diversity of ethnotaxa names within Ayahuasca-using communities reflects the immense cultural and biological diversity present in countries that share the Amazon rainforest. Interestingly, more ethnotaxa are reported among religious communities than Indigenous groups, possibly due to the proximity of Ayahuasca religions to Western science.

Despite this diversity, many ethnotaxa names are represented in herbaria only by incomplete collections. Gates (1982) noted that the high number of sterile *B. caapi* specimens in herbaria significantly hampers taxonomic correspondence studies.

The scarcity of studies on the phytochemistry of plants associated with Ayahuasca, except for *B. caapi* and *P. viridis,* is believed to stem from the limited knowledge of the vast diversity of species used in its preparation. This research reports, for the first time, 15 additional species employed in Ayahuasca preparation and 119 ethnotaxa for the vine.

An interesting finding of this research is that ethnotaxa exhibit distinct chemical compositions, raising the question of whether they all truly correspond to *Banisteriopsis caapi*. This hypothesis is supported by the findings of Langdon (1986) and Oliveira et al. (2023), who, through taxonomic identification and anatomical wood analysis, demonstrated that not all ethnotaxa belong to the *Banisteriopsis* genus but also include species from the *Diplopterys* genus.

Evidence suggests alternative mechanisms may be involved in Ayahuasca's psychoactive effects, as some authors have reported the absence of DMT (***4a***) in *P. carthagenensis* (Leal and Elisabetsky, 1996; McKenna et al., 1984). This raises the hypothesis that MAO inhibition may not be the only pathway responsible for these effects.

Although Rivier and Lindgren (Rivier and Lindgren, 1972) reported the presence of DMT in *P. carthagenensis*, this finding is believed to be related to misidentification, given the complex taxonomy of the *Psychotria* genus. The genus is exceptionally large, with approximately 1,820 described species (Razafimandimbison et al., 2014; Razafimandimbison and Rydin, 2024). Additionally, *P. carthagenensis* has 44 heterotypic synonyms, increasing the likelihood of taxonomic confusion with other species within the genus.

5. Conclusions

The complexity of Ayahuasca is evidenced by the significant number of species that constitute the brew. While this article highlights the importance of herbaria as a source of information on Ayahuasca's composition, many authors have neglected the necessity of including vouchers in collections. This need becomes even more evident given the existence of ethnotaxa within *B. caapi*, whose circumscription has been questioned by various researchers.

This lack of documentation, combined with conflicting phytochemical data across studies, leads us to assume that published analyses may have been conducted on misidentified plants. Finally, contrary to appearances, there are notable gaps in the phytochemical characterization of Ayahuasca, due to the scarcity of studies that consider the ethnotaxa of vines and the species of leaves used in its preparation.

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CHAPTER 2. Phytochemical Profiles of Ayahuasca: Insights from Different Ethnotaxa of Vines

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Abstract

Background: Ayahuasca is a psychoactive beverage traditionally prepared with *Banisteriopsis caapi* and *Psychotria viridis*. Despite its cultural relevance, the chemical variability associated with different *B. caapi* ethnotaxa remains poorly understood.

Objective: To compare the chemical composition of Ayahuasca preparations obtained from different ethnotaxa of *B. caapi* using chromatographic and spectrometric techniques.

Methods: Six Ayahuasca samples representing the ethnotaxa *Tucunacá, Caupuri, Ourinho, and Quebrador* were voluntarily donated by religious communities in Brazil. Samples were fractionated into methanol-soluble (E2) and methanol-insoluble (E3) phases. Chemical characterization was performed through Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD), and High-Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS/MS). Statistical analyses of chromatographic profiles were conducted using similarity matrices, Jaccard coefficients, and hierarchical clustering.

Results: TLC and HPLC-DAD revealed qualitative differences among samples, particularly in the methanol-soluble fraction. Jaccard similarity indices showed greater chemical variability in E2, while E3 fractions displayed higher homogeneity, suggesting conservation of structural compounds. HPLC-MS/MS confirmed the presence of canonical β-carbolines (harmine, tetrahydroharmine) and N,N-dimethyltryptamine, in addition to other indole derivatives and flavonoids not previously reported in Ayahuasca. Compound distribution varied among ethnotaxa, with *Tucunacá* and *Caupuri* exhibiting diversity.

Conclusions: The chemical composition of Ayahuasca is influenced by the *B. caapi* ethnotaxon used. While E3 fractions remain relatively conserved, the methanol-soluble fraction contributes most to the chemical and potentially pharmacological variability of the beverage.

