A Comparison of Bioactive Molecules in Three Sage Varieties

Une comparaison de molécules bioactives de trois variétés de sauge

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Abstract | Résumé

Sage belongs to the genus *Salvia* in the family Lamiaceae and is a large, globally distributed aromatic plant with a wide variety of species. This work explores and compares the chemical composition of raw plant material from common sage (*Salvia officinalis*), clary sage (*Salvia sclarea*) and white sage (*Salvia apiana*) using microwave-distilled hydrosol extraction, supercritical fluid carbon dioxide (sc-CO_2) extraction, and alcohol extraction. All extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The results show that the sc-CO_2 and alcohol extraction methods yield more chemical components than hydrosol extraction, such as epimanool, humulene, sclareol and linalyl acetate. The chemical composition of common sage closely resembles that of a commercially obtained dried white sage, while clary sage varies greatly depending on the extraction method. The powdered white sage does not show any components other than camphor and eucalyptol, likely due to its age and how it is dried or powdered.

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La sauge appartient au genre *Salvia* de la famille des Lamiacées et est une plante aromatique distribuée globalement ayant une vaste variété d'espèces. Ce travail explore et compare la composition chimique de matière première végétale provenant de la sauge commune (*Salvia officinalis*), la sauge sclarée (*Salvia sclarea*) et la sauge blanche (*Salvia apiana*) en utilisant l'extraction d'hydrolat par distillation assistée par micro-ondes, l'extraction au dioxyde de carbone en fluide supercritique (CO2 supercritique) et l'extraction par alcool. Tous les extraits ont été analysés par chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG-SM). Les résultats ont démontré que les méthodes d'extraction au CO2 supercritique (sc-CO2) et à l'alcool permettent d'obtenir un plus grand nombre de composants chimiques que l'extraction par hydrolat, particulièrement l'épimanool, l'humulène, la sclaréol et l'acétate de linalyle. La composition chimique de la sauge commune ressemblait étroitement celle de la sauge blanche séchée commerciales, alors que celle de la sauge sclarée variait grandement selon la méthode d'extraction. La sauge blanche en poudre ne démontre pas des composantes autres que le camphre et l'eucalyptol, probablement dû à son âge et à sa méthode de séchage ou de poudrage.

Keywords: Salvia officinalis; Salvia sclarea; Salvia apiana; GC-MS; supercritical CO_2 extraction; phytochemical analysis; bioactive compounds; essential oils; epimanool; α-humulene

Introduction

The earliest medicines came from natural products and were mostly derived from plants. For example, salicylic acid, the foundation of the aspirin we rely on today for a pain killer, was first discovered in willow bark, while the cancer-fighting agent taxol originates from the Pacific yew tree. Some drugs are extracted directly from plants, while others are created by modifying chemical compounds found in them. Though a few are synthesized from inorganic materials, many have their origins in research on the active compounds discovered in plants (1).

The genus *Salvia L.* is one of the best-known medicinal and aromatic plants of the Lamiaceae family, comprising 900 species, and found throughout Europe, Asia, and the Americas (2). Sage has a long history of being used as a seasoning and for health purposes. It is promoted for treating conditions such as sore throat, memory loss, diabetes and high cholesterol. *Salvia officinalis*, known as common sage, is a valuable industrial plant widely used in the food and pharmaceutical industries. It has been reported to be a potential therapeutic option for Alzheimer's disease based on its *in vitro* cholinergic binding properties and its role in regulating mood and improving cognitive performance in humans (3). Indigenous peoples across much of California incorporate both the seeds and green parts of white sage into their diets, medicines, and ceremonies (4). Although there is no historical record of its use in religious ritual during the pre-colonial period, the ritual smudging of white sage (*Salvia apiana*) is widely used in the Chumash religion for its sedative, diuretic, and common-cold healing properties (5).

