Chemical diversity and distribution of Ayahuasca metabolites

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Psychedelics have been used for humanity with religious and medicinal purposes for millennia. In this context, Ayahuasca, a brew utilized by more than seventy indigenous ethnicities concentrated in Amazon region, is prepared essentially from the liana *Banisteriospis caapi* (Mariri), although other plants could be present, such as *Psychotria viridis* (Chacrona), which contains the psychedelic *N*,*N*-dimethyltryptamine (DMT). Regarding the therapeutics of Ayahuasca, clinical trials have been revealed sustained and fast-acting antidepressive and anxiolytic properties in patients with resistant depression, and pre-clinical data indicates neuroprotective and neuroproliferative effects. Despite several phytochemical investigations involving both plants and also the beverage itself, comprehensive analysis using modern analytical methods, such as liquid chromatography-mass spectrometry (LC-MS)-based molecular networking, are still lacking. In this way, the aim of this work was to analyze the chemical content of Ayahuasca components and their distribution in *B. caapi* and *P. viridis* using a LC-MS metabolomic approach employing the Global Natural Products Social Molecular Networking (GNPS) environment, exploring the available spectral libraries and advanced analyses tools. The plant material was collected and registered in the UALF herbarium from Federal University of Alfenas-MG. One Ayahuasca traditional preparation was donated by a Santo Daime Church, and other was made from the collected plant material, based on academic articles and publications. Also, crude hydro-ethanolic extracts were prepared from *B. caapi* vines and from *P. viridis* leaves. All the samples were analyzed in an ultra-performance liquid chromatography system coupled to a quadrupole-Time of Flight mass spectrometer (Waters Xevo™ G2 Qtof) in both positive and negative ionization modes, in MS^e acquisition mode. The LC-MS^e data was processed using open access software (Waters2mzML v1.2.0, and MS-DIAL v4.9), and further analyzed by feature-based molecular networking (FBMN) and substructure topic modeling (MS2 Latent Dirichlet Allocation - MS2LDA) in GNPS environment. Networks were visualized using Cytoscape v3.10.2. The FBMN's revealed 178 and 321 spectral families, respectively, in positive and negative ionization data, with 48 putative annotations (level 2 of confidence accordingly the Metabolomics Standards Initiative) through a semi-automated library spectral matching against the GNPS public spectral libraries. The matches correspond mainly to glycosylated flavonoids, followed by phenylpropanoids, and alkaloids. The MS2LDA analyses revealed several motifs regarding glycosylated flavonols and phenylpropanoids (water, sugar, methyl, and carbon monoxide losses, as well flavonols, tyrosine, and ferulic and cinnamic acids substructures), and indole related substructures, among others. Flavonols are mainly from *P. viridis*, while lignoids and alkaloids come from *B. caapi*. Once synergist action of compounds in natural extracts have great importance in the biological activity profiles, flavonoids may have contribution in the therapeutic effects of Ayahuasca due to their widespread distribution in the brew and known antioxidant and anti-inflammatory properties. This is the first report of the chemical diversity of Ayahuasca by an untargeted approach, and despite the limited annotation, it reveals new insights into the chemical composition of this rediscovered ancestral medicine.

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