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HS-SPME-GC/MS-BASED VOLATILE PROFILING AND CHEMOMETRIC DISCRIMINATION OF SELECTED HERBAL TEAS

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Abstract: Herbal teas prepared from aromatic plants possess complex volatile profiles associated with their characteristic sensory properties. In this study, the headspace volatile profiles of aqueous infusions prepared from *Teucrium polium*, *Mentha pulegium*, *Rosmarinus officinalis*, *Lavandula stoechas*, and *Ocimum basilicum* were investigated using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS). Three independent infusions were prepared for each species, resulting in 15 individual volatile profiles, which were evaluated by principal component analysis (PCA) and hierarchical cluster analysis (HCA). A total of 115 volatile compounds were tentatively identified across the five species. Monoterpenoids were the predominant chemical group in *M. pulegium* (70.17 ± 0.20%), *R. officinalis* (81.80 ± 0.04%), *L. stoechas* (96.95 ± 0.08%), and *O. basilicum* (77.60 ± 0.35%), whereas *T. polium* showed a more heterogeneous profile comprising substantial proportions of monoterpenoids (49.95 ± 0.16%) and sesquiterpene hydrocarbons (31.32 ± 0.24%). The principal volatile constituents were carvacrol and germacrene D in *T. polium*; pulegone, piperitone oxide, limonene, and 1,8-cineole in *M. pulegium*; 1,8-cineole, borneol, and camphor in *R. officinalis*; fenchone and camphor in *L. stoechas*; and linalool, eugenol, and methyl chavicol in *O. basilicum*. The first two principal components accounted for 66.44% of the total variance. The three independently prepared infusions of each species formed compact groups in the PCA score plot, with *T. polium* showing the most distinct separation. PCA associated *M. pulegium* particularly with pulegone-, piperitone oxide-, and piperitenone oxide-related variation, whereas HCA grouped the three preparations of each species together and indicated the closest species-level similarity between *R. officinalis* and *L. stoechas*. Overall, HS-SPME-GC/MS combined with PCA and HCA provided a useful exploratory approach for the comparative characterization and species-related differentiation of herbal tea headspace volatile profiles under standardized infusion conditions.

Keywords: Herbal tea, Volatile compounds, HS-SPME-GC/MS, Chemometric analysis, Lamiaceae

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1. Introduction

Herbal teas are widely consumed due to their distinctive sensory properties, traditional uses, and plant-derived chemical composition (McKay and Blumberg, 2006; Poswal et al., 2019). Among the sensory attributes influencing consumer acceptance, aroma is of particular importance, as it is among the first characteristics perceived during preparation and consumption (Zhu et al., 2018). The aroma profile of herbal infusions is mainly associated with volatile organic compounds, including monoterpenes, sesquiterpenes, alcohols, aldehydes, ketones, esters, and other low-molecular-weight constituents (Yang et al., 2013; Zeng et al., 2025). The qualitative and quantitative composition of these compounds may vary depending on several factors, including plant species, chemotype, geographical origin, environmental conditions, plant part used, harvesting period, drying process, and preparation method (Figueiredo et al., 2008).

Plants belonging to the Lamiaceae family are particularly notable for their aromatic properties and rich volatile compound contents (Başer and Kırımer, 2018). *Ocimum basilicum* L., *Teucrium polium* L., *Mentha pulegium* L., *Rosmarinus officinalis* L., and *Lavandula stoechas* L. are aromatic plants used in different regions as culinary herbs, herbal teas, or components of traditional preparations (Uritu et al., 2018). Previous studies have shown that these species may contain diverse volatile constituents, particularly oxygenated monoterpenes and monoterpene hydrocarbons. Compounds such as linalool, estragole, eucalyptol, camphor, fenchone, pulegone, menthone, α-pinene, β-pinene, and terpinen-4-ol have frequently been reported in different members of this family (Bozin et al., 2007; Mahboubi and Haghi, 2008; Telci et al., 2006). However, the relative abundance of these compounds may vary considerably depending on the plant material, growing conditions, and analytical approach employed (Figueiredo et al., 2008).

