



Effect of extraction method and particle size of plant material on the yield of bioactive compounds and antioxidant activity of aqueous extracts from *Matricaria chamomilla* L. flower

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ABSTRACT

Chamomile (*Matricaria chamomilla* L.) is a widely used medicinal herb valued for its rich essential oil content and a broad spectrum of bioactive compounds with confirmed pharmacological properties. This study investigated the effect of different aqueous extraction techniques, cold-brew, hot-water, ultrasound-assisted extraction (bath and probe), pressurized hot water extraction, and sample origin in combination with three particle size fractions (whole flowers, 10 mm, and 4 mm) on the phytochemical content, antioxidant activity of chamomile extracts, and chemical composition of essential oils. Pressurized hot water extraction exhibited the highest recovery not only in total phenolic content and antioxidant activity but also in the yield of individual phenolic compounds and coumarins, followed by conventional hot-water extraction. Cold-brew and ultrasound-assisted methods were markedly less efficient for all evaluated parameters. Chlorogenic acid was determined as the dominant compound, with its concentration increasing with both temperature and decreasing particle sizes. In contrast, apigenin-7-glucoside exhibited an inverse trend; its yield decreased with fragmentation of the plant matrix. While the chemical composition of water chamomile extracts was strongly influenced by both sample origin and particle sizes, the particle size of the sample had minimal impact on the chemical profiling of chamomile essential oils, as revealed by multivariate statistics. The obtained results provide an applied perspective for the development of chamomile-derived products, emphasizing the need to align extraction technology and raw material quality to achieve reproducible phytochemical composition and biological efficiency.

1. Introduction

The chamomile plant (*Matricaria chamomilla* L.), commonly known as German chamomile, is a medicinal plant of the *Asteraceae* family, naturally widespread throughout Europe. Its use in traditional medicine dates back thousands of years and was well established in ancient civilizations, including those of Greece, Egypt, and Rome. The name “chamomile” derives from the Greek *khamaimēlon*, meaning “ground apple,” referring to the plant’s low growth habit and the apple-like scent of its flower heads. The botanical name *Matricaria* originates from the

Latin word *matrix* (uterus), highlighting its historical application in the treatment of menstrual and postpartum disorders (Dai et al., 2023; El Joumaa and Borjac, 2022; El Mihaoui et al., 2022; Craker and Simon, 1986).

Today, chamomile is one of the most popular medicinal herbs in the modern age; its tea is regularly consumed by a significant number of people around the world. In fact, it is included in the pharmacopoeias of 26 countries. It has long been recognized for its wide range of pharmacological effects. Its bioactive compounds exhibit antioxidant, antibacterial, antimicrobial, antiseptic, anti-inflammatory, anticancer, and

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antidepressant properties. Traditionally, it has been used for treating gastrointestinal disorders, skin conditions, and the common cold (Al-Dabbagh et al., 2019; El Joumaa and Borjac, 2022; El Mihaoui et al., 2022; Sah et al., 2022). A tincture of chamomile flowers treats childhood diarrhea, while poultices or hot fomentations are applied for external swellings, such as facial edema due to illness or abscess. Chamomile extracts are included in pharmacological preparations for toothaches, earaches, and neuralgia, and have also been reported as bath additives to relieve anogenital irritation. Inhalation of vaporized essential oils is recommended for sadness and anxiety, while chamomile oil is commonly used in aromatherapy and hair care products (Kyokong et al., 2002; Melnyk et al., 2024; Sharifi-Rad et al., 2018; Srivastava et al., 2010).

These therapeutic effects are attributed to its rich phytochemical profile, which includes organic acids, flavonoids, terpenoids, coumarins, phenolic acids, and others (Akram et al., 2024; Cvetanović Kljakić et al., 2023; Dai et al., 2023; Tsivelika et al., 2021). Among terpenes, α -bisabolol shows strong anti-inflammatory, analgesic, antimicrobial, and anticancer activity, while β -farnesene, germacrene D, and chamazulene contribute additional antimicrobial, antiviral, antispasmodic, and gastrointestinal protective effects (Akram et al., 2024; Aziz et al., 2018; Eddin et al., 2022; Stanojevic et al., 2016). Key flavonoids such as apigenin, luteolin, quercetin, and rutin exhibit anti-inflammatory, antioxidant, neuroprotective, cardioprotective, and antidepressant properties (Akram et al., 2024; Ganeshpurkar and Saluja, 2017; Jucá et al., 2020; Salehi et al., 2019). Other compounds, including chlorogenic acid, herniarin, geraniol, and umbelliferone, further enhance its antitumor, antimicrobial, and antioxidant potential, supporting chamomile's broad application in both traditional and modern medicine (Akram et al., 2024; Chen and Viljoen, 2010; Mazimba, 2017).

The chemical profile of chamomile is strongly influenced by environmental and agronomic factors such as geographical origin, cultivation conditions, and harvest time (Aqeel et al., 2023; Chauhan et al., 2021; Temerdashev et al., 2023). This variability highlights the necessity of monitoring the quality of raw materials to ensure consistent properties and suitability for specific industrial applications.

The extraction of bioactive compounds from chamomile represents a complex task due to the plant's diverse phytochemical composition, pronounced variability of raw materials, and constraints of current extraction methodologies. While traditional aqueous extractions, such as infusions and decoctions, are still widely employed for chamomile, modern green extraction techniques offer improved efficiency and preservation of thermolabile compounds. Methods such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized hot water extraction (PHWE) enable selective recovery of key hydrophilic constituents, including flavonoids, coumarins, and phenolic acids, while using water as a safe and environmentally friendly solvent (Adeeyo et al., 2023; Putra et al., 2025). The efficiency of extraction is also strongly influenced by the particle size of the plant matrix, as finer milling increases surface area and facilitates solvent penetration, thereby enhancing the release of bioactive compounds. Optimizing particle size is particularly important for industrial applications, where consistent extract yield and reproducible quality are essential for meeting product standards and regulatory requirements.

In this context, the aim of the present study was a comprehensive evaluation of five water-based extraction techniques (cold-brew, hot-water extraction, UAE using both bath and a probe, PHWE) to investigate their efficiency in recovering bioactive compounds from *Matricaria chamomilla* L. flower. This research uniquely combines extraction strategy with two critical variables, namely the degree of particle size reduction and the geographical origin of the chamomile samples. These factors were shown to substantially affect the phytochemical profile of the resulting extracts or chamomile essential oil composition. Moreover, all applied techniques utilize water as the extraction solvent, underscoring the green and sustainable character of the analytical approach.

Through the integration of chromatographic, spectrophotometric, and multivariate statistical methods, along with scanning electron microscopy (SEM), the study contributes practical insights into the optimization of eco-friendly extraction processes for consistent and high-quality production of chamomile-derived products.

2. Materials and methods

2.1. Chemicals

Acetonitrile, methanol, *n*-hexane, and formic acid, all of HPLC or GC-grade purity, were purchased from Riedel-de Haën (Seelze, Germany). Xylene, p. a., was obtained from Lach-Ner, s.r.o. (Neratovice, Czech Republic). Chlorogenic acid, apigenin, kaempferol, terpenes, and alkanes C8-C23 were purchased from Merck (Merck KGaA, Darmstadt, Germany). Herniarin, umbelliferone, and apigenin-7-glucoside were obtained from Biosynth s.r.o. (Bratislava, Slovakia). Water was purified with a reverse osmosis system Ultra Clear UV (Barsbüttel, Germany).