Keywords: *Ayahuasca, Banisteriopsis caapi; Ethnotaxa; β-carbolines; N,N-dimethyltryptamine (DMT)*

1. Introduction

Ayahuasca is an entheogenic beverage of South American origin, traditionally consumed by Indigenous peoples of the Amazon in Brazil, Bolivia, Colombia, Ecuador, and Peru, as well as in the Orinoco region of Venezuela and the Pacific coast of Colombia and Ecuador (Schultes and Hofmann, 1980). It is primarily used in rituals with spiritual and medicinal purposes. Starting in the 20th century in Brazil, Ayahuasca became central to the rituals of the ayahuasca religious groups Santo Daime, Barquinha, and União do Vegetal (UDV), which were founded in 1930, 1946, and 1961, respectively (CEBUDV, 2024; ICEFLU, 2024). The consumption of Ayahuasca in Brazil is regulated by Resolution No. 1 of January 25, 2010, issued by the National Council on Drug Policy (CONAD) (CONAD, 2010). Today, Ayahuasca use has expanded to countries in North America, Europe, and Asia (Labate et al., 2016; Labate and Jungaberle, 2011).

The beverage is also known by other names, including Caapi, Yagé, Pindé, Nixi Pae, Natema, Cadána, Iyona, Nepe, Honi, Daime, and Vegetal. The most widely known preparation of Ayahuasca involves the vine *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton (Malpighiaceae) and the leaves of *Psychotria viridis* Ruiz & Pav. (Rubiaceae). However, a review of herbarium vouchers and literature has identified that other vine species are also used, belonging to the genera *Alicia* W.R. Anderson, *Banisteriopsis* C.B. Rob., *Bronwenia* W.R. Anderson & C. Davis, *Callaeum* Small, *Christianella* W.R. Anderson, *Dicella* Griseb., *Diplopterys* A. Juss., *Glicophyllum* R.F. Almeida, *Heteropterys* Kunth, *Mascagnia* (Bertero ex DC.) Bertero, *Mezia* Schwacke ex Nied., *Niedenzuella* W.R. Anderson, *Stigmaphyllon* A. Juss., and *Tetrapterys* Cav (Saavedra-Saenz et. al, 2025, unpublished data).

Banisteriopsis caapi is widely cultivated. The species was first described by Richard Spruce, with the type specimen *Spruce, R. 2712* (K!, NY!, GH, MO) collected from a plant cultivated by Indigenous people in the Brazilian Amazon, as having smooth, open, striated, and atrophied branches with annular nodes and internodes 1 to 2 inches in length, along with bark marked by small lenticels (Eichler et al., 1840). However, communities that use the vine report approximately 119 vernacular names (Saavedra-Saenz et. al, 2025, unpublished data) which may reflect morphological variations, primarily differentiated by stem shape (Monteles, 2020; Oliveira et al., 2023; Schultes, 2000). These stems do not always exhibit the smoothness described in the type specimen *Spruce, R. 2712* (K!, NY!, GH, MO).

Oliveira et al. (2023) identified 18 vernacular names for *B. caapi*, referred to as ethnotaxa. Based on a multivariate analysis of stem anatomy, Oliveira et al. (2023) two main "generic" groups were established: the first includes vines with inflated nodes, collectively referred by folk taxonomy to as *Caupuri*, while the second groups vines with non-inflated nodes, known as *Tucunacá*. In line with this, Luz et al. (2022) using DNA barcodes from 20 *B. caapi* samples, reconstructed a phylogenetic tree in which the ethnotaxa group into three main categories: *Caupuri*, *Tucunacá*, and *Mariri* Nativo. Furthermore, Luz et al. (2022) suggest that the observed genetic distances may be related to the use of different species of *Banisteriopsis* in the preparation of Ayahuasca, and are not limited to *B. caapi* alone.

The physiological effects resulting from Ayahuasca consumption can vary depending on the *B. caapi* ethnotaxon used in its preparation. Oliveira et al. (2023) observed that vines classified as *Caupuri*, including ethnotaxa such as *Caupuri-long-nodes* and *Pajezinho*, are grouped within this category due to the effects they produce in consumers, even though some, like *Pajezinho*, do not exhibit the characteristic nodes of *Caupuri*. In this case, the perception of effects takes precedence over the morphology of the vine. These effects include tremors, diarrhea, imbalance, and a sensation of body cooling. In contrast, vines such as *Arara-vermelha*, *Ararinha*, *Quebrador*, *Ourinho*, *Caboquinho*, *Juruá*, *Pingo-de-ouro* and *Tucunacá*, are more commonly associated with less physical effects. Additionally, Langdon (1986) reported that the Siona Indigenous people recognize a potentially lethal ethnotaxon called *Tapir*, as well as other ethnotaxa that induce visions of different natures. Politi (2021) discusses that these effects may be influenced by cultural practices.

It is believed that the discrepancies in the effects experienced by individuals consuming Ayahuasca prepared with different ethnotaxa are correlated with chemical variations. In this regard, the ethnotaxa have been considered chemotypes of *B. caapi* (Gates, 1982).

The most commonly reported chemical compounds in the beverage are β-carbolines: harmine, harmaline, and tetrahydroharmine (THH), alkaloids derived from the vine (*B. caapi*), and the indole alkaloid N,N-dimethyltryptamine (DMT), derived from the leaves (*P. viridis*) (Callaway, 2005; Gambelunghe et al., 2008; McKenna et al., 1984; Pires et al., 2009; Rivier and Lindgren, 1972; Rodríguez et al., 2022; Santos et al., 2020; Schultes and Hofmann, 1980). The synergy between the aforementioned alkaloids generates a psychoactive effect in users, as the β-carboline alkaloids inhibit the degradation of monoamine oxidase (MAO), thus allowing the activation of DMT (McKenna, 2004; Ott, 1999; Riba et al., 2012).