Most of the available studies on these plants have focused on the analysis of essential oils obtained by hydrodistillation or extracts prepared using organic solvents (Bakkali et al., 2008; Maggio et al., 2016; Bahramikia et al., 2022). However, the volatile fraction recovered from herbal infusions can exhibit qualitative and semiquantitative differences when compared with the essential oil obtained directly from the corresponding plant material (Tschiggerl and Bucar, 2010). During brewing, the transfer of volatile constituents from plant material to the aqueous infusion and subsequently to the headspace may be affected by brewing temperature and time, the solid-to-water ratio, infusion matrix composition, and compound-specific physicochemical properties (Wright et al., 2007; Sánchez-López et al., 2020; Göksu Sürücü et al., 2024). Therefore, the direct characterization of volatile compounds in herbal infusions is important for providing a more realistic representation of the chemical profile encountered by consumers during tea preparation and consumption.

Headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS) is a widely used and suitable analytical approach for the characterization of volatile compounds in complex food and plant matrices (Kataoka et al., 2000). This technique enables the solvent-free extraction of compounds present in the headspace, requires limited sample preparation, and provides high sensitivity for the detection of a broad range of volatile and semi-volatile compounds (Chen et al., 2020; Djebili et al., 2022). The interpretation of chromatographic datasets containing numerous volatile compounds can be enhanced by multivariate statistical analysis. Principal component analysis (PCA) enables the visualization of similarities and differences among samples according to their multivariate volatile profiles, whereas hierarchical cluster analysis (HCA) groups samples on the basis of compositional similarity. In studies combining HS-SPME-GC/MS with chemometric analysis, PCA and HCA have been successfully used to differentiate samples with distinct volatile profiles and to identify variables associated with sample separation through PCA loading patterns (Lin et al., 2013; Duru et al., 2021). Therefore, the combined application of HS-SPME-GC/MS, PCA, and HCA may provide a useful framework for revealing species-related volatile patterns among herbal tea samples.

Although the volatile compositions of several Lamiaceae species have previously been investigated individually, direct comparative studies of aqueous infusions prepared from *O. basilicum*, *T. polium*, *M. pulegium*, *R. officinalis*, and *L. stoechas* under a unified experimental protocol remain limited. The originality of the present study lies in the side-by-side evaluation of these five herbal teas collected from Muğla Province using identical brewing and HS-SPME-GC/MS conditions, with three independently prepared infusions analyzed for each species. This standardized design reduces methodological variation and enables both within-species consistency and interspecies differences in the headspace volatile profiles to be evaluated. Therefore, the present study aimed to characterize the volatile profiles of the five herbal teas and to determine the volatile compounds most strongly associated with their species-related differentiation using PCA and HCA.

2. Materials and Methods

2.1. Plant Materials

The aerial parts of *Ocimum basilicum* L., *Teucrium polium* L., *Mentha pulegium* L., *Rosmarinus officinalis* L., and *Lavandula stoechas* L. were collected from selected locations in Muğla Province, Türkiye, including Menteşe, and Köyceğiz, between May and July 2024. Following collection, the plant materials were dried in the shade at room temperature. The dried samples were stored in a dry environment until further analysis.

2.2. Preparation of Herbal Infusions

Herbal infusions were prepared under standardized conditions. For each plant species, three independent infusions were prepared from separate aliquots of the dried material (n = 3). For each preparation, 10.0 g of dried plant material was mixed with 100 mL of distilled water preheated to 80 °C. The mixtures were covered and allowed to infuse for 10 min and were then filtered through Whatman No. 4 filter paper. Each freshly prepared infusion was analyzed individually by HS-SPME-GC/MS immediately after filtration.

2.3. Headspace Solid-Phase Microextraction of Volatile Compounds

Volatile compounds of the herbal infusions were extracted using headspace solid-phase microextraction (HS-SPME), adapted from previously reported procedures for plant-derived matrices (Djebili et al., 2022). Freshly prepared infusion (5.0 mL) was placed in a 20 mL headspace vial, followed by the addition of sodium chloride (1.0 g). Each vial was closed with a PTFE/silicone septum cap and equilibrated at 50 °C for 15 min under magnetic stirring. After equilibration, a DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA) was introduced into the vial headspace and maintained above the sample for a further 15 min at 50 °C while stirring was continued. The fiber was then transferred directly to the GC injector, where the retained analytes were thermally desorbed. Each of the 15 independently prepared infusions was subjected to one HS-SPME-GC/MS measurement. Before use, the fiber was conditioned according to the manufacturer's recommendations, and procedural blanks were included to assess background signals and carry-over.

2.4. GC-MS Analysis

Chromatographic separation and mass spectrometric detection were carried out using a Varian Saturn 2100T gas chromatograph coupled with an ion-trap mass spectrometer (Varian Inc., Walnut Creek, CA, USA). The system was fitted with an Rxi-5Sil MS fused-silica capillary column (Restek, Bellefonte, PA, USA; 30 m × 0.25 mm internal diameter, 0.25 µm film thickness).