2.2. Plant material

The *Matricaria chamomilla* L. flower samples were collected in a fully state of maturity during the growing season in June 2024 from four different sources in the Czech Republic and Slovakia. All sources were from certified ecological or sustainable production and were selected based on their traceable origin and post-harvest processing standards: (i) Herbona s.r.o. (a commercial supplier in Hradec Králové, originally intended for herbal tea production), (ii) agricultural company Úsovsko a.s. (chamomile was grown on six hectares under certified organic farming conditions), (iii) Rajecká dolina region in Slovakia, (RAJ), (a certified area for harvesting wild medicinal plants), and (iv) certified organic farm ELTO-BIO s.r.o. (Pardubice Region, cultivated without synthetic fertilizers or pesticides). All plant materials were authenticated as *Matricaria chamomilla* L. flower based on pharmacopoeial macroscopic and morphological characteristics of the inflorescences, in accordance with the European Pharmacopoeia. The identification criteria included a flower 10–25 mm in diameter with a conical, hollow receptacle, white ligulate female florets and yellow tubular hermaphroditic florets, as well as a characteristic aromatic odor and slightly bitter taste. Microscopic examination confirmed the presence of an epidermis with anomocytic stomata, secretory canals containing essential oil, and pollen grains typical of the *Asteraceae* family (European Pharmacopoeia Commission, 2025). Species identification was further confirmed by a qualified botanist. As the samples originated from different producers and collection sites, they may differ at the cultivar or genotype level; however, genetic uniformity was not a prerequisite for this study.

Drying of the plant material was carried out at a maximum temperature of 40 °C to a final moisture content of approximately 8% (EST-03S, Konel s.r.o., Czech Republic). After that, each sample was homogenized and then divided into three fractions. One fraction was kept as whole flowers (unprocessed), while the remaining material was ground using an IKA MF 10 basic laboratory mill (IKA®-Werke GmbH & Co. KG, Germany) at 4000 rpm and subsequently sieved to obtain particle size fractions of 10 mm and 4 mm. The grinding time (15–60 s) was adjusted to obtain the desired particle size fractions. All samples were stored in a cool, dark, and dry place until analysis. To standardize quantitative calculations, the dry matter content was determined using a standardized procedure by drying a representative subsample (whole flowers) at 105 °C to constant weight (Zbiral, 2005). This dry matter value was subsequently used as the reference for calculating the concentrations of chemical constituents and extraction yields on a dry weight basis (DW).

2.3. Extraction of essential oils (EOs)

The EOs from dried *Matricaria chamomilla* L. flower samples were extracted by hydrodistillation, following the method described in the Czech Pharmacopoeia (2017). A portion (30 g) of dried plant material at varying degrees of comminution (whole flowers, 10 mm, and 4 mm fractions) was placed into a distillation flask and mixed with 500 mL of distilled water. The distillation was performed using a Clevenger-type apparatus for 4 h.

At the beginning of the distillation, 0.5 mL of xylene was added to the graduated tube with a stoppered side arm using a pipette. During the process, the characteristic blue essential oil containing chamazulene was visibly captured in the organic layer. After completion of the 4-hour distillation, the heating was stopped, and after 10 min, the total volume of liquid in the graduated tube was noticed. The initial volume of xylene (0.5 mL) was subtracted from this value, and the final EO content was then recalculated and expressed in milliliters per 100 g of dried drug (mL/100 g DW). The distillation process was carried out in three replicates.

2.4. Extraction of phenolic compounds

2.4.1. Cold-brew and hot-water extraction

Cold-brew and hot-water aqueous extracts of *Matricaria chamomilla* L. flowers were prepared by the maceration of 3.0 g of dried plant material in 250 mL of deionized water. The cold-brew extraction was performed at 25 °C for 20 min, while the hot-water extract was prepared at 80 °C for 15 min. The extraction conditions were selected based on general recommendations by manufacturers for the preparation of herbal tea beverages. The extracts were then filtered, adjusted to a final volume of 250 mL in a volumetric flask with deionized water, and stored in the fridge until further analysis. The extraction process of each sample was performed in triplicate.

2.4.2. Ultrasound-assisted extraction using ultrasonic bath

A portion (0.5 g) of dried plant material was mixed with 40 mL of deionized water and sonicated for 15 min using ultrasound bath Elma-sonic S 30 H (Elma Schmidbauer GmbH, Singen, Germany) and a frequency of 35 kHz. The temperature was kept below 35 °C. The extracts were then filtered, transferred to a volumetric flask, adjusted to a final volume of 50 mL with deionized water, and stored in the fridge until further analysis. All extractions were performed in triplicate (Burdějová et al., 2025).

2.4.3. Ultrasound-assisted extraction using ultrasonic homogenizer probe

The extracts were prepared using a SONOPULS MS 72 ultrasonic homogenizer probe (Bandelin Ultraschall Apparatebau GmbH, Berlin, Germany), which was connected to a Bandelin GM 3100 sonicator. A portion (0.5 g) of dried plant material was placed into a 60 mL vial with 40 mL of deionized water and fixed on a stand inside a soundproof box. The probe was immersed in the sample that there was no contact between the probe and the bottom or walls of the vial. The top of the tube was sealed with a parafilm foil to prevent the escape of the extract. The vial with the sample was further immersed in a beaker of water containing ice to prevent overheating of the sample. The sonication parameters were set as follows: amplitude 50%, ultrasonic pulse off, and total sonication time 5 min. The obtained extract was filtered, transferred to a volumetric flask, adjusted to a final volume of 50 mL with deionized water, and stored in the fridge until further analysis. The extraction process of each sample was repeated in triplicate (Burdějová et al., 2025).

2.4.4. Pressurized hot water extraction (PHWE)

PHWE at static mode was carried out using unique laboratory-made instrumentation described in detail in Pol et al. (2007). A portion (0.5 g) of dried plant material was mixed with 5 mL of inert material (glass

beads 600–800 µm), placed to the 33 mL stainless steel extraction cell, and extracted at a pressure of 15 MPa, temperature of 100 °C, and extraction time 1×15 min. During the extraction process, a stream of gaseous helium passed through the extraction solvent to prevent degradation of analytes by air oxygen. A 60 s rinsing time and two minutes nitrogen purge time were set at the end of each extraction run. The obtained extracts were adjusted to a volume of 50 mL in a volumetric flask with the deionized water and kept in the fridge until further analysis. The extraction process of each sample was repeated in triplicate.

2.5. Total polyphenol content (TPC)

TPC of the obtained extracts was determined by the Folin–Ciocalteu method (Slinkard and Singleton, 1977) with some modification. Briefly, 100 µL of each extract sample was mixed with 7.9 mL of distilled water and 500 µL of Folin–Ciocalteu reagent. Within the time interval between 30 s and 8 min, the 1500 µL of sodium carbonate (1.89 mol.L^{-1}) was added, and the mixture was incubated for 60 min at room temperature in the dark. Finally, the absorbance was measured at 765 nm against a blank. A calibration curve was prepared using gallic acid as a standard with various concentrations ranging from 0 to 500 mg.L⁻¹. TPC was calculated as milligrams of gallic acid equivalent per gram of dry weight (mg GAE.g⁻¹ DW). The experiment was performed in triplicate using a UV–Vis spectrophotometer Cecil CE 9500 Super Aquarius (Cecil Instruments Ltd, Cambridge, UK).