Other β-carbolines, such as harmol, harmalol (McKenna et al., 1984) and tetrahydronorharmine (Rodríguez et al., 2022), have also been reported in the beverage, as well as tryptamine alkaloids like bufotenine and N-methyltryptamine (Rodríguez et al., 2022). In Ayahuasca preparations from European countries, the presence of medications (moclobemide: MAO inhibitor) and hallucinogens (psilocybin: alkaloid derived from psilocybin mushrooms, or yuremamine: indole alkaloid from *Mimosa tenuiflora* (Willd.) Poir.) has also been detected (Kaasik et al., 2021).

Harmine is the compound with the highest selective MAO-inhibitory activity, and it also inhibits dual specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) and acetylcholinesterase (ACHe) (Li et al., 2018; Royal Society of Chemistry, 2025). Due to these properties, various pharmacological activities have been attributed to harmine, including antioxidant, anti-inflammatory, antitumor, antidepressant, antileishmanial, and antidiabetic effects (Huang et al., 2022; Wang et al., 2015; Zhang et al., 2014).

It is believed that the β-carbolines present in the beverage are chemically stable over time, as Arbouche et al. (2023) analyzed samples of the beverage stored for over 100 years, detecting concentrations of harmine, harmaline, and tetrahydroharmine. However, DMT was not identified in the beverage, and the authors suggested this could be due to the absence of *Psychotria* leaves in the preparation; nevertheless, Verotta et al. (2023) reported that some species of this genus do not contain detectable levels of DMT. Studies by Callaway (2005), Byrska et al. (2022) and Rodríguez et al. (2022) have shown that the chemical composition of the beverage varies depending on the religious group preparing it.

It is still uncertain why beverages prepared with different ethnotaxa of *B. caapi* produce varied physiological effects in consumers. To date, the first study evaluating Ayahuasca prepared with different ethnotaxa was conducted by Rivier & Lindgreen (1972), who analyzed various preparations used in the Peruvian Amazon, such as *Black Ayahuasca*, *Boiled Black Ayahuasca*, *Crude Black Ayahuasca*, *Kamalampi*, *Red Ayahuasca* and *Shuri*. However, the authors did not discuss whether the physiological effects varied among the types of Ayahuasca consumed. Despite this, it was observed that the β-carboline and DMT content differed between preparations made with different ethnotaxa. Subsequently, Santos et al. (2020) analyzed 33 samples of Ayahuasca from Brazil and detected the presence of the β-carbolines harmine, harmaline, and THH, along with the alkaloid DMT. However, the samples made exclusively with *Banisteriopsis caapi* vine did not contain DMT.

Two studies focused exclusively on the analysis of vine ethnotaxa, rather than the beverage, have also been found. Santos et al. (2020) analyzed 159 vine samples from the following ethnotaxa: *Amarelinho* (11), *Caupurí* (18), *Híbrido* (7), *Ourinho* (10), and *Tucunacá* (113), and found no statistically significant differences in β-carboline content. On the other hand, Callaway (2002) reported that vines from the Caupuri group exhibited a higher β-carboline content compared to those from the *Tucunacá* group, and therefore, *Caupuri* causes more digestive discomfort than *Tucunacá*, although the author did not specify the number of samples analyzed or whether the results were statistically significant.

The aim of this article is to compare the chemical composition of Ayahuasca prepared with different ethnotaxa, applying various chromatographic techniques, including Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD), Liquid Chromatograph Coupled to a Mass Spectrometer (HPLC-MS/MS), Preparative Chromatography (Prep-LC), Reverse-Phase Column Chromatography (RP-CC), and Nuclear Magnetic Resonance Spectrometry (NMR).

2. Methods and Materials

2.1. Collection of Material

Six samples of Ayahuasca from the ethnotaxa *Caupuri, Ourinho, Quebrador* and *Tucunacá* were analysed, which were donated by members of the communities that consecrate the beverage. The research participants signed the Informed Consent Form (TLC) (see APPENDIX 1) and were interviewed to describe the preparation method of the beverage. Considering that there are reports of variations in production among the groups, as well as physical effects following the consecration, related to the ethnotaxon of vine used. When possible, a sample of the vine was collected for voucher preparation, which was deposited in the herbarium and wood collection of the University of Brasília (UB, UBw, respectively). The beverage samples were properly identified and characterized.

2.2. Chemical Analyses

To prevent microbial growth in the samples, 95% ethanol (Dinâmica) was added in a 1:1 ratio. The samples were evaporated under low pressure using a Hei-VAP Value rotary evaporator at 60°C and 200 rpm until a pellet with low water content was obtained. To each pellet, 150 mL of methanol was added, followed by agitation for 48 hours, allowing the separation of the phase containing soluble compounds, named E2, from the phase containing insoluble compounds, named E3. The resulting fractions were then separated by filtration.