The objective of this study is to explore and analyze the chemical composition of the hydrosol, supercritical-CO2, and alcohol extracts of common sage (*Salvia officinalis*), clary sage (*Salvia sclarea*) and white sage (*Salvia apiana*).

Figure 1. (a) Common sage (*Salvia officinalis*), (b) Clary sage (*Salvia sclarea*), (c) White sage powder (*Salvia apiana*) and (d) hydroponic white sage (*Salvia apiana*).

Figure 2. Microwave-distilled hydrosol apparatus and a bottle of common sage hydrosol.

Methods

Common sage (*Salvia officinalis*) was collected from a garden patch of sage which is 3 years old and thriving in a sunny exposure in a 5-acre gardening zone, south of Ottawa, Canada (Fig. 1a). Clary sage (*Salvia sclarea*) was harvested from the same area from plants that were two years old and in full flower (Fig. 1b). White sage (*Salvia apiana*) was obtained in dried powder from Mountain Herb (USA) (Fig. 1c) and from a hydroponics system in Calgary, Canada (NuLeaf Inc.) (Fig. 1d).

Microwave-Distilled Hydrosol Extraction

The roots and stems of the fresh common and clary sage were removed. The leaves were washed, torn into small pieces, weighed to 100 g and 615 g respectively (due to availability), and placed at the bottom of the jar in a circle around the beaker along with some water to have prevented over-drying during microwaving. The common sage was then microwaved four times for a total of 32 minutes (Fig. 2). The clary sage was microwaved seven times for a total of 50 minutes. Previous experience in the lab has demonstrated that further cycling does not result in increased analyte concentrations. The powdered white sage was weighed to 100 g and soaked in 500 mL of boiled water overnight. The slurry was then microwaved five times for a total of 39 minutes.

Solid Phase Extraction (SPE)

Chemical constituents from water extracts were removed via SPE to prepare them for instrumental analysis. The C-18 SPE cartridge was wetted with 1 mL of methanol and 1 mL of deionized water to activate the solid phase before loading the hydrosol. 10 mL and 3 mL of common sage and white sage hydrosol, respectively, were loaded and passed through the cartridge. 100 mL of clary sage hydrosol was used due to the low concentration of the chemical species present. After the water and other unwanted materials were removed, four aliquots of 250 µL 50:50 MeOH:CH_3CN were used to elute the analytes retained on the solid phase, resulting in 1 mL of eluted product to be analyzed by GC-MS.

Supercritical Fluid Carbon Dioxide (sc-CO_2) Extraction

To extract both polar and non-polar compounds and thus produce a more comprehensive overview of the plant material, Sc-CO_2 was used. Washed common sage and clary sage leaves were freeze-dried to minimize moisture content and ground into powder. The ground common sage, clary sage, and both white sages (Powder Mountain Herb & Nuleaf) were placed in the extraction chamber of the Supercritical Fluid Technologies Inc. SFT-250 SFE/SFR System. The flow rate of CO_2, pressure (300 bar), and extraction temperature (45°C) were controlled to ensure successful extraction and maximize the yield. The dissolved compounds were carried by sc-CO_2 into a separator vessel where the pressure was lowered, causing the sc-CO_2 to return to its gaseous state and the compounds were collected at the end. 5-minute intervals of soaking the plant material in CO_2 and then extracting were repeated between 4 and 6 times. The process was stopped when there was no extract entering the collection vial. The collected common sage, clary sage, Nuleaf and Mountain Herb white sage extracts were each diluted in 1 mL of ethyl acetate. 35 µL of diluted freeze-dried common sage, 0.036 µL of diluted clarysage, 10 µL of diluted white sage (Nuleaf Dried), 5 µL of diluted white sage powder (Mountain Herb) extracts were diluted to 1 mL with ethyl acetate for GC-MS analysis.

Alcohol Extraction

9 mL of methanol was added to each of 0.3 g of common sage, Mountain Herb white sage powder and NuLeaf white sage, and each was vortexed for 10 minutes. The supernatants from all the extracts were filtered several times using syringe filters before being analyzed by GC-MS.