Helium was supplied as the carrier gas at a constant flow rate of 1.4 mL/min. Thermal desorption of the SPME fiber was performed in the injector operated at 220 °C under splitless conditions. The oven was programmed from 60 °C, held for 5 min, to 280 °C at 4 °C/min, followed by a final hold of 5 min. Electron ionization was applied at 70 eV. The ion-source and transfer-line temperatures were maintained at 200 °C and 290 °C, respectively. Full-scan mass spectra were recorded across an m/z range of 28–650 using a scan duration of 0.5 s and an interscan interval of 0.1 s.

Volatile compounds were identified by comparison of their mass spectra with the NIST 14 and Wiley mass spectral libraries. The proposed identifications were further supported by comparison of the experimentally calculated linear retention indices (LRIs) with literature values reported by Adams (Adams, 2017) and, where available, by co-injection with authentic reference standards. Linear retention indices were calculated using an n-alkane standard series ranging from C7 to C30. When available, the identity of selected compounds was additionally confirmed by co-injection with authentic standards.

2.5. Data Preprocessing and Chemometric Analysis

The chemometric data matrix consisted of 15 observations and 115 variables. Each observation represented one independently prepared infusion, corresponding to three preparations for each of the five plant species, whereas each variable represented one volatile compound listed in Supplementary Table 1. Because the relative peak areas within each chromatographic profile had already been normalized to a total of 100%, no additional total-sum normalization was applied.

Compounds that were not detected in a given infusion and compounds recorded at trace level (“tr”) without a reliable numerical peak-area value were assigned a value of zero solely for chemometric analysis. Trace-level compounds were retained as “tr” in the descriptive data presented in Supplementary Tables 1 and 2.

Before multivariate analysis, the relative peak-area values were transformed using log10(x + 1) to reduce the disproportionate influence of highly abundant constituents while retaining the semiquantitative abundance structure of the volatile profiles. For PCA, the transformed variables were mean-centered. Autoscaling was not applied because it would have disproportionately weighted trace and very low-abundance compounds.

PCA was performed using scikit-learn version 1.8.0 in Python 3.13.5. The PCA score plot was used to evaluate the distribution and within-species consistency of the independently prepared infusions, whereas the loading coefficients were examined to identify volatile compounds associated with sample separation. For visual clarity, the PCA biplot displayed the 12 compounds with the largest loading magnitudes in the PC1–PC2 plane.

HCA was performed on the same log10(x + 1)-transformed data matrix using Euclidean distance and Ward’s linkage method in SciPy version 1.17.0. PCA scores and loadings are provided in Supplementary Tables 3 and 4, respectively.

3. Results

3.1. Volatile Compound Profiles of Herbal Tea Samples

The headspace volatile profiles of independently prepared herbal infusions of *Teucrium polium*, *Mentha pulegium*, *Rosmarinus officinalis*, *Lavandula stoechas*, and *Ocimum basilicum* were determined by HS-SPME-GC/MS. Three independent infusions were prepared for each species, resulting in 15 individual volatile profiles. Across the five species, a total of 115 volatile compounds were identified, including monoterpene hydrocarbons, monoterpenoids, sesquiterpene hydrocarbons, sesquiterpenoids, and other volatile constituents. At the species level, the number of detected compounds ranged from 39 in *R. officinalis* to 54 in *T. polium*. A total of 48, 50, and 51 compounds were detected in *M. pulegium*, *L. stoechas*, and *O. basilicum*, respectively. Mean relative peak areas and standard deviations are presented in Suplementary Table 1.

Monoterpenoids represented the predominant chemical class in the volatile profiles of *M. pulegium* (70.17 ± 0.20%), *R. officinalis* (81.80 ± 0.04%), *L. stoechas* (96.95 ± 0.08%), and *O. basilicum* (77.60 ± 0.35%). In contrast, *T. polium* exhibited a more heterogeneous volatile composition, comprising substantial proportions of monoterpenoids (49.95 ± 0.16%) and sesquiterpene hydrocarbons (31.32 ± 0.24%). The mean relative peak areas of sesquiterpene hydrocarbons were 4.95 ± 0.15% in *O. basilicum*, 2.64 ± 0.09% in *R. officinalis*, 1.14 ± 0.04% in *M. pulegium*, and 0.65 ± 0.03% in *L. stoechas*. Monoterpene hydrocarbons ranged from 0.40 ± 0.04% in *O. basilicum* to 22.47 ± 0.05% in *M. pulegium*.