Subsequently, Thin Layer Chromatography (TLC) was performed for each of the fractions obtained in the previous step, using silica as the stationary phase. For the E2 fractions, the mobile phase consisted of a solution of chloroform and methanol in a 9:1 ratio. For the E3 fractions, the mobile phase used was a solution composed of acetic acid, water, and acetone.

The E2 fractions were adjusted to a concentration of 2 mg/mL in HPLC-grade methanol (Merck), while the E3 fractions were dissolved in deionized water. Both fractions were filtered using 0.45 µm syringe filters. Following the methodology proposed by Leite et al. (2013), each fraction was analyzed by HPLC-DAD, using a Hitachi equipment with a LiChroCART 150-4.6 Purospher STAR RP-18e (5 µm) column and a LichroCART 4-4.6 Purospher STAR RP 18e (5 µm) pre-column, maintained at 25°C, with a flow rate of 0.6 mL/min and an analysis time of 55 minutes. The eluents used were a 1% phosphoric acid solution (Tedia) in pump A and acetonitrile (Tedia) in pump B, in a gradient elution system as follows: 90% A and 10% B at 0 min, 70% A and 30% B at 40 min, 50% A and 50% B at 50 min, 90% A and 10% B at 55 min. The detection range was from 230 to 400 nm, with data extraction at 280 and 354 nm.

The fractions were diluted to a final concentration of 0.5 mg/mL and filtered through a 0.45 µm syringe filter. E2 fractions were prepared in methanol (Merck), whereas E3 fractions were prepared in deionized water. Samples were analyzed on an Agilent 1260 Infinity II liquid chromatograph coupled to an AB Sciex TripleTOF 5600+

Q-TOF mass spectrometer using HPLC-MS/MS. Data analysis was performed with Sirius software version 5.7.0.

2.3. Statistical Analysis

Python was used to calculate and visualize the similarity between the peaks observed in the chromatograms of the soluble and insoluble phases in methanol of the Ayahuasca samples. To do this, the data were organized into a presence/absence matrix of peaks based on their appearance times, structured into a DataFrame using the pandas library, where the appearance time was used as an index.

A similarity matrix between the samples was calculated using the dot product between the columns of the DataFrame, with the diagonal removed to avoid self-comparison. Additionally, the Jaccard coefficient was used to measure the overlap between the samples, and the results were stored in a new matrix for clearer visualization. For a more in-depth analysis, hierarchical clustering was applied using the Ward method, generating a dendrogram that shows the hierarchy of the formed clusters. Finally, the matplotlib and seaborn libraries were used to graphically represent both the similarity matrix and the dendrogram, facilitating the interpretation of the relationships between the samples.

3. Results

Detailed information for each of the collected samples is presented in Table 1. A total of six samples were obtained, all voluntarily donated by religious communities that use the brew in their practices in the states of Goiás (GO), Federal District (DF), Rio de Janeiro (RJ), and Tocantins (TO).

Table 1. Characteristics of the Collected and Identified Ayahuasca Samples. The samples were named "AY" followed by the number assigned at the time of entry into the laboratory.

Sample Name	**AY2**	**AY3**	**AY4**	**AY5**	**AY6**	**AY7**
Ethnotaxa	*Tucunacá*	*Tucunacá*	*Caupuri*	*Ourinho (T)*	*Caupuri*	*Quebrador (C)*

Additional Species	*P. viridis*	*P. viridis*	None	None	*P. viridis*	*P. viridis*
Volume	40	40	25	25	40	40
Color	Café claro	Café claro	Laranja	Amarelo	Café escuro	Café escuro
Origin	Palmas – TO	Palmas - TO	Rio de Janeiro - RJ	Rio de Janeiro - RJ	São Gabriel - GO	Brasília - DF
Voucher	YCS-S s.n (UBw!)	YCS-S s.n (UBw!)	-	-	YCS-S 14 (UB!)	-

An overview of the thin-layer chromatography (TLC) profiles obtained for the methanol-soluble (E2) and methanol-insoluble (E3) fractions is provided in Figure 1. In the E2 fraction, band intensity varies among samples, with AY6 lacking certain bands and AY2 exhibiting distinct fluorescence in its terminal bands. In the E3 fraction, the overall band coloration is generally consistent, except for AY2 and AY3, which display a more intense initial band. These results indicate qualitative differences among the analyzed samples.

