Evaluation of the metabolic production of staminate and pistillate individuals of *Clusia lanceolata* (Clusiaceae) occurring in Ombrophilous Forest and Restinga

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Introduction

Clusia lanceolata Cambess., belonging to the Clusiaceae family, is a species native and endemic to Brazil, occurring in Rio de Janeiro and São Paulo. It is a dioecious species, with staminate and pistillate flowers occurring on different individuals. Chemical and chemosystematic studies of the genus *Clusia* highlight the occurrence of terpenes (sesquiterpenes and triterpenes), polyisoprenylated benzophenones and flavonoids[1]. Some of these substances have already been isolated from *C. lanceolata*, such as the terpenes α and β-amyrin[2], the benzophenones weddellianone A and lanceolatone[3], and the flavonoids vitexin, isovitexin, isovitexin-2-O-rhamnoside, vitexin-2-O-rhamnoside, isoorientin and orientin[2]. The extraordinary variety and complexity of secondary metabolites biosynthesized by plants are involved, among other things, in signaling and defense mechanisms. However, the biosynthesis and concentration of these metabolites are affected by various biotic or abiotic factors. Phenolic substances, such as flavonoids, can vary in response to environmental factors such as nutrient availability and light intensity[4]. *Clusia lanceolata* is a species that occurs in Ombrophilous Forest and Restinga regions in the Southeast. The Ombrophilous Forest is located in mountainous elevations with high rainfall rates, providing shadier environments. The Restinga is located along the coastal sea plains and is characterized by a brighter and drier environment. In this way, the same species can have different metabolic production depending on where it is located. Considering that many secondary metabolites are related to the therapeutic effects attributed to plants and that these show considerable variability, their evaluation is necessary.

Material and Methods

Leaves of staminate specimens of *C. lanceolata* were collected at Private Natural Heritage Reserve, Vale do Paraíso, Lumiar, Nova Friburgo, Rio de Janeiro. A second collection of leaves from staminate and pistillate specimens was made at the Restinga de Barra de Maricá APA, Rio de Janeiro. A voucher of each specimen was deposited in the Niterói Herbarium (NIT), at the Fluminense Federal University. The leaves were dried, reduced to fragments and then extracted by static maceration with ethanol. The solvent was reduced in a rotary evaporator to obtain the crude extracts. The extracts were then solubilized in methanol (1mg/mL) and analysed by High Performance Liquid Chromatography (HPLC) using a Shimadzu Vp Series Quaternary HPLC System chromatograph with ultraviolet detector, equipped with Lab Solution software, Neumann-Neader C18 column (250 mm x 4.6 mm, 5µm). The mobile phase consisted of solvent A (water: 0.1% formic acid) and solvent B (methanol). Elution was carried out at a flow rate of 0.6 mL/min with a gradient program: 35-80% B (0-30 min); 80-100% B (30-32 min); 100% B (32-42 min); 100-35% B (42-45 min); 35% B (45-55 min). The injection volume of the extracts was 20µL and the chromatograms were obtained at 350 nm, a selective wavelength for the flavonoid class. The flavonoid standards isovitexin (Sigma®), vitexin-2-O-rhamnoside (Sigma®) and isoorientin (HWI group®) were also analyzed under the same chromatographic conditions, at concentrations of 0.5mg/mL and 20µg/mL with an injection volume of 1µL.

Results and Discussion

The analyses by High Performance Liquid Chromatography using ultraviolet radiation detection (HPLC/UV) showed some similarities between the staminate individuals from the Ombrophilous Forest and the staminate and pistillate individuals from the Restinga, in terms of metabolic production. No qualitative differences were observed in the chemical profile between staminate and pistillate individuals from the same location. The flavonoid standards isovitexin, vitexin-2-O-rhamnoside and isoorientin had retention times of 21.0, 19.5 and 17.6 minutes, respectively. Signals with the same retention time were observed in the staminate leaves of *C. lanceolata* from the Ombrophilous Forest and in the staminate and pistillate leaves of *C. lanceolata* from the Restinga, indicating the presence of these flavonoids in the extracts from these individuals, corroborating other studies in the literature. However, there is an indication of a quantitative difference in these flavonoids between the individuals occurring in the Ombrophilous Forest and the Restinga, evidenced by a considerable variation in the intensity/area of the chromatographic signal. The staminate and pistillate leaves from the Restinga specimens showed more intense signals than the staminate leaves from the Ombrophilous Forest, which suggests a higher concentration of isovitexin, vitexin-2-O-rhamnoside and isoorientin in the individuals from the Restinga. This behavior may be associated with the fact that flavonoids are substances that have an effective protective action against oxidative processes and can act as photoprotectors, having their biosynthesis stimulated due to increased exposure to radiation, especially UV radiation[5]. Restinga plants are found in an environment with a higher incidence of sunlight, compared to Ombrophilous Forest plants, where the vegetation is denser, with more shaded environments.

Conclusion

The presence of the flavonoids isovitexin, vitexin-2-O-rhamnoside and isoorientin was verified in staminate and pistillate individuals of *C. lanceolata* found in Ombrophilous Forest and Restinga. While metabolic production between pistillate and staminate individuals occurring in the Restinga shows qualitative similarity, it was observed that staminate individuals occurring in different localities show qualitative and quantitative variation, suggesting the influence of abiotic factors on the metabolic production of these individuals.

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Bibliographic References

[1] Virginio, M. C. A. S. Chemosystematic study of the genus *Clusia* L. and chemical-biological prospection of *Clusia fluminensis* Planch. & Triana and *Clusia lanceolata* Cambess. (Clusiaceae). Thesis (PhD in Natural Products Chemistry) - Federal University of Rio de Janeiro, Rio de Janeiro. 2015, p. 27-41.

[2] Ferreira, R. O., Carvalho Junior, A. R. D., Riger, C. J., Castro, R. N., Silva, T., & Carvalho, M. G. D. Chemical constituents and in vivo antioxidant activity of flavonoids isolated of *Clusia lanceolata* (Clusiaceae). Química Nova, 2016, 39, p. 1093-1097.

[3] Porto, A. L., Machado, S. M., de Oliveira, C. M., Bittrich, V., Maria do Carmo, E. A., & Marsaioli, A. J. Polyisoprenylated benzophenones from *Clusia* floral resins. Phytochemistry, 2000, 55(7), p. 755-768.

[4] Verma, N., & Shukla, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. Journal of Applied Research on Medicinal and Aromatic Plants, 2015, 2(4), pp 105-113.

[5] Liu, Y., Fang, S., Yang, W., Shang, X., & Fu, X. Light quality affects flavonoid production and related gene expression in *Cyclocarya paliurus*. Journal of Photochemistry and Photobiology B: Biology, 2018, 179, p. 66-73.