The volatile profile of *T. polium* was characterized primarily by carvacrol (13.19 ± 0.11%), germacrene D (12.40 ± 0.09%), terpinen-4-ol (8.75 ± 0.09%), α-terpineol (5.93 ± 0.12%), caryophyllene oxide (4.17 ± 0.09%), trans-sabinene hydrate (4.04 ± 0.04%), verbenone (3.63 ± 0.02%), and β-caryophyllene (3.60 ± 0.05%). The simultaneous occurrence of substantial proportions of oxygenated monoterpenes and sesquiterpene hydrocarbons distinguished the *T. polium* profile from those of the other investigated species.

In *M. pulegium*, pulegone was the principal volatile constituent, with a mean relative peak area of 23.69 ± 0.10%. Other major constituents included piperitone oxide (15.44 ± 0.08%), limonene (13.97 ± 0.06%), 1,8-cineole (12.90 ± 0.05%), piperitenone oxide (8.55 ± 0.05%), β-pinene (4.23 ± 0.04%), linalool (3.57 ± 0.07%), and menthone (2.28 ± 0.07%). This profile was characterized by a combination of pulegone and several piperitone- and piperitenone-related oxygenated monoterpenes.

The volatile profile of *R. officinalis* was dominated by 1,8-

cineole (17.80 ± 0.03%), borneol (17.38 ± 0.04%), and camphor (16.76 ± 0.04%). Bornyl acetate (8.27 ± 0.06%), linalool (7.40 ± 0.04%), camphene (4.12 ± 0.03%), α-pinene (3.81 ± 0.06%), and verbenone (3.16 ± 0.05%) were also present at notable relative levels. Overall, oxygenated monoterpenes accounted for the major proportion of the *R. officinalis* headspace volatile profile.

Lavandula stoechas exhibited a distinctive fenchone-dominant profile. Fenchone accounted for 43.26 ± 0.07% of the mean relative peak area, followed by camphor (15.63 ± 0.07%), 1,8-cineole (10.18 ± 0.04%), bornyl acetate (10.15 ± 0.12%), fenchol (5.31 ± 0.10%), and fenchyl acetate (3.20 ± 0.13%). These compounds collectively represented the majority of the volatile profile and contributed to the particularly high proportion of monoterpenoids observed in this species.

The *O. basilicum* infusions were characterized by a pronounced predominance of linalool, which accounted for 65.25 ± 0.19% of the mean relative peak area. Eugenol (7.36 ± 0.07%), methyl chavicol (5.50 ± 0.05%), 1,8-cineole (4.42 ± 0.08%), β-caryophyllene (2.35 ± 0.13%), myroxide (1.62 ± 0.09%), guaiyl acetate (1.60 ± 0.06%), α-terpineol (1.58 ± 0.04%), bornyl acetate (1.34 ± 0.11%), and nerol (1.03 ± 0.06%) were also among the principal constituents. The high relative abundance of linalool clearly distinguished the *O. basilicum* profile from those of the other investigated herbal teas.

3.2. Chemometric Analysis of Volatile Compound Profiles

The relative peak-area data obtained from the 15 independently prepared herbal infusions were subjected to principal component analysis (PCA) and hierarchical cluster analysis (HCA) to evaluate within-species consistency and interspecies differences in the volatile profiles. The first two principal components explained 66.44% of the total variance, with PC1 and PC2 accounting for 35.98% and 30.47%, respectively (Figure 1A).

The PCA score plot showed compact grouping of the three independent preparations of each species in the PC1–PC2 plane. The three *T. polium* preparations were positioned on the positive side of PC1 and were clearly separated from the remaining species. The *M. pulegium* preparations formed a distinct group in the lower-left region and exhibited the most negative PC2 scores. The *R. officinalis* and *L. stoechas* preparations formed separate but relatively close groups in the upper-left region, whereas the *O. basilicum* preparations formed a distinct group in the lower-left region (Figure 1A). The compact within-species grouping indicated that the differences among species were greater than the variability among the independently prepared infusions under the conditions examined.