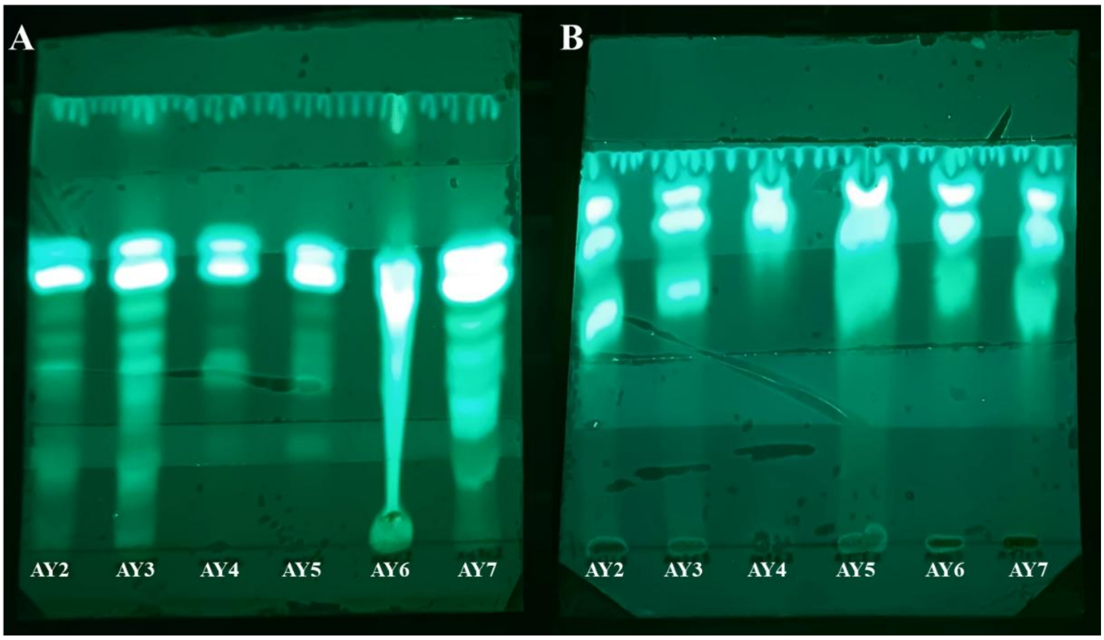

Figure 1. Thin-Layer Chromatography (TLC) of the collected Ayahuasca samples. (A) TLC of the methanol-soluble fractions (E2). (B) TLC of the methanol-insoluble fractions (E3). The samples correspond to the following ethnotaxa: AY2, Tucunacá; AY3, Tucunacá without leaf; AY4, Caupuri; AY5, Ourinho; AY6, Caupuri; and AY7, Quebrador.

The results obtained by HPLC, the chromatograms of the E2 and E3 fractions, are presented in Figure 2. It is observed that the samples share some peaks. However, the presence and absence of peaks over time in each sample are detailed in Table 2, where the value 1 indicates the presence of the peak in the chromatogram, and 0 indicates its absence.

