Abstract: Ayahuasca is a psychoactive beverage prepared traditionally from a mixture of the leaves and stems of *Psychotria viridis* and *Banisteriopsis caapi*, respectively, being originally consumed by indigenous Amazonian tribes for ritual and medicinal purposes. Over the years, its use has spread to other populations as a means to personal growth and spiritual connection. Also, the recreational use of its isolated compounds has become prominent. The main compounds of this tea-like preparation are N,N-dimethyltryptamine (DMT), β-Carbolines, and harmala alkaloids, such as harmine, tetrahydroharmine, and harmaline. The latter are monoamine-oxidase inhibitors and are responsible for DMT psychoactive and hallucinogenic effects on the central nervous system. Although consumers defend its use, its metabolic effects and those on the central nervous system are not fully understood yet. The majority of studies regarding the effects of this beverage and of its individual compounds are based on in vivo experiments, clinical trials, and even surveys. This paper will not only address the toxicological aspects of the ayahuasca compounds but also perform a comprehensive and critical review on the analytical methods available for their determination in biological and non-biological specimens, with special focus on instrumental developments and sample preparation approaches.

Keywords: ayahuasca; toxicity; analytical determination

1. Introduction

Ayahuasca is an entheogenic beverage that has been consumed for centuries, originally by South American populations, more specifically, by Amazonian indigenous groups, in spiritual rituals and ceremonies in the hope of obtaining further knowledge and divine invigoration [1–3].

The term “Ayahuasca” has a Quechua origin; “aya” means “spirit”, and “waska” means “vine”, that is to say, “vine of the souls” [4]. Although sometimes known as ayahuasca, it can also be referred as hoasca, caapi, daime, yagé, natema and several other local names in Brazil, Bolivia, Equator, and Peru [5]. The word itself can be applied to either the beverage or the vine that is used to produce the beverage [6].
Over time, specific churches devoted to the consumption of ayahuasca were created in order to hold shamanic ceremonies to non-indigenous Amazonian populations. The most dominant are Santo Daime and União do Vegetal (UDV), among others. These religions are a fusion of Christianism, Spiritualism, and other religions with African-Brazilian bases. The diffusion of these religions through Northern America, Europe, and Asia as also led to an increase of ayahuasca consumption, and thus concern for public health, toxicity, and possible therapeutic potential have risen [1,7–9].

The beverage itself is a tea-like/decocion preparation that commonly consists of a brew mixture of *Banisteriopsis caapi* vine and *Psychotria viridis* leaves [10]. Nevertheless, there are analogues which can be used for the same purpose, for example, instead of *P. viridis*, *Psychotria carthagenensis*, *Brugmansia suaveolens*, *Nicotiana tabacum*, *Malouetia tamarquina*, *Tabernaemontana* spp., *Brunfelsia*, spp., *Datura suaveolens*, *lochroma fuchsioides*, *Juanulloa* spp. can be used. [11]. In substitution of *B. caapi*, besides the natural analogue *Peganum harmala* (Syrian Rue), some synthetic compounds can be used, such as harmine freebase/HCl, tetrahydroharmine freebase/HCl, and moclobemide [3,12,13]. In addition, some of the analogues used in the blending of ayahuasca can contain different psychotropic substances, such as nicotine and caffeine [14].

A scientific analysis of the *B. caapi*, a vine from the Malpighiaceae family, showed that it is rich in β-carbolines alkaloids (B-CA), essentially harmine (HMN), tetrahydroharmine (THH), and harmaline (HML) [14,15]. Diversely, *P. viridis* belongs to the Rubiaceous family and contains N,N-dimethyltryptamine (DMT) [5,16]. The chemical structures of ayahuasca compounds are shown in Figure 1.

![Figure 1. Chemical structures of N,N-dimethyltryptamine (DMT), tetrahydroharmine (THH), harmaline (HML), and harmine (HMN).](image)

Depending on the origin and development of the plants used in ayahuasca beverage preparation, the chemical composition may differ, both in quantity and in quality. In addition, the preparation procedure of the beverage, done by religious or non-religious groups, can also impact on ayahuasca’s composition and ultimate effects [17]. Moreover, depending on the dose, the alkaloids present in ayahuasca beverage can also trigger an effect on their own [12].

As previously mentioned, ayahuasca consumption has been increasing and spreading all over the world, in some cases due to its reported health benefits [14,18]. In fact, some studies have described that, following a single dose of the drink, a rapid decrease in depressive symptoms is observed, which can last up to three weeks [19,20]. Other studies revealed that ayahuasca intake can significantly diminish anxiety and panic states [19,21]. In circumstances of drug and alcohol abuse, it has also been stated that the beverage can reduce the abuse of such substances [6,22,23], reduce attention disorders and lack of focus [24], as well as lower physical pain, insomnia, irritability, and obsessive symptoms [25].
Nonetheless, less beneficial and adverse effects have also been described when ayahuasca is consumed, for instance diarrhea, nausea, and vomiting [26] or increases in heart rate, blood pressure, and rectal temperature [27]. Particularly, DMT can also induce psychological disorders, such as emotional suffering, visual hallucinations, and changes of perception, cognition, and affection [26,28]. Moreover, ayahuasca consumption over a large period of time can also lead to episodes of psychosis [26].

Regarding the legal status of ayahuasca, there is some controversy on the matter due to the fact that neither the beverage nor the plants used to prepare it are mentioned in the Convention on Psychotropic Substances of United Nations. Nonetheless, the use of ayahuasca compounds is controlled in some European countries, namely, France, where DMT, THH, HML, and HMN are illegal. In several other European countries, however, only DMT is illegal, according to a review by Horák et al. [29].

2. N,N-Dimethyltryptamine

DMT is a simple molecule of low molecular weight (188.27 g/mol) and hydrophobic character (logP = 2.573) [26]. It is similar to naturally occurring molecules in the body, such as serotonin and melatonin, rapidly crossing the blood–brain barrier [30]. This compound has high affinity for some neuroreceptors, binding to them and triggering very robust responses [26]. DMT is the main psychoactive component of ayahuasca and can be present in a large number of plants [26,31,32]. As previously stated, DMT is more common in the leaves of P. viridis, where the concentration of DMT varies between 0.1% and 0.66% of the leave dry weight, depending also on the plant and the time of the day in which they are collected [33,34]. Generally, each preparation of ayahuasca can contain between 8.8 mg and 42 mg of DMT, causing hallucinogenic effects with doses higher than 0.2 mg/kg [26,33]. Intraperitoneal LD_{50} in rats is reported as 47 mg/kg. In the case of intravenous administration, the LD_{50} in rats is 32 mg/kg, while in humans, it is estimated to be approximately 1.6 mg/Kg [33]. With regard to DMT when ingested through ayahuasca preparations, the LD_{50} estimated is 8 mg/Kg, since not all the amount consumed will be bioavailable [33].

2.1. DMT Pharmacokinetics

The effects of DMT vary greatly depending on how it is administered. Smoking is the preferred route for its recreational consumption, although the intravenous route is widely used [26,35]. When consumed by the latter modality, the psychoactive effects of this substance are rapid, reaching a maximum intensity 5 min after injection and decreasing in the next 30 min [26]. DMT is rapidly metabolized by the enzyme monoamine-oxidase A (MAO-A) present in the liver, the half-life of this substance being approximately 5 to 15 min [36,37]. Due to this rapid metabolization, only about 1.8% of the dose of DMT injected into the bloodstream can be measured. Likewise, only 0.16% of the injected dose is detected in urine [26]. When this substance is smoked or inhaled, identical psychoactive effects manifest [26,33,38]. They begin to manifest immediately, peaking in just a few minutes and disappearing about 30 min later. However, data on the effects of DMT consumed in the smoked form remains scarce [35]. When taken orally, DMT is rapidly degraded by MAO-A present in the intestine and liver, which prevents its access to the bloodstream [38–41]. As previously mentioned, whenever DMT is ingested along with MAO-A inhibitors, such as in the case of ayahuasca tea admixtures, it is able to access the bloodstream and rapidly reach the brain, thus exerting its psychoactive effects [26,33]. The main metabolites identified in this mixture are indoleacetic acid, 2-methyl-1,2,3,4-tetrahydro-β-carboline, DMT-N oxide, N-methyltryptamine, 1,2,3,4-hydro-β-carboline, and tryptamine [42–47].
2.2. DMT Pharmacodynamics

DMT has high affinity for receptors that are part of the serotonergic system. Many of its effects are due to interactions with such receptors [26]. Serotonin 1A (5-HT 1A) receptors are coupled to Gi proteins that mediate inhibitory neurotransmission and are usually expressed in serotonergic neurons as well as in specific cells of the cortical and subcortical regions [48,49]. DMT binds with some affinity to this type of receptors, acting as an agonist [50]. Agonists of these receptors, such as DMT, have been demonstrated to have antidepressant and anxiolytic activity [51,52]. Possibly, these effects are a result of the desensitization of these receptors during chronic consumption of these agonist substances [26]. In contrast, Serotonin 2A (5-HT2A) receptors are the best-characterized receptors to date; they are coupled to the Gq protein and mediate excitatory neurotransmission [26]. DMT leads to the stimulation of this receptor, acting as an agonist [26,44]. According to the literature, the hallucinogenic effects of DMT are due to this agonist effect on 5-HT2A18 receptors [53]. Studies conducted by Aghajanian and collaborators [54,55] showed that DMT is responsible for the stimulation of 5-HT2A receptors, leading to an increased response produced by spontaneous excitatory post-synaptic currents in cortical pyramidal neurons.

