Derivatization of Rosemary is Integral to its Analysis

La dérivatisation du romarin est intégrale à son analyse

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Abstract | Résumé

Plants of the Lamiaceae family, such as rosemary (*Salvia rosmarinus*), common sage (*Salvia officinalis*), and white sage (*Salvia apiana*), are known for their bold flavours, fragrant leaves, and rich sources of bioactive compounds. A key characteristic shared by these plants is their high content of carnosic acid, a prominent phenolic diterpene, and its derivatives. However, the analysis of these compounds via gas chromatography–mass spectrometry (GC-MS) is challenging and not frequently explored due to their high polarity and low volatility. Derivatization techniques like trimethylsilylation and appropriate solvent selection can address these limitations by enhancing compound volatility, allowing for effective GC-MS identification through characteristic fragmentation patterns. We employed trimethylsilyl derivatization of acetonitrile extracts of rosemary, white and common sage, and quantified carnosic acid, rosmanol, and 12-O-methylcarnosic acid. Rosemary contained the highest amount of carnosic acid at 3.4 mg/mL, followed by common sage at 2.0 mg/mL and white sage at 0.7 mg/mL. Rosemary contained the most rosmanol at 2.2 mg/mL followed by common sage (0.3 mg/mL), and white sage contained minimal amounts (0.003 mg/mL). Additionally, both common sage and rosemary were found to contain 12-O-methylcarnosic acid, with concentrations of 1.3 mg/mL and 0.4 mg/mL, respectively. Thus, we demonstrated that simple solvent extraction and TMS derivatization is an effective approach to quantifying these bioactive compounds in plants.

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Les plantes de la famille des Lamiacées, tel le romarin (*Salvia rosmarinus*), la sauge commune (*Salvia officinalis*) et la sauge blanche (*Salvia apiana*) sont connues pour leurs saveurs prononcées, leurs feuilles parfumées et leurs sources riches de composés bioactifs. Une caractéristique clé partagée par ces plantes est leur teneur élevée en acide carnosique, un diterpène phénolique important, ainsi que ses dérivées. Cependant, l'analyse de ces composés via la chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG-SM) pose un défi et n'est pas fréquemment explorée dû à leur polarité élevée et leur faible volatilité. Les techniques de dérivatisation telles la triméthylsilylation (TMS) et la sélection de solvant approprié peuvent remédier à ces limitations en améliorant la volatilité des composés, ainsi permettant à une identification par CPG-SM efficace par des modèles de fragmentation caractéristiques. Nous avons utilisé la dérivatisation triméthylsilylée d'extraits d'acétonitrile de romarin, de sauge blanche et de sauge commune, et avons quantifié l'acide carnosique, le rosmanol et l'acide 12-O-méthylcarnosique. Le romarin contenait le montant le plus élevé d'acide carnosique à 3,4 mg/mL, suivi par la sauge commune à 2,0 mg/mL et la sauge blanche à 0,7 mg/mL. Le romarin contenait le plus de rosmanol à 2,2 mg/mL suivi par la sauge commune (0,3 mg/mL) et la sauge blanche qui contenait des montants minimes (0,003 mg/mL). De plus, la sauge commune et le romarin se sont révélés contenir de l'acide 12-O-méthylcarnosique, avec des concentrations de 1,3 mg/mL et de 0,4 mg/mL respectivement. Ainsi, nous avons démontré que la simple extraction de solvant et la dérivation TMS sont des approches efficaces pour quantifier ces composés bioactifs dans les plantes.

Keywords: rosemary; common sage; white sage; carnosic acid; rosmanol; phenolic diterpenes; trimethylsilylation; GC-MS derivatization; GC-MS; phytochemical analysis

Introduction

The Lamiaceae family is recognized worldwide for its diversity, distinctive flavours, and high versatility. While many of these plants are widely used in culinary applications, they also hold an important place in traditional medicine worldwide. In ancient Greece and Rome, rosemary (*Salvia rosmarinus*) have been traditionally used to alleviate migraines, muscle pain, respiratory conditions, and insomnia (Figure 1a) (1). In North America, common sage (*Salvia officinalis*) and white sage (*Salvia apiana*) have been used to relieve respiratory and digestive issues (Figure 1b,c). A key characteristic shared by these plants is their high content of carnosic acid, a prominent phenolic diterpene, and its derivatives. These compounds demonstrate a wide range of therapeutic properties, including anti-inflammatory, antimicrobial, and antioxidant effects, Nieto et al. explored the inhibitory effects of rosemary, finding the primary bioactive species to be carnosic acid, rosmanol, and related molecules (1).

