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Integrated dual-split high capacity sorptive extraction and chemometrics reveal extraction-dependent volatile signatures in *Hypericum perforatum*

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ABSTRACT

Hypericum perforatum (L.), commonly known as St. John's Worth, is a medicinal plant rich in bioactive compounds including volatile organic ones, which contribute to its antimicrobial, anti-inflammatory, and wound-healing properties. Optimising the extraction and analysis of these compounds is essential for their application in pharmaceutical, cosmetic and nutraceutical industries.

This study employed high-capacity extraction (HiSorb) probes and Gas Chromatography-Mass Spectrometry (GC–MS) to profile volatile organic compounds (VOCs) from hypericum flowering aerial parts. Four HiSorb phases were evaluated, with the double-phase CWR/PDMS demonstrating superior performance by capturing the broadest range of compounds and yielding the highest total peak area. A dual-split strategy was implemented to prevent detector saturation and ensure retention of low-abundance analytes, enabling comprehensive VOC profiling from a single sample. VOCs were analysed across various extract types, including alcoholic, water infused and oil-based preparations. A total of 108 VOCs were putatively identified, including terpenes, alcohols, and aldehydes, which contribute to the plant's aromatic and therapeutic properties. Chemometric analyses, including Principal Component Analysis (PCA), hierarchical clustering and Partial least square-discriminant analysis (PLS-DA), identified shared and discriminatory components across extract types. These findings support the selection of optimal extraction strategies tailored to specific industrial applications and highlight the chemical diversity and functional potential of *Hypericum perforatum*.

1. Introduction

Hypericum perforatum (L., St. John's Wart) is a highly recognised medicinal herb used extensively in traditional and modern remedies for the treatment of mild to moderate depression and anxiety, mainly due to its complex profile of bioactive phytochemicals [1]. The plant's therapeutic efficacy is primarily attributed to bioactive compounds like hypericin and hyperforin [2], but its unique aroma and biological effects, such as anti-inflammatory and antimicrobial properties, are linked to a rich mixture of volatile organic compounds (VOCs), including various monoterpenes and sesquiterpenes [3].

Given the widespread use of *H. perforatum* extracts in pharmaceutical, cosmetic and nutraceutical industries, the specific chemical composition of the final product and the preservation of both volatile and non-volatile components are essential to ensure consistent efficacy and quality [4]. The final chemical profile of *H. perforatum* extracts is highly dependent on the initial extraction procedure, which typically involves methods such as Soxhlet extraction, maceration and steam distillation [5]. While these extraction methods are effective, the resultant extracts often vary significantly in their VOCs abundance and composition due to volatility losses, thermal degradation, or matrix co-extraction [5]. Furthermore, modern industrial and consumer

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preparations frequently rely on simpler, milder, and greener techniques, such as water infusion or alcoholic maceration [6]. However, a detailed, comparative understanding of how these common procedures alter the full spectrum of VOCs relative to the natural state of the Hypericum flower remains limited [5,7]. In this context, traditional analysis of VOCs in complex matrices frequently relies on techniques like Headspace Solid-Phase Microextraction (HS-SPME), a well-established technique valued for its simplicity, solvent-free operation, and relatively low cost [8]. However, despite these advantages, HS-SPME can present certain limitations, particularly in applications requiring comprehensive VOC coverage [8]. Its limited sorptive phase volume may restrict the recovery of low-abundant or high-volatility compounds, and competitive adsorption among analytes of different polarity may compromise reproducibility [9]. These constraints underscore the need for alternative high-capacity sorptive extraction tools that offer larger sorbent volumes and improved sensitivity for both abundant and trace VOCs.

To ensure VOC profiling is comprehensive, the sorptive phase must be carefully optimised before proceeding with comparative extraction analyses. The choice of phase directly influences the range and abundance of VOCs recovered, as different coatings exhibit selective affinities for compounds of varying polarity and volatility. Without this preliminary optimization, certain analytes particularly trace-level or highly volatile compounds may be underrepresented, compromising the accuracy and reproducibility of the volatilome characterization.

Recent developments include stir-bar sorptive extraction (SBSE) [10], SPME Arrow [11–14],thin-film SPME (TF-SPME) [15], and HiSorb probes [16–20], which differ mainly in sorbent volume and extraction efficiency. These high capacity sorptive extraction tools were designed to overcome the limitations of traditional SPME by increasing the sorptive phase volume, which is positively correlated with extraction yield. For instance, conventional SPME fibers typically have a sorbent volume of about 0.6 µL, whereas SPME Arrow provides intermediate volumes between 3.8 and 11.8 µL, TF-SPME counts a volume of 40 µL, while HiSorb probes reach approximately 63 µL and SBSE offers a much larger range of 24–126 µL. While some tools offer different sorbent combinations, these options are not universally available, which further limits adaptability for diverse analytical needs [21,22]. Furthermore, the lack of automation requires manual handling steps that increase variability and reduce throughput.

Among all extraction tool, HiSorb is a probe-like tool designed for headspace or immersive sampling that combines a significantly larger sorptive phase and the support of multiple coatings, including Polydimethylsiloxane (PDMS), PDMS/ Divinylbenzene (DVB), PDMS/ Carbowax Resin (CWR), and DVB/CWR/PDMS [17,19,23,24]. Furthermore, HiSorb can be automated on a thermal desorption extraction and enrichment platform, namely Centri, reducing analytical errors due to manual sampling and improving reproducibility. This probe-like tool has been recently implemented in various analytical fields, from food to environmental applications, demonstrating its versatility for comprehensive VOC profiling [17,19,20,23,24]. Therefore, we initially evaluated multiple commercially available HiSorb phases to identify the coating that provided the broadest chemical coverage and highest extraction efficiency for *H. perforatum*. Following the optimisation of HiSorb phases, a comparative analysis was conducted on the flowering aerial parts of *H. perforatum* under several industrially and commercially relevant conditions, including aqueous infusion, oil maceration and alcoholic extraction using gas chromatography coupled to mass spectrometry (GC–MS). This step was essential to evaluate how commonly applied processing methods influence the plant's volatilome and to identify extraction strategies that preserve its aromatic and bioactive integrity.

The primary objective of this research was to characterise and compare the VOC composition of *H. perforatum* flowering aerial parts subjected to these treatments, contrasting them with the native, untreated volatile profile. Our findings demonstrate significant and characteristic alterations in the volatile profile, highlighting the critical need for accurate monitoring and optimization of extraction processes. This study provides essential data to improve the efficiency and consistency of industrial formulations based on *Hypericum perforatum*, ensuring both product quality and functional performance.

2. Materials and methods

2.1. Plant material, standards and chemicals

Dried plant material of *Hypericum perforatum* L. was kindly provided by the Department of Pharmacy of the University of Medicine, Tirana, Albania. Hypericum plant flowering aerial parts were collected during the full-bloom stage in the mountains of Krraba (Albania) in the summer of 2023. The plant flowering parts were naturally dried in a dark, well-ventilated room, separated from external impurities, packed in plastic bags and sealed with a vacuum machine, and stored in a cold, dark place until the extraction process. Dried flowering aerial parts (DFAP) were used both for extract preparation and for direct VOC analysis of the unprocessed sample. This allowed the characterisation of the native volatile profile without solvent interference. LC-MS grade water (H2O) and methanol (MeOH) were purchased from Carlo Erba Reagents (Milan, Italy). Ethanol (EtOH) was obtained from Merck Sigma-Aldrich. An alkane mixture (C7-C30) was purchased from MilliporeSigma (USA). The internal standard (IS) mixture (1-Bromo-4-fluorobenzene; 1,2-Dichloroethane-d4; Toluene D8) was obtained from Restek (USA, 30,004).

2.2. Sample preparation

All the extraction methods were designed to mimic the industrially and traditionally relevant processing procedures to favour the transfer from lab to commercial contexts [25] Dried flowering aerial parts of *H. perforatum* were manually homogenised using a mortar and pestle to ensure uniform particle size before extraction. For all extraction types, 1 % (w/v) ratio of homogenised sample was used. Hydroalcoholic extracts (methanol-water or ethanol-water mixtures, 80:20 % v/v) were prepared using ultrasound-assisted extraction (UAE) at 50/60 Hz and 550 W (Elmasonic S100H) for 30 min at 20 °C. The procedure was repeated for a total of five times using the same sample. Water infusions were similarly prepared using UAE under identical conditions, followed by overnight infusion at 4 °C. Water decoctions were obtained by adding 1 % (w/v) of the sample to water and allowed to simmer at 100 °C for 10 min in a closed glass container, after which the mixture was cooled to room temperature.

All extracts were centrifuged at room temperature under moderate speed (5000 rpm) for 5 min in fully capped tubes (NEYA 8; Remi Elektrotechnik Limited, REMI Lab World, India), and the supernatant was collected. To minimise photodegradation of light-sensitive constituents, aluminium foil was used throughout the extraction process. Each extraction was performed in three biological replicates. The resulting extracts were aliquoted into 20 mL amber glass vials, filled completely and cupped with airtight caps to minimise headspace and prevent evaporative losses, then stored at −20 °C in the dark until analysis.

Oil-based extracts were prepared in the Department of Pharmacy of the University of Medicine, Tirana, Albania. Dried flowering parts of the plant were used for maceration at a ratio of 10 % (w/v) in corn oil. The material was placed in a heat-resistant glass beaker and completely covered with the oil. The beaker was then positioned in a water bath maintained at 40–45 °C for 4 hours, with intermittent gentle stirring to facilitate extraction. After heating, the mixture was allowed to cool to room temperature before separating the oil phase from the plant residue by filtration through muslin cloth applying gentle pressure to recover the maximum amount of oil. The resulting macerated oil was stored in a cool, dry place until further analysis. Oil extracts were stored at controlled room temperature (20–22 °C) in the dark.