In another study, Mckenna and coworkers [56] found that DMT has small methyl groups that are responsible for a high affinity towards 5-HT2A receptors. Over time, this group of receptors maintains its sensitivity to DMT, which may justify the fact that the human body does not develop tolerance to this substance [26]. Another type of serotonergic receptor coupled to Gq proteins is 5-HT2C. DMT has only low affinity for these receptors, acting as a partial agonist [26]. In addition, over time, 5-HT2C receptors lose sensitivity to DMT [53]. The affinity of DMT for other serotonergic receptors, such as 5-HT6, 5-HT7, and 5-HT1D, is also described [50,57,58]. However, further studies are required to understand the possible effects of this affinity. A study by Bunzow and coworkers [59] suggested that DMT also interacts with the trace amine-associated receptor 1 (TAAR1). In this study, they resorted to the HEK293 cell line expressing TAAR1 and demonstrated that DMT activates TAAR1 by increasing cAMP production [59].

DMT has also affinity for the sigma-1 receptor. This affinity is about 100 times less than that for the above-mentioned 5-HT 2A receptors; nevertheless, this substance is one of the few known endogenous agonists of such receptor [60]. Szabo and coworkers [61] have recently shown that DMT triggers a mechanism through the sigma-1 receptor that protects cortical neurons from the effects of oxidative stress. Sigma-1 receptors are also targeted in the treatment of anxiety and depression [62]. Given that DMT produces antidepressant responses, it is possible that this receptor is involved in the mechanism that triggers these effects [26].

The effects of DMT on the cholinergic and dopaminergic systems have also been investigated, but on a smaller scale than those on the serotonergic system. Therefore, the information available is scarce [26]. DMT has a reduced binding affinity for dopamine receptors, compared to other substances [63]. Back in the 1970s, a study by Smith [64] suggested that DMT leads to increased dopamine production. Yet, in a different study by Haubrich and coworkers [65], it was shown that the dopamine levels in rat brains decreased after DMT administration. In the same study, it was also possible to demonstrate that when DMT was administered to rats, acetylcholine levels decreased in the striatum, but no change was observed in the cortex [65]. In a more recent study, where 18 volunteers were administered between 0.6 and 0.85 mg DMT per kg body weight, it was possible to verify that DMT exerts an agonistic effect on dopamine receptors [66].

Also the serotonin transporter (SERT) and the vesicular monoamine transporter (VMAT) are affected by DMT. Sangiah and colleagues [67] examined rat brain slices and found DMT accumulation achieved through an active transport mechanism. In another study by Cozzi and coworkers [68], it was possible to conclude that DMT acts as a substrate for both transporters.
2.3. Adverse Effects of DMT

When DMT is consumed, some physical effects such as nausea, vomiting, and diarrhea are common [26,33]. Also, increased heart rate and blood pressure were observed [27]. A study by Riba and coworkers [69] showed that an oral dose of 1 mg/kg DMT is sufficient for the occurrence of these effects. Otherwise, if DMT is administered intravenously, only doses between 0.1 and 0.2 mg/kg are required to produce these effects. Another study showed that 2 min after intravenous DMT administration, systolic blood pressure increased by about 35 mmHg and diastolic blood pressure increased by about 30 mmHg [27]. In the same study, there was also an increase in heart rate by about 26 beats per minute [27].

Other symptoms commonly manifested when consuming DMT are visual hallucinations and delirium [26,33]. DMT can cause emotional distress and may lead to psychosis or even schizophrenia [26,33]. Studies have shown that in patients with schizophrenia, the amount of DMT in urine and blood is above normal [70,71]. Years later, Ciprian-Ollivier and coworkers [72] hypothesized that DMT would lead to a gradual degradation of the cognitive processes. However, these findings are somewhat controversial, since another study concluded that increased levels of DMT have a calming effect and suppress psychotic activity [73]. Also, a sensation of relaxation caused by DMT has been described in the literature. Gillin and coworkers [74] carried out a clinical trial with intramuscular administration of DMT to healthy patients and reported that a great part of the participants experienced a feeling of relaxation. The same results were obtained in another study by Strassman and Qualls [27] with intravenous administration of DMT to healthy patients. Other studies indicate that the action of DMT on serotonergic neurons results in depression and anxiety [21,75–77]. However, there is, once again, some controversy in these results, since other studies suggest that DMT has anti-depressant properties and enhances a positive mood [18,27,33,74].

Psychedelic substances are commonly associated with dependence, but this does not apply to DMT, considering that, to the present date, there are no reports in the literature that this substance shows withdrawal symptoms when it is not consumed [26,33].

3. β-Carbolines Alkaloids

B-CA derive from tryptophan amino acid, presenting a heterocyclic and dehydrogenated structure. They are synthetized through the condensation of indolamines along with aldehydes or α-keto acids [4,78]. According to Moura and coworkers [79], B-CA have a broad spectrum of action in human organs and can be found not only in animals, but also in many plants and fungi [80]. Besides, they can function as endogenous compounds in some mammalian species [4].

Harmaline was first isolated from the seeds and roots of P. harmala L. (Zygophyllaceae), in which it appeared to be the major compound (5.6% w/w on the seeds). However, this plant also contains HMN, harmalol (HLOL), and THH, mainly in the seeds and roots [81], in quantities varying between 2% and 5% [82].

In 1999, Ott decided to evaluate the combined effects of DMT and HMN on his own body. Therefore, he started by taking 20 mg of DMT and 40 mg of HMN and then increased HMN doses, having discovered that 1.5 mg/kg of this compound exerted effects when combined with DMT, whereas DMT alone had little or no effects [83].
One of the mechanisms of action of B-CA is the reversible inhibition of MAO activity. These enzymes, present in the mitochondria membrane, are of two types: MAO-A and MAO-B. Their main functions are the oxidation of endogenous and exogenous amine substrates such as mono- and polyamines, and the modification of amino acids within proteins [84,85]. They can be found in the brain, kidney, spleen, and intestines [86]. In the brain, MAO-B is more abundant then MAO-A, but both are responsible for the oxidative deamination of several neurotransmitters, such as dopamine, serotonin, and norepinephrine [87,88], although these compounds have been reported to be specific substrates of MAO-A [52].

On the one hand, B-CA can undergo an N-methylation reaction on the second position, and the derived compound is an analogue of the 1-methyl-4-phenylpyridinium ion (MPP+), which can have mitochondrial cytotoxic effects, since its accumulation can lead to oxidative stress, which blocks the complex I of the mitochondrial electrons transport chain [89]. Diversely, some researchers suggested that B-CA has anti-oxidant properties against reactive oxygen species (ROS), possibly reducing cell damage [78].

When comparing the effects of harmine with those of other drugs (Lysergic Acid Diethylamide (LSD) and mescaline) in humans and animals, Naranjo found that harmine oral administration (20–50 mg) produced psychedelic effects beginning after 20–30 min and lasting up to 6–8 h, with the greatest effect after about 30 min to 1 h. The same did not occur when harmine was administered through intramuscular injection (10–20 mg) [90]. In this case, the effects started 5–10 min and lasted up to 3–5 h, reaching a maximum 30 min after administration. Compared to the effects of the other drugs, the effects of harmine were very much similar on a qualitative basis, but with different magnitude [90], so that harmine was described as the most “horrible” and “paranoid” drug, causing mydriasis, salivation, lacrimation, hyperthermia, hyperglycemia, hypotension, and other manifestations. All compounds tested caused aggressive behaviors in both humans and animals, as well as psychedelic and emotional effects [91].

Later studies found that harmaline psychedelics effects were achieved only when intravenous doses were higher than 1 mg/kg and were practically immediate after injection; in contrast, they required oral doses higher than 4 mg/kg, manifesting about one hour after harmaline consumption [91]. Some of the subjective effects reported by the author were nausea and vomiting, different types of vision, feeling of floating in air. Although this study was essential to uncover the potential effects of B-CA, it is important to consider that the method it used was archaic compared to today’s technology and methodology. Despite all existing researches, more studies on B-CA need to be performed to understand in depth its action mechanism and its biochemical effects on the human body.

