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# ANALYTICAL METABOLITE PROFILING OF *Halimium halimifolium* INFUSION BY UHPLC-PDA-ESI-HRMS METHODS

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## Abstract

The emergence of dereplication strategies as a new tool for the rapid identification of the natural products from complex natural extracts. The present work deals with the development of the metabolic profile of *Halimium halimifolium* infusion traditionally consumed in Algerian folk medicine, and prepared from dried *H. halimifolium* aerial parts. This study based on UHPLC-PDA-ESI/ MSn method and the structures of compounds were confirmed by NMR analysis after the isolation procedure from a hydroalcoolic extract.

## 1. Introduction

*Halimium halimifolium* (L.) Willk. (Cistaceae) is a shrub up to 120 cm tall with tomentose leaves and large yellow flowers, growing in the Mediterranean regions [1]. Infusions of aerial parts are used in Algerian folk medicine as antispasmodic remedies

## 2. Materials and Methods

- The infusion was prepared by adding 100 mL of boiling distilled water to 3 g of dried chopped aerial parts of Hh and the mixture was left to stand for 10 min. Then, the preparation was filtered through filter paper (0.45 µm) and freeze-dried (Alpha 1–2 LD freeze dryer Martin Christ). The infusion procedure was per- formed in triplicate.
- UHPLC separation was performed by a Kinetex C18 (50 × 2.1 mm, i. d., 1.7 µm) column protected by a C18, The HRMS and HRMS/MS were performed with an ESI source in the negative and positive ion

## 4. Results and discussion

In the HRMS analysis, the results showed that most of the detected compounds exhibited a higher sensibility of detection in the neg- ative ion mode. The UHPLC conditions were optimized to obtain maximal chromatographic resolution and MS signal. Nine major peaks (1–9) were detected for Hh-A (▶ Fig. 1) and their retention times

Secondary metabolite assignments were made by HRMS and UV spectra, tandem mass spectrometry experiments (MSn), and, when possible, by comparing retention times and MS spectra with corresponding authentic standards, or with chemo-taxonomic da- ta reported in the literature and databases.

All detected compounds were identified as flavonol glycocon- jugates, particularly, kaempferol (2, 6–9), myricetin (3 and 4), and quercetin (5) derivatives. They showed similar UV spectra with ab- sorption maxima (λ max) between 330 and 360 nm (banda I) and at 265–280 nm (banda II). These UV spectra are characteristic of 3-*O*-substituted flavonols [19]. Compound 2 was identified as kaempferol 3-O-glucopyranoside in the MS2 spectra and showed the classic loss of 162 Da, suggesting the presence of a hexose residue. Compounds 3–8 in the MS2 spectra showed the product ions with *m/z* 285.0401, 301.0343, and 317.0303 corresponding to the aglycons kaempferol (6–8), quercetin (5), and myricetin (3 and 4), respectively, and the characteristic loss of 146 Da sug- gested the presence of a coumaroyl residue. Furthermore, the aglycones were confirmed by MS3 spectra. Based on this evidence, 3 and 4 were identified as myricetin 3-O-(6″-*p*-coumaroyl) gluco- pyranoside isomers, 5 as quercetin 3-O-(6″-*p*-coumaroyl) gluco- pyranoside, and 6–8 as tiliroside isomers. Their structures were subsequently confirmed by NMR analysis.

Finally, a dicoumaroyl flavonol hexoside (9) was characterized in Hh-A, and the structure of kaempferol 3-O-(dicoumaroyl)-glu- copyranoside was hypothesized by analysis of the HRMS/MS data. The MS2 spectrum of [M – H]− ion at *m/z* 739.1657 ($C_{39}H_{32}O_{15}$) showed fragments at *m/z* 285.0392 ($C_{15}H_9O_6$, 0.9 ppm) and 453.1181 ($C_{24}H_{21}O_9$, 0.5 ppm) produced by glycosidic bond cleavage and corresponding to [Aglycone–H]− and [dicoumaroyl-hexose–$H_2O$–H]− ions, respectively.

## 5. Conclusion

this study allowed us the development of chemometric tools for the classification of spectroscopic data and dereplication of *Halimium* genus metabolites.