For all extraction conditions, blank samples were prepared by

incubating the corresponding solvent under identical conditions using the same laboratory consumables as those used for the test samples.

Although the extraction protocols differ in solvent composition, temperature and energy input, they were intentionally selected to reflect the diversity of preparation strategies most commonly employed for medicinal and aromatic plants in analytical, traditional, and commercial contexts [25]. Therefore, their comparison aims to evaluate how realistic processing conditions modulate the volatile chemical profile rather than to directly compare extraction efficiency under identical parameters. For instance, an overnight infusion step was used to the aqueous extract to compensate for the limited solubility and slower diffusion of hydrophobic constituents, thereby improving compound transfer into the solvent. In contrast, the hydroalcoholic mixture provides higher solvating power and faster extraction kinetics, enabling excellent extraction without the need for prolonged infusion [25] .

This experimental design is compatible with the multivariate statistical approach adopted, which focuses on relative compositional patterns and class discrimination rather than absolute quantitative yield comparisons.

2.3. VOC extraction

Headspace VOC extraction was performed using a Centri sample extraction and enrichment platform (Markes International Ltd, UK), equipped with an electrically cooled focusing trap. Four different commercially available HiSorb probes were supplied pre-coated by Markes International Ltd. (Bridgend, UK) and evaluated for extraction efficiency. Each HiSorb probe contains 65 µL of sorptive material made of polydimethylsiloxane (PDMS; H1-AXABC), PDMS/divinylbenzene (PDMS/DVB; H3-AXABC), PDMS/Carbowax resin (PDMS/CWR; H2-AXABC), and DVB/CWR/PDMS (H4-AXABC). The outer phase dimensions for HiSorb probes are approximately 8 × 3 mm (PDMS-only) or 8.5 × 3 mm (other phases), as specified by the manufacturer. HiSorb probes were conditioned according to the manufacturer's recommendations: 10 min at 50 °C followed by 90 min at 270 °C. Extraction conditions were adapted from [26] with minor modifications to account for differences in sample matrix, dilution level, and probe configuration. Briefly, 100 mg of the DFAP sample or 3 mL of a 1:5 aqueous dilution of DFAP extracts were transferred into 20 mL Headspace crimp vials (C-VCC20; Markes International), spiked with 5 µg/ mL of IS, and sealed with crimp caps (C—HSPCCS; Markes International). Samples were equilibrated at 55 °C for 10 min to allow the VOCs to saturate the headspace. The HiSorb probe was then inserted and exposed to the headspace for 20 min at 55 °C under controlled agitation (10 *sec* at 250 rpm followed by 2 *sec* rest), to accelerate mass transfer and improve analytical repeatability [16]. Following extraction, the HiSorb probe was thermally desorbed in the Centri injector at 260 °C for 10 min under a helium flow (50 mL/min), and the released volatiles were refocused on an electrically cooled trap (U-T12ME-2S, Markes int.) held at 0 °C. After a one-minute purge with helium (50 mL/min), the trap was heated to 280 °C and held for 3 min. The split flow of 40 mL/min was applied (high-split), and the effluent was recollected into a thermal desorption (TD) tube (C2-EAXX-5314; Inert Safelok Tube, MARKES International Ltd).

To enhance detection of low-abundance compounds, the TD tubes were subsequently re-desorbed. Conditions included a dry purge of 1 min at 50 mL/min, desorption at 280 °C for 10 min with a flow of 50 mL/min on a refocusing trap set at 0 °C. After one min helium purge at 50 mL/min, the trap was heated to 280 °C and held for 3 min with a reduced split flow of 10 mL/min (low-split). Carryover effect was assessed by running re-desorption after representative samples under the same analytical conditions, and no residual VOCs were detected (Supplementary Material, Figure S1). To prevent cross-contamination, both HiSorbs and TD tubes were reconditioned after each sample. Probes were reconditioned for 15 min at 260 °C, and TD tubes for 10 min at 300 °C.

2.4. GC–MS analysis of VOCs

Volatile organic compounds were analysed using a gas chromatograph coupled to a single quadrupole mass spectrometer (GC–MS; 7890B-5977B, Agilent Technologies, USA), following the methodology described by [26] with minor modification. Separation was achieved using a Rxi-5MS capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness; Restek). Helium was used as the carrier gas at a constant flow rate of 1.3 mL/min. The oven temperature was set at the initial temperature of 35 °C, held for 2 min, ramped to 200 °C at 3 °C/min, then increased to 260 °C at 25 °C/min. A post-run step was performed at 320 °C for 5 min. The MS operated at an ion source temperature of 230 °C, MS quadrupole of 150 °C and a transfer line of 280 °C. The scan range was from 45 to 350 *m/z* and an acquisition energy of 70 eV.

2.5. Data elaboration and statistical analysis

Raw chromatographic data were processed using ChromSpace version 2.2 (SepSolve Analytical, Peterborough, UK). To ensure consistent alignment across all samples, an enriched 'reference' chromatogram was generated using the external background addition feature. This approach allowed retention of low-abundance signals and improved alignment accuracy across datasets acquired at different time points throughout the year (Supplementary Material, Figure S2). Chromatograms were aligned using a spectral-based algorithm that automatically corrected retention time drift without requiring additional preprocessing. Peak integration was performed on the 'reference' chromatogram using ChromSpace's proprietary deconvolution algorithm, which resolves co-eluting compounds based on their mass spectral profiles. Putative compound identification was achieved by matching integrated peaks against the NIST20 spectral library, using a minimum match factor threshold of 80 % and a linear retention index (LRI) tolerance of ± 20 units relative to library value. Interferences co-extracted from each extraction medium (blank sample) were subtracted from the sample extracts, to eliminate medium-derived background features. For HiSorb phases comparison and selection, each compound ion abundance was normalised to the IS (1-bromo-4-fluorobenzene) to minimise variability associated with extraction phase. Selecting a universally appropriate internal standard is challenging in volatilome analysis of complex flowery matrices containing chemically diverse VOCs, and total ion based normalisation is commonly used. Therefore, for VOC profiling of *H. perforatum* preparations, total ion abundance normalisation was applied to each compound, followed by square-root transformation to reduce the influence of highly abundant compounds and improve statistical power [27–32] . Compounds were filtered out if they did not appear in multiple replicates for any condition, if they were a known contaminant and if the relative standard deviation (RSD) was greater than 20 %. Recurrent components that did not show either the required mass spectral fit or RI match were added as 'unknown'.

To ensure comprehensive VOC profiling, data from both high- and low-split chromatograms were evaluated for signal detection or saturation (representative examples are provided in the Supplementary Material, Figure S3). Relative abundances from low-intensity compounds (detected only by low-split, Supplementary Table S2) were merged with those from highly intense profiles to generate unified aroma fingerprints for each sample.

Univariate and Multivariate statistical analyses were conducted using MetaboAnalyst version 6.0 software [33]. These included univariate analysis of variance (ANOVA) and Permutational multivariate ANOVA (PERMANOVA) with a significance level of $p \leq 0.05$, followed by Tukey's post-hoc test. Multivariate analysis included principal component analysis (PCA) as an unsupervised method to assess overall variance without prior class information. Partial least square-discriminant analysis (PLS-DA) was employed as a supervised approach to enhance group separation and identify discriminant

variables based on variable importance in projection (VIP) score greater than one and calculation of overall classification performance based on Accuracy coefficient of explained variance (R^2) and cross-validated coefficient of Prediction (Q^2). Two-dimensional Hierarchical Clustering Analysis (HCA) was performed using the *hclust* function in R stat and visualised within MetaboAnalyst.

3. Results and discussion

3.1. HiSorb phases comparison and selection

To ensure comprehensive profiling of VOCs from *H. perforatum*, the efficiency of four high-capacity HiSorb coatings, namely PDMS, CWR/PDMS, DVB/PDMS, and DVB/CWR/PDMS, was systematically evaluated, for the first time, under identical headspace (HS) extraction and GC–MS conditions. The comparison aimed to identify the coating providing the highest extraction yield, reproducibility, and chemical coverage across diverse VOC classes of the DAFP sample. Statistical analysis of total internal standard normalised VOC abundance demonstrated significant differences among the coatings ($p < 0.001$), as shown in Fig. 1 and Supplementary Table S1. The CWR/PDMS exhibited the highest median and mean values, reaching approximately 115–120 units of total VOCs IS normalised abundance, and was statistically distinct from all other coatings. This superior performance reflects its combined polarity, which enhances retention of both oxygenated semi-volatiles and non-polar terpenes. The DVB/CWR/PDMS coating ranked second with median values ranging 75–80 units and a narrow interquartile range, indicating consistent extraction but significantly lower efficiency compared to CWR/PDMS. The PDMS coating showed intermediate performance, with median values around 65–70 of relative abundance, slightly lower but not statistically different performance compared to DVB/CWR/PDMS. The DVB/PDMS coating provided the significantly lowest extraction efficiency, with median values near 40 units of relative abundance, confirming its reduced suitability for comprehensive VOC profiling in this matrix.