The PCA biplot indicated that the separation of *T. polium* along the positive PC1 direction was primarily associated with germacrene D, carvacrol, and terpinen-4-ol. The negative PC2 direction, toward which the *M. pulegium* preparations were oriented, was associated with pulegone, limonene, piperitone oxide, and piperitenone oxide. The upper-left region containing the *R. officinalis* and *L. stoechas* groups was associated mainly with camphor, fenchone, bornyl acetate, and 1,8-cineole. The position of the *O. basilicum* group in the lower-left region was oriented particularly toward linalool (Figure 1B). For visual clarity, Figure 1B displays the 12 volatile compounds with the largest loading magnitudes in the PC1–PC2 plane.

The HCA dendrogram supported the species-related grouping pattern observed in PCA (Figure 2). The three independently prepared infusions of each species initially clustered together, confirming the consistency of the species-level volatile profiles. At the species level, *R. officinalis* and *L. stoechas* formed the closest cluster, indicating comparatively similar overall volatile compositions. A second cluster was formed by *M. pulegium* and *O. basilicum*. These two broader clusters subsequently joined, whereas the *T. polium* cluster merged with the remaining species at a higher linkage distance, confirming that *T. polium* possessed the most distinct volatile profile among the species examined.

4. Discussion

The present study demonstrated that three independently prepared infusions of five Lamiaceae species produced distinct and internally consistent headspace volatile profiles under standardized infusion conditions. Although the investigated plants belong to the same botanical family, marked differences were observed in the relative distribution of monoterpenoids, sesquiterpenes, and other volatile constituents. The compact grouping of the independent preparations of each species in the chemometric analyses indicated that the observed interspecies differences were greater than the variability associated with infusion preparation. Nevertheless, the present results represent the volatile fraction released from aqueous infusions into the headspace rather than the composition of hydrodistilled essential oils. Direct quantitative comparison with essential-oil studies is therefore not appropriate because infusion conditions, aqueous-matrix effects, and headspace partitioning may alter the relative representation of individual compounds.

Teucrium polium showed the most distinct volatile profile among the investigated herbal teas, as demonstrated by the separation of all three independent preparations along the positive PC1 direction and their later clustering with the remaining samples in HCA. Carvacrol, germacrene D, terpinen-4-ol, α-terpineol, caryophyllene oxide, and β-caryophyllene were among the principal compounds associated with this differentiation.