Since the interest in these compounds is increasing with the increase of ayahuasca consumption and because DMT is responsible for ayahuasca psychedelic effects, publications regarding the pharmacokinetics and dynamics of isolated B-CA remain scarce in the literature. Yet, it is already known that DMT and B-CA behave synergistically.

4. Methods of Quantification of Ayahuasca

As the consumption of ayahuasca tea and similar plants increases, new challenges and the development of new techniques in the forensic and toxicological fields become of utmost importance. One of the objectives of this review is a compilation of various existing and published procedures for ayahuasca quantification. This review reports the analysis on biological specimens such as urine and plasma, after consumption of ayahuasca teas or preparations.
The first work developed for the quantification of DMT and B-CA was performed by Yritia and coworkers. [92]. In this work, DMT present in plasma was extracted using liquid–liquid extraction (LLE) with n-pentane and was quantified using a gas chromatograph coupled to a nitrogen–phosphorus detector. Retentions of 74% and a quantification limit (LOQ) of 1.6 ng/mL were obtained. On the other hand, harmine, harmaline, and tetrahydroharmine, as well as harmine and harmaline C-demethylation metabolites, were quantified in the plasma using high-performance liquid chromatography (HPLC) coupled to a fluorescence detector, having previously performed a solid-phase extraction (SPE). Recoveries above 87% and an LOQ of 0.5 ng/mL for harmine, 0.3 ng/mL for harmaline, 0.3 ng/mL for harmol and harmalol, and 1.0 ng/mL for THH were obtained. In both processes, a good linearity was observed in the concentration ranges evaluated for DMT (2.5–50 ng/mL) and for β-carbolines (0.3–100 ng/mL). Later, in 2008, Pires and coworkers [93] developed a new method for the simultaneous quantification of DMT and B-CA. The alkaloids were extracted by SPE (C18) and quantified by gas chromatography with nitrogen–phosphorus detection. The method performance was linear in the concentration range of 0.02 to 4.0 mg/mL ($r^2 > 0.99$), with LOQ being 0.02 mg/mL. In 2012, Oliveira and coworkers [94] quantified the constituents of ayahuasca (DMT and B-CA) in human plasma. For this purpose, the analytes were extracted by SPE (C18) and quantified using liquid chromatography coupled to mass spectrometry (LC–MS/MS), determining LOQs lower than 0.5 ng/mL for all analytes. The following year, Gaujac and coworkers [3] combined a solid-phase microextraction technique (SPME) in headspace mode with gas chromatography coupled to ion trap mass spectrometry (GC–IT–MS) in order to quantify DMT. The method showed accuracy values between 71% and 109% and good linearity (1.56 to 300 mg/L, $r^2 = 0.9975$). The LOQ was 9.5 mg/L, and the limit of detection (LOD) was 0.78 mg/L. Finally, in 2014, Pichini and coworkers [95] developed a method for the detection of various substances, including DMT, in hair samples, using ultra-high-pressure liquid chromatography–tandem mass spectrometry. Initially, the hair was washed with methyl alcohol and diethyl ether, and internal standards were subsequently added. The samples were then treated with VMA-T M3 reagent (acidic aqueous buffer) for 1 h at 100 °C, and, after cooling, 100 µL of M3 extract was diluted with 400 µL of water, and 10 µL was injected into the apparatus. A reverse-phase column maintained at room temperature was used, and elution was performed in linear gradient with 0.3% formic acid in acetonitrile ammonium formate (5 mM pH 3). The method performance was linear from LOQ of 0.03–0.05 ng/mg up to 10 ng/mg of hair. Recovery was between 79.6% and 97.4%. Table 1 summarizes the methods published about the determination of ayahuasca components in several human biological matrices and plant materials.
Table 1. Bioanalytical procedures for the determination and quantification of the major compounds of ayahuasca in biological samples and ayahuasca teas or preparations.

| Analyte                | Matrix          | Sample Preparation | Detection Mode                  | Stationary and Mobile Phase                        | Recovery (%) | LOD; LOQ             | Concentrations of the Compounds | Reference |
|------------------------|-----------------|--------------------|---------------------------------|---------------------------------------------------|--------------|----------------------|-------------------------------|-----------|
| DMT, HMN, HML, THH     | Plasma          | HPLC–NPD: Protein precipitation; GC–NPD: LLE (n-buthylchloride) | HPLC–FLD (HMN, HML, THH); GC–NPD (DMT) | HPLC–NPD: Supercosil LC-DB-8 (15.0 × 4.6 mm i.d., 5 mm); Mobile Phase: Methanol/acetonitrile/ammonium acetate 0.1M pH = 6.9 (HPLC–FLD); GC–NPD: DB-1 and DB-17 | n.a          | 0.5 ng/mL; 5 ng/mL (DMT); 0.1 ng/mL; 2.00 ng/mL (HMN) | 222.3 ng/mL (HMN); 9.4 ng/mL (HML); 134.5 ng/mL (THH) | [96]      |
| DMT, HMN, HML, THH and HLOL | Plasma | LLE (n-pentane) (DMT); SPE (HMN, HML, THH and THH O-demethylation metabolites) | GC–NPD (DMT); HPLC–FLD (HMN, HML, THH and THH O-demethylation metabolites) | GC–NPD: 5% phenyl-methylsilicone (12 m × 30.2 mm × 0.33µm film thickness) (DMT) HPLC–FLD: Kromasil 100 C18 (5 µm, 150 × 34 mm); Mobile phase: Solvent A: mixture ammonium acetate buffer (50 mM, pH 8.0) (63:37 v/v) and acetonitrile/methanol (20:30 v/v) and Solvent B: mixture of acetonitrile/methanol (20:30 v/v) (HMN, HML and THH); Solvent A: mixture ammonium acetate buffer (50 mM, pH 6.3) (73:27 v/v) and acetonitrile/methanol (20:30 v/v); Solvent B: acetonitrile/methanol (20:30 v/v) (harmol and HLOL) | n.a; 1.6 ng/mL (DMT) | 0.5 ng/mL (HMN), 0.3 ng/mL (HML), 1.0 ng/mL (harmol and harmala) | 0.53 mg/mL (DMT); 0.9 mg/mL (HMN); 0.06 mg/mL (HML); 0.72 mg/mL (THH) | [92]      |
| DMT, THH, HML and HMN  | Plasma          | SPE (C18)           | LC–MS/MS (ESI)                   | Phenomenex Synergi Hydro-RP80A (50 × 2.0 mm, 4 µm); Mobile phase: Solvent A: mixture of aqueous solution of ammonium formate (5 mmol/L) with formic acid (0.1%); Solvent B: methanol and formic acid (0.1%) | 88.4–107.7 | 0.1 ng/mL; 0.2–0.4 ng/mL | 1.2–19.8 ng/mL (DMT); 1.0–15.6 ng/mL (HMN); 2.7–15.7 ng/mL (HML) and 27.1–71.4 ng/mL (THH) | [94]      |
Table 1. Cont.