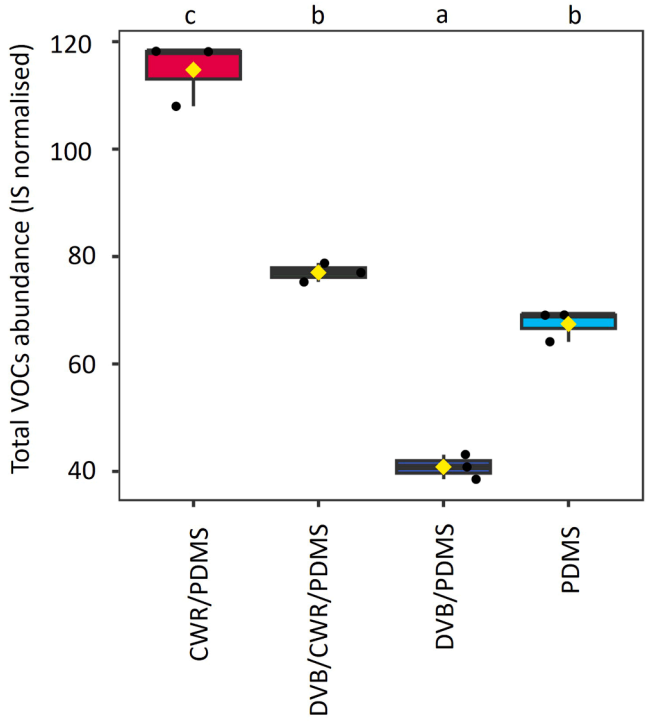

Fig. 1. Boxplot displaying the distribution of total VOC IS normalised abundance extracted using four HiSorb phases. Each box shows the interquartile range (IQR), the horizontal lines represent the median, and the diamond indicates the mean. The phase named CWR/PDMS presents the most stable, consistent and highest extraction for VOCs. The internal standard (IS) is 1-bromo-4-fluorobenzene. Different letters (a–c) above the boxes indicate Tukey HSD groupings; groups sharing the same letter are not significantly different ($p > 0.05$).

Comparative evidence from previous studies supports the matrix-dependent nature of HiSorb phase performance. Optimal recovery has been reported for DVB/PDMS coatings in matrices dominated by low-molecular-weight polar acids, such as hard seltzers, whereas triphasic DVB/CWR/PDMS coatings have been shown to provide broader coverage in complex aqueous matrices containing both polar and non-polar constituents, including goat cheese whey wastewater [19,23]. Similarly, Schincaglia et al. [24] highlighted the advantage of multi-phase coatings for lipid-rich matrices, such as pistachio samples, where DVB/CWR/PDMS improved classification accuracy in GC × GC workflows. These findings reinforce the principle that sorptive phase selection should be guided by analyte polarity and matrix composition, and further highlight the superior sensitivity of HiSorb relative to conventional SPME in complex food and dairy systems due to its larger sorbent volume and enhanced sensitivity [34].

A more detailed analysis was conducted to investigate compositional patterns across volatility ranges and chemical families among the selected sorptive coatings. Overall results indicated that the CWR/PDMS coating enhances recovery of both early-eluting alkanes and mid- to late-eluting terpenes and aromatics, yielding broader coverage and higher intensities than other phases (Fig. 2, Supplementary Materials Table S1). In the high volatile range, representative branched and straight alkanes such as 2-methyl-octane (C1) and nonane (C2) show marked increases with CWR/PDMS relative to the other coatings, with 3-methylnonane (C6) following a similar trend. Progressing to monoterpenes on the RT window spanning 11 to 16 min, compounds such as α-pinene (C25), β-myrcene (C8), m-cymene (C12) and D-limonene (C13) exhibit consistently higher signal with CWR/PDMS, confirming balanced retention of hydrocarbon and oxygenated terpenoids on this volatility window. At intermediate to late RTs, the CWR/PDMS coating shows an increased recovery of heavier hydrocarbons and aromatics, including undecane (C16), 2-methyl-dodecane (C18), and 1-methyl-naphthalene (C118). In the sesquiterpene region, compounds such as caryophyllene (C20), humulene (C76), (E)-β-farnesene (C77), γ-cadinene (C82), and cadina-1(10),4-diene (C85) are substantially higher with CWR/PDMS, indicating improved capture of late eluting sesquiterpenes. Collectively, these examples illustrate that CWR/PDMS enhances intensity across alkanes, monoterpenes, sesquiterpenes and aromatics over a wide RT.

In addition to analytical performance, several compounds enriched by CWR/PDMS are annotated in the Human Metabolome Database (HMDB) as exogenous metabolites detected in human biofluids, which support their inclusion as analytically meaningful targets while avoiding any inference of causality (Supplementary Materials Table S2) [35]. Among them one can find β-myrcene which reduces chronic arthritis pain/inflammation via a cannabinoid-receptor-dependent mechanism [36,37]. Furthermore, β-caryophyllene acts as a selective cannabinoid receptor type two agonist with immunomodulatory/anti-inflammatory properties [38,39] and D-limonene is tolerable in humans with evidence of tumour biomarker modulation [40,41].

This contextual information reinforces the analytical importance of selecting a coating that maximises recovery of structurally diverse VOCs, as it enables downstream studies on both functional and pharmacological implications without compromising the primary chromatographic objectives.

Overall, the present results and literature comparisons confirm that CWR/PDMS offers the most effective solution for exhaustive VOC profiling for *Hypericum perforatum*, combining high extraction abundance and broad chemical coverage. This analytical advantage underpins its selection for subsequent analyses and reinforces the importance of tailoring extraction method and coating phase choice to the physicochemical characteristics of the target matrix.

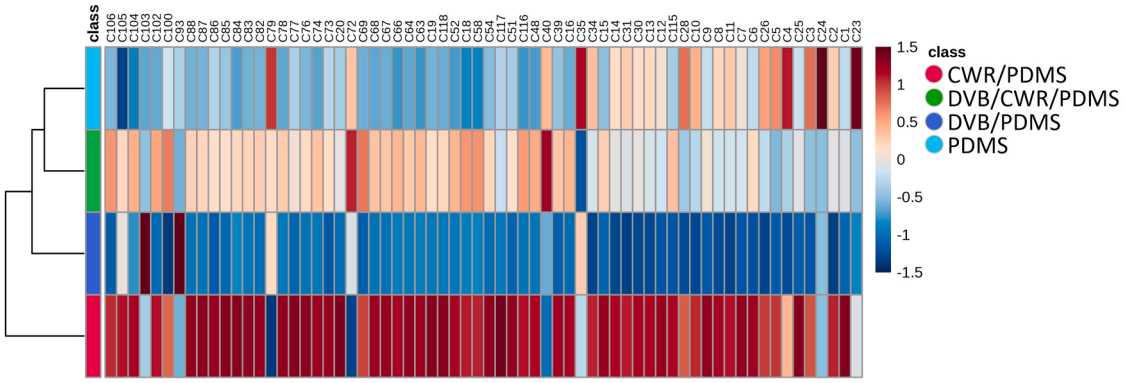

Fig. 2. Heatmap and hierarchical clustering analysis of four HiSorb sorptive coatings based on targeted volatile compounds detected in the *H. perforatum* DFAP sample. Each coloured cell represents the mean normalised abundance of each compound (IS normalised) across three replicates, following Log_{10} transformation to improve comparability of signal intensities. Red and blue colours indicate relatively higher and lower abundances, respectively. Compound codes (Cn) correspond to those reported in Table S2, where full compound identification and details are provided.

3.2. VOC profiling of H. perforatum preparations

After defining the most suitable HiSorb sorptive coating, the native DFAP and five extracts of *Hypericum perforatum* were analysed for their VOC composition. Across the six sample types, a total of 113 VOCs were detected (Supplementary Material Table S2). The largest number belonged to the terpene and terpenoid class (63), including 17 monoterpenes, 20 sesquiterpene, 8 monoterpenoids and 18 sesquiterpenoids. The remaining VOCs were distributed among branched and linear alkanes (11), non-acetate esters (8, which included benzoate and fatty acid esters), alcohols (6), aromatic hydrocarbons (5), aldehydes (2), one ether, and 17 recurrent but unidentified compounds classified as unknowns. Similar patterns have been reported in previous research studies on *H. perforatum*, which consistently report terpenes and terpenoids as the principal contributors to its volatilome, alongside minor fractions of alkanes, aldehydes, esters and aromatic hydrocarbons [3,7]. Hydroalcoholic extracts (ethanol:water and methanol:water) and native DAFP exhibited the highest overall VOC abundance and chemical diversity (106, 107 and 104 compounds, respectively), with profiles dominated by monoterpenes and sesquiterpenes (Fig. 3). This enrichment pattern closely aligns with earlier findings from hydrodistillation and HS-SPME studies demonstrating that *H. perforatum* intrinsic terpene-rich fingerprint is preserved when solvents of moderate polarity are used [5]. This dominance of terpenes indicates that hydroalcoholic solvents efficiently solubilise *H. perforatum* non-polar volatilome, maintaining a chemical profile comparable to intact or hydrodistilled herb. Dudek et al. (2022) [3] similarly showed that monoterpenes and sesquiterpenes remain stable across drying methods, suggesting that these compounds constitute a robust chemical core that persists across both pre- and post-extraction treatments. In contrast, water-based infusion and decoction favoured mono- and sesqui-terpenoids, esters and aldehydes, with a total number of VOCs of 89 and 88, respectively. Similar shifts toward more polar VOC subclasses have been documented in *H. perforatum* hydrosols, where monoterpenoids and sesquiterpenoids dominate due to increased solubility in water [7]. Our findings thus extend the aqueous polarity effect to infusion and decoction, which reproduce a similar extraction patten but under milder, consumption relevant conditions. The oil maceration showed the lowest overall chemical diversity (29 compounds) and selectively enriched non-polar branched and linear alkanes, with hydrophobic properties, reflecting solvent polarity and partitioning principles (Fig. 3 and Supplementary Material Table S3). This alkane enrichment sharply contrasts with the hydro-distilled essential oil of *H. perforatum*, where sesquiterpenes remain predominant and alkanes represent a smaller fraction. This discrepancy reflects differences in solvent partitioning, in fact whereas hydrodistillation extracts VOCs based on volatility, oil maceration stabilises lipophilic hydrocarbons and enhances their subsequent headspace release. These findings have not been reported before, and show how lipidic solvents strongly reshape the *H. perforatum* volatilome by enriching chemical classes that are poorly represented in more volatile-dependent extraction techniques. Principal Component Analysis (PCA) was performed on 108 reproducible VOCs (RSD < 20 %). The first two principal components explained the 87,1 % of the total variance in the data set (PC1=47,5 %, PC2=39,6 %), indicating robust dimensionality reduction and clear discrimination among extraction types (Fig. 4). Oil maceration formed a distinct cluster driven by alkanes. Water-based extracts (infusion and decoction) though separated from each other, grouped on the positive site of PC1 and PC2, the grouping is associated with terpenoids, alcohols and esters. While DAFP partially overlapped with hydroalcoholic extracts (methanol:water and ethanol:water) consistent with their terpenic profile dominated by monoterpenes and sesquiterpenes. PerMANOVA analysis (F-value= 2381.9; R2= 0.99899; $p < 0.001$) confirmed that extraction type accounted for over 99 % of the variance in VOCs composition, underscoring solvent polarity as the primary driver of volatilome differentiation.