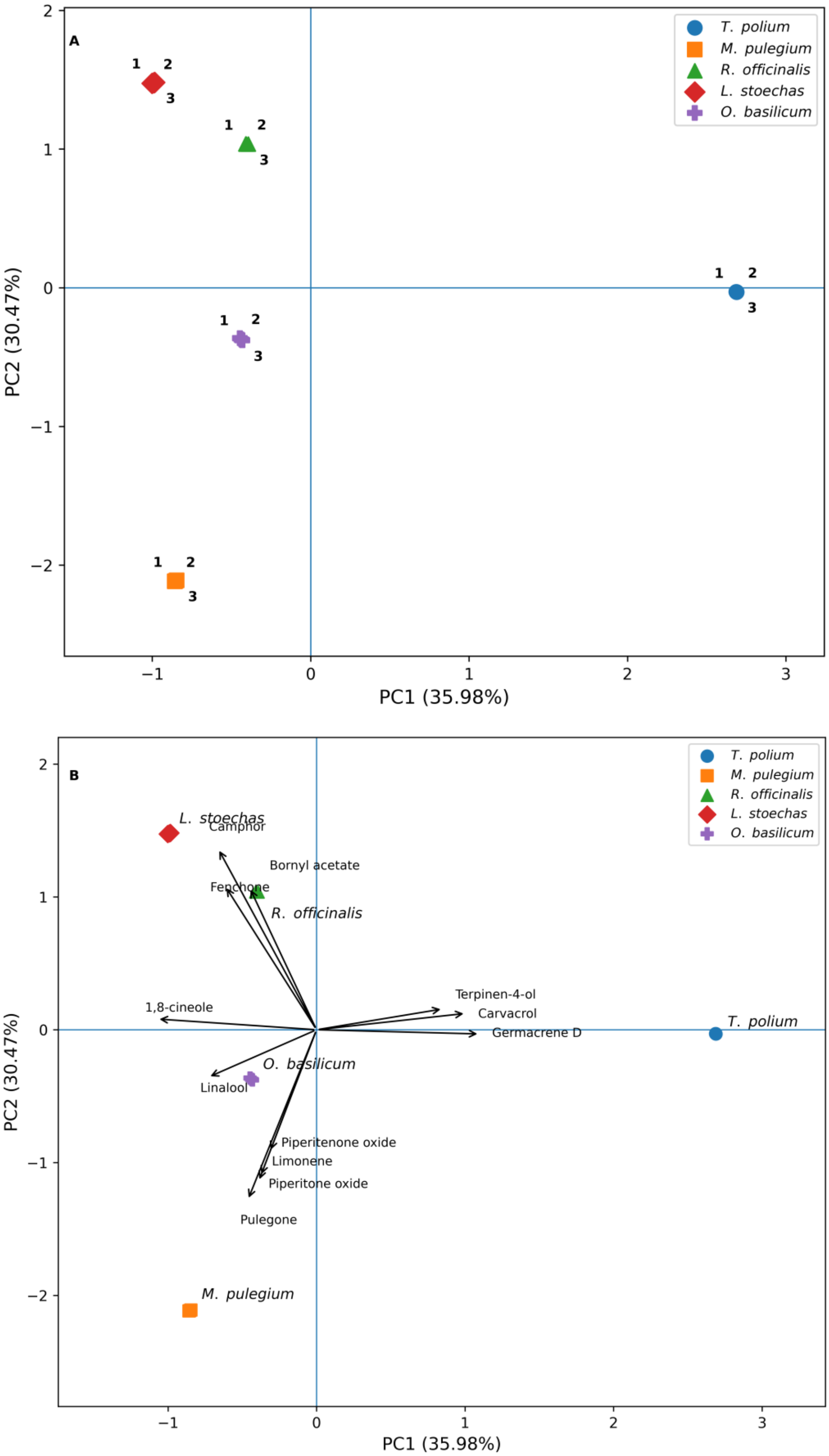

Figure 1. Principal component analysis of the headspace volatile profiles of 15 independently prepared herbal infusions. (A) PCA score plot showing three independent preparations for each plant species. (B) PCA biplot displaying the 12 volatile compounds with the largest loading magnitudes in the PC1–PC2 plane. PC1 and PC2 explained 35.98% and 30.47% of the total variance, respectively, accounting together for 66.44% of the total variance.

