Over Ten Year´s Research with the Amazonian adaptogen plant Saracura-mirá: *Ampelozizyphus amazonicus* Ducke.

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Ampelozizyphus amazonicus Ducke (Rhamnaceae) is an Amazonian medicinal plant popularly known as "saracuramirá" that is found in the Amazon forest territories of Brazil, Venezuela, Colombia, Peru, and Ecuador. In Brazil, it is restricted to the states of Amazonas, Pará, and Roraima and grows mainly in the "terra firme" forests near waterfalls or "igarapés"[1]. An aqueous drink with reported tonic and antimalarial properties can be prepared from the bark and roots of *A. amazonicus*. This drink has a very bitter taste and forms abundant foam when shaken, due to the high saponin content in the species, which gives rise to its other popular name "cervejade-índio"[1]. Our interest in this plant as an adaptogen arose because ethnopharmacological studies indicate both stimulatory and energetic properties for *A. amazonicus*. Therefore, our research group engaged in the study of its immunomodulatory properties, its chemistry and its biotechnological applications. Due to the growing interest in dietary supplements with adaptogen properties and to provide a new functional ingredient, barks from *A. amazonicus* were extracted. The water extract from the barks was spray dried without drying adjuvants, resulting in a powder (SARF), which was characterized by its physicochemical properties and proximate, mineral and saponin contents. The SARF particles tended to have a spherical shape and a unimodal size distribution [3,4]. The particles also had good rehydration characteristics and high saponin content (33%) [4]. The effect of SARF on the immune response was investigated by measuring immunoglobulin production induced by immunization with the antigen TNP-Ficoll in *Plasmodium chabaudi*-infected mice, and also by measuring the levels of anti-ovalbumin, anti-LPS and anti-dextran IgM and IgG antibodies in immunized and unimmunized mice [2,4]. Our data confirmed that SARF possesses immunomodulatory properties, inducing an in vivo modification of the B lymphocyte response and anti-inflammatory properties, which are partly due to a reduction in cell migration and are most likely due to an inhibition of the production of inflammatory mediators [2]. SARF also increased the basal levels of anti-ovalbumin, anti-LPS and anti-dextran IgM antibodies, and the anti-dextran IgG antibodies in unimmunized mice. No increase in antibody titers was observed after SARF treatment in immunized mice [4]. The SARF saponins were isolated into different groups by countercurrent chromatography (CCC) and characterized by off-line ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry (HPLC-$HRMS^n$) analysis [6]. Group 1 presented mainly oleane type saponins, and group 3 showed mainly jujubogenin glycosides, keto-dammarane type triterpene saponins and saponins with C_{31} skeleton. A further purification of group 3 by CCC and HPLC-RI allowed obtaining these unusual aglycones in pure form [6]. More recently, we initiated the study of a water extract from the woods, which are discarded in the traditional process of beverage preparation, but generate saponin-rich extracts as well. A methodology developed to dereplicate aqueous extracts from both bark and wood by high resolution (HRMS) precursor and tandem mass spectrometry (MS/MS) will be discussed. Taken together, these results suggest that SARF could be an interesting new functional ingredient for food applications or pharmaceutical products.

References:

[1] Oliveira, DR; et al. Estudo etnofarmacognóstico da saracuramirá (*Ampelozizyphus amazonicus* Ducke), uma planta medicinal usada por comunidades quilombolas do Município de Oriximiná-PA, Brasil. *Acta Amazonica*, v. 41, p. 383-392, 2011.

[2] Peçanha, LMT; et al.,Immunobiologic and Antiinflammatory Properties of a Bark Extract from *Ampelozizyphus amazonicus* Ducke. *Journal of Biomedicine and Biotechnology*, (2013), 1-11, 2013.

[3] Freitas, D.G C.; et al. Development of an acceptable beverage based on 'Saracura-mirá' plant. In: 10th Pangborn Sensory Science Symposium, 2013, Rio de Janeiro.

[4] Simen, TJM; et al., Spray-dried extract from the Amazonian adaptogenic plant *Ampelozizyphus amazonicus* Ducke (Saracura-mirá): Chemical composition and immunomodulatory properties. *Food Research International*, v. 90, p. 100-110, 2016.

[5] Simen, TJM; Leitao, SG; LEITÃO, GG; Oliveira, DR; Freitas, DGC; Finotelli, PV; Moura, MRL; FERNANDES, PD; Peçanha, LMT; Pierucci, APTR. **Patent**: Registry Number: BR10201402089, Instituto Nacional da Propriedade Industrial, Universidade Federal do Rio de Janeiro, Deposited: 25/08/2014, published in 2016.

[6] Figueiredo F.S.; et al. Countercurrent chromatography separation of saponins by skeleton type from *Ampelozizyphus amazonicus* by off-line ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry analysis and characterization. *Journal of Chromatography A*, v.1481, p. 92-100, 2016.