



**OPTIMIZATION OF AN ECOLOGICAL ANALYTICAL PROCEDURE FOR  
METABOLIC PROFILING *Theobroma grandiflorum* AND *Theobroma cacao***

**Maria Gabriela de Albuquerque Santiago<sup>1\*</sup>**, Danili Teixeira Piedade de Oliveira, Lucas Gomes de Brito<sup>1</sup>, Leonardo de Oliveira Sartori<sup>1</sup>, Martin Kássio Leme da Silva<sup>1</sup>, Cristiano Soleo de Funari<sup>1</sup>

m.santiago@unesp.br

1-GreenBiotech Network, DBB, UNESP, Av. Universitária, 3780, Botucatu, SP, Brazil.

The *Theobroma* spp. is composed of a group of fruit trees native to the Amazon with great economic and cultural relevance. *T. cacao* L. (cocoa) stands out as it is widely cultivated in Brazil and used in the food, cosmetic, and pharmaceutical industries, followed by *T. grandiflorum* (cupuaçu)<sup>[1]</sup>. This work aimed to develop a highly efficient and green analytical procedure for analyzing parts of these species from a multivariate approach. The separation by UHPLC-DAD/UV-MS was optimized from a four-factor Doehlert design, while the extraction was from a two-factor central rotational compound design from a mixture of cupuaçu parts (leaf, husk, seed, and pulp). An optimal separation ( $R^2 = 0.85$ ) was achieved with 5% of 50% MeOH in 20 min, using 0.2% HCOOH in the mobile phase, which resulted in  $80.5 \pm 2.4$  peaks at  $\lambda = 280$  nm. Then, an optimal extraction ( $R^2 = 0.80$ ) was achieved using dynamic maceration with 30% ethanol for 60 min, resulting in  $88.8 \pm 2.8$  peaks at  $\lambda = 280$  nm. The full optimized condition, from sample preparation to separation, was applied to each part of the cupuaçu separately. Leaves had the highest number of peaks ( $85.0 \pm 0.0$ ), followed by husks ( $80.5 \pm 0.7$ ), seeds ( $65.5 \pm 2.1$ ) and pulp ( $42.0 \pm 2.8$ ). For comparison, the developed method was also satisfactorily applied to different parts of cocoa, resulting in  $83.0 \pm 2.8$ ,  $59.5 \pm 2.1$  and  $70.0 \pm 1.4$  in leaves, husks and seeds, respectively. The adopted multivariate approach led to a high level of chemical information for individual parts of both cupuaçu and cocoa, while employing only recommended solvents<sup>[2]</sup>. This globally optimized analytical procedure will be applied for compound annotation using UHPLC-QtoF-MS/MS. The authors would like to thank CNPq (131186/2024-8 and 03675/2021-7) and CAPES (88887.172509/2025-00).

**Keywords:** Green natural products chemistry, design of experiments, metabolomics, green analytical chemistry

[1] Mar, J. M. et al. *Theobroma* Spp.: A Review of It's Chemical and Innovation Potential for the Food Industry. *Food Chem. Adv.* **4**, 100683 (2024)

[2] Funari, C. S. et al. Reaction of the Phytochemistry Community to Green Chemistry: Insights Obtained Since 1990. *J. Nat. Prod.* **86**, 440 (2023)