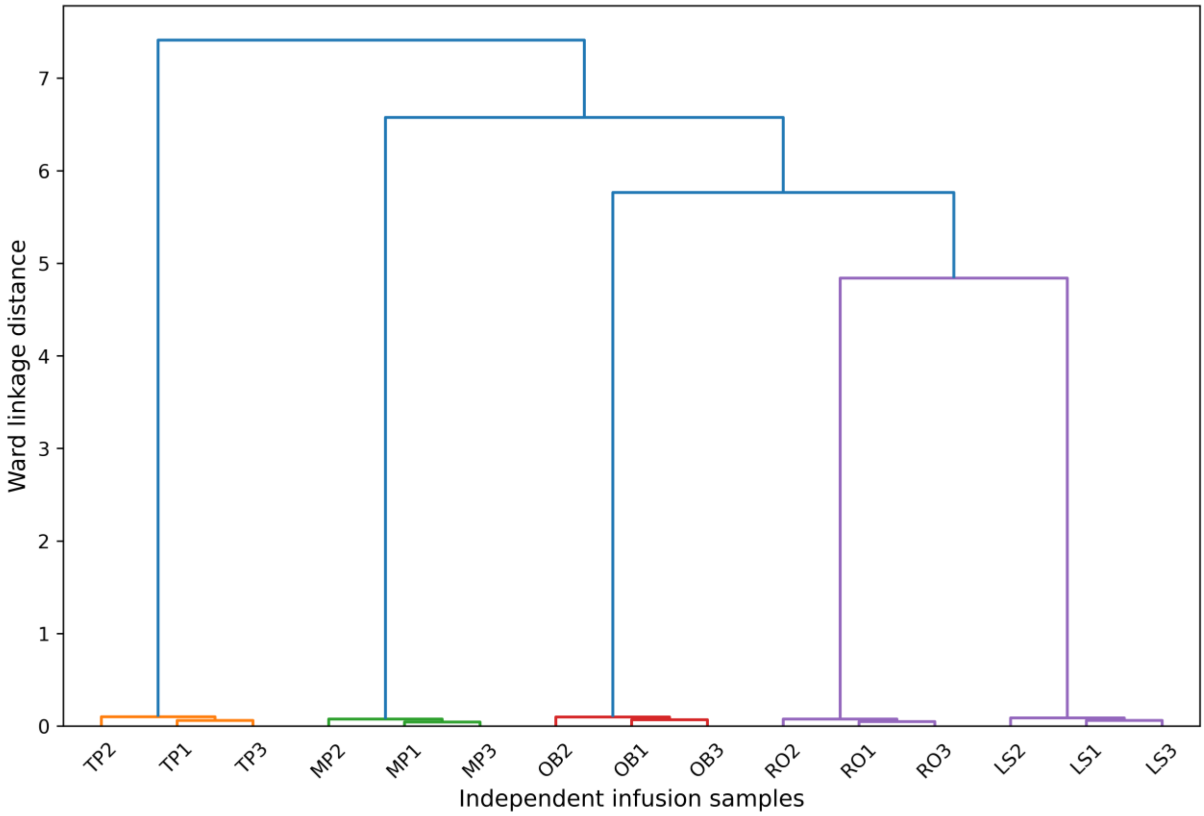

Figure 2. Hierarchical cluster analysis (HCA) dendrogram of the headspace volatile profiles of 15 independently prepared herbal infusions. The analysis was performed on log10(x + 1)-transformed relative peak-area data using Euclidean distance and Ward's linkage method. Sample codes: TP, *Teucrium polium*; MP, *Mentha pulegium*; RO, *Rosmarinus officinalis*; LS, *Lavandula stoechas*; and OB, *Ocimum basilicum*. The numbers 1–3 indicate the three independently prepared infusions of each species.

Previous studies have demonstrated substantial variability in the volatile composition of *T. polium*, with carvacrol, caryophyllene-type sesquiterpenes, germacrene derivatives, and oxygenated monoterpenes reported at different relative levels depending on population, plant material, chemotype, and extraction procedure (Candela et al., 2021; Chabane et al., 2021). The heterogeneous profile observed in the present herbal tea was therefore consistent with the chemical variability reported for this species. Carvacrol is of particular interest because antimicrobial and antioxidant properties have frequently been reported for this phenolic monoterpenoid and for carvacrol-containing *Teucrium* essential oils (Candela et al., 2021; Ultee et al., 1999). However, the occurrence of carvacrol and other potentially bioactive constituents in the infusion headspace should not be interpreted as direct evidence of biological activity because antimicrobial and antioxidant effects were not evaluated in the present study.

Mentha pulegium was characterized primarily by pulegone, piperitone oxide, piperitenone oxide, limonene, and 1,8-cineole, which were oriented toward the negative PC2 direction in the PCA biplot. Pulegone is frequently reported as a major constituent of *M. pulegium* essential oils, although limonene-, piperitone-, and piperitenone-related profiles have also been described in different populations (El Omari et al., 2025). The predominance of oxygenated monoterpenes in the present infusion was consistent with this general compositional tendency. The pulegone-, piperitone oxide-, piperitenone oxide-, and limonene-rich profile may also contribute to the strong mint-like and penetrating aromatic character generally associated with pennyroyal. Although *M. pulegium* has a history of traditional use as an aromatic herbal preparation, the predominance of pulegone is toxicologically relevant because hepatotoxic effects have been reported in experimental models at sufficiently high exposure levels (Zárybnický et al., 2018). Nevertheless, the relative headspace peak-area values obtained in the present study do not represent absolute concentrations, ingested doses, or consumer exposure and therefore cannot be used to draw conclusions regarding the safety of the prepared infusion.

The close positioning of *Rosmarinus officinalis* and *Lavandula stoechas* in both PCA and HCA reflected their generally oxygenated-monoterpene-rich volatile profiles. Nevertheless, the two species retained clearly distinguishable dominant compositions. *R. officinalis* was characterized mainly by 1,8-cineole, borneol, camphor, and bornyl acetate, whereas *L. stoechas* showed a pronounced fenchone-dominant profile together with camphor, 1,8-cineole, bornyl acetate, and fenchyl acetate. Similar terpenoid groups have frequently been reported in rosemary and *L. stoechas* essential oils, although their relative proportions may vary according to geographical origin, plant part, phenological stage, and extraction conditions (Rafya et al., 2024; Sevindik et al., 2025). The abundance of 1,8-cineole, camphor, borneol, fenchone, and bornyl acetate is consistent with the fresh, penetrating, woody, and camphoraceous aromatic characteristics commonly associated with rosemary and *L. stoechas* (Benabdelkader et al., 2011; Tschiggerl and Bucar, 2010). Several of these oxygenated monoterpenes have also been investigated for biological properties. In particular, isolated 1,8-cineole has been studied for its anti-inflammatory effects, while rosemary- and lavender-derived preparations have been associated with antimicrobial and antioxidant activities (Juergens et al., 2003; Rafya et al., 2024; Sevindik et al., 2025). However, these literature-based associations cannot be directly extrapolated to the present infusions because neither biological activity nor absolute compound concentrations were determined.