Gas Chromatography-Mass Spectrometry

An Agilent 7820A Gas Chromatography coupled to a 5975C Mass

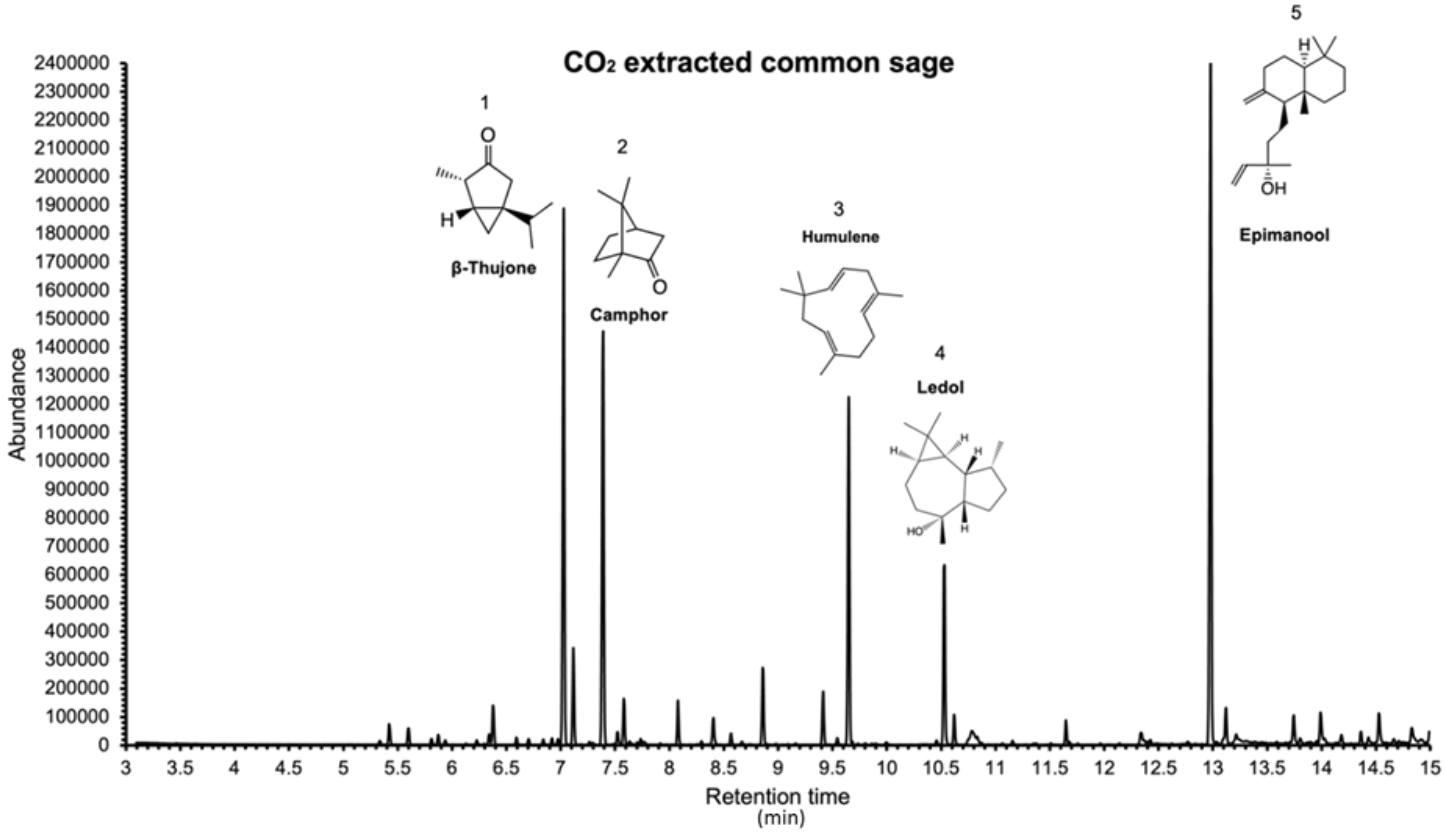

Figure 3. Major chemical components of the CO_2 extracted common sage and their molecular structures.