2.6. DPPH radical scavenging activity

The radical scavenging activity of the obtained extracts was evaluated based on the scavenging of •DPPH by antioxidants present in the extracts. The assay was assessed as described by Brand-Williams et al. (1995) with some modifications. Briefly, 1 mL of extract (diluted 5, 10 or 20x) was mixed with 3 mL of •DPPH in ethanol (0.1 mmol.L^{-1}) and the mixture was incubated in a dark place at room temperature for 30 min. Then the absorbance was measured at 515 nm against the blank. A solution of •DPPH was prepared daily by dissolving •DPPH in ethanol and protected from the light. Trolox was employed as the reference standard, and radical scavenging activity of the samples was expressed as mg of Trolox equivalent per g of dry weight (mg TE.g⁻¹ DW). All experiments were performed using a UV–Vis spectrophotometer Cecil CE 9500 Super Aquarius (Cecil Instruments Ltd, Cambridge, UK) in triplicate.

2.7. GC-MS analysis of EOs

GC-MS analyses of chamomile EOs were performed using a Thermo Trace GC Ultra system equipped with a TriPlus autosampler, coupled with a Polaris Q ion-trap mass spectrometer operating in electron ionization (EI) mode at 70 eV (Thermo Fisher Scientific Inc., USA), and equipped with a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent J&W Scientific, USA). The temperature program was set as follows: an initial hold at 45 °C for 2 min, followed by an increase to 250 °C at 4 °C/min, then to 300 °C at 30 °C/min, and a final hold at 300 °C for 2 min. Helium was used as the carrier gas at a flow rate of 0.8 mL/min. A volume of sample (1 µL) dissolved in *n*-hexane was injected in splitless mode. The mass scan range was *m/z* 50–650 amu, with data acquired in full scan mode. Data analysis was performed using Xcalibur 2.2 software (Thermo Fisher Scientific Inc., Waltham, MA, USA). Individual components were identified by comparison with co-injected pure standards, MS fragmentation patterns, commercial mass spectral libraries (NIST MS Search 2.0 library; National Institute of Standards and Technology, USA), and literature data (Adams, 2007). The relative amounts (%) of compounds were calculated based on the GC peak areas.

2.8. HPLC-DAD analysis of phenolic compounds and coumarins

The extracts were analyzed using an Agilent 1200 series HPLC-DAD system (Agilent Technologies, USA) equipped with a Kinetex Polar C18 column (4.6 mm × 150 mm, 2.6 µm particle size; Phenomenex, USA), maintained at 30 °C. The mobile phase was consisted of acetonitrile (A) and 0.1% formic acid (B), following the gradient program: 0 min: 5% A; 30 min: 60% A; 34 min: 100% A; 34–40 min: 100% A. Prior to each run, the column was equilibrated with 5% A for 10 min. The flow rate was set at 0.6 mL/min and the injection volume was 5 µL. The analytes were detected at 280 nm. Target compounds were identified by comparing their retention times with those of authentic standards and also using the standard addition method. Quantitative analysis was performed by the external standard method. The analytical conditions were based on a previously published method (Baranová et al., 2024) and modified to achieve efficient separation of the target compounds.

Calibration solutions at five concentration levels (4.5–100 µg.mL⁻¹) were prepared by serial dilution of a stock mixture of individual standards (1 mg.mL⁻¹) and each calibration level was analyzed in triplicate. All compounds demonstrated good linearity within the tested range, with correlation coefficients (r^2) greater than 0.997. The limits of detection (LOD, $s/n = 3$) and quantification (LOQ, $s/n = 10$) were in the range of 0.30–2.00 µg.mL⁻¹ and 1.00–6.67 µg.mL⁻¹, respectively. Method repeatability, expressed as relative standard deviation (RSD, %), was below 1% for all analytes.

2.9. Scanning electron microscopy (SEM)

SEM was employed to gain detailed insights into the surface morphology of the chamomile flower samples. Following the extraction process, the dried chamomile flower specimens were carefully sliced in half with a sharp instrument to expose the inner structures. The resulting halves were then mounted perpendicularly onto a supportive dural block using specialized double-sided conductive adhesive tape to ensure a stable platform for imaging. The dural block, with the attached chamomile specimens, subsequently underwent gold sputtering using a Bal-tec/SCD 500 Sputter Coater. A thin layer of gold (12–20 nm) was deposited through plasma discharge in a vacuum environment to achieve sufficient conductivity. The gold-sputtered samples were placed on a handling stage and introduced into the chamber of a Mira3 electron microscope (Tescan, Brno, Czech Republic). Imaging was performed at an operating voltage of 5 kV, with a working distance (WD) of 6 mm and beam intensity of 10 pA by a secondary electrons (SE) detector at 34x magnification.

2.10. Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2019 (Microsoft, Redmond, WA, USA), STATISTICA.CZ version 14 (StatSoft, Prague, Czech Republic), and Python with the scikit-learn and SciPy libraries. Experimental data were evaluated by two-way analysis of variance (ANOVA), and statistically significant differences between mean values were assessed using Fisher's least significant difference (LSD) post hoc test at a significance level of $p \leq 0.05$. The applied statistical approach is reflected in the tables, where values followed by the same letter are not statistically different according to Fisher's LSD test.

Multivariate data analysis was conducted to explore relationships among samples based on their chemical composition and antioxidant-related parameters. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were applied after data standardization. PCA biplots, HCA dendrograms, and heatmaps were used for data visualization and interpretation of sample grouping and variable contributions.

3. Results

3.1. Effect of extraction technique on phenolic profile of chamomile samples determined by HPLC-DAD

The Herbona s.r.o. sample was used as the model sample for all extraction methods. A total of four phenolic compounds, namely chlorogenic acid, apigenin-7-glucoside (apig-7-glc), apigenin, and kaempferol, as well as two coumarins, umbelliferone and herniarin, were identified and quantified in individual aqueous extracts based on HPLC-DAD analysis. It was noticed that the content of individual target compounds was strongly influenced by the extraction method and particle size $p < 0.05$.

3.1.1. Cold-brew and hot-water extraction of chamomile samples

The total sum of individual target compounds increased as the particle size decreased in both the cold-brew and hot-water extracts (Table 1). In the cold-brew extract, the total sum of individual target compounds varied from 1.202 mg.g⁻¹ DW in whole flowers to 3.063 mg.g⁻¹ DW in the 4 mm fraction. The total sum of individual target compounds in the hot-water extract was two times higher than in the same fraction of the cold-brew extract, ranging from 4.272 mg.g⁻¹ DW in whole flowers to 6.274 mg.g⁻¹ DW in 4 mm fraction.

Among the individual compounds, chlorogenic acid and apig-7-glc were the most abundant compounds in both extracts. Chlorogenic acid consistently exhibited significantly higher content in hot-water extracts (0.509–4.254 mg.g⁻¹ DW) compared to cold-brew extracts (0.203–1.864 mg.g⁻¹ DW) across all particle sizes. Furthermore, decreasing the particle size enhanced chlorogenic acid recovery under both methods, with the highest levels observed in the 4 mm fraction (1.864 mg.g⁻¹ DW for cold-brew and 4.254 mg.g⁻¹ DW for hot-water extraction). In contrast, apig-7-glc showed an inverse relationship with particle size. Although hot-water extraction also led to generally higher yields (0.851–3.019 mg.g⁻¹ DW) compared to cold-brew extraction (0.371–0.513 mg.g⁻¹ DW), the apig-7-glc content decreased with smaller particle sizes. The highest concentration was observed in hot-water extract of whole flowers, 3.019 mg.g⁻¹ DW, while the 4 mm fraction yielded only 0.851 mg.g⁻¹ DW under the same conditions. Umbelliferone, apigenin, herniarin, and kaempferol were present in lower amounts but, like chlorogenic acid, showed significant increases with both decreasing particle size and higher temperature.