| Analyte | Matrix | Sample Preparation | Detection Mode | Stationary and Mobile Phase | Recovery (%) | LOD/LOQ | Concentrations of the Compounds | Reference |
|---------|--------|-------------------|---------------|-----------------------------|--------------|---------|--------------------------------|-----------|
| DMT     | Hair   | Hydrolysis (M3 reagent) | UHPLC-MS/MS (ESI) | Acquity UHPLC HSS C18 (2.1 mm × 150 mm, 1.8 µm); Mobile phase: solvent A: formic acid in acetonitrile (0.3%); Solvent B: ammonium formate (5 mM, pH 3) | 79.6–97.4 | 0.01–0.02 ng/mg; 0.03–0.05 ng/mg | 5.6 ng/mg | [95] |
| DMT, THH, HML and HMN | Ayahuasca preparations | SPE (C18) | GC-NPD | HP Ultrasphere C18 (25 m ×0.2 mm × 0.33 µm) and Solvent A: formic acid in acetonitrile (0.3%); solvent B: ammonium formate (5 mM, pH 3) | 68.4–99 | 10000 ng/mL; 20000 ng/mL | 0.31–0.73 mg/mL (DMT); 0.37–0.83 mg/mL (HML); 0.64–1.72 mg/mL (HMN) and 0.21–0.67 mg/mL (THH) | [93] |
| DMT     | Ayahuasca beverages | SPME (polydimethylsiloxane/divinylbenzene fiber) | GC–IT-MS (EI) | Supelco SLB-5 MS (30 m × 0.25 mm, 0.25 mm film thickness) | 71–109 | 780 ng/mL; 950 ng/mL | 0.17–1.14 mg/mL | [3] |
| DMT, THH, HML and HML | Ayahuasca beverages | Dilution with methanol/water (1:1) and direct injection | LC–MS/MS (ESI) | Acquity™ UPLC BEH C18 (50 mm × 2.1 mm, 1.7 µm); Mobile phase: water (90%); solvent B: methanol (10%) | n.a | n.a | 6.4; 210 ng/mL (DMT); n.a; 350 ng/mL (THH); n.a; 100 ng/mL (HMN) and n.a;100 ng/mL (HML) | [97] |
| DMT; HML; HMN | Ayahuasca beverage | LLE (10 mL diethyl ether) | GC–MS (ion trap) (EI) | Chrompack CP–SIL 8CB-MS (30 m × 0.25 mm × 0.25 µm) | n.a | n.a | 0.24 mg/mL (DMT); 0.06 mg/mL (HML); 0.34 mg/mL (HML) | [98] |
| DMT; THH; HML; HLOL, harmol and metabolites | Ayahuasca preparations | Dilution with mobile phase and direct injection | LC–MS/MS (ESI) | Zorbax Eclipse Plus HT C18 (1.8 µm × 4.6 × 50 mm (i.d.)); Mobile phase: Solvent A: formic acid (0.1% in water); Solvent B: Formic acid (0.1% in acetonitrile) | n.a | 6.4; 210 ng/mL (DMT); 0.5; 210 ng/mL (THH); 0.5; 100 ng/mL (HMN); 2.8; 220 ng/mL (HML); 34.3;510 ng/mL (HLOL) | 0.13–3.19 mg/mL (DMT); 1.22–11.90 mg/mL (THH); 0.91–16.14 mg/mL (HML); 0.2186–1.35 mg/mL (HLOL); 0.0026–0.0310 mg/mL (harmol); 0.0009–0.0633 mg/mL (N-methyltryptamine) | [99] |
| Analyte | Sample Preparation | Detection Mode | Stationary and Mobile Phase | Recovery (%) | LOD; LOQ | Concentrations of the Compounds |
|---------|-------------------|----------------|-----------------------------|--------------|----------|--------------------------------|
| DMT; THH; HMN; HML; HLOL; harmol and various metabolites | Urine | Enzymatic hydrolysis (B-glucuronidase/sulfatase) of urine, dilution with mobile phase and direct injection | LC–MS/MS (ESI) | n.a | 0.12; 5.00 ng/mL (DMT); 0.21; 5.00 ng/mL (THH); 0.18; 5.00 ng/mL (HMN); 0.07; 5.00 ng/mL (HML); 0.18; 5.00 ng/mL (HLOL) | 0–0.6 µg/mL (DMT); 0–6.3 µg/mL (THH); 0–0.21 µg/mL (HMN); 0–0.53 µg/mL (HML); 0–14.16 (HLOL); 0.04–126.18 µg/mL (harmol) |
| DMT; THH; HMN; HML | blood | Protein precipitation 96-well plates, dilution with mobile phase and direct injection | LC–MS/MS (ESI) | 60.28–76.31 | 0.45; 1 ng/mL (DMT); 0.36; 1 ng/mL (THH); 0.25; 1 ng/mL (HMN); 0.22; 1 ng/mL (HML); 0.38; 1 ng/mL (HLOL); 0.3; 1 ng/mL (harmol) | 0–15.09 ng/mL (DMT); 0–55.44 ng/mL (THH); 0–5.18 ng/mL (HMN); 0–4.53 ng/mL (HML); 0–3.27 ng/mL (HLOL); 0–5.55 ng/mL (harmol) |
| DMT; THH; HMN; HML | Ayahuasca preparation | direct injection | DART–HRMS | n.a | n.a | n.a |
| DMT; THH; HMN; HML | Leaves of Psychotria viridis | n.a | CE–LIF–MS (ESI) | n.a | n.a | 70 |
| DMT; THH; HMN; HML | Ayahuasca beverage | n.a | NMR | n.a | 12,500; 12,500 ng/mL | 400 µg/mL |
| DMT; THH; HMN; HML; HLOL; harmol and metabolites | Urine | Dilution with mobile phase and direct injection | LC–MS/MS (ESI) | n.a | n.a; 5 ng/mL for all compounds | n.a |

CE: capillary electrophoresis; DART–HRMS: direct analysis in real-time–high-resolution mass spectrometry; DMT: N,N-dimethyltryptamine; EI: electron ionization; ESI: electrospray ionization; GC–FT-MS: Gas chromatography coupled to ion trap mass spectrometry; GC–NPD: Gas chromatography coupled to a Nitrogen–Phosphorous Detector; HESI: heated electrospray ionization; HLOL: Harmalol; HML: Harmaline; HMN: Harmine; HPLC–FLD: High-performance liquid chromatography coupled to fluorescence detection; LC–MS/MS: liquid chromatography coupled to tandem mass spectrometry; LIF: laser-induced fluorescence; LOD: Limit of detection; LOQ: Limit of quantification; n.a: not available; NMR: Nuclear magnetic resonance of proton; SPE: solid-phase extraction; SPME: solid-phase microextraction; THH: tetrahydroharmine; UHPLC–MS/MS: ultra-high-pressure liquid chromatography tandem mass spectrometry; CE–LIF–MS: Capillary electrophoresis-laser induced fluorescence-electrospray ionization-mass spectrometry.
5. In Vivo and In Vitro Studies of Ayahuasca Compounds

For many years, studies on ayahuasca or its chemical compounds have been focused on humans and have been mainly based on inquiries or clinical trials. Therefore, the results obtained are subjective, and little is known about the biochemical and physiological effects. Some of the symptoms reported after constant ayahuasca consumption are feelings of confidence and optimism and, on a psychiatric level, a reduction on the lack of concentration, sleep deprivation, irritability, and depression [25,105].

In a recent study, whose main goal was to understand the possible reproductive effects of the beverage in male Wistar rats [106], researchers used the same dose (1×) and doses 2, 4, and 8 times higher (2×, 4×, 8×) than the dose used in a ritual. The content of the 1× dose was 0.146 mg/mL of DMT, 0.12 mg/mL of harmaline, and 1.56 mg/mL of harmine. It was observed that only the animals exposed to the 8× dose suffered from stress, showing vocalization during the gavage procedure, piloerection, tremors, and weight loss. A decrease of the will to eat was mostly observed in animals treated with the 4× or 8× dose. Indeed, two animals treated with the 8× dose died. They found that such results indicate that exposure to higher doses could be representative of chronic toxicity in rats. Moreover, for the 4× dose, it was observed a reproductive toxic effect, and for the 8× dose, a reduction of the testis size in male rats, without further morphological changes.

In vitro studies concerning the effects or therapeutics potential of ayahuasca and/or its major compounds are scarce. Moreover, to the best of our knowledge, no in vitro study has been done evaluating the effects of traditional ayahuasca (B. caapi + P. viridis) or other plants used as analogues, such as P. harmala or M. tenuiflora. In addition, the published works only focus on one of the compounds and do not report the overall ayahuasca effects. Still, we tried to make a synthetic review of what has been published so far.

In 2010, Samoylenko and coworkers [107] conducted a study with different mammalian cellular lines, including human cancer cell lines (SK-MEL, KB, BT-549, and HepG2), non-human primate kidney fibroblasts (VERO), and pig kidney’s epithelial cells (LLC-PK1). The cytotoxicity at a concentration of 100 µg/mL was tested toward different enzymes, namely, acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and catechol-O-methyl transferase (COMT). No negative effects on SK-MEL, BT-549, and monkey’s VERO cell line were observed [107]. These authors also studied MAO inhibition by HMN and HML, concluding that these compounds may have therapeutic potential in the treatment of Parkinson’s Disease [107].

In a different study using just B. caapi alkaloids, conducted by Morales-García and coworkers, it was found that HMN, THH, and HML can stimulate adult neurogenesis, which is the mechanism that develops new functional neurons from progenitor cells. This was grounded on the fact that these B-CA have an anti-depressant effect and are associated with the capacity of this drugs to stimulate neurogenesis. It was concluded that these chemical substances can promote proliferation, migration, and differentiation of progenitor cells from the sub-ventricular zone to the sub-granular zone, which are the main areas of the brain where neurogenesis happens [14].

6. Conclusions and Further Perspectives

Over the years, the consumption of ayahuasca all over the world has increased not only in shamanic and religious rituals, but also for recreational purposes. Therefore, many psychological studies have been conducted in order to evaluate ayahuasca effects on a mental and subjective level. Given that, clinical and in vivo studies have also increased, evaluating ayahuasca biological effects particularly in the CNS, one of its main targets.

Even though it is known that the components of the beverage, mainly B-CA and DMT, exert their effects by inhibiting MAO-A (B-CA) and through DMT actions after entering the bloodstream, little is known of the effects at the cellular level, and few in vitro studies have been conducted. The ones considered in this review describe positive effects on proliferation and development of new brain cells (neurogenesis). Nonetheless, one may wonder about the cytotoxicity of ayahuasca, not only because the beverage is consumed for long periods of time, as the rituals take place twice a month, but also
because recreational use of this beverage is becoming more prominent in western populations. Thus, further investigation in this sense is needed. The main goal of this article was to review in vitro studies done with these compounds and to try to understand their effects on cells.