Spectrometer was used to identify the major compounds in all the extracts of the sages. The inlet was 200°C and the oven ramped from 40-250°C at a rate of 15°C per minute for hydrosol extraction and essential oil, with a 2-minute hold time at the beginning. The oven ramped from 40-300°C at a rate of 15°C per minute for sc-CO_2 and alcohol extraction, with a 1-minute hold time at the beginning and end of the run. 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. The mass spectrometer was calibrated weekly using PFTBA, (Sigma Millipore 77299).

Retention times were calibrated using a C7-C40 saturated alkane standard (Supelco 49452-U) to create the Kovats Retention Indices (RI) using the equation:

$$RI = 100 * \left[n + (N - n)\frac{(tr(unknown) - tr(n))}{tr(N) - tr(n)}\right] \quad \text{(Eq. 1)}$$

where n is the number of the alkane below the unknown, N is the number of the alkane above the unknown, tr (unknown) is the retention time of the analyte, tr(N) is the retention time for the alkane above the unknown, and tr(n) is the retention of the alkane below the unknown.Compounds were identified by the NIST 2 database. Retention Indices were calculated and compared to the NIST Chemistry WebBook for the MS 5 Column. Percentage abundances were used to compare the amounts of compounds present in different extracts.

Results and Discussion

sc-CO_2 Common Sage Extracts

An example of the results obtained is shown in Fig. 3 for common sage extracted by sc-CO_2. The main compounds present are determined to be β-thujone (RT = 7.029 min, 1); camphor (RT = 7.391 min, 2); α-humulene (RT = 9.651 min, 3); ledol (RT = 10.531 min, 4); and 13-epimanool (RT = 12.981 min, 5).

Comparison of Common Sage Using Different Extraction Methods

The results show that the main bioactive molecules in all common sage extracts are α/β-thujone, camphor, ledol, humulene and epimanool (Fig. 4). However, the hydrosol lacks humulene but contains a small amount of carvacrol (2.2%). The chemical components of both CO_2 and alcohol extracts appear similar, while the hydrosol shows fewer peaks due to the steam-distilled method, which extracts only more volatile and water-soluble compounds; some heat-sensitive compounds might also be lost during the processing.

Studies typically showed a variety of cyclic monoterpenes such as eucalyptol, α- and β-pinene, isothujone, camphene, sabinene,