3.3. Shared VOCs across plant preparations

Across all preparations, 23 VOCs were consistently detected, encompassing monoterpenes, sesquiterpenes, terpenoids, aldehydes and alkanes. We focused on the 23 VOCs shared across all preparations because these compounds represent the most stable and extraction-independent portion of the volatilome. This avoids bias from solvent-specific compound loss, particularly in low-yield preparations such as oil maceration. The 23 VOCs include bioactive constituents such as α-pinene (C25), camphene (C4), α-thujene (C3), D-limonene (C13), γ-terpinene (C14), α-campholenal (C17), β-caryophyllene (C20), and α-selinene (C22). Their aroma descriptors range from citrus and herbal to woody and balsamic (Supplementary Material Table S2, VOCs in bold), defining the characteristic fragrance of Hypericum species and reported in essential oil and hydrosol extracts [3,26]. Several monoterpenes (e.g., D-limonene and γ-terpinene) are GRAS-listed (Generally Recognised as Safe), supporting their use in food, fragrance and cosmetic formulations [42]. These VOCs exhibit multifunctionality activity. Monoterpenes such as α-pinene and D-limonene are documented for anti-inflammatory, antioxidant, antimicrobial and immunomodulatory effects, with α-pinene suppressing the common inflammatory signalling pathways of Mitogen-activated protein kinase (MAPK) and NF-κB[36,37] and β-myrcene which, as already mentioned, reduces chronic pain via cannabinoid receptor modulation [43,44]. Sesquiterpenes like β-caryophyllene act as selective cannabinoid type 2 (CB2) receptor agonists, conferring immunomodulatory and analgesic

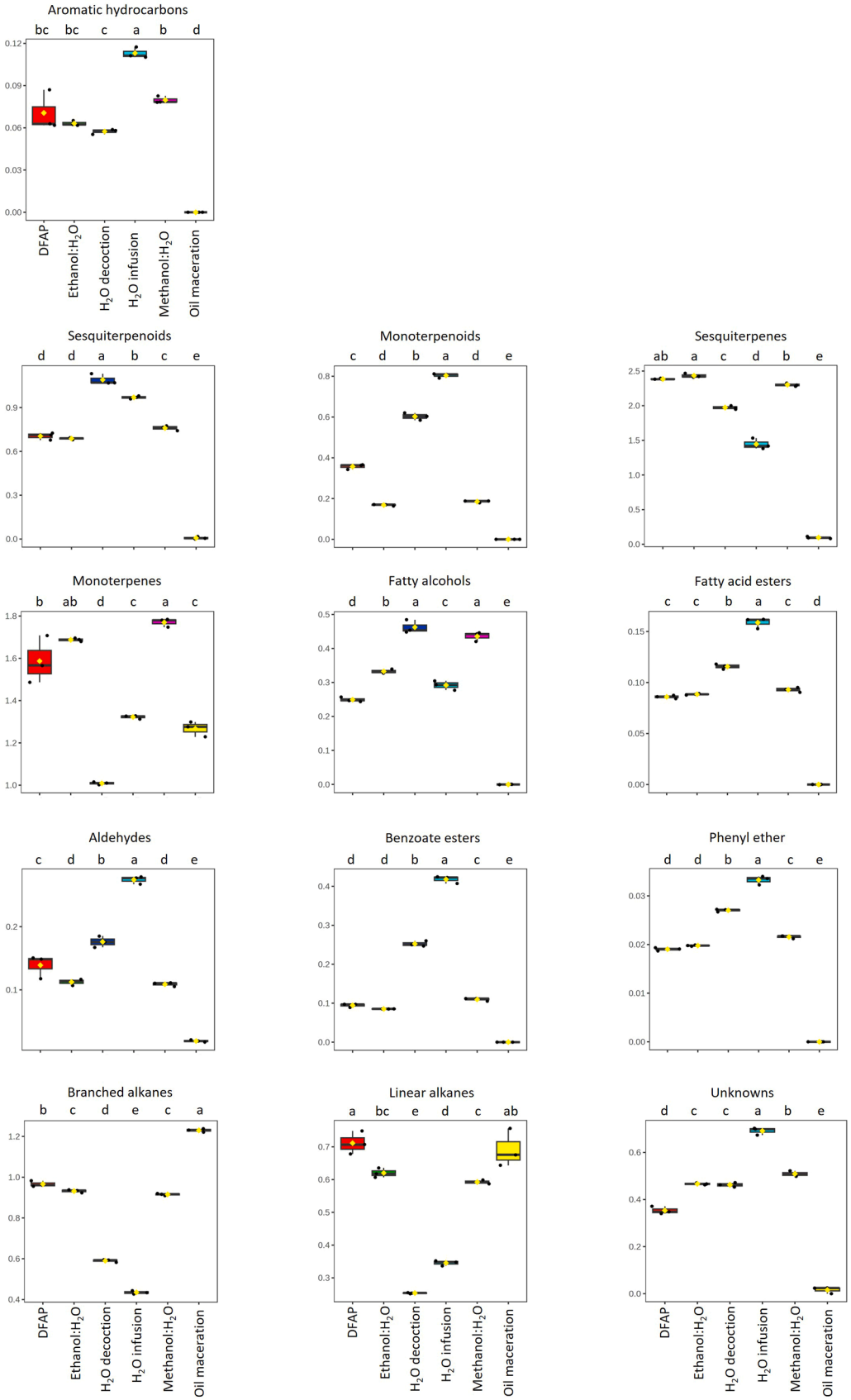

Fig. 3. Boxplot displaying the distribution of VOC classes normalised to the abundance extracted using four HiSorb phases and transformed by Squareroot. Each box shows the interquartile range (IQR), the horizontal lines represent the median, and the diamond indicates the mean. Different letters (a–e) above the boxes indicate Tukey HSD groupings; groups sharing the same letter are not significantly different ($p > 0.05$).

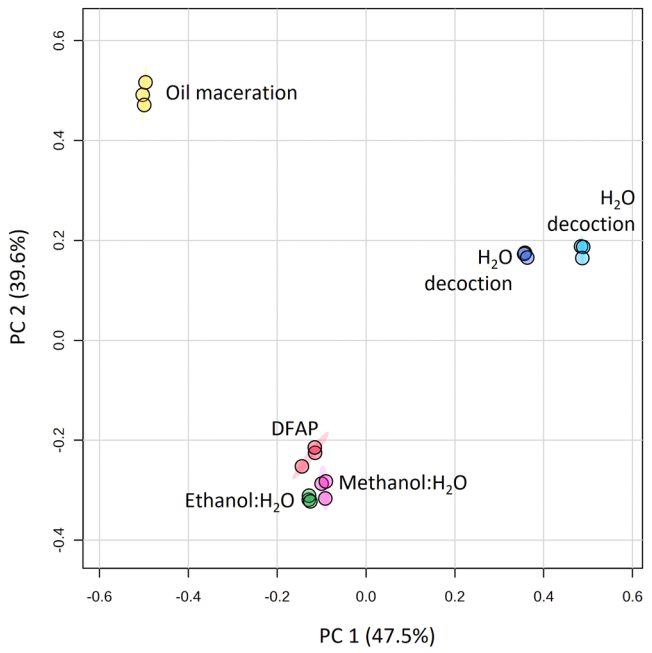

Fig. 4. PCA score plot (PC1 and PC2) of VOC profiles with 95 % confidence interval, showing variation among different solvent-based procedures.

properties [39,45]. Conversely, alkanes such as nonane and 2-methyldecane, while relevant for lipid-based formulations, pose flammability and irritancy risks, limiting their suitability for oral applications [46]. Heatmap clustering (Fig. 5) provides a comprehensive visualization of relative abundance and clustering patterns of the 23 compounds shared across extraction preparations. Hydroalcoholic extracts (ethanol:water and methanol:water) cluster together and are closely related to the native DAPF profile confirming the results shown by means of PCA of the entire volatilome dataset (Fig. 3). Those extracts show significantly higher abundance for monoterpenes such as α-thujene (C3), camphene (C4), and γ-terpinene (C14) ($p < 0.05$; Figure S2) confirming their strong affinity for semi-polar terpenes. Oil infusion forms a distinct cluster significantly enriched in non-polar alkanes (e.g., 2-methyloctane (C1), nonane (C2), and 2-methyldecane (C15)). Water-based infusions cluster together in the heatmap, although they show difference in the abundance of specific shared VOCs. Cold infusion favoured 3-carene (C11), m-cymene (C12), and α-campholenal (C17). In contrast, decoction was more enriched in β and α -selinene (C21 and C22). This integrated analysis demonstrates that solvent polarity and thermal conditions drive selective recovery of VOC classes as expected. Identifying the 23 shared compounds provides a set of potential chemical markers that remain stable across extraction preparations. Therefore, they may serve as foundation for quality control and guidance for extraction strategies across industrial applications.