It is of outmost importance to have highly sensitive and selective analytical methods in order to identify these compounds in several biological specimens. This is crucial in the clinical field, mainly in the countries that consume ayahuasca regularly, since it can help differentiate ayahuasca compounds intoxication from intoxications due to other drugs of abuse. In the same way, it is important to continue developing studies both in vitro and in vivo to acquire further knowledge on the biological behavior of ayahuasca compounds.

Still, more studies need to be performed to establish whether ayahuasca effects are beneficial or if they can cause severe and irreversible damages after long ayahuasca exposure/intake.

**Author Contributions:** A.Y.S. was responsible for research and writing the manuscript concerning the introduction section, information about the compounds, as well as in vivo and in vitro studies. J.G. was responsible for articles search and writing of the manuscript concerning modes of use, pharmacodynamics, pharmacokinetics, and toxic effects. The authors A.Y.S. and J.G. contributed equally to this paper. A.P.D. was responsible for revising the section of the components of ayahuasca. M.B. was responsible for research, writing the section of analytical methodologies, and revising the manuscript. A.C.C. was responsible for designing the study and revising the section on in vivo and in vitro studies in the manuscript. E.G. was responsible for designing the study, organization, and revising the manuscript.

**Funding:** This work was supported by FEDER funds: POCI-COMPETE 2020-Operational Programme Competitiveness and Internationalization in Axis I–Strengthening Research, Technological Development and Innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT-Fundação para a Ciência e a Tecnologia (UID/Multi/00709/2019). J. Gonçalves acknowledges Program Santander-Totta Universidades in the form of a fellowship (Bolsa BID/UBI-Santander Universidades/2018).

**Conflicts of Interest:** The authors confirm that this article content has no conflict of interest.

**Abbreviations**

| Acronym | Full Form |
|---------|-----------|
| 5-HT receptors | 5-Hydroxytriptamine receptors |
| AChE | acetylcholinesterase |
| B-CA | beta-carbolines alkaloids |
| BT-549 | human breast cancer cell line |
| BuChE | butyrylcholinesterase |
| cAMP | cyclic adenosine monophosphate |
| CE | capillary electrophoresis |
| COMT | catechol-O-methyl transferase |
| CNS | central nervous system |
| DART-HRMS | direct analysis in real-time–high-resolution mass spectrometry |
| DMT | N, N-dimethyltriptamine |
| EI | electron ionization |
| ESI | electrospray ionization |
| GC | gas chromatography |
| GC-IT-MS | gas chromatography coupled to ion trap mass spectrometry |
| GC-MS | gas chromatography coupled to mass spectrometry |
| GC-NPD | gas chromatography coupled to nitrogen phosphorous detector |
| HEK293 | human embryonic kidney 293 cells |
| HepG2 | human liver cancer cell line |
| HESI | heated electrospray ionization |
| HLOL | harmalol |
| HML | harmaline |
| HMN | harmine |
| HPLC | high-performance liquid chromatography |
| HPLC-FLD | high-performance liquid chromatography coupled to fluorescence detector |
| IS | internal standard |
| KB | human HeLa contaminant carcinoma cell line |
LC-MS/MS  liquid chromatography coupled to tandem mass spectrometry
LD50    lethal dose 50
LIF     laser-induced fluorescence
LLC-PK11 pig kidney’s epithelial cells
LLE     liquid–liquid extraction
LOD     limit of detection
LOQ     limit of quantification
LSD     lysergic acid diethylamide
MAO     monoamine oxidase
n.a.    not available
NMR     nuclear magnetic resonance of proton
MS      mass spectrometry
R2/r2   determination coefficient
SERT    serotonin transporter
SK-MEL  human melanoma cell Line
SPE     solid-phase extraction
SPME    solid-phase microextraction
TAAR1   trace amine-associated receptor 1
THH     tetrahydroharmine
UDV     União do Vegetal
UHPLC-MS/MS  ultra-high-pressure liquid chromatography–tandem mass spectrometry
VERO    monkey kidney cell line
VMAT    vesicular monoamine transporter

References

1. Labate, B.C.; Feeney, K. Ayahuasca and the process of regulation in Brazil and internationally: Implications and challenges. *Int. J. Drug Policy* **2012**, *23*, 154–161. [CrossRef] [PubMed]
2. Andrade, T.S.; de Oliveira, R.; da Silva, M.L.; Von Zuben, M.V.; Grisolia, C.K.; Domingues, I.; Caldas, E.D.; Pic-Taylor, A. Exposure to ayahuasca induces developmental and behavioral alterations on early life stages of zebrafish. *Chem. Biol. Interact.* **2018**, *293*, 293–302. [CrossRef] [PubMed]
3. Gaujac, A.; Dempster, N.; Navickiene, S.; Brandt, S.D.; Andrade, J.B. de Determination of N,N-dimethyltryptamine in beverages consumed in religious practices by headspace solid-phase microextraction followed by gas chromatography ion trap mass spectrometry. *Talanta* **2013**, *106*, 394–398. [CrossRef] [PubMed]
4. McKenna, D.J. Clinical investigations of the therapeutic potential of ayahuasca: Rationale and regulatory challenges. *Pharmacol. Ther.* **2004**, *102*, 111–129. [CrossRef]
5. Callaway, J.C.; McKenna, D.J.; Grob, C.S.; Brito, G.S.; Raymon, L.P.; Poland, R.E.; Andrade, E.N.; Andrade, E.O.; Mash, D.C. Pharmacokinetics of Hooasca alkaloids in healthy humans. *J. Ethnopharmacol.* **1999**, *65*, 243–256. [CrossRef]
6. Grob, C.S.; McKenna, D.J.; Callaway, J.C.; Brito, G.S.; Neves, E.S.; Oberlaender, G.; Saide, O.L.; Labigalini, E.; Tacla, C.; Miranda, C.T.; et al. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J. Nerv. Ment. Dis.* **1996**, *184*, 86–94. [CrossRef]
7. Anderson, B.T.; Labate, B.C.; Meyer, M.; Tupper, K.W.; Barbosa, P.C.R.; Grob, C.S.; Dawson, A.; McKenna, D. Statement on ayahuasca. *Int. J. Drug Policy* **2012**, *23*, 173–175. [CrossRef]
8. Malcolm, B.J.; Lee, K.C. Ayahuasca: An ancient sacrament for treatment of contemporary psychiatric illness? *Ment. Health Clin.* **2018**, *7*, 39–45. [CrossRef]
9. Pic-Taylor, A.; da Motta, L.G.; de Morais, J.A.; Junior, W.M.; Santos Ade, F.; Campos, L.A.; Mortari, M.R.; von Zuben, M.V.; Caldas, E.D. Behavioural and neurotoxic effects of ayahuasca infusion (*Banisteriopsis caapi* and *Psychotria viridis*) in female Wistar rat. *Behav. Processes* **2015**, *118*, 102–110. [CrossRef]
10. Dos Santos, R.G.; Bouso, J.C.; Hallak, J.E.C. Ayahuasca, dimethyltryptamine, and psychosis: A systematic review of human studies. *Ther. Adv. Psychopharmacol.* **2017**, *7*, 141–157. [CrossRef]
11. Ramachandran, P.; Zhang, N.; McLaughlin, W.B.; Luo, Y.; Handy, S.; Duke, J.A.; Vasquez, R.; Ottesen, A. Sequencing the vine of the soul: Full Chloroplast genome sequence of *Banisteriopsis caapi*. Genome Announc. 2018, 6, e00203-18. [CrossRef] [PubMed]

12. Frison, G.; Favretto, D.; Zancaano, F.; Fazzin, G.; Ferrara, S.D. A case of beta-carboline alkaloid intoxication following ingestion of *Peganum harmala* seed extract. Forensic Sci. Int. 2008, 179, e37–e43. [CrossRef] [PubMed]

13. Sklerov, J.; Levine, B.; Moore, K.A.; King, T.; Fowler, D. A fatal intoxication following the ingestion of 5-methoxy-N,N-dimethyltryptamine in an ayahuasca preparation. J. Anal. Toxicol. 2005, 29, 838–841. [CrossRef] [PubMed]

14. Morales-Garcia, J.A.; de la Fuente Revenga, M.; Alonso-Gil, S.; Rodriguez-Franco, M.I.; Feilding, A.; Perez-Castillo, A.; Riba, J. The alkaloids of *Banisteriopsis caapi*, the plant source of the Amazonian hallucinogen Ayahuasca, stimulate adult neurogenesis in vitro. Sci. Rep. 2017, 7, 5309. [CrossRef]

15. McKenna, D.J. Ayahuasca: An ethnopharmacologic history. In *Sacred Vine of Spirits: Ayahuasca*; Park Street Press: Rochester, VT, USA, 1999; pp. 40–62.