The *Ocimum basilicum* herbal tea was distinguished by its high relative abundance of linalool, together with methyl chavicol and eugenol. Linalool-rich and methyl chavicol-rich basil chemotypes are widely described in the literature, and eugenol- or methyleugenol-containing profiles further contribute to the chemical diversity of *O. basilicum* (Dharsono et al., 2022; Wirtu et al., 2024). In the PCA biplot, the three *O. basilicum* preparations were oriented toward linalool, whereas HCA placed the *O. basilicum* cluster closer to the *M. pulegium* cluster than to the other species. This pattern indicates that HCA reflects the overall similarity structure of the complete volatile dataset, whereas the PCA biplot highlights the variables contributing most strongly to variation along the first two principal components. Linalool is widely associated with floral, sweet, and citrus-like odor characteristics and is considered an important contributor to the aroma of linalool-rich basil chemotypes. Eugenol and methyl chavicol may additionally contribute clove-like, spicy, sweet, and anise-like notes (Brindisi et al., 2025; Patel et al., 2021). Although linalool and eugenol have been associated with several biological activities, their presence in the headspace profile does not by itself demonstrate the biological efficacy of the prepared herbal infusion.

Overall, PCA and HCA provided complementary exploratory tools for evaluating both within-species consistency and interspecies differences among the herbal tea samples. The PCA score plot showed compact grouping of the three independent preparations of each species, while the biplot associated *T. polium* with carvacrol-, germacrene D-, and terpinen-4-ol-related variation; *M. pulegium* with pulegone- and piperitone-related variation; *R. officinalis* and *L. stoechas* with cineole-, camphor-, fenchone-, and bornyl acetate-related variation; and *O. basilicum* with linalool-related variation. HCA similarly grouped the three independent preparations of each species together and indicated the closest species-level similarity between *R. officinalis* and *L. stoechas*. Nevertheless, the chemometric findings should still be considered exploratory because the samples represented a single geographical region and harvest period, and no external sample set was available for validation.

The present study has several methodological limitations. A single standardized brewing condition (80 °C for 10 min) was applied to all samples to enable direct interspecies comparison; however, the effects of alternative brewing temperatures and infusion times on the extraction and headspace partitioning of individual volatile compounds were not evaluated. In addition, although three independent infusions were prepared for each species, each infusion was subjected to a single HS-SPME-GC/MS measurement, and analytical repeatability could therefore not be evaluated separately from preparation variability. The relative peak-area data were semiquantitative, and neither absolute concentrations, odor activity values, nor sensory responses were determined. Future studies should examine different temperature–time combinations, replicate HS-SPME measurements, multiple independent plant collections, broader geographical and seasonal sampling, targeted quantification, and sensory or GC–olfactometry analyses.

5. Conclusion

This study demonstrated that standardized infusion preparation combined with HS-SPME-GC/MS and chemometric analysis can reveal distinct headspace volatile profiles among herbal teas prepared from five Lamiaceae species. The independently prepared infusions of each species showed compact grouping in PCA and HCA, indicating that the observed interspecies differences exceeded the variability associated with infusion preparation under the conditions examined. *Teucrium polium* was differentiated mainly by carvacrol-, germacrene D-, and terpinen-4-ol-related variation; *Mentha pulegium* by pulegone and piperitone derivatives; *Rosmarinus officinalis* and *Lavandula stoechas* by oxygenated monoterpenes such as 1,8-cineole, camphor, fenchone, and bornyl acetate; and *Ocimum basilicum* by its linalool-dominant profile.

The combined analytical and chemometric approach may be useful for comparative characterization and preliminary batch-level assessment of herbal tea volatile profiles. However, the findings are limited to the investigated samples, geographical origin, harvest period, and brewing conditions and should not be interpreted as validation of species authentication markers. Future studies should include multiple independent plant collections, broader geographical and seasonal sampling, replicate HS-SPME/GC-MS measurements, different brewing temperature–time combinations, targeted quantitative analysis, and sensory or GC–olfactometry evaluation.

As the present chemometric evaluation was based on the analyzed profiles of the selected samples, further studies including independent biological replicates, different harvest periods, geographical origins, and sensory evaluation would be valuable for confirming the stability and practical relevance of the observed volatile patterns.

Author Contributions

The percentages of the author's contributions are presented below. The author reviewed and approved the final version of the manuscript.

	S.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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