Overall, oil maceration was the least enriched both in total VOC number and in specific compound classes, confirming its limited suitability for comprehensive volatilome recovery. Hydroalcoholic extracts (ethanol:water and methanol:water) present very similar aromatic patterns. While, despite clustering similarities between water-based preparations, significant differences persist between cold infusion and boiling decoction, particularly in oxygenated sesquiterpenes and aldehydes.

3.4. Comparison between food related preparations

A supervised statistical analysis was performed exclusively on the volatilomes of the two water-based preparations and the ethanol extract, as these methods are considered most suitable for food-related applications and, among hydroalcoholic solvents, ethanol is widely recognised as a more sustainable ("green") solvent (renewable bio-origin, low toxicity, recoverability). This aligns with green extraction practice for food and botanicals.

Partial Least Squares Discriminant Analysis (PLS-DA) model achieved an overall excellent classification performance (Accuracy = 1.00; $R^2 \approx 0.999$; $Q^2 \approx 0.999$) confirming absence of model overfitting [47]. VOCs selection relied on interpretable feature importance (VIP) greater than one (Fig. 6), a widely accepted threshold for identifying class-defining features in chemometrics [48]. Among the compounds not previously discussed in Section 3.2, several monoterpenes, oxygenated terpenoids, esters and sesquiterpenes strongly influence the aromatic and functional profiles of the extracts.

In detail, ethanolic extracts exhibited the highest diversity and abundance of monoterpenes and sesquiterpenes creating a citrus, woody, balsamic aromatic profile. These compounds are not only key to fragrance but also confer multifunctional bioactivities relevant for nutraceutical and pharmaceutical applications. For instance, the sesquiterpene (E)-β-farnesene (C77), imparts green notes, and beyond its ecological role as aphid alarm pheromone, its derivatives have demonstrated antioxidant properties, and emerging evidence suggests neuroprotective potential through modulation of oxidative stress pathways [49–51]. The cadinene-type sesquiterpenes, γ-cadinene (C82) and δ-cadinene (C85) contribute woody depth and the latter is associated with antimicrobial effects [52]. The compounds α-copaene (C67) and β-bourbonene (C68) provide woody, balsamic, honey nuances and have shown promise for neuroprotection, anti-cancer effects and cytotoxic activity, though further in vivo validation is needed for their therapeutic use [53–55]. Monoterpenes such as cis-β-ocimene (C31) and D-limonene (C13) deliver sweet-green and citrus top notes, complemented by β-myrcene (C8) for woody balsamic undertones. These compounds are widely recognized for antioxidant, anti-inflammatory and chemopreventive properties, with limonene documented for lipid metabolism modulation and β-myrcene for analgesic and anti-arthritic effects [7,37,44,56]. Functionally, ethanol's intermediate polarity enables efficient solubilization of both hydrocarbon terpenes and semi-polar monoterpenes, resulting in extracts rich in compounds with anti-inflammatory, antioxidant, antimicrobial and chemopreventive potential, supporting its suitability for tinctures and nutraceutical formulations.

Cold infusion preferentially recovered monoterpenoids and sesquiterpenoids, creating a fresh, woody, floral and spicy profile (Fig. 5, Supplementary Material Table S2). Among monoterpenoids, linalool (C38) and linalool oxide (C33) supported floral and citrus notes and are frequently associated with sedative/analgesic and anti-inflammatory effects; terpinen-4-ol (C49) contributed herbal and spicy facets with antimicrobial/antioxidant and therapeutic agent potential [57–60]. Whitin sesquiterpenoids, caryophyllene oxide (C94) gave a wood-fresh tone and is documented for anti-inflammatory and cytoprotective actions [61], additional oxygenated sesquiterpenoids, caryophylla-4(12),8(13)-dien-5β-ol (C101) one of the main components of Eugenia genus oils and Germacra-4(15),5,10(14)-trien-1β-ol (C107), point to a broader enrichment of polar sesquiterpene derivative in the cold-water preparation [62,63]. A distinct aromatic ester, methyl benzoate (C37), added cherry, floral nuances consistent with its fragrance and antiseptic properties [64,65]. The high abundance of pinocarvone (C47) (monoterpene ketone) rounds nicely with minty notes. Furthermore, the peaking of α-campholenal in the cold infusion, an aldehyde with green, herbal and citrus nuances, adds complexity to the cold-water preparation aroma and is associated with antioxidant and insect-repellent activities, making it relevant for food, pharmacological and preservative applications [66].

Altogether, the cold infusion pattern shows that oxygenated mono- and sesquiterpenes can be effectively retained under cold aqueous conditions, aligning with reports that such constituents contribute to immunomodulatory and anti-inflammatory properties in Hypericum [63].

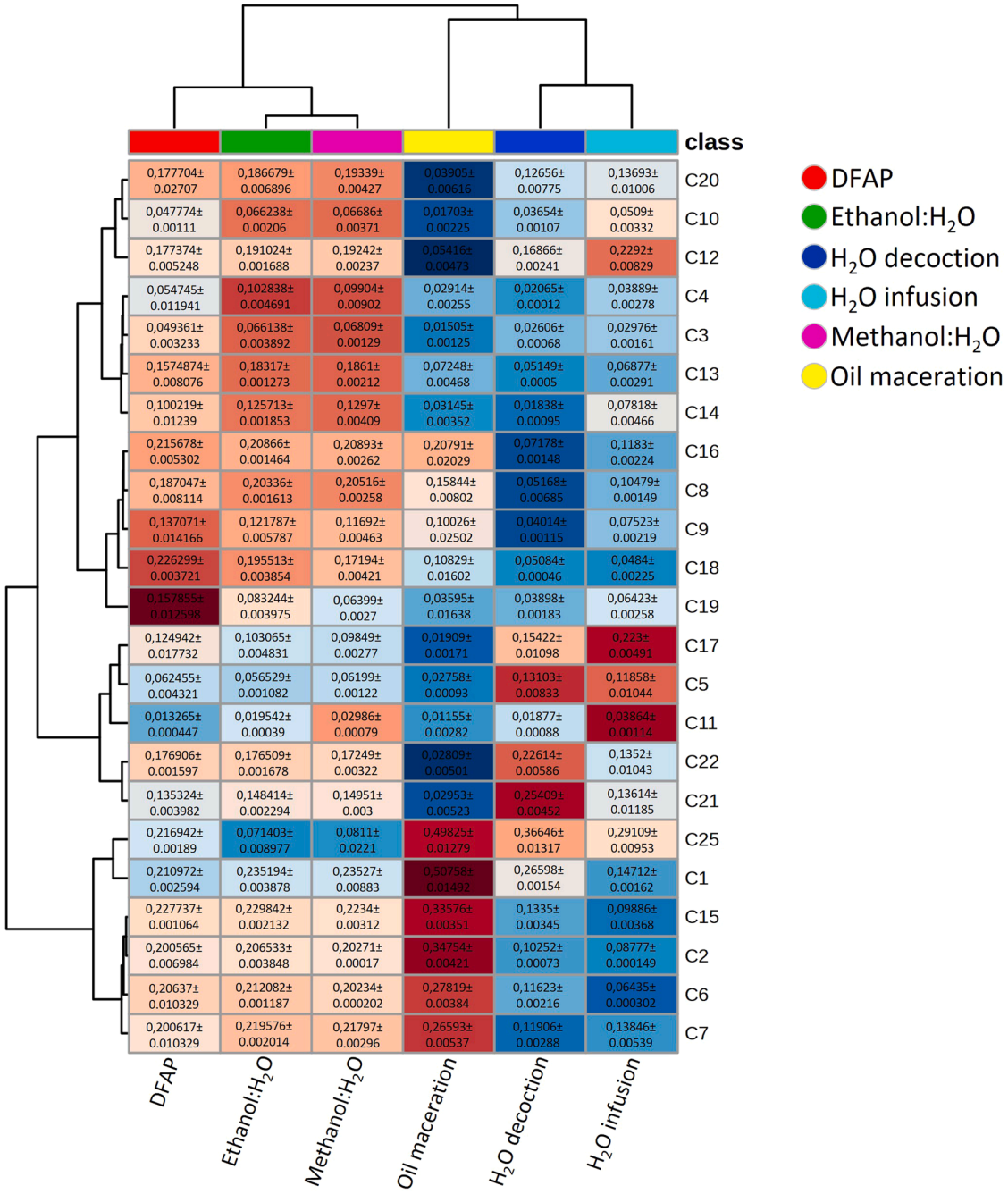

Fig. 5. Heatmap and hierarchical clustering analysis of the 23 VOCs shared among different *H. perforatum* sample. Each coloured cell represents the mean normalised abundance (annotated with ± SD) of each compound across three replicates, following Square-root transformation to improve comparability of signal intensities. Red and blue colours indicate relatively higher and lower abundances, respectively. Compound codes (Cn) correspond to those reported in **Table S2 in bold**.

Boiling decoction is distinguished by the oxygenated sesquiterpenoid neointermedeol (C103) and α-pinene (C25), which significantly peak in this preparation and play a central role in shaping its aromatic and functional identity. α-Pinene, a monoterpene with woody, herbal and pine notes, is widely recognised for its anti-inflammatory, bronchodilatory and antimicrobial properties, acting through NF-KB/MAPK pathway modulation. Its presence in decoction suggests that thermal conditions do not degrade this relatively stable monoterpene but may enhance its release from plant matrices. It is worth noting that boiling decoction retains notable amounts of compounds that peak in ethanol or cold infusion, such as linalool (C38), terpine-4-ol (C49), and caryophyllene oxide (C94). This intermediate abundance suggests that heat promotes release from bound forms without complete degradation, creating a hybrid profile that bridges aqueous and alcoholic extracts. Sesquiterpenes such as γ-Cadinene (C82) [52] and (E)-β-Farnesene (C77) [49–51], although highest in ethanol, remain at moderate levels in decoction, contributing woody-spicy depth and ecological signalling roles. This pattern reflects thermal hydrolysis and matrix disruption, enabling partial extraction of semi-polar volatiles while favouring oxygenated species. Overall, decoction offers a balanced aromatic identity and retains bioactive terpenes relevant for anti-inflammatory and antimicrobial uses.