16. Rivier, L.; Lindgren, J.-E. “Ayahuasca,” the South American hallucinogenic drink: An ethnobotanical and chemical investigation. Econ. Bot. 1972, 26, 101–129. [CrossRef]

17. da Motta, L.G.; de Morais, J.A.; Tavares, A.C.A.M.; Vianna, L.M.S.; Mortari, M.R.; Amorim, R.F.B.; Carvalho, R.R.; Paunggarten, F.J.R.; Pic-Taylor, A.; Caldas, E.D. Maternal and developmental toxicity of the hallucinogenic plant-based beverage ayahuasca in rats. Reprod. Toxicol. 2018, 77, 143–153. [CrossRef]

18. dos Santos, R.G.; Osório, F.L.; Crippa, J.A.S.; Hallak, J.E.C.; dos Santos, R.G.; Osório, F.L.; Crippa, J.A.S.; Hallak, J.E.C. Antidepressive and anxiolytic effects of ayahuasca: A systematic literature review of animal and human studies. Rev. Bras. Psiquiatr. 2016, 38, 65–72. [CrossRef]

19. Osório Fde, L.; Sanches, R.F.; Macedo, L.R.; dos Santos, R.G.; Maia-de-Oliveira, J.P.; Wichert-Ana, L.; de Araujo, D.B.; Riba, J.; Crippa, J.A.; Hallak, J.E.; et al. Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression: A preliminary report. Rev. Bras. Psiquiatr. 2015, 37, 13–20.

20. Nunes, A.A.; dos Santos, R.G.; Osório, F.L.; Sanches, R.F.; Crippa, J.A.S.; Hallak, J.E.C. Effects of Ayahuasca and its Alkaloids on Drug Dependence: A Systematic Literature Review of Quantitative Studies in Animals and Humans. J. Psychoactive Drugs 2016, 48, 195–205. [CrossRef]

21. Thomas, G.; Lucas, P.; Capler, N.; Tupper, K.; Martin, G. Ayahuasca-Assisted Therapy for Addiction: Results from a Preliminary Observational Study in Canada. Curr. Drug Abuse Rev. 2013, 6, 30–42. [CrossRef]

22. Oliveira-Lima, A.J.; Santos, R.; Hollais, A.W.; Gerardi-Junior, C.A.; Baldaia, M.A.; WuO-Silva, R.; Yokoyama, T.S.; Costa, J.L.; Malpezz-Marinho, E.L.A.; Ribeiro-Barbosa, P.C.; et al. Effects of ayahuasca on the development of ethanol-induced behavioral sensitization and on a post-sensitization treatment in mice. Physiol. Behav. 2015, 142, 28–36. [CrossRef] [PubMed]

23. Da Silveira, D.X.; Grob, C.S.; de Rios, M.D.; Lopez, E.; Alonso, L.K.; Tacla, C.; Doering-Silveira, E. Ayahuasca in Adolescence: A Preliminary Psychiatric Assessment. J. Psychoactive Drugs 2005, 37, 129–133. [CrossRef] [PubMed]

24. Barbosa, P.C.R.; Cazorla, I.M.; Giglio, J.S.; Strassman, R. A six-month prospective evaluation of personality traits, psychiatric symptoms and quality of life in ayahuasca-naive subjects. J. Psychoactive Drugs 2009, 41, 205–212. [CrossRef] [PubMed]

25. Cameron, L.P.; Olson, D.E. Dark Classics in Chemical Neuroscience: N,N-Dimethyltryptamine (DMT). ACS Chem. Neurosci. 2018, 9, 2344–2357. [CrossRef] [PubMed]

26. Strassman, R.J.; Qualls, C.R. Dose-response study of N,N-dimethyltryptamine in humans. I. Neuroendocrine, autonomic, and cardiovascular effects. Arch. Gen. Psychiatry 1994, 51, 85–97. [CrossRef] [PubMed]

27. dos Santos, R.G.; Bouso, J.C.; Alcázar-Córcules, M.A.; Hallak, J.E.C. Efficacy, tolerability, and safety of serotonergic psychedelics for the management of mood, anxiety, and substance-use disorders: a systematic review of systematic reviews. Expert Rev. Clin. Pharmacol. 2011, 4, 889–902. [CrossRef]

28. Horáčková, M.; Nováková, P.; Vozárková, W. Legal Aspects of the Ayahuasca Consumption in the European Union. In *Sborník příspěvků z Mezinárodní Vedecké Konference v Rozvoji Společnosti*; Mendel University: Brno, Czech Republic, 2016; pp. 276–283.

29. Pajouhesh, H.; Lenz, G.R. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx* 2005, 2, 541–553. [CrossRef]
30. Ott, J. Pharmacotheon: Entheogenic Drugs, Their Plant Sources and History, 2nd ed.; Natural Products Co.: Kennewick, WA, USA, 1993; ISBN 0961423439.

31. Halpern, J.H. Hallucinogens and dissociative agents naturally growing in the United States. Pharmacol. Ther. 2004, 102, 131–138. [CrossRef]

32. Gable, R.S. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. Addiction 2007, 102, 24–34. [CrossRef]

33. Callaway, J.C.; Brito, G.S.; Neves, E.S. Phytochemical analyses of Banisteriopsis Caapi and Psychotria Viridis. J. Psychoact. Drugs 2005, 37, 145–150. [CrossRef]

34. Cakic, V.; Potkonyak, J.; Marshall, A. Dimethyltryptamine (DMT): Subjective effects and patterns of use among Australian recreational users. Drug Alcohol Depend. 2010, 111, 30–37. [CrossRef] [PubMed]

35. Barker, S.A.; Monti, J.A.; Christian, S.T. N,N-dimethyltryptamine: An endogenous hallucinogen. Int. Rev. Neurobiol. 1981, 22, 83–110. [PubMed]

36. Sitaram, B.R.; Lockett, L.; Talomsin, S.; Blackman, G.L.; McLeod, W.R. In vivo metabolism of 5-methoxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine in the rat. Biochem. Pharmacol. 1987, 36, 1509–1512. [CrossRef]

37. Barbosa, P.C.R.; Mizumoto, S.; Bogenschutz, M.P.; Strassman, R.J. Health status of ayahuasca users. Drug Test. Anal. 2012, 4, 601–609. [CrossRef]

38. Riba, J.; Valle, M.; Urbano, G.; Yritia, M.; Morte, A.; Barbanoj, M.J. Human pharmacology of ayahuasca: Subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J. Pharmacol. Exp. Ther. 2003, 306, 73–83. [CrossRef]

39. Riba, J.; McHenney, E.H.; Valle, M.; Bouso, J.C.; Barker, S.A. Metabolism and disposition of N,N-dimethyltryptamine and harmala alkaloids after oral administration of ayahuasca. Drug Test. Anal. 2012, 4, 610–616. [CrossRef]

40. dos Santos, R.G.; Balthazar, F.M.; Bouso, J.C.; Hallak, J.E. The current state of research on ayahuasca: A systematic review of human studies assessing psychiatric symptoms, neuropsychological functioning, and neuroimaging. J. Psychopharmacol. 2016, 30, 1230–1247. [CrossRef]

41. Helsley, S.; Fiorella, D.; Rabin, R.A.; Winter, J.C. A comparison of N,N-dimethyltryptamine, harmaline, and selected congeners in rats trained with LSD as a discriminative stimulus. Prog. Neuro Psychopharmacol. Biol. Psychiatry 1998, 22, 649–663. [CrossRef]

42. Appel, J.B.; West, W.B.; Rolandi, W.G.; Alici, T.; Pechersky, K. Increasing the selectivity of drug discrimination procedures. Pharmacol. Biochem. Behav. 1999, 64, 353–358. [CrossRef]

43. Smith, R.L.; Canton, H.; Barrett, R.J.; Sanders-Bush, E. Agonist Properties of N,N-dimethyltryptamine at serotonin 5-HT2A and 5-HT2C receptors. Pharmacol. Biochem. Behav. 1998, 61, 323–330. [CrossRef]

44. Catch, M.B.; Rutledge, M.A.; Carbonaro, T.; Forster, M.J. Comparison of the discriminative stimulus effects of dimethyltryptamine with different classes of psychoactive compounds in rats. Psychopharmacology 2009, 204, 715–724. [CrossRef] [PubMed]

45. Cameron, C.; Kelly, S.; Hsieh, S.-C.; Murphy, M.; Chen, L.; Kotb, A.; Peterson, J.; Coyle, D.; Skidmore, B.; Gomes, T.; et al. Triptans in the acute treatment of migraine: A systematic review and network meta-analysis. Headache J. Head Face Pain 2015, 55, 221–235. [CrossRef] [PubMed]

46. Karila, D.; Freret, T.; Bouet, V.; Boulouard, M.; Dallemagne, P.; Rochais, C. Therapeutic potential of 5-HT6 receptor agonists. J. Med. Chem. 2015, 58, 7901–7912. [CrossRef] [PubMed]