3.1.2. Ultrasound-assisted extraction of chamomile samples (UAE)

UAE, performed in a bath system and with a probe, showed that the decreasing particle size resulted in a higher total sum of individual target compounds for both UAE systems, and the bath setup was more effective than the probe arrangement (Table 2). The highest total sum of individual target compounds was observed in the 4 mm fraction extracted using the UAE bath (3.509 mg.g⁻¹ DW), while the lowest content was observed in the whole flowers fraction obtained by the UAE probe (1.026 mg.g⁻¹ DW). Chlorogenic acid was the most abundant compound in the extracts, followed by apig-7-glc. The level of chlorogenic acid increased as particle size decreased, regardless of the UAE setup. Its highest concentration was observed in the 4 mm fraction extracted by the UAE bath (2.180 mg.g⁻¹ DW), followed by the same fraction using the probe (1.850 mg.g⁻¹ DW). Consistent with previous observation, apig-7-glc was the most efficiently extracted from the whole flowers (0.830 mg.g⁻¹ DW) using the bath compared to the probe at the same fraction (0.440 mg.g⁻¹ DW), with recovery decreasing as particle size reduced. The recovery of the other compounds (umbelliferone, apigenin, herniarin, and kaempferol) increased as particle size decreased in both UAE arrangements, with no significant difference in their yields between these setups.

3.1.3. PHWE of chamomile samples

The optimization of the basic PHWE parameters (pressure, time,

Table 1

Effect of cold-brew and hot-water extraction on the phenolic compounds and coumarins profile of the chamomile samples at different particle sizes.

	chlorogenic acid	umbelliferone	apig-7-glc	herniarin	apigenin	kaempferol	TOTAL SUM
	[mg.g ⁻¹ DW]						
<i>cold-brew extraction</i>							
whole flowers	0.203a ± 0.003	0.108a ± 0.003	0.513ab ± 0.008	0.181c ± 0.001	0.198c ± 0.001	n.d.	1.202a ± 0.006
10 mm fraction	1.489c ± 0.051	0.225b ± 0.006	0.338a ± 0.024	0.122a ± 0.001	0.108ab ± 0.020	0.164b ± 0.016	2.446b ± 0.105
4 mm fraction	1.864d ± 0.002	0.304c ± 0.007	0.371a ± 0.012	0.136b ± 0.002	0.166b ± 0.020	0.223c ± 0.022	3.063c ± 0.050
<i>hot-water extraction</i>							
whole flowers	0.509b ± 0.003	0.123a ± 0.002	3.019d ± 0.269	0.306d ± 0.019	0.315d ± 0.003	n.d.	4.272d ± 0.287
10 mm fraction	3.697e ± 0.160	0.394d ± 0.021	0.709bc ± 0.049	0.163bc ± 0.004	0.085a ± 0.004	0.303c ± 0.008	5.351e ± 0.230
4 mm fraction	4.254 f ± 0.113	0.508e ± 0.012	0.851c ± 0.067	0.170bc ± 0.006	0.117b ± 0.007	0.375d ± 0.027	6.274 f ± 0.233

Note: Data are expressed as means ± standard deviation (n = 3). The total sum was calculated as the sum of individual compounds based on HPLC-DAD analysis. Means followed by the same letter are not statistically different at $p \leq 0.05$ according to Fisher's LSD test. n.d. – not detected.

Table 2

Influence of UAE techniques (probe sonication vs. ultrasonic bath) on the phenolic compounds and coumarins profile of the chamomile sample at different particle sizes.

	chlorogenic acid	umbelliferone	apig-7-glc	herniarin	apigenin	kaempferol	TOTAL SUM
	[mg.g ⁻¹ DW]						
<i>UAE probe</i>							
whole flowers	0.230a ± 0.012	0.121a ± 0.005	0.440b ± 0.013	0.117b ± 0.004	0.118a ± 0.012	n.d.	1.026a ± 0.013
10 mm fraction	1.390c ± 0.006	0.107a ± 0.001	0.286a ± 0.030	0.098a ± 0.003	0.146a ± 0.048	0.207b ± 0.041	2.234c ± 0.031
4 mm fraction	1.850d ± 0.002	0.126a ± 0.004	0.339a ± 0.029	0.122b ± 0.002	0.289 cd ± 0.052	0.258c ± 0.017	2.984e ± 0.037
<i>UAE bath</i>							
whole flowers	0.426b ± 0.033	0.134a ± 0.010	0.830c ± 0.080	0.154c ± 0.002	0.227bc ± 0.021	n.d.	1.771b ± 0.034
10 mm fraction	1.770d ± 0.034	0.182b ± 0.021	0.332a ± 0.025	0.125b ± 0.006	0.180ab ± 0.003	0.200b ± 0.001	2.789d ± 0.020
4 mm fraction	2.180e ± 0.096	0.191b ± 0.018	0.312a ± 0.001	0.145d ± 0.001	0.319d ± 0.026	0.362d ± 0.010	3.509 f ± 0.099

Note: Data are expressed as means ± standard deviation (n = 3). The total sum was calculated as the sum of individual compounds based on HPLC-DAD analysis. Means followed by the same letter are not statistically different at $p \leq 0.05$ according to Fisher's LSD test. n.d. – not detected.

number of extraction steps, time, and temperature) was performed with the Herbona s.r.o. sample at a particle size of 10 mm. Based on our previous experience, the extraction pressure was maintained at 15 MPa throughout all experiments without further optimization, as this setting kept the solvent in the liquid state and also ensured the tightness of the extraction system in our experimental arrangement. The initial phase of optimization focused on the effect of extraction time, which was evaluated at a constant temperature of 60 °C using one extraction cycle. Time periods of 5, 10, 15, and 20 min were tested, with 15 min yielding the most efficient recovery of target compounds. Subsequently, the influence of the number of extraction cycles (1–3) was examined; however, no significant improvement in extraction recovery was observed after the 15 min period (Table S1, Supplementary material). The influence of extraction temperature on the recovery of phenolic compounds and coumarins was investigated within the temperature range of 40–160 °C at 20 °C steps. A substantial increase in the total sum of individual target compounds was observed with rising temperature, reaching a maximum of 9.221 mg.g⁻¹ DW at 160 °C (Table 3). Chlorogenic acid and

apig-7-glc, the most abundant compounds, showed a continuous increase in concentration up to 100 °C (4.510 mg.g⁻¹ DW) and 140 °C (1.950 mg.g⁻¹ DW), respectively. At higher temperatures, a slight but statistically significant decrease in their yields was observed. Similarly, the extraction yields of umbelliferone, kaempferol, and apigenin increased steadily with temperature, with their highest values between 140 °C and 160 °C. Significant temperature dependence was noticed for herniarin, a key coumarin derivate present in chamomile. Its yield increased gradually up to 120 °C, followed by a marked rise from 0.431 mg.g⁻¹ DW at 140 °C to 1.760 mg.g⁻¹ DW at 160 °C. Although the highest overall yields were achieved at 140–160 °C, these conditions negatively affected the sensory and visual quality of the extracts. Temperatures above 120 °C led to noticeable browning and the development of an unpleasant odor, suggesting the onset of thermal degradation or the formation of undesirable by-products. To ensure both sufficient extraction efficiency and acceptable extract quality, 100 °C was selected as the optimal extraction temperature for further experiments.

The influence of particle size on extraction efficiency under

Table 3

Effect of extraction temperature on the yield of phenolic compounds and coumarins from chamomile samples using PHWE.