4. Conclusions

This study provides a comprehensive and methodologically robust

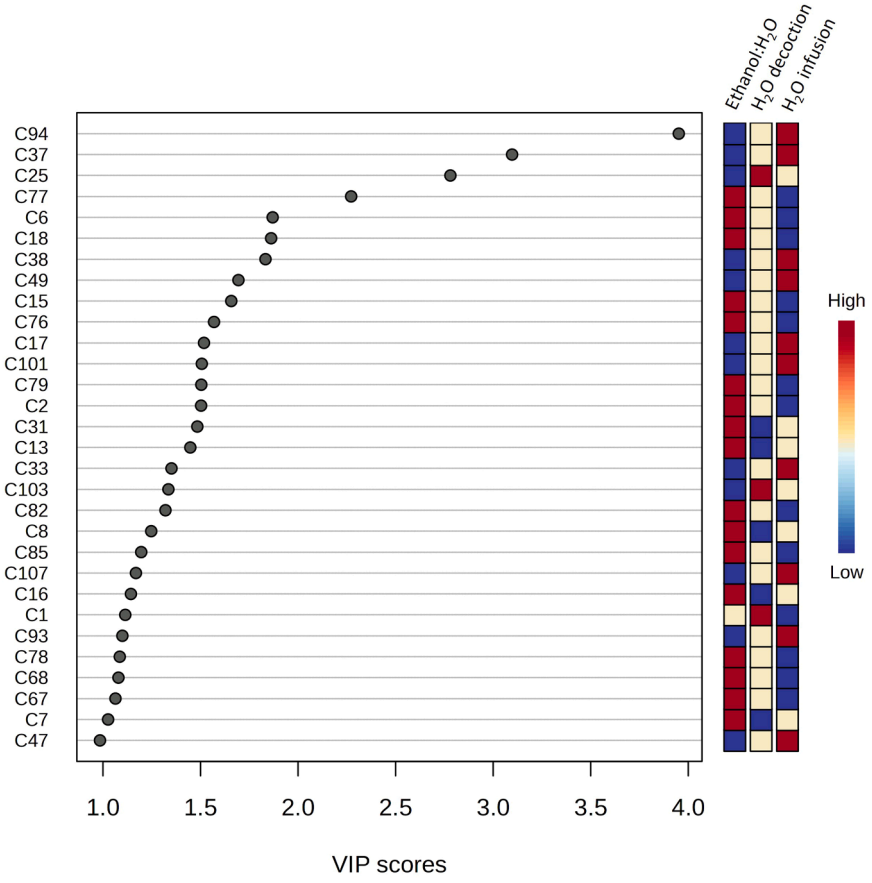

Fig. 6. Important features identified by PLS-DA. The coloured boxes on the right indicate the relative abundance of the corresponding VOC in ethanol:water, decoction and cold infusion.

characterisation of the volatilome of *Hypericum perforatum* flowering aerial parts by combining HiSorb with GC–MS and advanced chemometric analyses. For the first time in this complex botanical matrix, multiple HiSorb coatings were systematically evaluated under identical analytical conditions, demonstrating that CWR/PDMS offers superior extraction efficiency and chemical coverage. Its balanced polarity enhanced recovery across a wide volatility range, encompassing hydrocarbons, mono- and sesquiterpenes and oxygenated derivatives, thereby overcoming intrinsic limitations associated with conventional HS extraction approaches. The implementation of a dual-split analytical strategy further strengthened the analytical workflow, preventing detector saturation while preserving low-abundance compounds. By integrating high- and low-split databases, comprehensive volatilome fingerprints were generated from each sample without compromising data consistency. This approach significantly improves sensitivity and dynamic range and represents a practical solution for the analysis of complex plant matrices rich in both high and low-abundance VOCs collectively, these methodological advances contribute a scalable, sensitive, and reproducible platform for volatilomic studies of medicinal plants, with clear advantages for both research and quality control applications.

Comparative analysis of industrially and traditionally relevant processing procedures demonstrated that solvent polarity and thermal conditions are the primary drivers of VOC recovery and selectivity. Hydroalcoholic extracts most closely resembled the native volatilome, exhibiting the highest diversity and abundance of mono- and sesquiterpenes associated with aromatic quality and multifunctional bioactivity. Cold aqueous infusion selectively enriched oxygenated terpenoids and aldehydes, while boiling decoction produced an intermediate profile, retaining thermally stable monoterpenes alongside oxygenated sesquiterpenoids. In contrast, oil infusion resulted in limited VOC diversity and preferential and preferential enrichment of non-polar hydrocarbons, highlighting its reduced suitability for comprehensive volatilome recovery.

The identification of a core set of VOCs conserved across all preparations provides a chemically meaningful fingerprint of the aromatic identity of *H. perforatum*, independent of processing conditions. These shared compounds represent promising markers candidates for quality control, batch to batch consistency, and authenticity assessment of commercial Hypericum products. Furthermore, the integration of uni- and multivariate unsupervised and supervised chemometric tools (ANOVA, PCA, HCA and PLS-DA) demonstrated excellent discriminatory power, reinforcing the suitability of volatilomic fingerprints for rational selection and optimisation of extraction strategies tailored to specific end-use applications. Building from these findings, future studies will focus on a reduced panel of discriminatory VOCs, for which a fully quantitative method will be developed to support targeted applications.

This work underscores the importance of pre-analytical optimisation of sorptive phase chemistry for untargeted analysis and highlights the added value of high-capacity extraction tools in complex botanical matrices. In future work we aim to transfer the workflow to other aromatic and medicinal plants, supporting a broader range of applicability in food, pharmaceutical, cosmetics and nutraceutical research.

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CRediT authorship contribution statement

Valentina Braghin: Writing – original draft, Investigation, Formal analysis, Data curation. **Sadia Kanwal:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Davide Barboni:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Brunilda Myftari:** Writing – review & editing, Writing – original draft, Conceptualization. **Nicoletta Bianchi:** Writing – review & editing, Writing – original draft, Conceptualization. **Paola Tedeschi:** Writing – review & editing, Investigation, Funding acquisition. **Chiara De Luca:** Writing – review & editing, Formal analysis. **Alberto Cavazzini:** Writing – review & editing, Funding acquisition. **Simona Felletti:** Writing – review & editing, Writing – original draft, Validation, Formal analysis. **Martina Catani:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Natasha D. Spadafora:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Martina Catani is in the Advisory Editorial Board of Journal of Chromatography Open. Other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcoa.2026.100339.

Data availability

Data will be made available on request.

References

[1] M.C. Otero, F. Ceric, S. Miranda-Rojas, C. Carreño, R. Escares, M.J. Escobar, C. Saracini, C. Atala, R. Ramírez-Barrantes, F. Gordillo-Fuenzalida, Documentary analysis of Hypericum perforatum (St. John's Wort) and its effect on depressive disorders, Pharmaceuticals 17 (2024), https://doi.org/10.3390/ph17121625.

[2] S.Z. Nobakht, M. Akaberi, A.H. Mohammadpour, A. Tafazoli Moghadam, S. A. Emami, Hypericum perforatum: traditional uses, clinical trials, and drug interactions, Iran. J. Basic Med. Sci. 25 (2022) 1045–1058, https://doi.org/10.22038/IJBMS.2022.65112.14338.

[3] K. Dudek, M.J. Pietryja, S. Kurkiewicz, M. Kurkiewicz, B. Błońska-Fajfrowska, S. Wilczyński, A. Dzierżęga-Lęcznar, Influence of the drying method on the volatile component profile of Hypericum perforatum herb: a HS-SPME-GC/MS study, Processes 10 (2022) 2593, https://doi.org/10.3390/pr10122593.

[4] V.V. Milevskaya, M.A. Statkus, Z.A. Temerdashev, N.V. Kiseleva, T.S. Butyl'skaya, E.A. Shil'ko, Extraction and determination of biologically active components of St. John's wort and its pharmaceutical preparations, J. Anal. Chem. 71 (2016) 741–747, https://doi.org/10.1134/S1061934816070133.

[5] V. Ion, I. Ielciu, A.-G. Cârje, D.L. Muntean, G. Crişan, R. Păltinean, Hypericum spp.—An overview of the extraction methods and analysis of compounds, Separations 9 (2022) 17, https://doi.org/10.3390/separations9010017.

[6] M. Tegtmeier, L. Knierim, A. Schmidt, J. Strube, Green manufacturing for herbal remedies with advanced pharmaceutical technology, Pharmaceutics 15 (2023) 188, https://doi.org/10.3390/pharmaceutics15010188.

[7] E. Vuko, V. Dunkić, M. Ruščić, M. Nazlić, N. Mandić, B. Soldo, M. Šprung, Ž. Fredotović, Chemical composition and new biological activities of essential oil and hydrosol of Hypericum perforatum L. ssp. Veronense (Schrank) H, Lindb, Plants 10 (2021) 1014, https://doi.org/10.3390/plants10051014.

[8] C. Lancioni, C. Castells, R. Candal, M. Tascon, Headspace solid-phase microextraction: fundamentals and recent advances, Adv. Sample Prep. 3 (2022) 100035, https://doi.org/10.1016/j.sampre.2022.100035.

