Dereplication of Brazilian Plants from Cerrado and Atlantic Forest Using Hyphenated Techniques and NMR

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Introduction
The need for new and innovative analytical methods that may shed information towards complex natural matrices composition is a keystone of bioprospecting programs. Dereplication methodologies associated to state of the art spectroscopic techniques have been successful in the selection of biologically active extracts. Our research group “NuBBE” (Nuclei of Bioassays, Biosynthesis and Ecophysiology of Natural Products), has incorporated the use of high performance liquid chromatography (HPLC) hyphenated with high-resolution mass spectrometry (HRMS) allowing us to analyze plant and endophytic fungi extracts and thus speeding up the selection of biologically active fractions. Innovative spectroscopic techniques using Nuclear Magnetic Resonance (NMR) recently applied on the study of dynamic complex matrices are being integrated with HPLC methods mainly due to their robustness, low sample amounts and spectroscopic versatilities. Prof. Arthur Edison’s group at the University of Florida (UF), USA, is nowadays a reference research center in the development of analytical methods using diverse NMR techniques. The goal of this proposal is to strengthen the collaboration between our research groups, using NMR aiming to increase the understanding of molecular relationships on dynamic natural matrixes and thus, establishing a rational approach for the study of bioactive natural products.

Experimental
Crude extracts from selected Brazilian plant species (A. lusoria, C. estrellensis, P. glabrata, P. sp, A. sp, R. viburnioidea, P. sp, J. sp and V. arenaria) are being analyzed using HPLC/DAD-HRMS and compared with in silico databases aiming to detect know plant metabolites. $^1$H NMR spectra are collected and analyzed according to the protocol established by Zhang and co-workers. 10 μL of the prepared solutions are transferred to a capillary tube (1mm x 10 mm, Norell Inc.) and analyzed using 500 or 600 MHz NMR spectrometers with conventional 5mm or 1mm probes, depending on the complexity of the matrixes and the availability of sample. The data acquisition is performed using the DIPSI-2 sequence and standard $^1$H, $^{13}$C, TOCSY, HSQC, HMBC, NOESY experiments. The data will be processed aiming for the construction of the metabolomic profile of each sample. Separations will be performed on an HPLC (Agillent 1200 Series, Waldbroom, Germany).

Results and Discussion
Three known flavonoids, from A. lusoria, were detected so far using UV and NMR data: 8-methyl-naringenine, 4',5,7-trihydroxy-3',6-dimethoxy-8-methylflavanone, 3',4',5,6,7-pentahydroxy-8-methyldihydroflavonol and the benzophenone bis (2-hydroxy-4,6-dimethoxy-3-methylphenyl) metanone. From C. pinnatifida we were able to detect 4 flavonoids such as 4',5,7-trihydroxy-3-methoxy-6,8-dimethylflavanone, 4',7-dihydroxy-5-methoxy-6-methyldihydroflavonol, 5,6,7-trihydroxy-3',4'-dimethoxy-8-methylflavanone and 3',4'-dimethoxy-5,7-dihydroxy-6,8-dimethyldihydroflavonol.

Conclusions and future perspectives
As an ongoing work we recently received samples from Sacharrum officinarum (Sugarcane). Although partial chemical composition of this important plant has been described in the literature, the metabolomic profile as well as relationships between metabolic variation and ethanol production remain to be verified. We are designing an NMR virtual environment with all described secondary metabolites for S. officinarum in order to compare it with the real extract and thus, attempt to simplify the matrix using NMR interferogram subtraction techniques, TOCSY and DOSY experiments.

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References