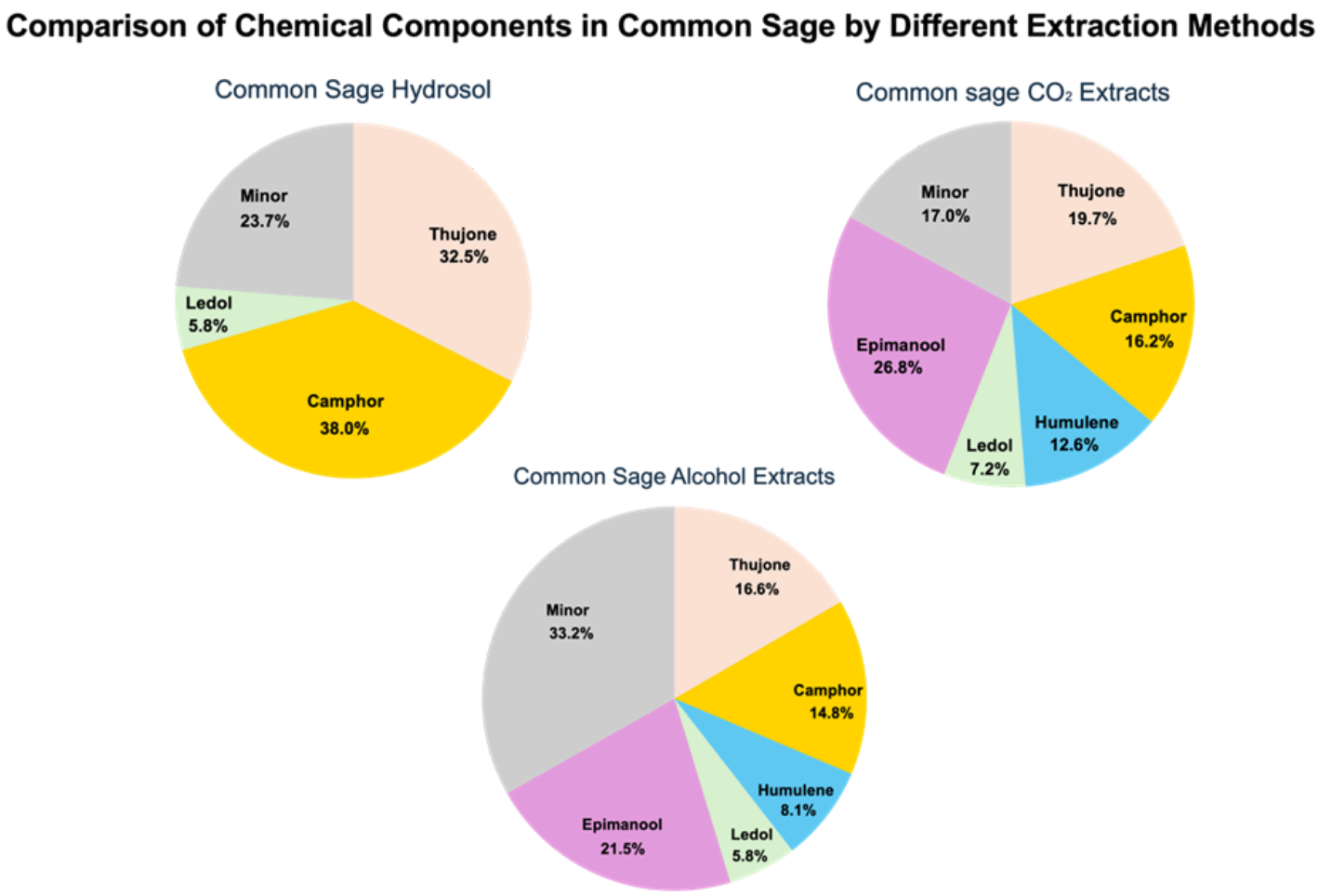

Figure 4. Major chemical components of common sage obtained using different extraction

limonene, myrcene, camphor and (+)-bornyl diphosphate. The wide variety of acyclic, monocyclic, and bicyclic monoterpenes are attributed to the activity of various synthases, which converted the common substrate geranyl diphosphate into multiple products (6). Among these chemical constituents, the most dominant was eucalyptol (62.0%), followed by camphor (8.0%), β-pinene (6.0%), borneol (5.0%), α-pinene (3.7%), β-myrcene (3.0%) and (–)-camphene (2.6%) (7). The chemical composition of common sage leaf essential oil from three different global regions suggested that eucalyptol (26.9%) is again the major component, followed by α-thujone (17.2%), camphor (12.8%), camphene (5.2%), α-pinene (5.0%), β-caryophyllene (4.9%) and β-pinene (4.1%). Notably, α-humulene (5.7% in a sample from Mexico) was detected in the essential oil, as does manool, a stereoisomer of epimanool (8). Eucalyptol was only observed as a minor component (~ 1-2 %) in the current study. Although ledol is not typically reported in common sage, it was detected in all three extracts analyzed in this experiment. It is a sesquiterpenoid alcohol commonly found in plants like wild rosemary, sage and wormwood.

