



HPLC-DAD METHOD VALIDATION FOR AMENTOFLAVONE AND BRASIMARIN B IN CALOPHYLLUM BRASILIENSE ETHANOLIC EXTRACT.

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INTRODUCTION

Calophyllum brasiliense Cambess., a native Brazilian plant, possesses extracts rich in bioactive compounds. Amentoflavone (3',8''-biapigenin; CAS 1617-53-4), a biflavonoid, and brasimarin B (CAS 514214-46-1), a coumarin, exhibit pharmacological potential. Validated analytical methods for quantifying these compounds in plant extracts are crucial for quality control, standardization, and further research. This study validated a High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) method for amentoflavone and brasimarin B in 90% ethanolic extract of *C. brasiliense*, aiming at their quantification in extractive solutions and dry extract.

MATERIAL AND METHODS

In the method validation, the parameters of linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) were evaluated. Calibration curves were prepared with standard solutions in methanol, prepared in triplicate, at concentrations of 2, 4.66, 20, 50, 100, 200, and 1000 µg/mL for amentoflavone and 1.33, 3.33, 6.66, 33.33, 166.66, 333.33, and 1000 µg/mL for brasimarin B. Each concentration was injected three times. HPLC-DAD analyses were performed at 280 nm.

RESULTS

The method demonstrated adequate linearity ($R^2 > 0.99$), precision within the acceptable range, as well as accuracy and recovery. The LOD was 2.00 and 1.33 µg/mL, and the LOQ was 6.00 and 4.02 µg/mL for amentoflavone and brasimarin B. The equations for the curves were: $y = 21890x + 32221$, $R^2 = 0.9996$ (amentoflavone) and $y = 22888x + 528550$, $R^2 = 0.9967$ (brasimarin B). The method quantified amentoflavone and brasimarin B in the extractive solution, under optimized conditions, and in the dry extract of *C. brasiliense*.

CONCLUSIONS

The validated HPLC-DAD method demonstrated efficiency and reliability for the quantification of amentoflavone and brasimarin B in extracts of *Calophyllum brasiliense*, serving as a useful tool for quality control and for future research on the species..

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