47. Pazos, A.; Probst, A.; Palacios, J.M. Serotonin receptors in the human brain—III. Autoradiographic mapping of serotonin-1 receptors. Neuroscience 1987, 21, 97–122. [CrossRef]

48. Sotelo, C.; Cholley, B.; El Mestikawy, S.; Gozlan, H.; Hamon, M. Direct immunohistochemical evidence of the existence of 5-HT1A autoreceptors on serotoninergic neurons in the midbrain raphe nuclei. Eur. J. Neurosci. 1990, 2, 1144–1154. [CrossRef] [PubMed]

49. Keiser, M.J.; Setola, V.; Irwin, J.J.; Laggner, C.; Abbas, A.I.; Hufefisen, S.J.; Jensen, N.H.; Kuijer, M.B.; Matos, R.C.; Tran, T.B.; et al. Predicting new molecular targets for known drugs. Nature 2009, 462, 175–181. [CrossRef]

50. Dos Santos, R.G.; Osório, F.L.; Crippa, J.A.S.; Riba, J.; Zuardi, A.W.; Hallak, J.E.C. Antidepressive, anxiolytic, and antiaddictive effects of ayahuasca, psilocybin and lysergic acid diethylamide (LSD): A systematic review of clinical trials published in the last 25 years. Ther. Adv. Psychopharmacol. 2016, 6, 193–213. [CrossRef]
51. Domínguez-Clavé, E.; Soler, J.; Elices, M.; Pascual, J.C.; Álvarez, E.; de la Fuente Revenga, M.; Friedlander, P.; Feilding, A.; Riba, J. Ayahuasca: Pharmacology, neuroscience and therapeutic potential. *Brain Res. Bull.* **2016**, *126*, 89–101. [CrossRef]

52. Carbonaro, T.M.; Eshleman, A.J.; Forster, M.J.; Cheng, K.; Rice, K.C.; Gatch, M.B. The role of 5-HT2A, 5-HT2C and mGluc2 receptors in the behavioral effects of tryptamine hallucinogens N,N-dimethyltryptamine and N,N-diisopropyltryptamine in rats and mice. *Psychopharmacology* **2015**, *232*, 275–284. [CrossRef]

53. Aghajanian, G.K.; Marek, G. Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology* **1997**, *36*, 589–599. [CrossRef]

54. Aghajanian, G.K.; Marek, G.J. Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. *Brain Res.* **1999**, *825*, 161–171. [CrossRef]

55. Mckenna, D.J.; Repke, D.B.; Lo, L.; Peroutka, S.J. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. *Neuropharmacology* **1990**, *29*, 193–198. [CrossRef]

56. Pierce, P.A.; Peroutka, S.J. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology* **1989**, *97*, 118–122. [CrossRef] [PubMed]

57. Heuring, R.E.; Peroutka, S.J. Characterization of a novel 3H-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.* **1987**, *7*, 894–903. [CrossRef]

58. Bunzow, J.R.; Sonders, M.S.; Arttamangkul, S.; Harrison, L.M.; Zhang, G.; Quigley, D.I.; Darland, T.; Suchland, K.L.; Pasumamula, S.; Kennedy, J.L.; et al. Amphetamine, 3,4-methylenedioxyamphetamine, lsergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Mol. Pharmacol.* **2000**, *60*, 1181–1188. [CrossRef]

59. Saavedra, J.M.; Axelrod, J.; Hajipour, A.R.; Cozzi, N.V.; Jackson, M.B.; Ruoho, A.E. Psychotomimetic N-methylated tryptamines: Formation in brain in vivo and in vitro. *Science* **1972**, *175*, 1366–1368. [CrossRef]

60. Szabo, A.; Kovacs, A.; Riba, J.; Djurovic, S.; Rajnavolgyi, E.; Frecska, E. The endogenous hallucinogen and trace amine N,N-dimethyltryptamine (DMT) displays potent protective effects against hypoxia via sigma-1 receptor activation in human primary iPSC-derived cortical neurons and microglia-like immune cells. *Front. Neurosci.* **2016**, *10*, 423. [CrossRef]

61. Hayashi, T. Sigma-1 receptor: The novel intracellular target of neuropsychotherapeutic drugs. *J. Pharmacol. Sci.* **2015**, *127*, 2–5. [CrossRef]

62. Rickli, A.; Moning, O.D.; Hoener, M.C.; Liechti, M.E. Receptor interaction profiles of novel psychoactive indolealkylamines. *Eur. Neuropsychopharmacol.* **2016**, *26*, 1327–1337. [CrossRef]

63. Smith, T.L. Increased synthesis of striatal dopamine by N,N-dimethyltryptamine. *Life Sci.* **1977**, *21*, 1597–1601. [CrossRef]

64. Haubrich, D.R.; Wang, P.F.L. N,N-dimethyltryptamine lowers rat brain acetylcholine and dopamine. *Brain Res.* **1977**, *131*, 158–161. [CrossRef]

65. Riba, J.; Anderer, P.; Morte, A.; Urbano, G.; Jané, F.; Saletu, B.; Barbanoj, M.J. Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Br. J. Clin. Pharmacol.* **2002**, *53*, 613–628. [CrossRef] [PubMed]

66. Sangiah, S.; Gomez, M.V.; Domino, E.F. Accumulation of N,N-dimethyltryptamine in rat brain cortical slices. *Biol. Psychiatry* **1979**, *14*, 925–936. [PubMed]

67. Cozzi, N.V.; Gopalakrishnan, A.; Anderson, L.L.; Feih, J.T.; Shulgin, A.T.; Daley, P.F.; Ruoho, A.E. Dimethyltryptamine and other hallucinogenic tryptamines exhibit substrate behavior at the serotonin uptake transporter and the vesicle monoamine transporter. *J. Neural Transm.* **2009**, *116*, 1591–1599. [CrossRef] [PubMed]

68. Riba, J.; Rodriguez-Fornells, A.; Urbano, G.; Morte, A.; Antonijoan, R.; Montero, M.; Callaway, J.C.; Barbanoj, M.J. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology* **2001**, *154*, 85–95. [CrossRef] [PubMed]

69. Checkley, S.A.; Murray, R.M.; Oon, M.C.; Rodnight, R.; Birley, J.L. A longitudinal study of urinary excretion of N,N-dimethyltryptamine in psychotic patients. *Br. J. Psychiatry* **1980**, *137*, 236–239. [CrossRef]

70. Lipinski, J.F.; Mandel, L.R.; Ahn, H.S.; Vanden Heuvel, W.J.; Walker, R.W. Blood dimethyltryptamine concentrations in psychotic disorders. *Biol. Psychiatry* **1974**, *9*, 89–91.

71. Ciprian-Ollivier, J.; Cetkovich-Bakmas, M.G. Altered consciousness states and endogenous psychoses: A common molecular pathway? *Schizophr. Res.* **1997**, *28*, 257–265. [CrossRef]

72. Jacob, M.S.; Presti, D.E. Endogenous psychoactive tryptamines reconsidered: An anxiolytic role for dimethyltryptamine. *Med. Hypotheses* **2005**, *64*, 930–937. [CrossRef]
73. Gillin, J.C.; Kaplan, J.; Stillman, R.; Wyatt, R.J. The psychedelic model of schizophrenia: The case of N,N-dimethyltryptamine. *Am. J. Psychiatry* 1976, 133, 203–208.

74. Santos, R.G.; Landeira-Fernandez, J.; Strassman, R.J.; Motta, V.; Cruz, A.P.M. Effects of Ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. *J. Ethnopharmacol.* 2007, 112, 507–513. [CrossRef] [PubMed]

75. Fortunato, J.J.; Réus, G.Z.; Kirsch, T.R.; Stringari, R.B.; Kapczinski, F.; Pinto, J.P.; Hallak, J.E.; Zuardi, A.W.; Crippa, J.A.; et al. Acute harmine administration induces antidepressive-like effects and increases BDNF levels in the rat hippocampus. *Prog. Neuro Psychopharmacol. Biol. Psychiatry* 2009, 33, 1425–1430. [CrossRef] [PubMed]

76. Fortunato, J.J.; Réus, G.Z.; Kirsch, T.R.; Stringari, R.B.; Fries, G.R.; Kapczinski, F.; Hallak, J.E.; Zuardi, A.W.; Crippa, J.A.; Quevedo, J. Effects of β-carboline harmine on behavioral and physiological parameters observed in the chronic mild stress model: Further evidence of antidepressant properties. *Brain Res. Bull.* 2010, 81, 491–496. [CrossRef] [PubMed]

77. De Lima Osório, F.; Ribeiro, L.; De Macedo, H.; Machado De Sousa, J.P.; Pinto, J.P.; Quevedo, J.; Alexandre De Souza Crippa, J.; Hallak, J.E.C. 5. The Therapeutic Potential of Harmine and Ayahuasca in Depression: Evidence From Exploratory Animal and Human Studies; Rafael dos Santos, Ed.; Transworld Research Network: Kerala, India, 2011; ISBN 9788178955261.

