

## CONSERVATION AND SUSTAINABLE USE OF CERRADO AND ATLANTIC FOREST DIVERSITY: CHEMICAL DIVERSITY AND POTENTIAL DRUGS PROSPECTION – PHASE II

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The main goal of this thematic project is to search lead molecules from plant species and endophytic fungi occurring in the State of São Paulo, especially in the Cerrado and Atlantic Forest. The project was conceived to identify antioxidant, anti-inflammatory, antifungal, anticancer, antimalarial and acetylcholinesterase inhibition lead compounds from plant species of these biomes. With a strong multi-disciplinary relationship, this is the second phase of the bioprospecting program, initiated four years ago, when Biota-FAPEESP was created as a permanent FAPEESP's Program. Besides the main goals on drug discovery, biodiversity conservation, and sustainable economic growth, this project also is supported on advanced natural product chemistry knowledge such as: i) to evaluate, correlate and develop chemical methodologies to study intra- and inter-specific variability in some selected bioactive species; ii) to develop micropropagation and cell cultures of rare plants producing active compounds; iii) to study biosynthetic pathways and particularly determine the activity of phenyltransferases, terpene cyclases and cytochrome P-450 oxido-reductases for kinetic studies and further proteomic and functional analysis. This should lead to the cloning of genes that eventually can be used for recombinatorial biosynthesis of heterologous overexpression in other plants, to conduct studies on structure activity relationships (SAR) of some lead compounds previously identified and to sustain and maintain the virtual database (data mine) already initiated in phase I.



*Seedlings of Senna spectabilis*

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Around 800 species belonging to 87 families of plants were collected. All stored extracts (ca. 1,700) were subjected to antifungal, antimalarial, antitumoral, and acetylcholinesterase inhibition bioassays aiming to select bioactive extracts/fraction for further guided-fractionation. *In vivo* test for assessment of activity in the central nervous system, have been performed with some pure compounds. From all species assayed, ca. of 5% showed promising activity in one of the targets tested, and were selected for guided-fractionation and detailed evaluation of the toxicological and pharmacological properties. More than 150 compounds of plants and ca. of 100 from endophytic fungi were isolated and tested for confirmation of activity, in the trials already described. Four plant species biosynthesize secondary metabolites with potential for further investment as prototypes. The main goal of this project is looking for bioactive compounds. Two semi-synthetic derivatives of alkaloids isolated from plants were selected as prototypes of acetylcholinesterase inhibitors, and it is in pre-clinical trials aiming to reach the pharmaceutical chain to Alzheimer drugs (national and international patent protected). This pharmaceutical innovation has been licensed for a national pharmaceutical industry, and the patent deposited in USA, Japan, China, and Europe. Two species of plants with potential for cosmetic products have been also licensed, with contract of royalties already defined and agreed upon between the Paulista State University (Unesp), Federal University of Rio de Janeiro (UFRJ) and FAPESP. Two species of plants showed potential as herbal medicines, and toxicology studies are on advanced stage. It is important to emphasize that besides the main goal of this project, which is dedicated in searching for new natural products as lead molecule, the state-of-art of natural product chemistry is being developed taking in account the last advances on phytochemistry and molecular biology. In addition, with the standardized methodology to screen large amount of plant species, our lab gained good experience to carry out dereplication methodology, which is fundamental for the success of bioprospecting. Studies of biosynthesis involving isolation of key enzymes in the metabolic process of pharmacological substances of interest is a gap in the national phytochemical research, and has been achieved with success. The isolation, synthesis and function of biological peptides of plants are unprecedented, and the partial results are very promising.

## MAIN PUBLICATIONS

Newton GC, Rodrigo SC, Pimentel LS, Danuello A, Romeiro NC, Viegas Jr. C, Barreiro EJ, Fraga CAM, Bolzani VS, Rocha MS. 2008. CNS-selective noncompetitive cholinesterase inhibitors derived from the natural piperidine alkaloid(-) - spectaline. *European Journal of Pharmacology*. **580**:339-349.

Bolzani VS, Flausino Jr, Otavio Aparecido, De Avila S, Nunes-Souza. 2007. Anxiolytic effects of new and known erythrinian alkaloids from *erythrina mulungu*. *Journal of Natural Products*. **70**:48-53.

Telascrea M, de Araújo CC, Marques MOM, Moraes PLR, Falcalani R, Cavalheiro AJ. 2007. Essential oil from leaves of *Cryptocarya mandioccana* Meisner (Lauraceae): composition and intraspecific chemical variability. *Biochemical Systematics and Ecology*. **35**:222-232.

Moraes PLR, Nehme CJ, Alves MC, Derbyshire MTVC, Cavalheiro AJ. 2007. Chemical composition of flavonoids and styrylpyrones and the genetic variability of isozymes in natural populations of *Cryptocarya mandioccana* Meisner (Lauraceae). *Biochemical Systematics and Ecology*. **35**(4):233-244.

Navickiene HMD, Miranda JE, Bortoli SA, Kato MJ, Bolzani VS, Furlan M. 2007. Toxicity of extracts and isobutyl amides from *Piper tuberculatum*: potent compounds with potential for the control of the velvetbean caterpillar, *anticarsia gemmatilis*. *Pest Management Science*. **63**:399-403.

Viegas Jr. C, Danuello A, Bolzani VS, Barreiro EJ, Fraga CA. 2007. Molecular hybridization: a useful tool in the desing of new drug prototypes. *Current Medicinal Chemistry (Hilversum)*. **14**:1829-1852.

Lopes AA, Baldoqui DC, Lopez SN, Kato MJ, Bolzani VS, Furlan M. 2007. Biosynthetic origin of the isoprene units of gaudichaudianic acid in *Piper*. *Phytochemistry*. **68**:2053-2058.

Silva VC, Faria AO, Bolzani VS, Lopes MN. 2007. A new ent-kaurane diterpene from stems of *Alibertia macrophylla* F-Schum. (Rubiaceae). *Helvetica Chimica Acta*. **90**:1781-1785.

Araújo AR, Teles HL, Silva GH, Bolzani VS, et al. 2006. Cadinane s sesquiterpenoids of *Phomopsis cassiae*, an endophytic fungus associated with *Cassia spectabilis* (Leguminosae). *Phytochemistry*. **67**:1964-1969.

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