[9] M.L. Williams, S. Cretnik, L. Lüthy, T. Flug, M. Stebler, E. Gionfriddo, Comparison of solid phase microextraction geometries for effective preconcentration of volatile per- and polyfluoroalkyl substances, Analyst 150 (2025) 4630–4637, https://doi.org/10.1039/D5AN00758E.

[10] E. Baltussen, P. Sandra, F. David, C. Cramers, Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles, J. Microcolumn Sep. 11 (1999) 737–747, https://doi.org/10.1002/(SICI)1520-667X(1999)11:10737::AIDCS73.0.CO;2-4.

[11] A. Helin, T. Rönkkö, J. Parshintsev, K. Hartonen, B. Schilling, T. Läubli, M.-L. Riekkola, Solid phase microextraction Arrow for the sampling of volatile amines in wastewater and atmosphere, J. Chromatogr. A 1426 (2015) 56–63, https://doi.org/10.1016/j.chroma.2015.11.061.

[12] J.S. Herrington, G.A. Gómez-Ríos, C. Myers, G. Stidsen, D.S. Bell, Hunting molecules in complex matrices with SPME arrows: a review, Separations 7 (2020) 12, https://doi.org/10.3390/separations7010012.

[13] A. Kremser, M.A. Jochmann, T.C. Schmidt, PAL SPME Arrow—Evaluation of a novel solid-phase microextraction device for freely dissolved PAHs in water, Anal. Bioanal. Chem. 408 (2016) 943–952, https://doi.org/10.1007/s00216-015-9187-z.

[14] K.H. Scheuler, C. Schilling, Extraction device, US20140220701A1, 2014.

[15] J.B. Wilcockson, F.A.P.C. Gobas, Thin-film solid-phase extraction to measure fugacities of organic chemicals with low volatility in biological samples, Environ. Sci. Technol. 35 (2001) 1425–1431, https://doi.org/10.1021/es001561t.

[16] D. Eggermont, N.D. Spadafora, J. Aspromonte, G. Purcaro, Unraveling the impact of the capsule material on the aroma of brewed coffee by headspace analysis using a HiSorb probe followed by reverse fill/flush flow modulation GC×GC-MS, Anal. Bioanal. Chem. 415 (2023) 2511–2521, https://doi.org/10.1007/s00216-022-04457-x.

[17] D. Eggermont, N.D. Spadafora, J. Aspromonte, R. Pellegrino, G. Purcaro, Exploring different high-capacity tools and extraction modes to characterize the aroma of brewed coffee, Anal. Bioanal. Chem. 415 (2023) 4501–4510, https://doi.org/10.1007/s00216-023-04654-2.

[18] H. Faulkner, T.F. O'Callaghan, S. McAuliffe, D. Hennessy, C. Stanton, M. G. O'Sullivan, J.P. Kerry, K.N. Kilcawley, Effect of different forage types on the volatile and sensory properties of bovine milk, J. Dairy Sci. 101 (2018) 1034–1047, https://doi.org/10.3168/jds.2017-13141.

[19] L. Hearn, R. Cole, N.D. Spadafora, R. Szafnauer, Volatile and semi-volatile compounds in flavoured hard seltzer beverages: comparison of high-capacity sorptive extraction (HiSorb) methods, Adv. Sample Prep. 3 (2022) 100032, https://doi.org/10.1016/j.sampre.2022.100032.

[20] N.D. Spadafora, S. Mascrez, L. McGregor, G. Purcaro, Exploring multiple-cumulative trapping solid-phase microextraction coupled to gas chromatography–mass spectrometry for quality and authenticity assessment of olive oil, Food Chem 383 (2022) 132438, https://doi.org/10.1016/j.foodchem.2022.132438.

[21] C. Blasco, M. Fernández, Y. Picó, G. Font, Comparison of solid-phase microextraction and stir bar sorptive extraction for determining six organophosphorus insecticides in honey by liquid chromatography–mass spectrometry, J. Chromatogr. A 1030 (2004) 77–85, https://doi.org/10.1016/j.chroma.2003.11.037.

[22] M. He, Y. Wang, Q. Zhang, L. Zang, B. Chen, B. Hu, Stir bar sorptive extraction and its application, J. Chromatogr. A 1637 (2021) 461810, https://doi.org/10.1016/j.chroma.2020.461810.

[23] S. Elia, M. Stylianou, A. Agapiou, Advanced micro-extraction techniques (SPME, HiSorb) for the determination of goat cheese whey wastewater VOCs, J. Environ. Manage. 351 (2024) 119934, https://doi.org/10.1016/j.jenvman.2023.119934.

[24] A. Schincaglia, L. Pasti, A. Cavazzini, G. Purcaro, M. Beccaria, Optimization of headspace high-capacity tool coupled to two-dimensional gas chromatography–mass spectrometry for mapping the volatile organic compounds of raw pistachios. A proof-of-concept on the classification ability by geographic origin, Food Chem 460 (2024) 140702, https://doi.org/10.1016/j.foodchem.2024.140702.

[25] S. Hlatshwayo, N. Thembane, S.B.N. Krishna, N. Gqaleni, M. Ngcobo, Extraction and processing of bioactive phytoconstituents from widely used South African medicinal plants for the preparation of effective traditional herbal medicine products: a narrative review, Plants 14 (2025), https://doi.org/10.3390/plants14020206.

[26] A. Balea, M. Pojar-Fenesan, I. Ciotlaus, Traceability of volatile organic compounds from Hypericum perforatum in fresh and dried form and in essential oil, Rev. Chim. 71 (2020) 59–65, https://doi.org/10.37358/RC.20.6.8170.

[27] Q. Wen, A. Myridakis, P.R. Boshier, S. Zuffa, I. Belluomo, A.G. Parker, S.T. Chin, S. Hakim, S.R. Markar, G.B. Hanna, A complete pipeline for untargeted urinary volatolomic profiling with sorptive extraction and dual polar and nonpolar column methodologies coupled with gas chromatography time-of-flight mass spectrometry, Anal. Chem. 95 (2023) 758–765, https://doi.org/10.1021/acs.analchem.2c02873.

[28] G. Lubes, M. Goodarzi, Analysis of volatile compounds by advanced analytical techniques and multivariate chemometrics, Chem. Rev. 117 (2017) 6399–6422, https://doi.org/10.1021/acs.chemrev.6b00698.

[29] Y. Nolvachai, M.S.S. Amaral, R. Herron, P.J. Marriott, Solid phase microextraction for quantitative analysis – Expectations beyond design? Green Anal. Chem. 4 (2023) https://doi.org/10.1016/j.greeac.2022.100048.

[30] D. Aros, N. Spadafora, M. Venturi, G. Núñez-Lillo, C. Meneses, L. Methven, C. T. Müller, H. Rogers, Floral scent evaluation of segregating lines of alstroemeria