Comparison of Clary Sage Components Using Different Extraction Methods

The chemical composition of clary sage extracts varied greatly depending on the extraction method (Fig. 5). The hydrosol contains β-thujone, camphor, carvacrol, germacrene D and caryophyllene oxide. In contrast, the sc-CO_2 extract only showed two main peaks, linalyl acetate (13.3%) and sclareol (86.1%). This is due to the use of high pressure and temperature during the CO_2 extraction process, which separated the less volatile compounds. Other main components present in the hydrosol were found in minor proportions in the CO_2 extracts.

Among various parts and regional sources of clary sage, the essential oil extracted from leaves of Slovak Republic and flowers of clary sage was found to contain linalool (18.9%), sclareol (15.7%), linalyl acetate (13.7%), α-terpineol (6.5%), germacrene D (5.0%), and geranyl acetate (4.3%) as the most abundant components (9). According to another study, the most significant difference between steam distillation and sc-CO_2 extraction was the amount of sclareol in the extract. Steam distillation yielded nearly no sclareol, whereas sc-CO_2 extraction concentrated it up to 50%, consistent with our current results. It is found that changing the extraction conditions from 90 bar/50 °C to 100 bar/40 °C increased the sclareol concentration from 0 to 25.3% while linalyl acetate decreased from 13.4% to 2.5%, selectively reducing other components while increasing the amounts of sclareol (10). In this

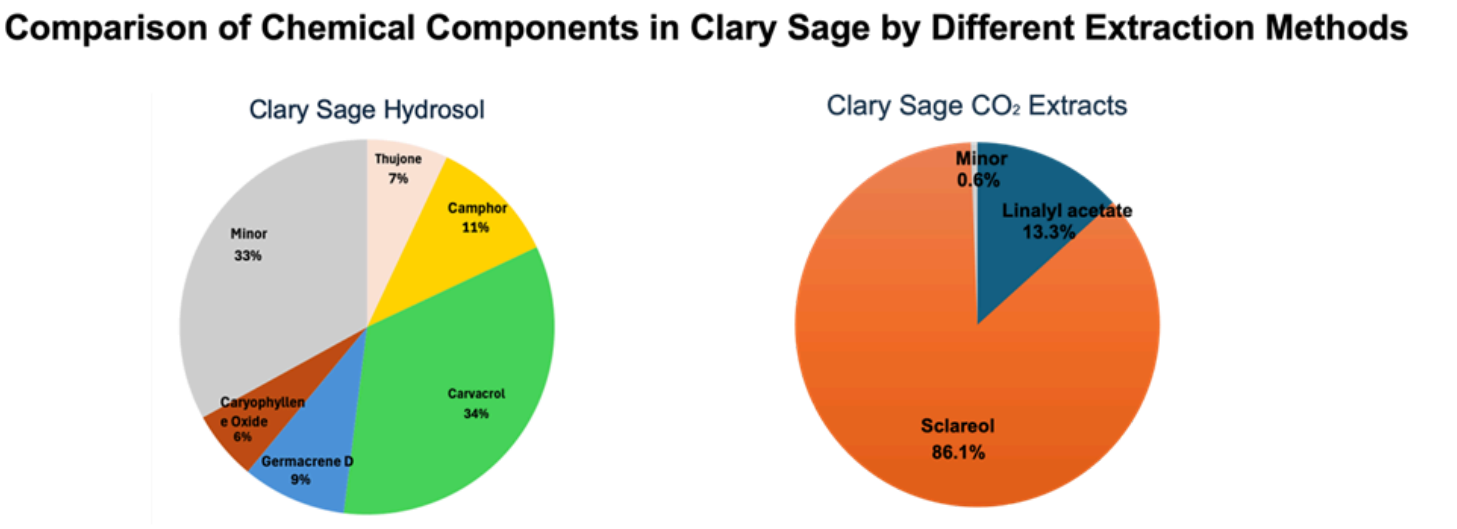

Figure 5. Major chemical components of clary sage obtained using different extraction methods.