These compounds interact with the cell membrane, leading to disruptions in electron transport and leakage of cellular components. Similarly, Vegara et al. reported that carnosic acid demonstrates greater efficacy against pathogenic bacteria compared to any other major extract components (2). Nonetheless, its effectiveness and related applications are still under active investigation. Traditionally, high-performance liquid chromatography (HPLC) has been the primary method for analyzing carnosic acid. However, very few studies have explored its analysis using gas chromatography–mass spectrometry (GC-MS). A 2016 study by Islamčević Razboršek, and Ivanović investigated several extraction techniques and adapted GC-MS methods for diterpene analysis (3). Despite this, there remain limitations such as prolonged wait times and extra sample preparation steps. The present study aims to develop a unique method to analyze carnosic acid and related molecules in a rapid and efficient manner and quantify the amounts of each molecule across various natural sources using GC-MS.

Methods

Dried plant material was purchased from Mountain Rose Herbs Inc. (Eugene, Oregon) for rosemary, common sage, and white sage. One gram (1 g) of dried plant material was ground into a fine powder and placed in 10 mL of acetonitrile and left to sit at room temperature for two days. The extract underwent two rounds of filtration: first using a standard vacuum filtration apparatus and then using a microneedle filter to ensure no solid material entered the GC–MS. Notably, no further evaporation or drying steps were required before derivatization with the trimethylsilyl (TMS) reagent, Regisit-1%TMCS (bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane). The role of TMS in this experiment was to convert polar hydroxyl groups into non-polar derivatives. This transformation occurs through the substitution of each acidic proton with a trimethylsilyl, $Si(CH_3)_3$, group (Figure 2). Excess TMS reagent handled any residual moisture in the samples.

The reactivity of functional groups during derivatization is influenced by heating: carboxylic acid groups typically react first, followed by alcohol groups. For this experiment, 100 µL of acetonitrile extract was mixed with 100 µL of TMS reagent, then heated for 40 minutes at 70 °C, with shaking every 10 minutes to ensure complete derivatization of all acidic groups. The GC oven temperature was programmed from 80 °C to 300 °C, with a total run time of 18 minutes. The instrument used was an Agilent 7820A Gas Chromatography coupled to a 5975C Mass Spectrometer. The inlet was 200°C and the oven ramped from 40-250°C at a rate of 15°C per with a 2-minute hold time at the beginning. Helium gas ran at a constant flow of 1.3 ml min^{-1}. The column was a Rx 5 sil MS 30m x 0.25mm x 0.025µm.

Each extract was analyzed in triplicate. Quantification was performed using abietic acid as an external standard, with a calibration curve constructed from concentrations of 0.125 mg/mL, 0.25 mg/mL, and 0.5 mg/mL (Figure 3). Three points in the calibration curve necessarily limit the accuracy of this approach.

The identification of specific molecules was done through the testing of pure standards and library matches through NIST databases. Values are quoted for concentration in the derived hydrosol (mg/mL) and per gram of plant material (mg/g).

Figure 1. a) Rosemary (*Salvia rosmarinus*), b) common sage (*Salvia officinalis*), and c) white sage (*Salvia apiana*).

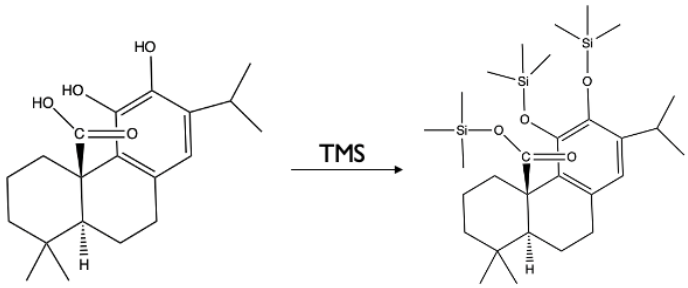

Figure 2. Carnosic acid before and after complete TMS derivatization. Derivatization increases the molecular weight from 332.4 g/mol to 548.4 g/mol and lowers the boiling point, allowing GC–MS detection.

Results and Discussion

Prior to derivatization, the chromatograms for all three plants resemble that in Figure 4, in which there is a large, undefined area of molecules from 11 to 13 minutes that cannot be analyzed directly.