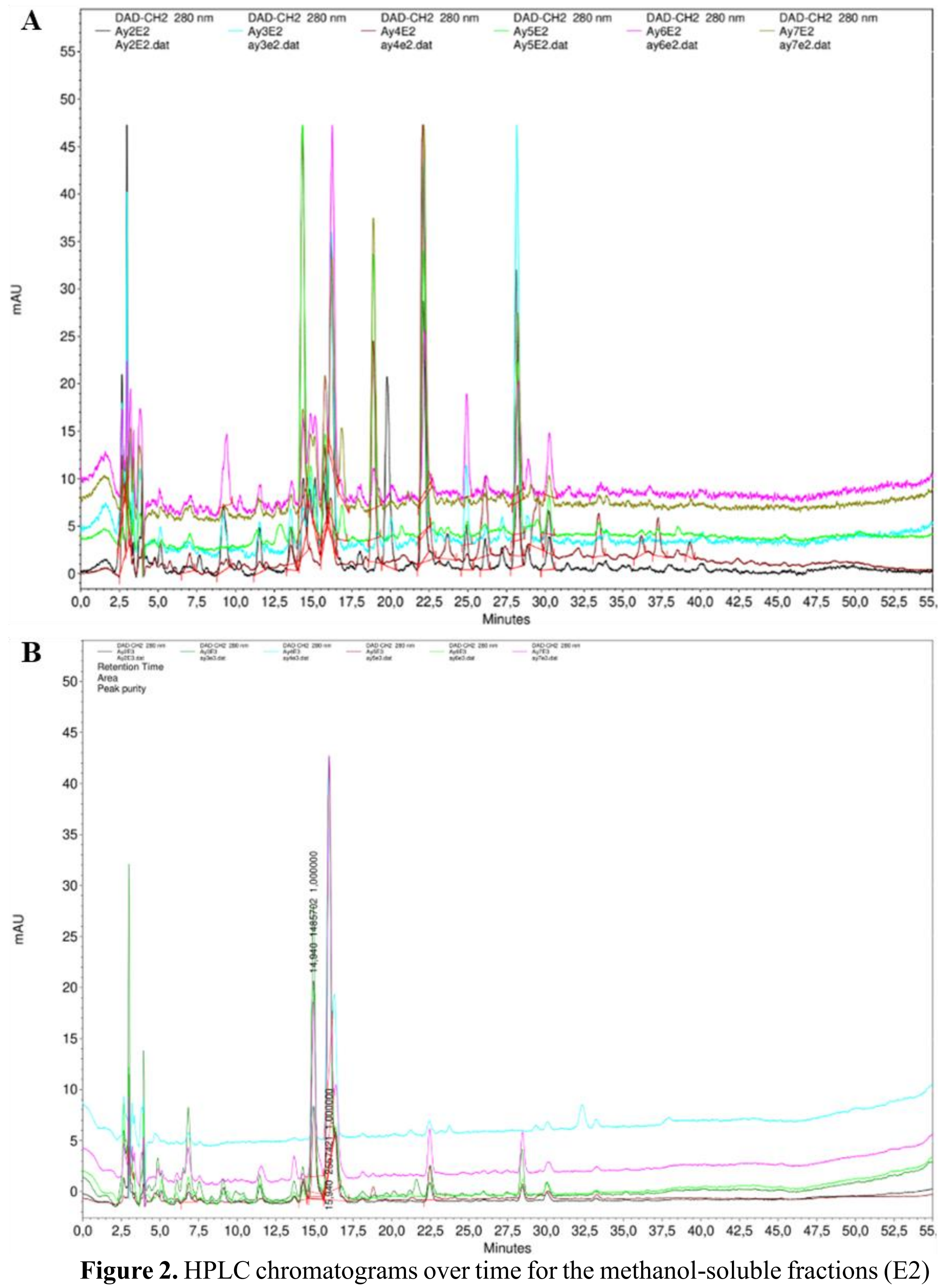

Figure 2. HPLC chromatograms over time for the methanol-soluble fractions (E2) and for the methanol-insoluble fractions (E3) of the samples AY2, AY3, AY4, AY5, AY6, and AY7. The samples correspond to beverages prepared with vines from the following ethnotaxa: AY2, *Tucunacá*; AY3, *Tucunacá only* AY4, *Caupuri*; AY5, *Ourinho*; AY6, *Caupuri*; and AY7, *Quebrador*.

Table 2. Presence and absence of peaks in the chromatograms of the Ayahuasca samples over time, where 1 indicates the presence of a peak and 0 indicates its absence. The samples correspond to beverages prepared with vines from the following ethnotaxa: AY2, *Tucunacá*; AY3, *Tucunacá only*; AY4, *Caupuri*; AY5, *Ourinho*; AY6, *Caupuri*; and AY7, *Quebrador*.

Time (minutes)	AY2	AY3	AY4	AY5	AY6	AY7
2.67	1	0	1	0	0	0
2.99	1	0	0	0	0	0
9.21	1	0	0	0	0	0
14.33	0	0	1	1	0	0
14.94	1	1	1	1	1	1
15.76	0	0	1	1	0	0
15.94	1	1	0	1	1	1
16.17	1	1	0	0	1	1
16.37	1	0	0	0	0	0
18.89	0	0	1	1	0	1
19.79	1	0	0	0	0	0
22.11	1	1	1	1	0	1
22.47	0	0	0	1	0	0
23.67	1	0	0	0	0	0
26.11	0	0	1	0	0	0
28.12	1	1	1	1	0	1

29.52	0	0	1	0	0	0
30.21	0	0	1	0	0	0
33.44	0	0	1	0	0	0
37.28	0	0	1	0	0	0

The Jaccard similarity matrices reveal distinct patterns in the composition of Ayahuasca samples and their methanol-soluble and methanol-insoluble fractions (See Figures 4 and 5). In the general matrix, moderate similarities are observed among some samples, with notably high values between AY3: Tucunacá only and AY7: Quebrador (0.83), suggesting a significant shared composition. In the methanol-soluble fraction, similarities are lower, with values not exceeding 0.75, indicating greater chemical variability in this fraction. The methanol-insoluble fraction exhibits the highest similarities, with values close to 1.0 in several comparisons, suggesting a higher conservation of structural compounds among the samples. These results indicate that the chemical variability among Ayahuasca samples is primarily attributed to the methanol-soluble fraction, while the insoluble fraction maintains a more homogeneous composition.