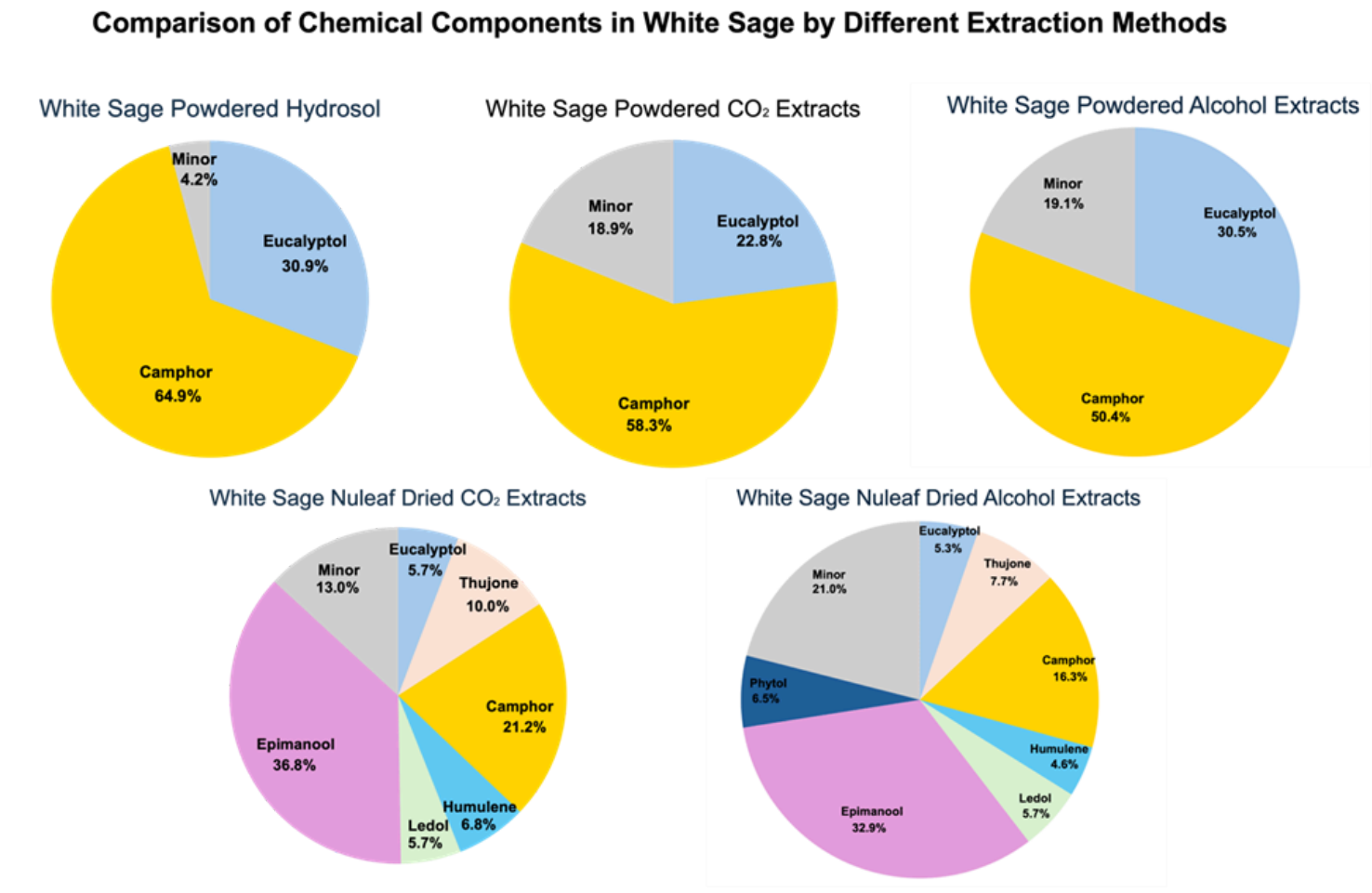

Figure 6. Major chemical components of white sage obtained using different extraction methods.

study, by conducting the sc-CO_2 extraction at 300 bar/45 °C, a yield of 86.1% sclareol was obtained.