The peaks become much more defined post-derivatization. The chromatograms obtained from the rosemary, common sage, and white sage derivatized acetonitrile extracts are shown in Figure 5.

From these results, it was observed that carnosic acid and rosmanol were present in varying concentrations across all products. Rosemary contained the highest amount of carnosic acid at 3.4 ± 0.15 mg/mL (34 ± 1.5 mg/g), followed by common sage at 2.0 ± 0.15 mg/mL (20 ± 1.5 mg/g), and white sage at 0.7 ± 0.15 mg/mL (7 ± 1.5 mg/g). A similar trend was seen with rosmanol: rosemary contained the most at 2.20 ± 0.15 mg/mL (22 ± 1.5 mg/g), common sage had 0.30 ± 0.15 mg/mL (3 ± 1.5 mg/g), and white sage contained only 0.003 ± 0.15 mg/mL (0.03 ± 1.5 mg/g). Additionally, both common sage and rosemary were found to contain 12-O-methylcarnosic acid, with concentrations of 1.3 ± 0.15 mg/mL (13 ± 1.5 mg/g) and 0.4 ± 0.15 mg/mL (4 ± 1.5 mg/g), respectively. This aligns with previous studies, as common sage tends to have higher concentrations of 12-O-methylcarnosic acid than rosemary (4). It should be noted that several unknown peaks appeared in each chromatogram within the 11 to 13-minute region, which are most likely phenolic diterpenes that could not be identified.

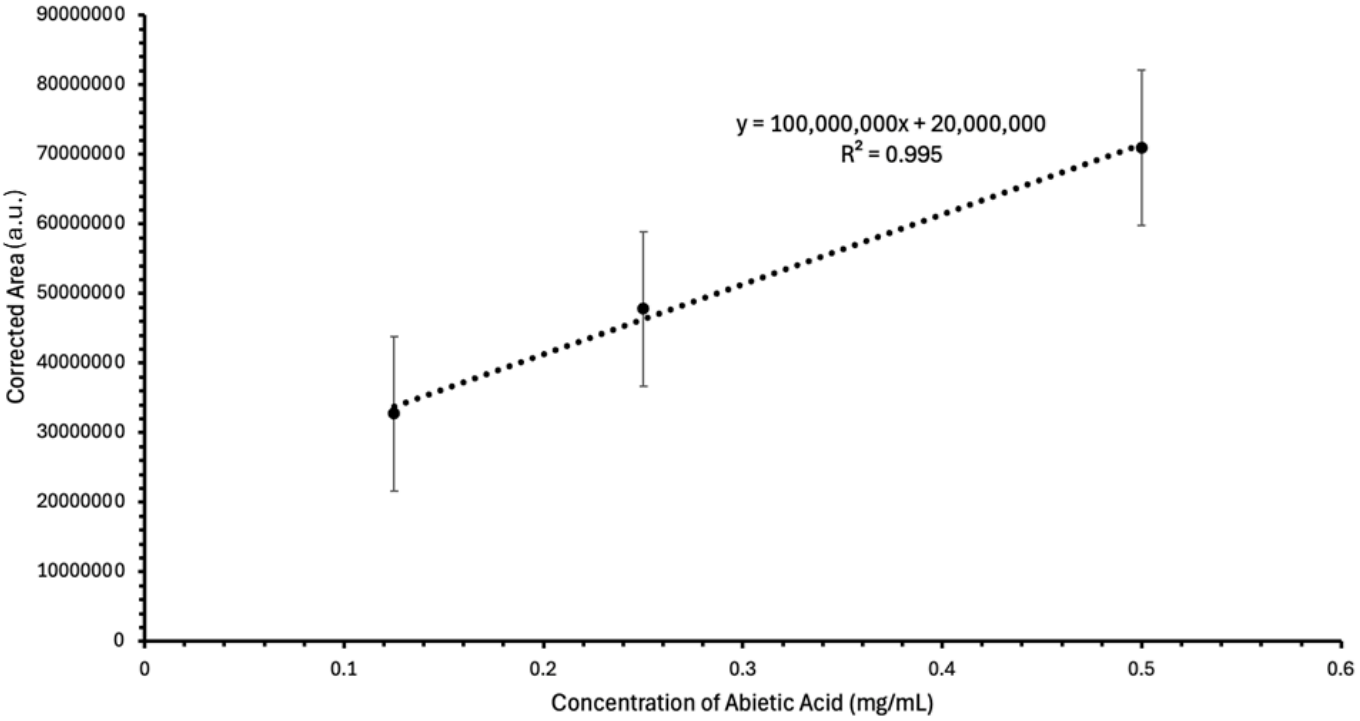

Figure 3. Calibration curve of the external standard, abietic acid, from concentrations of 0.125 mg/mL, 0.25 mg/mL, and 0.5 mg/mL.