caryophyllaea, Sci. Hortic. 185 (2015) 183–192, https://doi.org/10.1016/j.scienta.2015.01.014.
[31] N.D. Spadafora, D. Eggermont, V. Křešťáková, T. Chenet, F. Van Rossum, G. Purcaro, Comprehensive analysis of floral scent and fatty acids in nectar of Silene nutans through modern analytical gas chromatography techniques, J. Chromatogr. A (2023) 1696, https://doi.org/10.1016/j.chroma.2023.463977.
[32] N.D. Spadafora, S. Felletti, T. Chenet, T.M. Sirangelo, M. Cescon, M. Catani, C. De Luca, C. Stevanin, A. Cavazzini, L. Pasti, The influence of drying and storage conditions on the volatilome and cannabinoid content of Cannabis sativa L. inflorescences, Anal. Bioanal. Chem. 416 (2024) 3797–3809, https://doi.org/10.1007/s00216-024-05321-w.
[33] Z. Pang, Y. Lu, G. Zhou, F. Hui, L. Xu, C. Viau, A.F. Spigelman, P.E. Macdonald, D. S. Wishart, S. Li, J. Xia, MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation, Nucleic Acids Res 52 (2024) W398–W406, https://doi.org/10.1093/nar/gkae253.
[34] Z. Cheng, D.T. Mannion, M.G. O'Sullivan, S. Miao, J.P. Kerry, K.N. Kilcawley, Comparison of automated extraction techniques for volatile analysis of whole milk powder, Foods 10 (2021) 2061, https://doi.org/10.3390/foods10092061.
[35] D.S. Wishart, A. Guo, E. Oler, F. Wang, A. Anjum, H. Peters, R. Dizon, Z. Sayeeda, S. Tian, B.L. Lee, M. Berjanskii, R. Mah, M. Yamamoto, J. Jovel, C. Torres-Calzada, M. Hiebert-Giesbrecht, V.W. Lui, D. Varshavi, D. Varshavi, D. Allen, D. Arndt, N. Khetarpal, A. Sivakumaran, K. Harford, S. Sanford, K. Yee, X. Cao, Z. Budinski, J. Liigand, L. Zhang, J. Zheng, R. Mandal, N. Karu, M. Dambrova, H.B. Schiöth, R. Greiner, V. Gautam, HMDB 5.0: the Human Metabolome Database for 2022, Nucleic Acids Res 50 (2022) D622–D631, https://doi.org/10.1093/nar/gkab1062.
[36] J.J. McDougall, M.K. McKenna, Anti-inflammatory and analgesic properties of the cannabis terpene myrcene in rat adjuvant monoarthritis, Int. J. Mol. Sci. 23 (2022) 7891, https://doi.org/10.3390/ijms23147891.
[37] S. Surendran, F. Qassadi, G. Surendran, D. Lilley, M. Heinrich, Myrcene—What are the potential health benefits of this flavouring and aroma agent? Front. Nutr. 8 (2021) https://doi.org/10.3389/fnut.2021.699666.
[38] N.K. Jha, C. Sharma, H.M. Hashiesh, S. Arunachalam, M.N. Meeran, H. Javed, C. R. Patil, S.N. Goyal, S. Ojha, β-caryophyllene, A natural dietary CB2 receptor selective cannabinoid can be a candidate to target the trinity of infection, immunity, and inflammation in COVID-19, Front. Pharmacol. 12 (2021), https://doi.org/10.3389/fphar.2021.590201.
[39] C. Ricardi, S. Barachini, G. Consoli, D. Marazziti, B. Polini, G. Chiellini, Beta-caryophyllene, a cannabinoid receptor type 2 selective agonist, in emotional and cognitive disorders, Int. J. Mol. Sci. 25 (2024) 3203, https://doi.org/10.3390/ijms25063203.
[40] J.J. Chebet, J.E. Ehiri, D.J. McClelland, D. Taren, I.A. Hakim, Effect of D-limonene and its derivatives on breast cancer in human trials: a scoping review and narrative synthesis, BMC Cancer 21 (2021) 902, https://doi.org/10.1186/s12885-021-08639-1.
[41] J. Zhou, M. Azrad, L. Kong, Effect of limonene on cancer development in rodent models: a systematic review, Front. Sustain. Food Syst. 5 (2021), https://doi.org/10.3389/fsufs.2021.725077.
[42] S.M. Cohen, G. Eisenbrand, S. Fukushima, N.J. Gooderham, F.P. Guengerich, S. S. Hecht, I.M.C.M. Rietjens, M. Bastaki, J.M. Davidsen, C.L. Harman, M. M. McGowen, S.V. Taylor, FEMA GRAS assessment of natural flavor complexes: mint, buchu, dill and caraway derived flavoring ingredients, Food Chem. Toxicol. 135 (2020) 110870, https://doi.org/10.1016/j.fct.2019.110870.
[43] F. Francomano, A. Caruso, A. Barbarossa, A. Fazio, C. La Torre, J. Ceramella, R. Mallamaci, C. Saturnino, D. Iacopetta, M.S. Sinicropi, β-caryophyllene: a sesquiterpene with countless biological properties, Appl. Sci. 9 (2019) 5420, https://doi.org/10.3390/app9245420.
[44] A. Koziol, A. Stryjewska, T. Librowski, K. Salat, M. Gawel, A. Moniczewski, S. Lochynski, An overview of the pharmacological properties and potential applications of natural monoterpenes, Mini-Rev. Med. Chem. 14 (2015) 1156–1168, https://doi.org/10.2174/1389557514666141127145820.
[45] C. Sharma, J.M. Al Kaabi, S.M. Nurulain, S.N. Goyal, M. Amjad Kamal, S. Ojha, Polypharmacological properties and therapeutic potential of β-caryophyllene: a dietary phytocannabinoid of pharmaceutical promise, Curr. Pharm. Des. 22 (2016) 3237–3264, https://doi.org/10.2174/1381612822666160311115226.
[46] D.R. Joshi, N. Adhikari, An overview on common organic solvents and their toxicity, J. Pharm. Res. Int. (2019) 1–18, https://doi.org/10.9734/jpri/2019/v28i330203.
[47] L.C. Lee, C.Y. Liong, A.A. Jemain, Partial least squares-discriminant analysis (PLS-DA) for classification of high-dimensional (HD) data: a review of contemporary practice strategies and knowledge gaps, Analyst 143 (2018) 3526–3539, https://doi.org/10.1039/c8an00599k.
[48] N. Akarachantachote, S. Chadcham, K. Saithanu, Cutoff threshold of variable importance in projection for variable selection, Int. J. Pure Appl. Math. 94 (2014), https://doi.org/10.12732/ijpam.v94i3.2.
[49] S. Wang, J. Zhou, C. Zhan, J. Qiao, Q. Caiyin, M. Huang, Fine-tuning the function of farnesene synthases for selective synthesis of farnesene stereoisomers, J. Agric. Food Chem. 72 (2024) 27355–27364, https://doi.org/10.1021/acs.jafc.4c09515.
[50] I.A. Schepetkin, G. Özek, T. Özek, L.N. Kirpotina, A.I. Khlebnikov, R.A. Klein, M. T. Quinn, Neutrophil immunomodulatory activity of Farnesene, a component of Artemisia dracunculus essential oils, Pharmaceuticals 15 (2022) 642, https://doi.org/10.3390/ph15050642.
[51] Y. Qin, Z. Yang, D. Song, Q. Wang, S. Gu, W. Li, H. Duan, J. Zhou, X. Yang, Bioactivities of synthetic salicylate-substituted carboxyl (*E*)-β-farnesene derivatives as ecofriendly agrochemicals and their binding mechanism with potential targets in aphid olfactory system, Pest Manag. Sci. 76 (2020) 2465–2472, https://doi.org/10.1002/ps.5787.
[52] R. Qin, S. Yang, B. Fu, Y. Chen, M. Zhou, Y. Qi, N. Xu, Q. Wu, Q. Hua, Y. Wu, Z. Liu, Antibacterial activity and mechanism of the sesquiterpene δ-cadinene against Listeria monocytogenes, LWT 203 (2024) 116388, https://doi.org/10.1016/j.lwt.2024.116388.
[53] H. Turkez, B. Togar, A. Tatar, F. Geyıkoglu, A. Hacımuftuoglu, Cytotoxic and cytogenetic effects of α-copaene on rat neuron and N2a neuroblastoma cell lines, Biologia (Bratisl) 69 (2014) 936–942, https://doi.org/10.2478/s11756-014-0393-5.
[54] Z. Wang, F. Liu, J.-J. Yu, J.-Z. Jin, β-bourbonene attenuates proliferation and induces apoptosis of prostate cancer cells, Oncol. Lett. (2018), https://doi.org/10.3892/ol.2018.9183.
[55] Z. Alimoradi, F. Taghian, K.Jalali Dehkordi, Linalool, Cineole, and β-bourbonene coupled with aerobic training improved the PSEN1/APP/IL1β/CASP3 networks, oxidative capacity and miRNA-210 in mice with Alzheimer's disease, Arch. Razi Inst. (2024) 629–638, https://doi.org/10.32592/ARI.2024.79.3.629.
[56] J.M.S. de Sousa, T.A. de L. Nunes, R.R.L. Rodrigues, J.P.A. de Sousa, M. da C.A. Val, F.A. da R. Coelho, A.L.S. dos Santos, N.B. Maciel, V.M.R. de Souza, Y.A. A. Machado, P.S. de A. Sousa, A.R. de Araújo, J.A. Rocha, D.P. de Sousa, M.V. da Silva, D.D.R. Arcanjo, K.A. da F. Rodrigues, Cytotoxic and antileishmanial effects of the monoterpene β-ocimene, Pharmaceuticals 16 (2023) 183, https://doi.org/10.3390/ph16020183.
[57] Q. An, J.-N. Ren, X. Li, G. Fan, S.-S. Qu, Y. Song, Y. Li, S.-Y. Pan, Recent updates on bioactive properties of linalool, Food Funct 12 (2021) 10370–10389, https://doi.org/10.1039/D1FO02120F.
[58] J.I. Deen, A.N.M.S. Zawad, M. Uddin, M.A.H. Chowdhury, S.Q. Al Araby, Md. A. Rahman, Terpinen-4-ol, A volatile terpene molecule, extensively electrifies the biological systems against the oxidative stress-linked pathogenesis, Adv. Redox Res. 9 (2023) 100082, https://doi.org/10.1016/j.arres.2023.100082.
[59] E. Yadav, R. Rao, A promising bioactive component Terpinen-4-Ol: a review, Int. J. Pharmacogn. 3 (2016) 336–345.
[60] M.-G. Kim, S.-M. Kim, J.-H. Min, O.-K. Kwon, M.-H. Park, J.-W. Park, H.I. Ahn, J.-Y. Hwang, S.-R. Oh, J.-W. Lee, K.-S. Ahn, Anti-inflammatory effects of linalool on ovalbumin-induced pulmonary inflammation, Int. Immunopharmacol. 74 (2019) 105706, https://doi.org/10.1016/j.intimp.2019.105706.
[61] Y.V. Gyrdymova, S.A. Rubtsova, Caryophyllene and caryophyllene oxide: a variety of chemical transformations and biological activities, Chem. Pap. 76 (2022) 1–39, https://doi.org/10.1007/s11696-021-01865-8.
[62] J.Da Silva, E. Andrade, L. Barreto, N.Da Silva, A. Ribeiro, R. Montenegro, J. Maia, Chemical composition of four essential oils of eugenia from the brazilian Amazon and their cytotoxic and antioxidant activity, Medicines 4 (2017) 51, https://doi.org/10.3390/medicines4030051.
[63] I. Schepetkin, G. Özek, T. Özek, L. Kirpotina, A. Khlebnikov, M. Quinn, Chemical composition and immunomodulatory activity of Hypericum perforatum essential oils, Biomolecules 10 (2020) 916, https://doi.org/10.3390/biom10060916.
[64] A. Alemdar, B. Tan, O. Toksöz, G. Kurtuluş, C. Sesal, Z. Odabaş, Systematically investigation on the spectral, antioxidant and antibacterial properties of fragrant methyl benzoate esters containing electron withdrawing and electron releasing groups, J. Mol. Struct. 1291 (2023) 136100, https://doi.org/10.1016/j.molstruc.2023.136100.
[65] Y. Feng, A. Zhang, A floral fragrance, methyl benzoate, is an efficient green pesticide, Sci. Rep. 7 (2017) 42168, https://doi.org/10.1038/srep42168.
[66] M. Verdeguer, A.M. Sánchez-Moreiras, F. Araniti, Phytotoxic effects and mechanism of action of essential oils and terpenoids, Plants 9 (2020) 1571, https://doi.org/10.3390/plants9111571.