Material and Methods

Study Title: Bioprospection of the aromatic potential of species from the Atlantic Rainforest in São Paulo: occurrence, taxonomy and chemical, genetic and physiological characterization of plant populations

1.1 Biological samples collection and environmental data

Native plants were sampled from nine Atlantic rainforest locations (Adamantina, Campinas, Jundiaí, Mococa, Monte Alegre do Sul, Pariquera-Açú, Ribeirão Preto, Ubatuba, Votuporanga) for botanical identification, herbarium mounts and chemical analyses. Plants were marked and the coordinate reference determined by Global Positioning System (GPS). Botanical names are as in the list of species of Brazilian flora (Jardim Botânico do Rio de Janeiro, 2015). Voucher specimens were deposited at the Herbarium of Instituto Agronômico de Campinas (IAC) (http://herbario.iac.sp.gov.br/). Marked plants were resampled in two subsequent years and those failing to be located or in poor condition were excluded. Environmental data were obtained from meteorological stations at the locations. Monthly values were used to calculate the average seasonal data.

1.2. Essential oil extraction

Leaves were detached from woody stalks and air dried at room temperature, in the absence of direct light. Essentials oils were extracted from 54 to 1870 g of dry material depending on availability, using a Clevenger-type apparatus until total recovery. Oils were transferred to hermetically closed vials, and stored at -20°C. Yield is given by oil weight (g) per plant

material weight (g). Extractions were performed independently for each collection, and average values for two years are presented.

1.3. Chemical characterization and quantification of essential oils

Chemical composition of the essential oils was determined by GC-MS using a Shimadzu QP-5000, equipped with fused silica capillary column OV - 5 (30m x 0.25mm x 0.25μm, Ohio Valley Specialty Chemical, Inc., USA), operating with electron impact (70 eV) and the mass range of 40-450 Da. Helium was used as carrier gas at a constant flow rate of 1.0 mL min^{-1}. The chromatographic conditions were: injector temperature at 220 °C; detector at 230 °C; 1 μL injection volume; split ratio 1:30 and the oven program, 60 ^{0}C increased at 3 ^{0}C min^{-1} to 240 ^{0}C. The chemical components were identified by the comparative analyses of mass spectra against the system database (Nist 62.lib), literature (Adams, 1995; Adams, 2007; Nist WebBook) and retention indices (Adams, 1995; Adams, 2007), obtained from the injection of a mixture of *n*-alcanes (C_9H_{20}–$C_{25}H_{52}$, Sigma Aldrich, 99%) using the equation proposed by Van den Dool and Kratz (1963).

Quantification was performed by the area normalization, as triplicates, by gas chromatography with flame ionization detection (GC–FID) at a Shimadzu equipment, model GC-2010, equipped with fused silica capillary column OV - 5 (30m x 0.25mm x 0.25μm, Ohio Valley Specialty Chemical, Inc., USA), under the previously described operating conditions for GC-MS.

2. References

ADAMS, R.P. *Identification of Essential oil Components by Gas Chromatography/Mass Spectrometry*. 4 ed. Carol Stream, Illinois: Allured Publishing Corporation, 2007. 804p.

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Van den Dool, E.; Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr.*, 11(8), 463-471