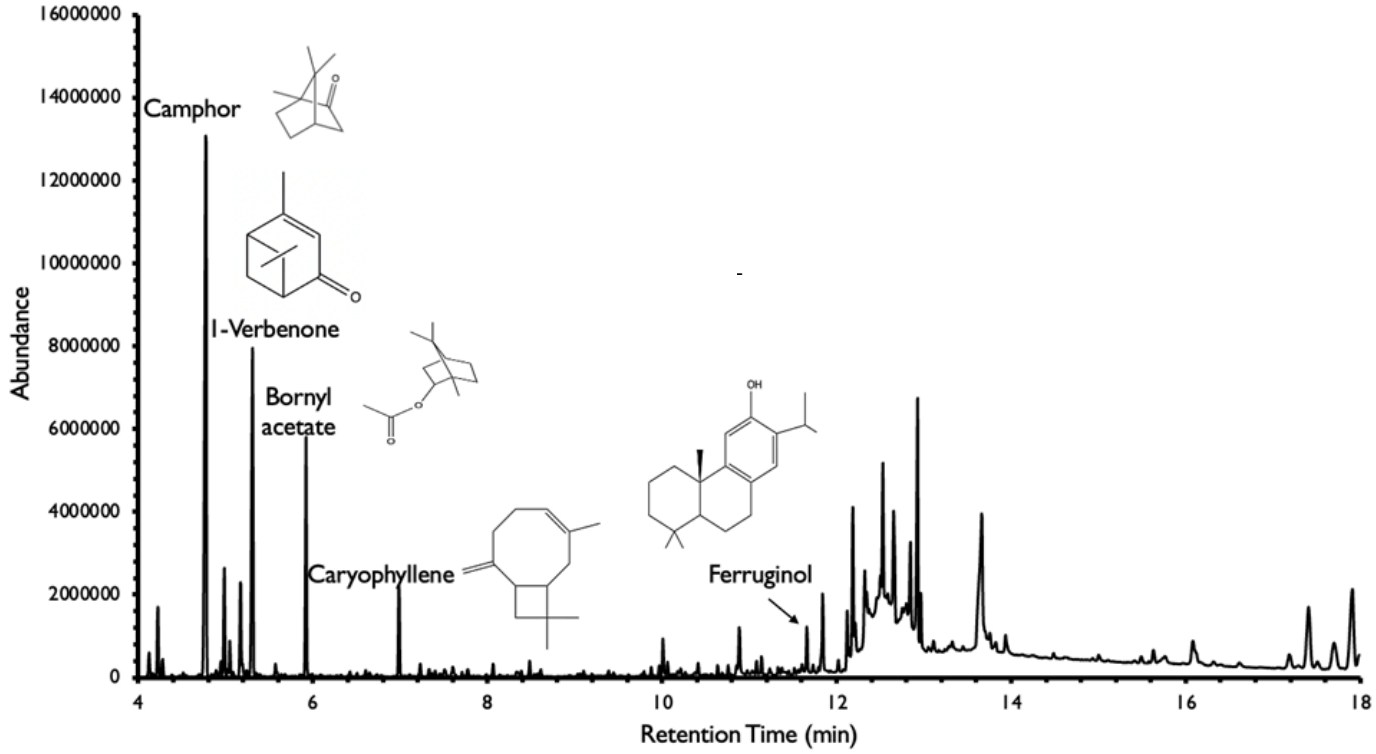

Figure 4. The chromatogram obtained from the GC-MS of an underivatized rosemary acetonitrile extract.