PHWE temperature	chlorogenic acid	umbelliferone	apig-7-glc	herniarin	apigenin	kaempferol	TOTAL SUM
	[mg.g ⁻¹ DW]						
40 °C	1.610a ± 0.009	0.194a ± 0.001	0.411a ± 0.012	0.143a ± 0.002	0.117c ± 0.002	0.213a ± 0.004	2.688a ± 0.005
60 °C	2.930b ± 0.127	0.277b ± 0.003	0.752b ± 0.021	0.173b ± 0.001	0.101b ± 0.002	0.275b ± 0.002	4.508b ± 0.103
80 °C	4.350d ± 0.024	0.524c ± 0.004	1.220c ± 0.095	0.198c ± 0.002	0.091a ± 0.001	0.405c ± 0.018	6.788c ± 0.130
100 °C	4.510 g ± 0.002	0.699b ± 0.001	1.190a ± 0.111	0.203a ± 0.003	0.100a ± 0.002	0.391b ± 0.009	7.093c ± 0.093
120 °C	4.420d ± 0.050	0.795 f ± 0.014	1.630d ± 0.070	0.213d ± 0.007	0.186d ± 0.001	0.467d ± 0.03	7.711e ± 0.152
140 °C	4.060c ± 0.138	0.820 g ± 0.010	1.950e ± 0.042	0.431e ± 0.004	0.222e ± 0.001	0.718e ± 0.02	8.201 f ± 0.163
160 °C	4.070c ± 0.013	0.658d ± 0.010	1.630d ± 0.033	1.760 f ± 0.007	0.254 f ± 0.004	0.849 f ± 0.02	9.221 g ± 0.020

Note: Data are expressed as means ± standard deviation (n = 3). The total sum was calculated as the sum of individual compounds based on HPLC-DAD analysis. Means followed by the same letter are not statistically different at $p \leq 0.05$ according to Fisher's LSD test.

optimized PHWE conditions (100 °C, 15 min) is summarized in Table 4. A clear particle size-dependent trend was observed, consistent with the general pattern described above. The 4 mm fraction yielded the highest total sum of individual target compounds (7.884 mg.g⁻¹ DW), mainly due to enhanced recovery of chlorogenic acid. Conversely, apig-7-glc exhibited a markedly higher concentration in whole flowers compared to ground fractions. Minor compounds such as herniarin, apigenin, and kaempferol followed a similar trend. Interestingly, despite the pronounced decrease in apig-7-glc with particle size reduction, the concentration of apigenin did not show a corresponding increase. This suggests that, rather than simple hydrolysis, more extensive degradation or structural transformation of the compounds may have occurred under the combined influence of mechanical disruption and elevated temperature and pressure.

3.2. Effect of extraction method and particle size on TPC

It was observed that both the extraction technique and particle size of the plant significantly influenced TPC yield (Table 5), with a statistically significant interaction between these two factors ($p < 0.001$). Among the tested methods, PHWE yielded the highest TPC across all particle size fractions (19.966–31.665 mg GAE.g⁻¹ DW), followed by conventional hot-water extraction (11.206–24.092 mg GAE.g⁻¹ DW); while cold-brew extraction (4.877–13.074 mg GAE.g⁻¹ DW) and UAE methods resulted in significantly lower TPC ($p < 0.001$). Slightly higher efficiency was observed for the ultrasonic bath (4.842–12.422 mg GAE.g⁻¹ DW) compared to the probe (3.513–11.587 mg GAE.g⁻¹ DW), although the difference was not statistically significant for all particle sizes ($p > 0.05$). For all extraction methods, a consistent trend of increasing TPC with decreasing particle size was observed, with the 4 mm fraction significantly higher than the 10 mm and whole flower fractions ($p < 0.001$).

3.3. Effect of extraction method and particle size on antioxidant activity of the chamomile extracts (DPPH radical assay)

A similar pattern was observed for the antioxidant activity of the extracts (Table 6), measured by the DPPH radical scavenging assay. The results showed that antioxidant activity was again statistically significantly affected by both the extraction method and particle sizes of the plant material ($p < 0.001$). PHWE resulted in the highest antioxidant activity, with a maximum value of 53.628 mg TE.g⁻¹ DW in the 4 mm fraction. Conventional hot-water extraction also yielded high activity (11.773–25.334 mg TE.g⁻¹ DW), whereas the cold-brew extraction (4.385–11.154 mg TE.g⁻¹ DW) and UAE methods were markedly less effective across all fractions ($p < 0.05$), with higher efficiency noticed for bath (6.740–15.580 mg TE.g⁻¹ DW) compared to probe (3.373–9.260 mg TE.g⁻¹ DW). The antioxidant capacity increased significantly with decreasing particle size across all extraction methods ($p < 0.001$), with the 4 mm fraction consistently showing the highest activity and the whole flowers the lowest.

3.4. Effect of extraction technique on the morphology of chamomile flowers

SEM (Fig. 1) revealed clear structural differences of chamomile flower heads after various water-based extraction methods. The control sample (a) shows an intact and compact inflorescence structure with well-preserved tissues. Similarly, cold-brew extraction (b) reveals mild surface disruption while the overall morphology remains largely preserved. On the other hand, the hot-water extraction (c) causes notable expansion and partial disruption of cellular layers, resulting in increased porosity. Samples treated by UAE using a bath (d) and a probe (e) demonstrate increasingly pronounced tissue disintegration; especially after probe treatment, the structure is highly fragmented and decomposed. Finally, PHWE at 100 °C (f) results in severe breakdown and collapse of floral tissues. These morphological modifications illustrate the progressive impact of each extraction method on the internal structure of the chamomile flower, which has important consequences for extraction efficiency and phytochemical profile.

3.5. Effect of sample origin and fractionation on phytochemical composition of chamomile aqueous extracts

PHWE was applied under optimized extraction (100 °C, 1 × 15 min, 15 MPa) conditions to compare the phytochemical content of various chamomile samples. As shown in Table 7, significant variability was observed among the samples obtained from different suppliers in terms of their phytochemical profiles, TPC, and antioxidant activity (DPPH radical scavenging). The HPLC-DAD chromatograms of individual samples are shown in Supplementary material (Figs. S1–S4). The Herbona s.r.o. sample was characterized by the highest content of chlorogenic acid and apig-7-glc with moderate levels of herniarin, kaempferol, and the total sum of individual target compounds (6.636–7.884 mg.g⁻¹ DW). Its TPC and antioxidant activity ranged from 19.966 to 31.665 mg GAE.g⁻¹ DW and 27.502–53.628 mg TE.g⁻¹ DW, respectively, depending on the extracted fraction. A similar profile was observed for the Úsovsko a.s. sample, with slightly higher levels of herniarin and umbelliferone, as well as a higher total sum of individual target compounds (6.558–9.145 mg.g⁻¹ DW). However, its TPC (18.926–26.377 mg GAE.g⁻¹ DW) and antioxidant activity (29.221–38.241 mg TE.g⁻¹ DW) were slightly lower compared to the Herbona s.r.o. sample. The RAJ sample consistently showed the lowest concentrations of most target analytes, resulting in the lowest total sum of individual target compounds (4.306–4.767 mg.g⁻¹ DW), TPC (18.122–20.341 mg GAE.g⁻¹ DW), and antioxidant activity (28.250–30.093 mg TE.g⁻¹ DW). In contrast, the ELTO-BIO s.r.o. sample demonstrated the most balanced phytochemical profile, with elevated levels of umbelliferone, herniarin, and kaempferol. Its total sum of individual target compounds reached 12.045–12.841 mg.g⁻¹ DW, more than twice that of the other samples. This was reflected in its higher TPC (28.038–29.906 mg GAE.g⁻¹ DW) and antioxidant activity (37.828–39.807 mg TE.g⁻¹ DW).