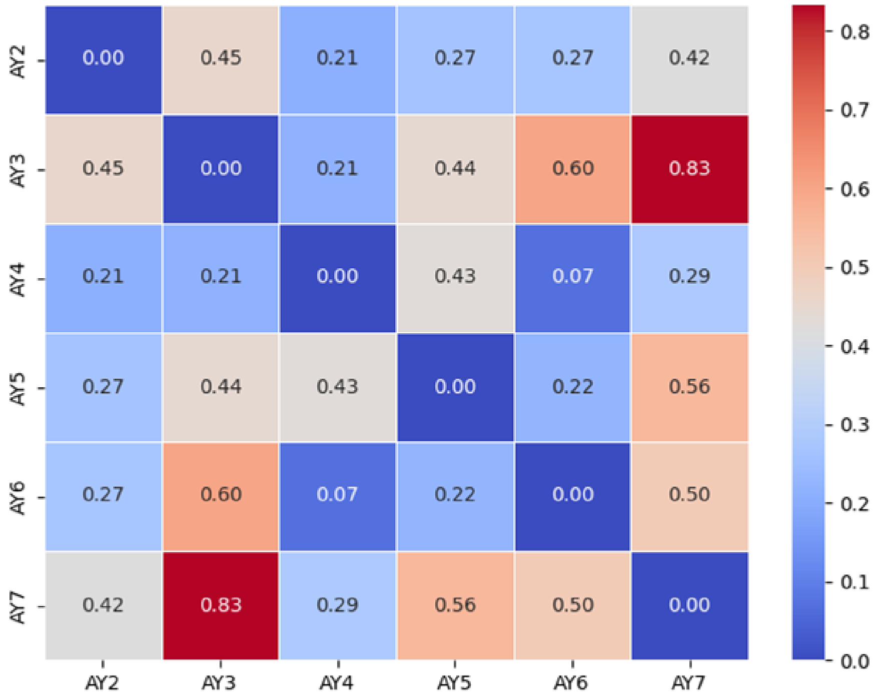

Figure 4. Jaccard matrix pattern for Ayahuasca samples: AY2 (*Tucunacá*), AY3 (*Tucunacá only*), AY4 (*Caupuri*), AY5 (*Ourinho*), AY6 (*Caupuri*), and AY7 (*Quebrador*). The color gradient represents similarity, where red denotes high similarity and blue indicates low similarity. A value of 0 signifies no similarity, while a value of 1 indicates identical samples..

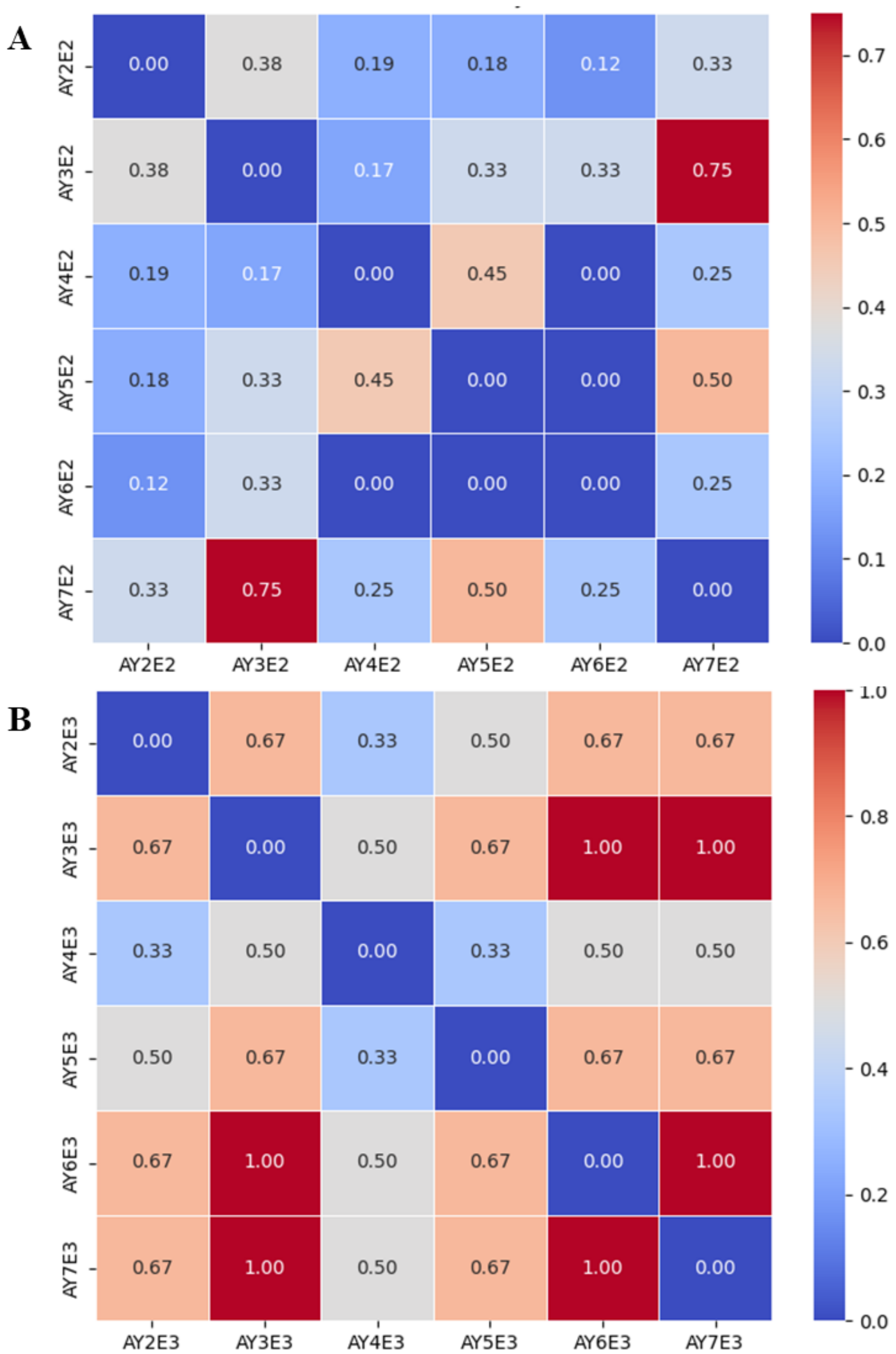

Figure 5. Jaccard Matrix for for the methanol-soluble fractions (E2) and for the methanol-insoluble fractions (E3) of the samples AY2, AY3, AY4, AY5, AY6, and AY7.