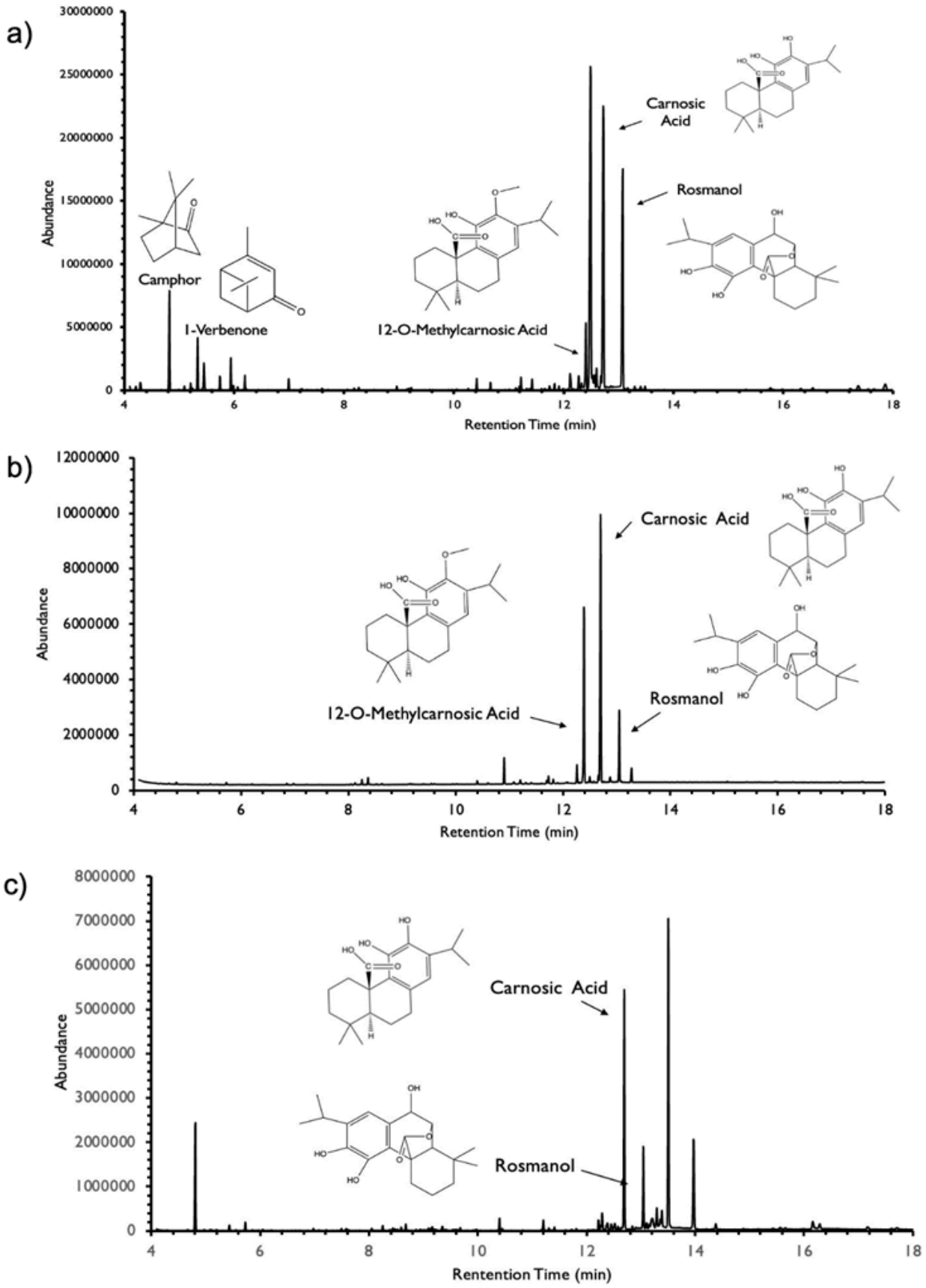

Figure 5. The chromatogram obtained from the GC-MS of derivatized rosemary, b) common sage, and c) white sage acetonitrile extracts.

Conclusion

A reliable, reproducible method has been developed to analyze carnosic acid in a quick and effective manner. The choice of solvent, acetonitrile, is optimal for direct derivatization without the need for an intermediate drying/evaporation step. While maceration occurs over a period of two days, the GC-MS run time is only 18 minutes and is still able to elucidate multiple phenolic diterpenes and other major constituents. Derivatization is an important step needed for the analysis of phenolic diterpenes with GC-MS instrumentation, as it increases their volatility, decreases polarity, and allows prominent, well-defined peaks to emerge. As expected, rosemary contained the highest amount of carnosic acid and rosmanol among the various plant samples, while white sage contained the lowest. Likewise, both common sage and rosemary contained 12-O-methylcarnosic acid, with common sage having the higher concentration.

Acknowledgments

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