To visualize the relationships between chamomile samples of different origins and processing fractions based on their chemical composition, the principal component analysis (PCA) was employed.

Table 4

Effect of particle size on the yield of phenolic compounds and coumarins in chamomile extracts obtained by PHWE at 100 °C.

Particle size	chlorogenic acid	umbelliferone	apig-7-glc	herniarin	apigenin	kaempferol	TOTAL SUM
	[mg.g ⁻¹ DW]						
whole flowers	1.210a ± 0.024	0.360a ± 0.008	4.100e ± 0.044	0.394bc ± 0.002	0.142b ± 0.010	0.430c ± 0.011	6.636bc ± 0.036
10 mm fraction	4.510 g ± 0.002	0.699b ± 0.001	1.190a ± 0.111	0.203a ± 0.003	0.100a ± 0.002	0.391b ± 0.009	7.093c ± 0.093
4 mm fraction	5.290 h ± 0.044	0.720b ± 0.013	1.170a ± 0.019	0.208a ± 0.012	0.101a ± 0.001	0.395b ± 0.005	7.884d ± 0.082

Note: Data are expressed as means ± standard deviation (n = 3). The total sum was calculated as the sum of individual compounds based on HPLC-DAD analysis. Means followed by the same letter are not statistically different at $p \leq 0.05$ according to Fisher's LSD test.

Table 5

Impact of extraction technique and particle size of the sample on TPC of the chamomile extracts.

Particle size	cold-brew extraction	hot-water extraction	UAE probe	UAE bath	PHWE
	[mg GAE.g ⁻¹ DW]				
whole flowers	4.877a ± 0.054	11.206a ± 0.701	3.513a ± 0.129	4.842a ± 0.333	19.966a ± 0.120
10 mm fraction	10.062b ± 0.270	20.775b ± 0.970	8.381b ± 0.208	9.505b ± 0.239	29.638b ± 0.322
4 mm fraction	13.074c ± 0.216	24.092c ± 1.240	11.587c ± 0.016	12.422c ± 0.372	31.665c ± 0.147

Note: Data are expressed as means ± standard deviation (n = 3). Means followed by the same letter are not statistically different at p < 0.05 according to Fisher's LSD test.

Table 6

Impact of extraction technique and particle size of the sample on antioxidant activity of the chamomile extracts.

Particle size	cold-brew extraction	hot-water extraction	UAE probe	UAE bath	PHWE
	[mg TE.g ⁻¹ DW]				
whole flowers	4.385a ± 0.056	11.773a ± 0.338	3.373a ± 0.046	6.740a ± 0.307	27.502a ± 0.313
10 mm fraction	9.060b ± 0.176	24.571b ± 0.778	8.852b ± 0.239	13.317b ± 0.085	37.547b ± 0.360
4 mm fraction	11.154c ± 0.326	25.334b ± 0.050	9.260b ± 0.043	15.580c ± 0.079	53.628c ± 0.130

Note: Data are expressed as means ± standard deviation (n = 3). Means followed by the same letter are not statistically different at p ≤ 0.05 according to Fisher's LSD test.

The resulting biplot (Fig. 2) showed that the first two components

account for more than 88% of the total variance in the dataset, with PC1 (56.9%) separating samples based on flavonoids and coumarins content, while PC2 (31.4%) reflects variation in TPC, antioxidant activity (*DPPH), and chlorogenic acid content. Samples originating from ELTO-BIO s.r.o. and RAJ exhibited a consistent chemical profile across different fractions, whereas samples from Herbona s.r.o. and Úsovsko a. s. displayed a clear separation based on the processing fraction, with the whole flowers shifted towards higher flavonoids and coumarins content.

Hierarchical cluster analysis (HCA) confirmed the presence of five stable clusters that reflected both sample origin and fraction type (Fig. 3). The strongest separation was observed between whole flowers and fractionated chamomile flowers. Whole flowers of Herbona s.r.o. and Úsovsko a.s. samples grouped together into a distinct cluster associated with higher apig-7-glc content, while Herbona s.r.o. sample fractions (4 mm, 10 mm) were characterized by elevated TPC, DPPH radical scavenging activity, and chlorogenic acid level. RAJ samples consistently clustered together and were defined by a lower total sum of individual target compounds. A similar tight grouping was observed for

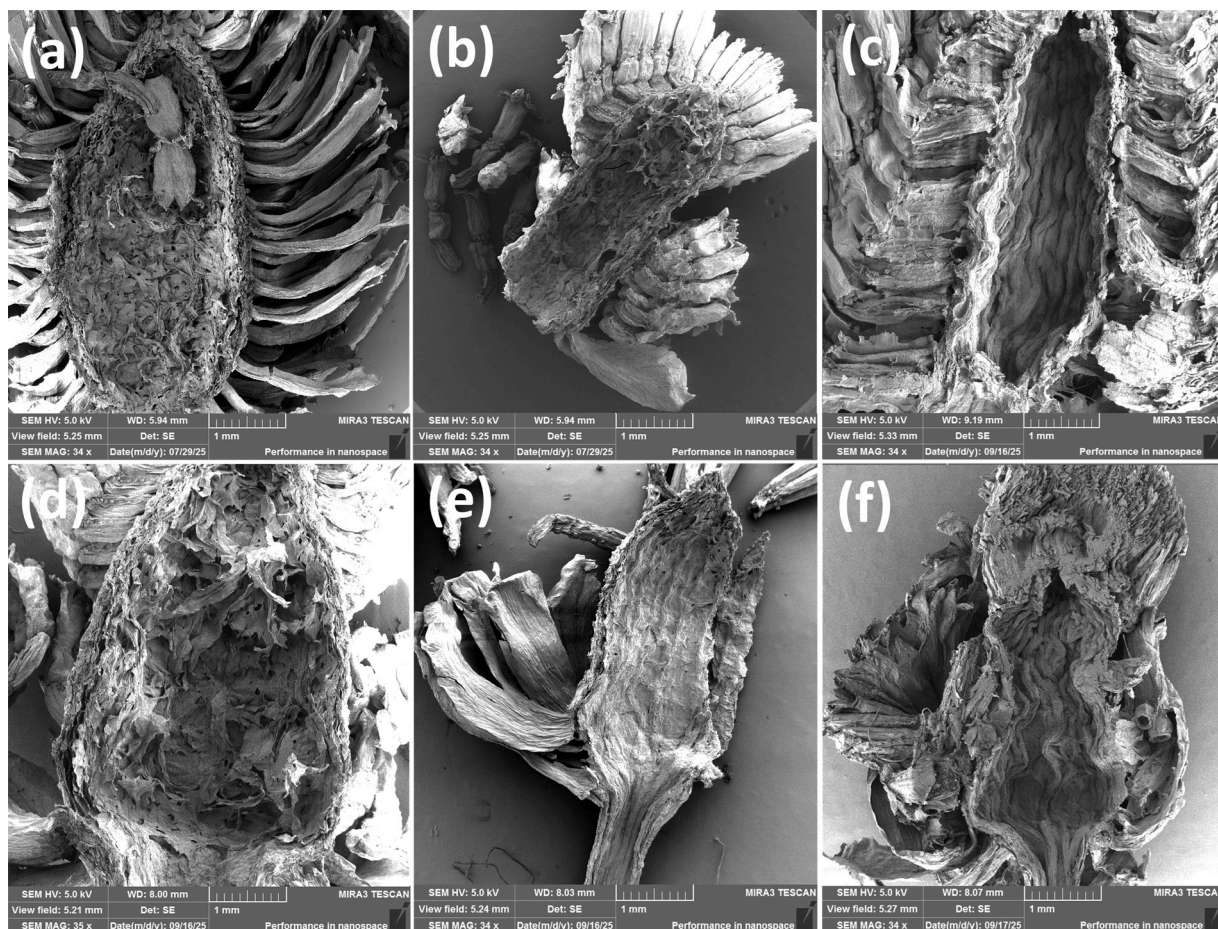


Fig. 1. Scanning electron microscopy (SEM) of chamomile flower after water extraction processes. (a) control; (b) cold-brew extraction; (c) hot-water extraction; (d) UAE bath; (e) UAE probe; (f) PHWE at 100 °C.