Considering the high flavonoids composition of *Hh* infusion traditionally drunk; the beverage has used as dietary supplement to improving human well-being and reducing the risk of chronic diseases, such as cardiovascular rdisorders, neurodegenerative pathologies and diabetes.

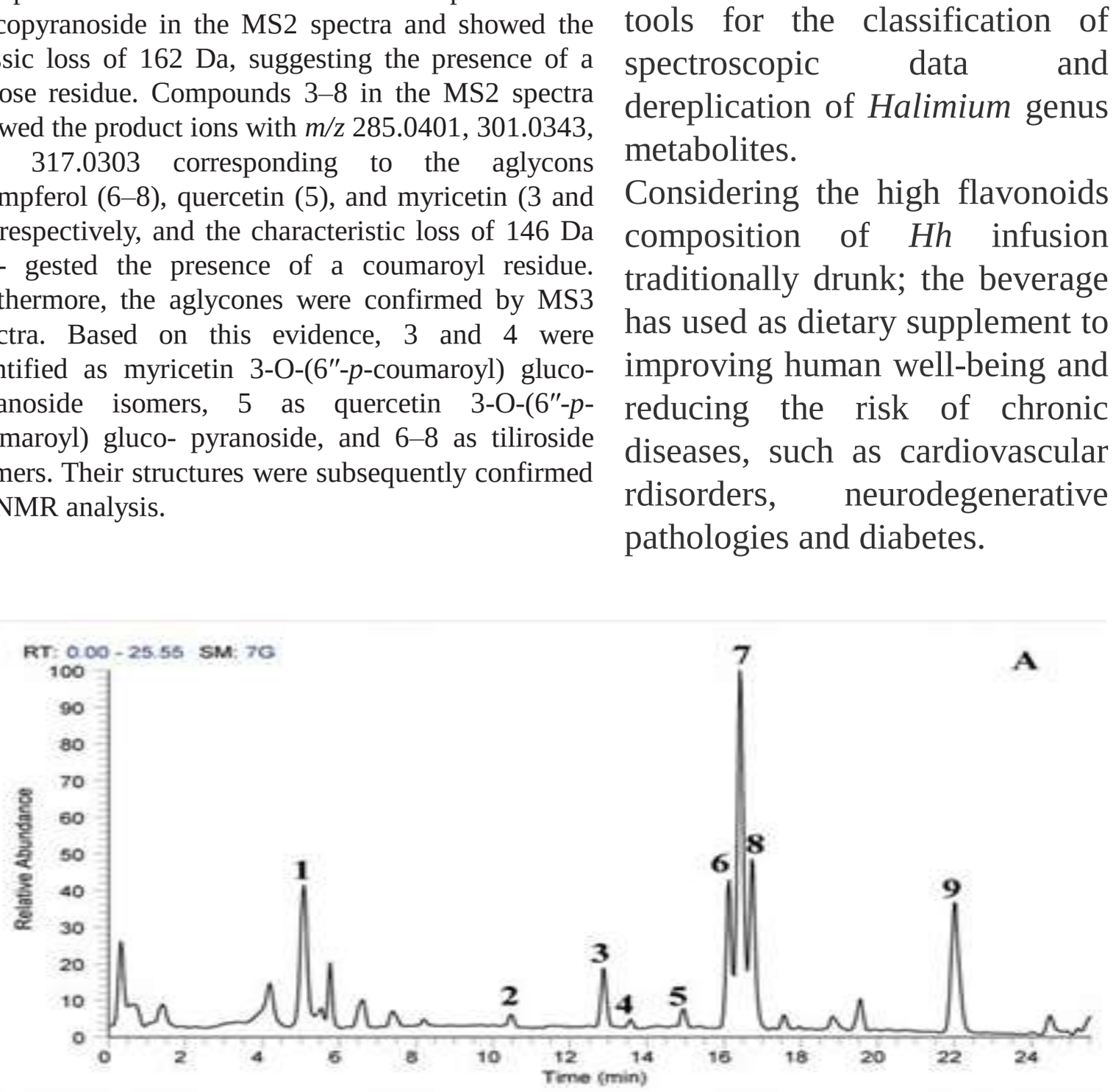


**Fig. 1 : UHPLC-UV (330 nm) profile of Hh.**

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