ULST Timisoara
Multidisciplinary Conference on Sustainable Development
15-16 May 2025

OPTIMIZATION OF EXTRACTION FROM AMBROSIA ARTEMISIIFOLIA OF SOME SESQUITERPENE DERIVATIVES FOR UPLC ANALYSIS

Constantina -Bianca Vulpe[1], Nicoleta Ianovici[2], Vasile Ostafe[2]

[1] *West University of Timisoara, Department of Biology, Institute for Advanced Environmental Research, West University of Timisoara, Oituz 4C, 300086, Timisoara, Romania*

[2] *West University of Timisoara, Faculty of Chemistry, Biology, Geography, Department of Biology, Pestalozzi 16, Timisoara, 300115, Romania*

Abstract: *A two-step UPLC-MS method was developed for the separation and identification of five compounds — germacrene D, spathulenol, longipinanol, ambrosin, and cumanin — extracted from Ambrosia artemisiifolia, even in the absence of analytical standards. In the first step, the MassLynx software was employed to generate isotopic model spectra, allowing identification of the target compounds based on the recorded spectra during chromatographic separation. In the second step, the retention times of the eluted peaks were correlated with the logP values of the target compounds, providing an additional layer of identification. Another key objective of this study was to optimize the solid–liquid extraction process of the target compounds from different plant organs (root, stem, leaf). To achieve this, a full factorial experimental design was generated using Minitab software, minimizing the number of experiments while maximizing the information obtained. By evaluating factors such as the organ origin of the plant material, extraction time, and the hydrophobicity and volume of the extraction solvent, the number of experiments was effectively reduced to 36. The results highlighted that solvent hydrophobicity and solvent volume significantly influenced the extraction yield. This method provides an efficient and reliable strategy for both the identificationand extraction optimization of compounds from A. artemisiifolia.*

• Introduction

Ambrosia species, including *A. artemisiifolia*, are rapidly spreading across Europe and Romania, producing highly allergenic pollen and causing significant health, ecological, and agricultural concerns. Despite their notoriety, *Ambrosia* plants are traditionally used in herbal medicine, owing to the presence of sesquiterpenes—compounds with antimicrobial, anti-inflammatory, and antitumor effects. This study optimized the extraction of five sesquiterpene derivatives from *A. artemisiifolia* using a factorial design and MS-based identification, aiming to support their potential pharmaceutical applications.

• Material and method

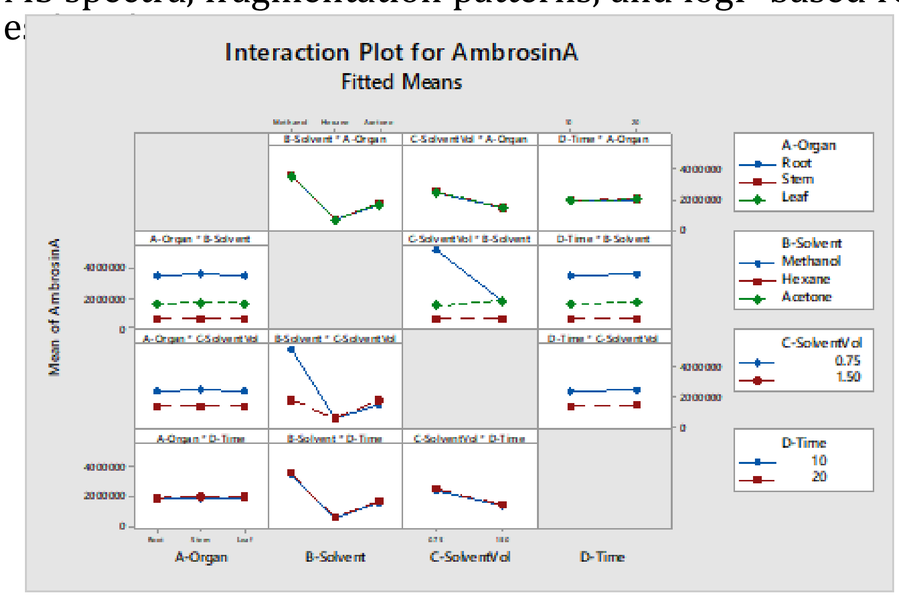
Sample Collection and Preparation:Entire Ambrosia artemisiifolia plants were collected in Timisoara (October 2013), air-dried, and separated by organ (root, stem, leaf). The materials were stored at 4°C until extraction.

Extraction Procedure:Dried plant parts were extracted with methanol, hexane, or acetone under different solvent volumes and extraction times. The design of experiments approach (using Minitab software) evaluated the impact of organ type, solvent, volume, and time on sesquiterpene extraction efficiency.

Sample Processing:Extracts were centrifuged, evaporated under nitrogen, redissolved in 50% methanol with formic acid, and filtered prior to analysis. The method ensured consistency and minimized compound degradation.

Analytical Method (UPLC-MS):Compounds were separated and identified on a Waters Acquity UPLC-MS system using gradient elution. Without standards, identification relied on MS spectra, fragmentation patterns, and logP-based retention e

• Results and discussions



Despite the complexity of the factorial design, extraction yield was primarily influenced by solvent type, volume, and their interaction—less polar solvents (higher logP) like hexane required smaller volumes than polar solvents like methanol.