Table 7
Phytochemical composition, TPC, and antioxidant activity of various chamomile samples extracted by PHWE at optimized conditions.

Sample	Particle size	chlorogenic acid	umbelliferone	apig-7-Glc	herniarin	apigenin	kaempferol	TOTAL SUM	TPC	*DPPH
		[mg.g ⁻¹ DW]							[mg GAE.g ⁻¹ DW]	[mg TE.g ⁻¹ DW]
Herbona s. r.o.	whole	1.210a ± 0.024	0.360a ± 0.008	4.100e ± 0.044	0.394bc ± 0.002	0.142b ± 0.010	0.430c ± 0.011	6.636bc ± 0.036	19.966a ± 0.120	27.502a ± 0.313
	flowers	4.510 g	0.699b ± 0.001	1.190a ± 0.111	0.203a ± 0.003	0.100a ± 0.002	0.391b ± 0.009	7.093c ± 0.093	29.638b ± 0.322	37.547b ± 0.360
	10 mm	fraction ± 0.002	0.001	0.111	0.003	0.002	0.009	0.009	0.322	0.360
	4 mm	5.290 h	0.720b ± 0.013	1.170a ± 0.019	0.208a ± 0.012	0.101a ± 0.001	0.395b ± 0.005	7.884d ± 0.082	31.665c ± 0.147	53.628c ± 0.130
	fraction	± 0.044	0.013	0.019	0.012	0.001	0.005	0.082	0.147	0.130
Úsovsko a. s.	whole	1.086a ± 0.004	0.363a ± 0.003	3.854de ± 0.522	0.587de ± 0.027	0.276d ± 0.012	0.393b ± 0.008	6.558b ± 0.538	18.926ab ± 0.166	29.221bc ± 0.205
	flowers	4.029 f	1.164c ± 0.070	2.711c ± 0.102	0.630e ± 0.010	0.140b ± 0.017	0.471d ± 0.003	9.145e ± 0.195	24.540d ± 0.139	34.647d ± 0.480
	10 mm	fraction ± 0.138	0.138	0.102	0.010	0.017	0.003	0.195	0.139	0.480
	4 mm	3.900 f	1.237c ± 0.004	2.673c ± 0.201	0.641e ± 0.003	0.157b ± 0.001	0.502d ± 0.021	9.110e ± 0.143	26.377e ± 0.610	38.241e ± 0.054
	fraction	± 0.044	0.044	0.201	0.003	0.001	0.021	0.143	0.610	0.054
RAJ	whole	1.546b ± 0.178	0.293a ± 0.018	2.086b ± 0.063	0.327b ± 0.008	0.143b ± 0.007	0.371b ± 0.007	4.767a ± 0.140	19.67bc ± 0.69	30.093c ± 1.282
	flowers	0.178	0.018	0.063	0.008	0.007	0.007	0.140	0.69	1.282
	10 mm	fraction ± 0.033	0.033	0.001	0.095	0.007	0.020	0.150	0.506	0.232
	4 mm	2.092c ± 0.007	0.305a ± 0.001	1.422a ± 0.011	0.447c ± 0.010	0.108a ± 0.015	0.308a ± 0.007	4.682a ± 0.022	20.341c ± 0.126	29.859c ± 0.004
	fraction	0.007	0.001	0.011	0.010	0.015	0.007	0.022	0.126	0.004
ELTO-BIO s.r.o.	whole	3.080d ± 0.059	3.075d ± 0.066	3.557d ± 0.124	2.280 f ± 0.103	0.306e ± 0.001	0.543e ± 0.028	12.841 g ± 0.042	28.049 f ± 0.278	39.688 f ± 0.685
	flowers	0.059	0.066	0.124	± 0.103	0.001	0.028	± 0.042	± 0.278	± 0.685
	10 mm	fraction ± 0.031	0.031	0.150	0.157	± 0.020	0.015	± 0.338	± 0.483	0.780
	4 mm	3.354d ± 0.057	3.070d ± 0.033	2.514c ± 0.026	2.348fg ± 0.014	0.222c ± 0.014	0.539e ± 0.011	12.045 f ± 0.155	29.906 g ± 0.609	39.807 f ± 1.410
	fraction	0.057	0.033	0.026	0.014	0.014	0.011	± 0.155	± 0.609	± 1.410

Note: Data are expressed as means ± standard deviation (n = 3). The total sum was calculated as the sum of individual compounds based on HPLC-DAD analysis. Means followed by the same letter are not statistically different at p ≤ 0.05 according to Fisher's LSD test.

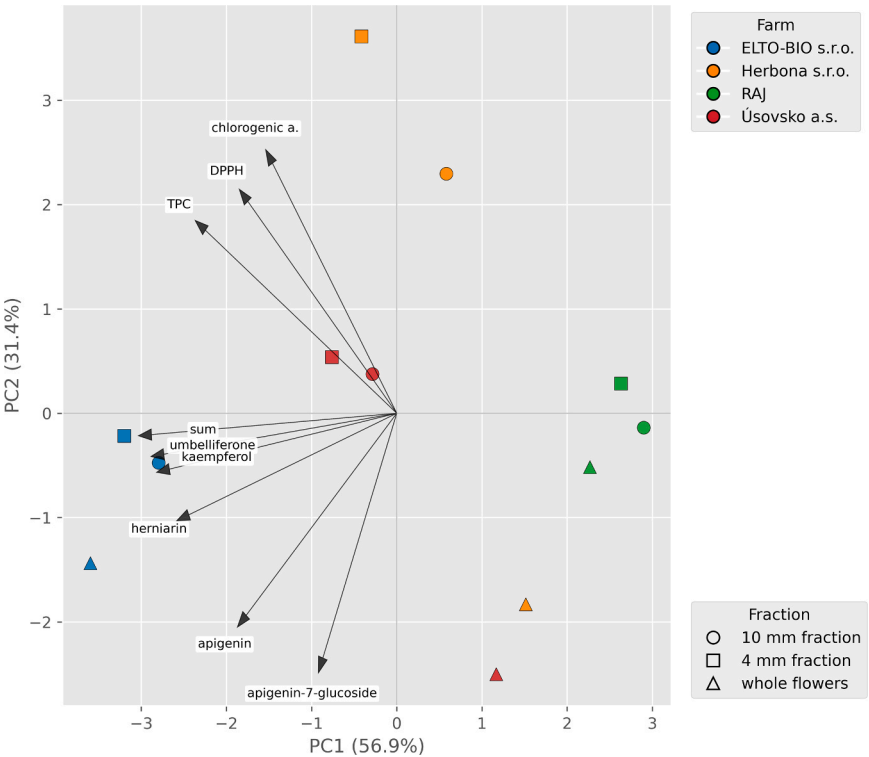


Fig. 2. PCA biplot of chamomile samples of various origins based on their chemical composition and processing fraction. Sum means the total sum of target compounds determined by HPLC-DAD.

