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

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**Sample name description**- (Example "E1_Aya0_sec_HILIC_NEG.mzML"). For each cellular fraction and sample nomenclature (general $E_{(1to3)}$ _intra or secretome fraction_Aya(0 to 20) _HILIC_ionization mode); 12 samples were included in the analysis where three (3) experimental samples ($E_{1to3}$, e.g., $E_1$= biological independent experiment one), from intra or secretome cell fraction (compartment), for each group treatment were identified as Aya0, Aya1, Aya10, or Aya20; correspond to DMT dose determinations, and five (5) for quality control (QC) samples, being four (4) QC related to dose-treatments and one (1) to all treatment groups. Observation:(AyaDMSO group was not used in metabolomics identifications)

**Fingerprint and Footprint metabolomics-based MS analysis.** Samples were resuspended in 150 µL of a mixture of $H_2O$: ACN (1:1). Chromatographic separation was performed in an ultra-high performance liquid chromatography system (ACQUITY UPLC™, Waters Corp., Milford, MA, USA). For the separation profile, Acquity BEH Amide column (2.1 mm x 150 mm x 1.7 µm, Waters Corp.) for hydrophilic interaction liquid chromatography (HILIC) was used. The sample injection volume was set to 5 µL.

For the HILIC method, the column temperature was kept at 45 °C. Separation was performed at a flow rate of 0.4 mL $min^{-1}$ under a gradient program in which the mobile phases consisted of: (A) 10 mmol $L^{-1}$ ammonium acetate in acetonitrile: water (95: 5) and (B) 10 mmol $L^{-1}$ ammonium acetate in acetonitrile: water (50:50). The gradient

started with 1 % B for 1 min, increasing to 100% B for 9 min and subsequently returning to 1 % B in 0.1 min. Over the next 3.9 min, the column was re-equilibrated before the next injection. Total execution time was 14 min.

Detection was performed using a XEVO-G2XS quadrupole time-of-flight (QTOF) mass spectrometer (Waters Corp., Manchester, UK) equipped with an electrospray ionization (ESI) source. Data were collected in both positive and negative ionization modes, separately. The acquisition was performed using data-independent analysis ($MS^E$), with the following parameters: capillary voltage +3.5 kV (positive mode) and -2.5 kV (negative mode), sample cone voltage at 40 kV, source temperature at 140 °C, desolvation temperature of 550 °C, desolvation gas flow of 900 L $h^{-1}$, and cone gas flow of 10 L $h^{-1}$ (positive mode) or 50 L $h^{-1}$ (negative mode). MS data were acquired in the centroid mode from *m/z* range of 50–1200 Da, using the acquisition rate of 0.5 s per scan. The $MS^E$ analysis was operated at 20 V for low collision energy and 50 V for high collision energy. During MS analysis, a leucine enkephalin (Waters®, molecular mass = 555.62; 200 pg $\mu L^{-1}$ in 1:1 ACN: $H_2O$) was continuously infused into MS at a flow rate of 30 µL $min^{-1}$ and the ions $[M-H]^-$ = 554.26 and $[M+H]^+$ = 556.27 were used as lock mass for accurate mass measurement. Data acquisition was controlled by MassLynx V4.2 (Waters®). Calibration was performed prior to sample analysis via infusion of 0.5 mmol $L^{-1}$ sodium formate solution, which was used for calibration procedures, both in positive and negative mode analysis. Samples were randomly analyzed and QC samples composed of pooled samples after resuspension were injected every 8 injections.

**Data processing and metabolite identification.** All $MS^E$ datasets were converted from the vendor-specific file format (.raw) into the Analysis Base File format (.abf) using the freely available Reifycs ABF converter (https://www.reifycs.com/AbfConverter/index.html). After conversion, the MS-DIAL software (version 5.0) was used for feature detection, spectral deconvolution, peak identification, and alignment between samples. MassBank databases were used for identification from MS-DIAL metabolomics MSP spectral kit containing ESI-MS and MS/MS (positive; 8,068 and negative; 4,782 compounds). (http://prime.psc.riken.jp/compms/msdial/main.html). The parameters for MS-DIAL were optimized based on their based on developer instructions. The UHPLC-MS raw data were processed with MS-DIAL version 4.8 [30]. Automatic feature detection was performed between 0.3 and 13 min for mass signal extraction between 100 and 1500 Da in positive and negative modes. $MS^1$ and $MS^2$ tolerance were set to 0.01 and 0.4 Da, respectively, in profile mode. For identification, it was used the default of accurate mass tolerance 0.01 Da for $MS^1$ and 0.05 Da for $MS^2$, and identification score cut off 80 %. For

the potential adducts, $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[M+NH_4]^+$, $[2M+H]^+$, $[M+2H]^{2+}$, $[M+2Na]^{2+}$, $[M+2K]^{2+}$, $[M+H+Na]^{2+}$, $[M+H+K]^{2+}$, $[M+H+NH_4]^{2+}$, $[M+2Na-H]^+$, $[M+2K-H]^+$, $[M+H-H_2O]^+$, and $[M+H-2H_2O]^+$ were considered for positively charged adducts, while $[M-H]^-$, $[M+Cl]^-$, $[M-H-H_2O]^-$, $[2M-H]^-$, $[M-2H]^{2-}$, $[M+Na-2H]^-$, $[M+K-2H]^-$, and $[M+FA-H]^-$ were considered for negatively charged. To generate matrix files for downstream statistical analysis, the aligned results were exported by selecting the area raw data matrix, that were restructured by applying a manual exclusion filter for unknown metabolites, and the negative and positive files were combined for statistical analysis in MetaboAnalyst 5.0 [31].