78. Kim, H.; Sablin, S.O.; Ramsay, R.R. Inhibition of monoamine oxidase A by β-Carboline derivatives. *Arch. Biochem. Biophys.* 1997, 337, 137–142. [CrossRef] [PubMed]

79. Moura, D.J.; Richter, M.F.; Boeira, J.M.; Pêgas Henriques, J.A.; Saffi, J. Antioxidant properties of beta-carboline alkaloids are related to their antimutagenic and antigenotoxic activities. *Mutagenesis* 2007, 22, 293–302. [CrossRef] [PubMed]

80. McKenna, D.J.; Towers, G.H.N. Biochemistry and pharmacology of tryptamines and β-carbolines A minireview. *J. Psychoactive Drugs* 1984, 16, 347–358. [CrossRef]

81. Moloudizargari, M.; Mikaili, P.; Aghajanshakeri, S.; Asghari, M.; Shayegh, J. Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. *Pharmacogn. Rev.* 2013, 7, 199. [CrossRef]

82. Asgarpanah, J.; Ramezanloo, F. Chemistry, pharmacology and medicinal properties of *Peganum harmala* L. *Afr. J. Pharm. Pharmacol.* 2012, 6, 1573–1580. [CrossRef]

83. Ott, J. Pharmahuasca: Human pharmacology of oral DMT plus harmine. *J. Psychoact. Drugs* 1999, 31, 171–177. [CrossRef]

84. Brierley, D.I.; Davidson, C. Developments in harmine pharmacology—Implications for ayahuasca use and drug-dependence treatment. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 2012, 39, 263–272. [CrossRef] [PubMed]

85. Liester, M.B.; Prickett, J.I. Hypotheses Regarding the mechanisms of Ayahuasca in the treatment of addictions. *J. Psychoact. Drugs* 2012, 44, 200–208. [CrossRef]

86. Gaweska, H.; Fitzpatrick, P.F. Structures and mechanism of the monoamine oxidase family. *Biomol. Concepts* 2011, 2, 365–377. [CrossRef] [PubMed]

87. Orlefors, H.; Sundin, A.; Fasth, K.J.; Oberg, K.; Långström, B.; Eriksson, B.; Bergström, M. Demonstration of high monoaminoxidase-A levels in neuroendocrine gastroenteropancreatic tumors in vitro and in vivo-tumor visualization using positron emission tomography with 11C-harmine. *Nucl. Med. Biol.* 2003, 30, 669–679. [CrossRef]

88. Lewis, A.; Miller, J.H.; Lea, R.A. Monoamine oxidase and tobacco dependence. *Neurotoxicology* 2007, 28, 182–195. [CrossRef] [PubMed]

89. Boulton, S.J.; Keane, P.C.; Morris, C.M.; McNeil, C.J.; Manning, P. Real-time monitoring of superoxide generation and cytotoxicity in neuroblastoma mitochondria induced by 1-trichloromethyl-1,2,3,4-tetrahydro-beta-carboline. *Redox Rep.* 2012, 17, 108–114. [CrossRef] [PubMed]

90. Naranjo, P. Estudio comparativo de la harmina, la dietilamida del acido lisérgico (LSD 25) y la mescalina. *Rev. Confefderación Médica Panam.* 1959, 6, 1–8.

91. Naranjo, C. Psychotropic properties of the harmala alkaloids. In *Ethnopharmacologic Search from Psychoactive Drugs*; Efron, D.H., Holmstedt, B., Kline, N.S., Eds.; United States Department of Health and Human Services: Washington, DC, USA, 1967; pp. 385–391.
92. Yritia, M.; Riba, J.; Ortúñ, J.; Ramirez, A.; Castillo, A.; Alfar, Y.; de la Torre, R.; Barbanoj, M.J. Determination of N,N-dimethyltryptamine and β-carboline alkaloids in human plasma following oral administration of ayahuasca. *J. Chromatogr. B* 2002, 779, 271–281. [CrossRef]
93. Pires, A.P.S.; De Oliveira, C.D.R.; Moura, S.; Dörr, F.A.; Silva, W.A.E.; Yonamine, M. Gas chromatographic analysis of dimethyltryptamine and β-carboline alkaloids in ayahuasca, an amazonian psychoactive plant beverage. *Phytochem. Anal.* 2009, 20, 149–153. [CrossRef]
94. Oliveira, C.D.R.; Okai, G.G.; da Costa, J.L.; de Almeida, R.M.; Oliveira-Silva, D.; Yonamine, M. Determination of dimethyltryptamine and β-carbolines (ayahuasca alkaloids) in plasma samples by LC–MS/MS. *Bioanalysis* 2012, 4, 1731–1738. [CrossRef]
95. Pichini, S.; Marchei, E.; García-Algar, O.; Gomez, A.; Di Giovannandrea, R.; Pacifici, R. Ultra-high-pressure liquid chromatography tandem mass spectrometry determination of hallucinogenic drugs in hair of psychedelic plants and mushrooms consumers. *J. Pharm. Biomed. Anal.* 2014, 100, 284–289. [CrossRef]
96. Callaway, J.C.; Raymon, L.P.; Hearn, W.L.; Mckenna, D.J.; Grob, C.S.; Brito, G.S.; Mash, D.C. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *J. Anal. Toxicol.* 1996, 20, 492–497. [CrossRef] [PubMed]
97. Souza, R.C.Z.; Zandonadi, F.S.; Freitas, D.P.; Tófoli, L.F.F.; Sussulini, A. Validation of an analytical method for the determination of the main ayahuasca active compounds and application to real ayahuasca samples from Brazil. *J. Chromatogr. B* 2019, 1124, 197–203. [CrossRef] [PubMed]
98. Gambelunghe, C.; Aroni, K.; Rossi, R.; Moretti, L.; Bacci, M. Identification of N,N-dimethyltryptamine and β-carbolines in psychotropic ayahuasca beverage. *Biomed. Chromatogr.* 2008, 22, 1056–1059. [CrossRef] [PubMed]
99. McIlhenny, E.H.; Pipkin, K.E.; Standish, L.J.; Wechkin, H.A.; Strassman, R.; Barker, S.A. Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca by liquid chromatography–electrospray ionization-tandem mass spectrometry. *J. Chromatogr. A* 2009, 1216, 8960–8968. [CrossRef]
100. McIlhenny, E.H.; Riba, J.; Barbanoj, M.J.; Strassman, R.; Barker, S.A. Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomed. Chromatogr.* 2011, 25, 970–984. [CrossRef]
101. McIlhenny, E.H.; Riba, J.; Barbanoj, M.J.; Strassman, R.; Barker, S.A. Methodology for determining major constituents of ayahuasca and their metabolites in blood. *Biomed. Chromatogr.* 2012, 26, 301–313. [CrossRef]
102. Lesiak, A.D.; Musah, R.A. Application of ambient ionization high resolution mass spectrometry to determination of the botanical provenance of the constituents of psychoactive drug mixtures. *Forensic Sci. Int.* 2016, 266, 271–280. [CrossRef]
103. Huhn, C.; Neusüß, C.; Polzing, M.; Pyell, U.; Mannhardt, J.; Pütz, M. Capillary electrophoresis-laser induced fluorescence-electrospray ionization-mass spectrometry: A case study. *Electrophoresis* 2005, 26, 1389–1397. [CrossRef]
104. Moura, S.; Carvalho, F.G.; de Oliveira, C.D.R.; Pinto, E.; Yonamine, M. qNMR: An applicable method for the determination of dimethyltryptamine in ayahuasca, a psychoactive plant preparation. *Phytochem. Lett.* 2010, 3, 79–83. [CrossRef]
105. Barbosa, P.C.R.; Giglio, J.S.; Dalgajarrondo, P. Altered states of consciousness and short-term psychological after-effects induced by the first ritual use of ayahuasca in an urban context in Brazil. *J. Psychoactive Drugs* 2005, 37, 193–201. [CrossRef]
106. Santos, A.D.F.A.; Vieira, A.L.S.; Pic-Taylor, A.; Caldas, E.D. Reproductive effects of the psychoactive beverage ayahuasca in male Wistar rats after chronic exposure. *Rev. Bras. Farmacogn.* 2017, 27, 353–360. [CrossRef]
107. Samoylenko, V.; Rahman, M.M.; Tekwani, B.L.; Tripathi, L.M.; Wang, Y-H.; Khan, S.I.; Khan, I.A.; Miller, L.S.; Joshi, V.C.; Muhammad, I. Banisteriopsis caapi, a unique combination of MAO inhibitory and antioxidative constituents for the activities relevant to neurodegenerative disorders and Parkinson’s disease. *J. Ethnopharmacol.* 2010, 127, 357–367. [CrossRef] [PubMed]