The samples correspond to beverages prepared with vines from the following ethnotaxa: AY2 (*Tucunacá*), AY3 (*Tucunacá only*), AY4 (*Caupuri*), AY5 (*Ourinho*), AY6 (*Caupuri*), and AY7 (*Quebrador*). The color gradient represents similarity, where red denotes high similarity and blue indicates low similarity. A value of 0 signifies no similarity, while a value of 1 indicates identical samples.

In the samples analyzed by liquid chromatography coupled to mass spectrometry (HPLC-MS/MS), it was possible to identify chemical compounds traditionally reported in Ayahuasca, including harmine, N,N-dimethyltryptamine, and tetrahydroharmine. In addition, other compounds not previously reported in the literature were also detected (Table 3 and Table 4).

Table 3. List of compounds identified in the methanol-soluble fractions (E2) of the Ayahuasca samples.

Compound	**Sample**
Harmine	*Tucunacá* (AY2), *Tucunacá* (AY3), *Caupuri* (AY4), *Ourinho* (AY5), *Caupuri* (AY6), *Quebrador* (AY7)
N,N-Dimethyltryptamine	*Caupuri* (AY6)
Tetrahydroharmine	*Caupuri* (AY6), *Quebrador* (AY7), *Ourinho* (AY5)
2-((3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl)oxy)-4,5-dihydroxy-6-(hydroxymethyl)oxan-3-yl 2-amino-3-(1H-indol-3-yl)propanoate	*Tucunacá* (AY3)
2-((2-carboxy-6-((2-carboxy-6-((2-carboxy-4,5,6-trihydroxyoxan-3-yl)oxy)-4,5-dihydroxyoxan-3-yl)oxy)-4,5-dihydroxyoxan-3-yl)oxy)-3,4-dihydroxy-3,4-dihydro-	*Tucunacá* (AY3)

2H-pyran-6-carboxylic acid	
3-ethenyl-5-methoxy-2-methyl-1H-indole	*Caupuri* (AY4), *Ourinho* (AY5)
C-glicosiltriptófano (2-amino-3-(1-(3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)-1H-indol-3-yl)propanoic acid)	*Ourinho* (AY5)
GIcA(a1-2)Fuc(a1-3)(GIcA(a1-2))Fuc4Ac(a1-3)Fuc	*Quebrador* (AY7)

Table 4. Compounds identified in the methanol-insoluble fractions (E3) of Ayahuasca samples

Compound	**Sample**
Harmine	Tucunacá (AY3)
2-((3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-y)oxy)-4,5-dihydroxy-6-(hydroxymethyl)oxan-3-yl 2-amino-3(1H-indol-3-yl)propanoate	Tucunacá (AY2)
Ormocarpin	Tucunacá (AY2)
7-o-beta-d-glucopyranosylchamaejasmin	Tucunacá (AY3)
Threonylglycylysine	Ourinho (AY5)
2-((3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl)oxy)-4,5-dihydroxy-6-(hydroxymethyl)oxan-3-yl 2-amino-3-(1H-indol-3-yl)propanoate	Caupuri (AY6)

Tetrahydrorobustaflavone	Caupuri (AY6)
GIcA(a1-2)Fuc(a1-3)(GIcA(a1-2))Fuc4Ac(a1-3)Fuc	Quebrador (AY7)

4. Discussion

The chemical analyses revealed considerable variability among the Ayahuasca samples, particularly in the methanol-soluble fractions (E2). TLC and HPLC profiles indicated qualitative and quantitative differences, with unique peaks and banding patterns distinguishing some ethnotaxa, while the insoluble fractions (E3) showed greater conservation. These findings suggest that the chemical diversity of Ayahuasca is largely driven by metabolites present in the soluble fraction, whereas the insoluble fraction is dominated by more stable structural compounds.

Mass spectrometry confirmed the presence of canonical alkaloids such as harmine, N,N-dimethyltryptamine, and tetrahydroharmine, validating the analytical approach and aligning with previous phytochemical reports. Notably, additional compounds not previously described in the literature were detected, including flavonoids and glycosylated tryptophan derivatives. The presence of these metabolites expands the known chemical diversity of Ayahuasca and points to potential contributions beyond the well-studied β-carbolines and tryptamines.

Overall, the variability observed across ethnotaxa reflects the influence of plant selection and preparation practices on the final chemical composition of the brew. These results underscore the importance of integrating ethnobotanical knowledge with modern analytical methods to fully characterize Ayahuasca and better understand the pharmacological and cultural significance of its diverse chemical profile.

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Author contributions

YCS-S took the lead in writing the original draft, conducting the investigation, and securing funding. OES contributed to the methodology. DA provided the necessary resources. RCO was responsible for the conceptualization, supervision, writing – review & editing, and funding acquisition of the study.

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