• Results and discussions

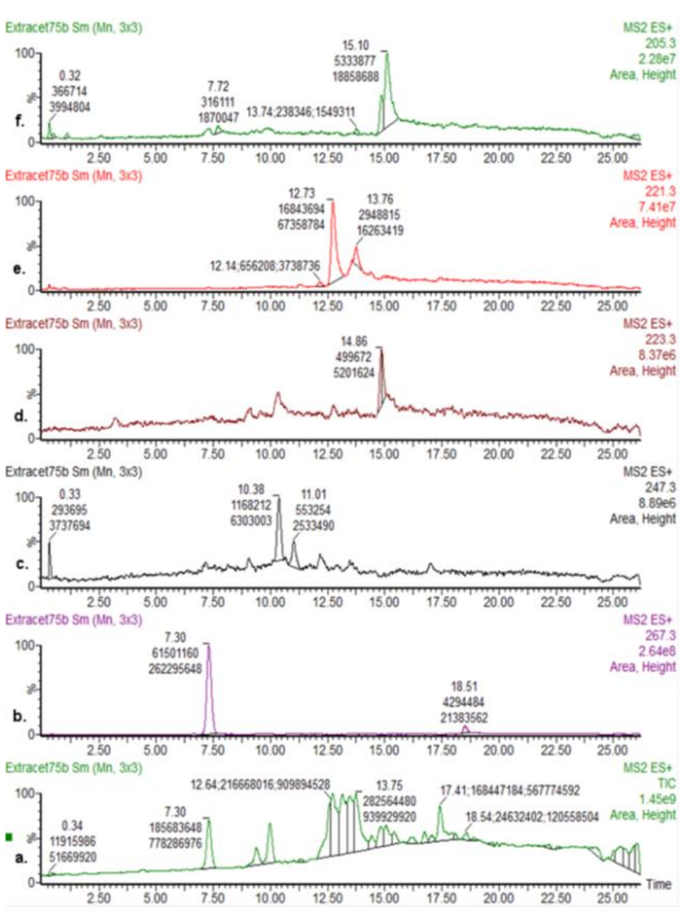


Figure 1. Chromatogram of an extract from leaf of Ambrosia sp. when 0.75 mL acetone was used. a. TIC MS range from 180 – 350 Da; b. Extracted chromatogram at 267.3 Da corresponding to cumanin; c. Extracted chromatogram at 247.3 Da corresponding to ambrosin; d. Extracted chromatogram at 223.3 Da corresponding to longipinanol; e. Extracted chromatogram at 221.3 Da corresponding to spathulenol and f. Extracted chromatogram at 205.3 Da corresponding to germacrene D

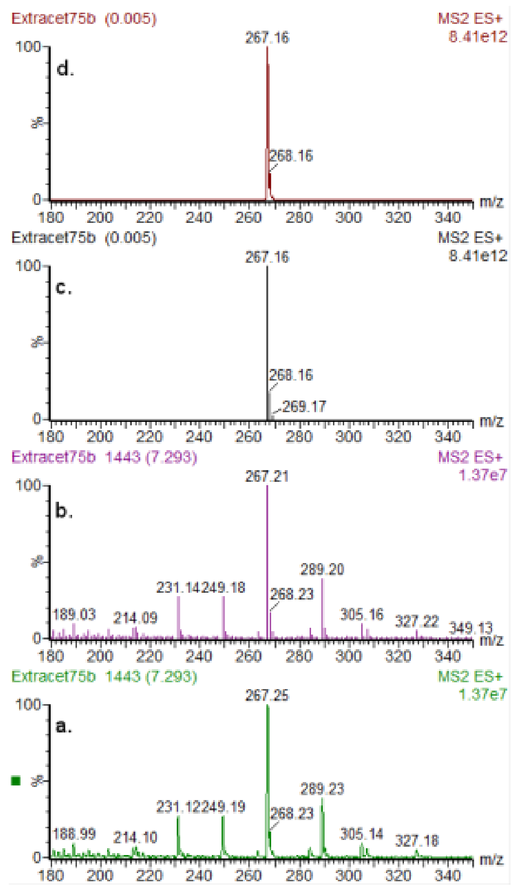


Figure 2. Spectral information for confirmation that the peak eluated at 7.3 min in chromatogram from Figure 1. b. belongs to cumanin. Panel a. The continuous MS spectrum of peak eluated at 7.3 min (Figure 1.a.); Panel b. The centroid MS spectrum of peak eluated at 7.3 min; Panel c. The isotopic elemental model of a compound with the molecular formula C15H22O4; Panel d. A model of a continuous MS spectrum of a compound with the molecular formula C15H22O4

Using Minitab software, a multilevel factorial design with four variables (plant organ, solvent type/logP, solvent volume, and extraction time) was applied to optimize extraction yield, resulting in 72 experiments and using peak area as the response variable.

• Conclusions

An optimized UPLC-MS method enabled the identification of five sesquiterpenes from Ambrosia artemisiifolia without analytical standards, using MS spectral matching and retention order correlated with logP values.A factorial design approach revealed that solvent hydrophobicity and volume significantly affect extraction yield, while the plant organ (root, stem, or leaf) had no measurable influence.