Anti-inflammatory Bioactive Markers of Ocotea (Lauraceae) Uncovered Through Concatenated UPLC/MS-NMR Metabolomics

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The Lauraceae family is well-known for its chemical diversity, traditional medicinal applications, and various biological activities. Within this family, the genus *Ocotea* has demonstrated promising anti-inflammatory properties. Inflammation plays a role in the pathophysiology of several diseases and involves complex biochemical pathways, including the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. These pathways are essential for the production of pro-inflammatory mediators, such as prostaglandin E2 (PGE2) and leukotriene B4 (LTB4). Concomitant dual inhibition of these pathways is a target mechanism of action for new anti-inflammatory drugs as may lead to fewer adverse drug reactions and possible higher efficacy. Thus, this study aimed to annotate biomarkers for inhibition of both COX and LOX pathways in *Ocotea* species . The research utilized concatenated metabolomics approaches to uncover these biomarkers and provided deeper chemical insights through complementary analytical techniques. Sixteen species from the *Ocotea* genus were selected for investigation. PGE2 and LTB4 were quantified using ultra-performance liquid chromatography in tandem mass spectrometry (UPLC-MS/MS) in an *ex-vivo* anti-inflammatory assay. The analyses were performed with a system (Shimadzu®) equipped with a triple-quadrupole mass analyzer operating in negative mode. The *Ocotea* extracts were analyzed using an ultra-performance liquid chromatography system coupled with a high resolution mass spectrometry (UPLC-HRMS) in both positive and negative ionization modes, under MS^e acquisition (Waters Xevo™ G2 Qtof). Likewise, ^{1}H-NMR (500 MHz) spectra were recorded using a Bruker-Biospin Ascend 500 (Avance III series). The output data from ^{1}H-NMR and UPLC-HRMS data processing were separately analysed and concatenated. To fuse disparate data types, block scaling was employed to prevent dominance due to differences in scale or variance. UPLC/MS data was defined as block 1 and ^{1}H-NMR data as block 2. The study utilized unsupervised and supervised multivariate statistical analysis, including statistical total correlation spectroscopy (STOCSY) for NMR and gas-phase molecular networking-based fragmentation reactions for UPLC/MS. Nine *Ocotea* species demonstrated the ability to inhibit both COX and LOX pathways, suggesting the presence of dual-action bioactive markers. The combined use of UPLC/MS-NMR data and multivariate statistical techniques, such as orthogonal partial least square (OPLS-DA) pointed key discriminant bioactive markers, including aporphine alkaloids and glycosylated flavonoids. These specialized metabolites were annotated with high confidence alongside their fragmentation pattern, indicating their potential role in the dual inhibition of PGE2 and LTB4 release. STOCSY correlation analysis further clarified the chemical shifts correlated with these metabolites, enhancing annotation accuracy. The results from each technique corroborated and

validated each other, increasing confidence in the annotation of the bioactive markers. This research supports the ethnopharmacological and anti-inflammatory potential of *Ocotea* species and underscores the effectiveness of concatenated UPLC/MS-NMR metabolomics approaches in Natural Product research.

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