The hydrosol contains a variety of compounds, including carvacrol, which is not typically seen in clary sage extracts. It is a monoterpenoid alcohol commonly found in plants such as thyme and oregano. Studies have shown that the use of this compound can effectively inhibit the growth of pathogenic bacteria, including Staphylococcus aureus, Escherichia coli, Salmonella enterica subsp. enterica serovar Typhimurium, Listeria monocytogenes, and Shigella sonnei (11). Germacrene D and caryophyllene oxide were otherwise spotted in small amounts in clary sage. Germacrene D is a sesquiterpene commonly found in plants such as cannabis, rosemary, ginger, lavender and black pepper. It has been found to isomerize under acidic conditions to produce a variety of naturally occurring sesquiterpenes, including cadinane, muurolane and amorphane (12).

The white sage powder extracts contained a significant amount of eucalyptol and camphor (Fig. 6). The powdered white sage was a year old when extracted, and it is unknown how it was dried or powdered. This could be responsible for the lack of chemical diversity. In contrast, the dried white sage derived from the Nuleaf hydroponic system displayed significantly more chemical variety, including eucalyptol, α/β-thujone, camphor, humulene, ledol and epimanool, in the alcohol and CO_2 extracts. Nuleaf white sage is chemically very similar to common sage, with the main difference being the presence of eucalyptol, which is unique to white sage.

Previous research on the aerial parts of white sage have demonstrated that the major chemical components are eucalyptol (34.5%), camphor (21.7%), β-pinene (7.4%), α-pinene (6.4%), camphene (3.9%), limonene (3.5%) and myrcene (3.2%) (13). In another study on white sage, α-humulene was detected only in trace amounts (0.1%) (14). In contrast, the current analysis found α-humulene present in both CO_2 and alcohol extracts of Nuleaf Dried white sage. α-humulene is a sesquiterpene commonly found in plants like hops, cannabis, and some spices like sage, ginseng, and basil. Epimanool, while not observed in white sage essential oil (15), was identified as a common compound in the other extracts. Based on available knowledge, this is the first time that epimanool has been identified in sage extracts and opens the door to further in-depth research, from isolation and purification to exploring its exact mechanism of action (16). Epimanool also has significant antifungal and anticancer properties, as demonstrated in studies involving human leukemia cells and human neuroblast cells (17).

Conclusions

In conclusion, this study focused on hydrosol, alcohol, and sc-CO_2 extracts of common, white and clary sages. One notable difference was the presence of eucalyptol, which was found only in both types of white sage as one of the major components. Contrarily, other studies have shown that eucalyptol was found in large quantities in common sage, whereas in our results it only accounts for less than 3% in minor parts. Despite its rare occurrence in the literature, this study identified ledol in both common sage and white sage. α-Humulene is commonly detected in common sage, though in minor amounts, but isnot consistently present in white sage. Epimanool has been detected in specific conditions in common sage but is rarely reported in white sage. In this study, both epimanool and α-humulene are found in the CO_2 extracts of both common and Nuleaf Dried white sage. Epimanool, being a less well-known diterpenoid alcohol, demonstrates significant biological activity.

A limitation of the present study was the diversity of the samples collected. From fresh to store-bought dried samples, this range of plant sources could almost certainly have affected the results. Future research could focus on further biological testing to validate the medicinal properties and applications of these identified compounds. Comparing and identifying the chemical components of different sage species provides valuable insights into their bioactive diversity, pharmacological and commercial potential.

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