Understanding ayahuasca effects in major depressive disorder treatment through *in vitro* metabolomics studies and bioinformatics

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Preparation of extra and intracellular metabolome fractions. From the *in vitro* experiments, 3 mL of secretome (extracellular fraction) were collected, centrifuged under 500 x *g* at room temperature (about 27 ºC) for 5 min, stored and conditioned at -80 °C until the time of metabolomics sample preparation. In parallel, to obtain the content of intracellular metabolites, cell monolayers were washed three times with 5 mL of ice-cold PBS to remove any excess of secretome. Subsequently, a mechanical detachment was performed for removal, followed by chemical rupture of the cell wall in 1.2 mL of ice-cold 80 % (v/v) methanol in PBS solution, to obtain an intracellular metabolite suspension [28]. The intracellular fractions, in methanol solution, were stored and conditioned at -80 °C. For metabolomics analysis, after being defrosted, the secretome samples volumes were reduced to 500 µL by centrifugation in an Amicon® Ultra-2 mL (Millipore) filter, according to the manufacturer specifications [29]. Then, quality control (QC) samples were prepared previously to the protein precipitation. Ice-cold 80 % (v/v) methanol was added to the concentrated secretome, kept at -80 °C, as well as the intracellular fraction, for the protein precipitation step for 24 h. After that period of time, all samples (extra and intracellular metabolite suspensions) were centrifuged at 16,000 x *g* at 4 °C for 15 min, the supernatants filtered in a 0.22 µm syringe filter, and dried in a vacuum concentrator. For each cellular fraction, 12 samples were included in the analysis, where three (3) experimental samples for each group were identified as Aya0, Aya1, Aya10, or Aya20, corresponding to DMT doses in µmol L^{-1}, and five (5) for QC samples, being four (4) QC related to dose-treatments and one (1) to all treatment groups.