EVALUATION OF THE CHEMICAL PROFILE OF FRUITS AQUEOUS EXTRACTS OF *Clusia fluminensis* COLLECTED IN DIFFERENT PERIODS

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Introduction

The quality control of natural products is still a great challenge, since the pharmacological action may not be associated with a single component, but rather be the result of a synergistic effect caused by different constituents. The use of fingerprints, which consists of obtaining the chemical profile of a complex sample through chromatographic, electrophoretic or spectroscopic techniques, without a specific target, and which can be performed without prior knowledge of the sample or use of reference standards, has been shown a viable alternative for checking the quality of natural products. Brazil is considered the country with the greatest plant biodiversity in the world and among the native species is *Clusia fluminensis*, whose biological potential to inhibit proteolytic and hemolytic activity of bothropic venom has already been reported [1-3]. The present study aimed to compare the chromatographic fingerprintings of aqueous extracts of *C. fluminensis* obtained from fruits collected in different periods.

Material and Methods

The tests were conducted with lyophilized aqueous extracts of *C. fluminensis fruits*, collected in May and August 2018 (samples ELFr0518 and ELFR0818) and March and June 2019 (samples ELFr0319 and ELFR0619). LC-MS analysis of the extracts was performed on a Dionex Ultimate 3000 chromatograph (UHPLC) coupled to a Q-Exactive Plus high resolution mass spectrometer (Thermo Scientific, USA). Chromatographic separations were obtained using a C18 ZORBAX 1.8 µm 2.1 × 50 mm column (Agilent, USA). The mobile phases were used as follows: phase A water with 0.1% formic acid and 5 mM ammonium formate and phase B methanol with 0.1% formic acid. The total running time was 12 min in a linear gradient. Mass spectra were acquired in positive and negative ionization modes through the electrospray ionization source (HESI).

Results and Discussion

To compare the fingerprints of freeze-dried aqueous extracts of *C. fluminenis* fruits, the samples were submitted to ultra-efficiency liquid chromatography. By visual inspection, the chemical profiles did not show evident differences, suggesting reprodutibility. Signal intensity variations were observed in the ELFR0518 sample. Several factors can affect the content of secondary metabolites in plants and, among them, storage. The ELFr0518 sample came from the first collection performed. The fruits used in the sample preparation was stabilized and, as well as the dry extract, kept in conditions considered adequate for storage. However, the sample was stored for a longer period than the others, which may have influenced the less intense signal (Figure 1). Seasonal variation can also be suggested to explain less intense peaks, since the amount of active constituents is not constant throughout the year and studies reported this influence on *Digitalis obscura* leaves that have the lowest concentrations of cardenolides in spring, accumulation in summer and decrease in autumn [4]. Variations in metabolites content were also observed in *Hypericum perforatum*, where hypericin concentrations increased from about 100 ppm in winter to more than 3000

ppm in summer [5]. A study with plant species sampled in their natural habitat showed qualitative and quantitative constancy of its chemical components during the two years of the study, demonstrating, therefore, that in some cases the secondary metabolism remains stable, corroborating the result found in this work [6].

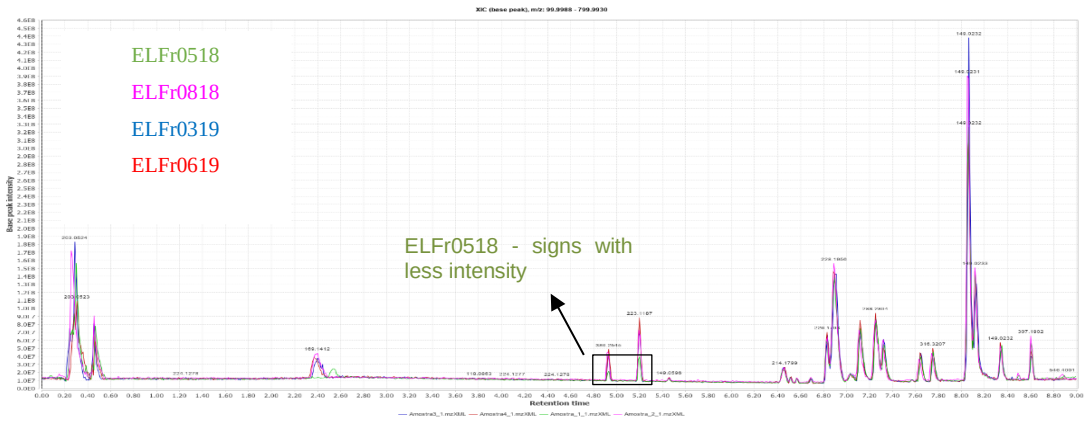

Figure 1- Chromatograms of samples of aqueous extracts of *Clusia fluminensis* fruits (ELFr0518, ELFr0818, ELFr0319 and ELFr0619) by UHPLC-MS. C18 column 2.1 x 50mm ZORBAX 1.8µm. Mobile phase A: water / 0.1% formic acid and 5 mM ammonium formate. Mobile phase B: methanol / 0.1% formic acid. Column and sample chamber 40 °C and 7 °C, respectively.

Conclusion

Fingerprintings comparison is a simple and effective qualitative analytical method that can be used in quality control of *C. fluminensis* fruit extracts. In addition to being an important control tool, it can help identify adulterations and detect the best periods for collection, contributing to obtain active plant inputs with assured therapeutic properties.

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