ELTO-BIO s.r.o., with all fractions clustering at a low linkage distance and characterized by elevated coumarin levels (umbelliferone, herniarin). At a higher hierarchical level, RAJ and ELTO-BIO s.r.o. samples merged into one branch, while Herbona s.r.o. and Úsovsko a.s. samples formed a separate branch, highlighting broader differences between

these two sample origin groups. Overall, both PCA and HCA results demonstrate that the phytochemical profile of chamomile is determined by both the sample origin and the fractionation of chamomile flowers, with whole flowers consistently displaying a distinct phytochemical signature compared to fractionated material. The conclusions from PCA

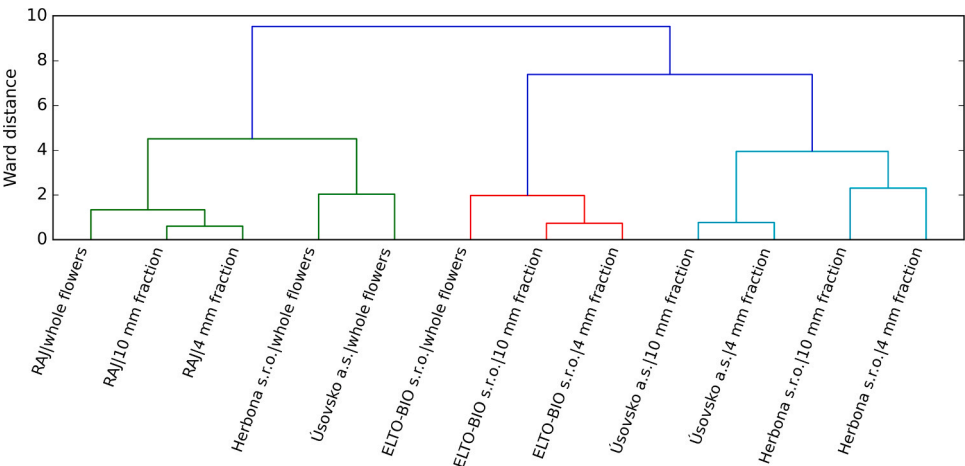


Fig. 3. Hierarchical clustering of chamomile samples according to phytochemical profiles.

and HCA were further supported by the heat map of standardized values (Z-score), which confirmed the sample origin-specific phytochemical profile and its consistent differences between unprocessed whole flowers and their fractions (Fig. S5, Supplementary material).

3.6. GC/MS profile of EOs of various chamomile samples at different fractionation

In addition to the analysis of phenolic compounds and coumarins, the chemical profile of chamomile EOs was evaluated to provide a more comprehensive insight into the bioactive composition of the chamomile samples. The results revealed considerable differences in both the yield and composition of EOs among the samples (Table 8a,b). The GC/MS chromatograms of individual samples are shown in Supplementary material (Figs. S6-S9). The EO yield varied from 0.150 to 0.400 mL/100 g DW, with the highest values noticed for the ELTO-BIO s.r.o. sample (0.300–0.400 mL/100 g DW) and the lowest for the Herbona s.r.o. (0.150–0.200 mL/100 g DW) sample. Particle size reduction generally resulted in a higher EO yield. α -Bisabolol was the dominant compound in the ELTO-BIO s.r.o. sample, with concentrations ranging from 44.790% to 57.464%, depending on particle size. In comparison, other samples showed significantly lower levels, between 1.381% and 3.884%. Conversely, the Herbona s.r.o. sample exhibited the highest levels of α -bisabolol oxide A (37.616–41.653%), whereas ELTO-BIO s.r.o. contained only trace amounts of this compound (0.670–0.964%). Similarly, α -bisabolol oxide B was most abundant in Herbona s.r.o., RAJ

and Úsovsko a.s. (14.692–29.494%), but was almost absent in the ELTO-BIO s.r.o. sample (1.487–2.427%). α -Bisabolone oxide A followed a similar trend, with the highest levels observed in RAJ (17.257–18.074%) and the lowest in ELTO-BIO s.r.o. (0.084–0.184%). The highest values of chamazulene, a characteristic compound of chamomile oil, were found in Úsovsko a.s. (22.963–30.288%), while the lowest was found in Herbona s.r.o. (7.424–8.408%). Other sesquiterpenes, such as farnesene, germacrene D, and spathulenol, showed moderate variation among samples, with farnesene reaching the highest levels in the ELTO-BIO s.r.o. sample (10.461–27.902%). Minor constituents such as *cis*- and *trans*-spiroethers were detected under 3.411% across samples. The grinding of the plant matrix primarily affected the relative abundance of farnesene, germacrene D, and chamazulene, as their concentrations increased in the finer fractions. On the other hand, grinding did not enhance the recovery of the other components; their highest concentrations were observed in unprocessed whole chamomile flowers.

These compositional patterns are reflected in the PCA biplot (Fig. 4), which shows a clear separation of chamomile samples based on their EO profiles. The first two principal components explain 73.3% of the total variance (PC1: 46.2%, PC2: 27.1%). Úsovsko a.s. samples are characterized by a higher level of chamazulene, germacrene D, and spiroethers, while those from Herbona s.r.o. are more associated with α -bisabolol oxide A. ELTO-BIO s.r.o. samples were positioned on the negative side of PC1 and linked to higher levels of α -bisabolol and farnesene, whereas RAJ samples showed a stronger association with α -bisabolone oxide A,

Table 8a
Chemical composition of EO of various chamomile samples obtained by hydrodistillation.

Compounds	RI lit.	RI cal.	Herbona s.r.o.			Úsovsko a.s.		
			Whole flowers	10 mm fraction	4 mm fraction	Whole flowers	10 mm fraction	4 mm fraction
			[rel. %]					
farnesene	1454	1452	5.109b ± 0.142	8.633 f ± 0.061	7.575e ± 0.035	14.393 h ± 0.032	21.817j ± 0.109	24.327k ± 0.123
germacrene D	1480	1482	0.320a ± 0.028	0.398ab ± 0.026	0.355a ± 0.036	2.315 f ± 0.120	2.792 g ± 0.144	3.336 g ± 0.162
spathulenol	1577	1578	1.530a ± 0.042	1.768b ± 0.130	1.595ab ± 0.021	2.445c ± 0.050	2.401c ± 0.169	2.534c ± 0.063
α -bisabolol oxide B	1656	1655	28.945 g ± 0.064	27.803e ± 0.222	27.858e ± 0.172	16.484d ± 0.150	15.087c ± 0.203	14.692c ± 0.073
α -bisabolone oxide A	1684	1682	8.951c ± 0.087	9.642d ± 0.088	8.448b ± 0.054	16.405 g ± 0.050	11.906e ± 0.122	12.620 f ± 0.056
α -bisabolol	1685	1685	3.603d ± 0.075	3.884e ± 0.133	3.732de ± 0.074	1.691b ± 0.059	1.381a ± 0.058	1.537ab ± 0.103
chamazulene	1730	1730	7.648a ± 0.096	8.408b ± 0.124	7.424a ± 0.091	22.963 h ± 0.060	30.288j ± 0.139	25.088i ± 0.153
α -bisabolol oxide A	1748	1749	41.653j ± 0.089	37.616 h ± 0.121	40.870i ± 0.142	17.942 g ± 0.115	11.315c ± 0.092	12.455d ± 0.107
<i>cis</i> -spiroether	1879	1878	1.539b ± 0.115	1.251a ± 0.126	1.441ab ± 0.112	3.411 f ± 0.211	1.916c ± 0.104	2.199d ± 0.172
<i>trans</i> -spiroether	1890	1892	0.702a ± 0.040	0.597a ± 0.074	0.702a ± 0.125	1.952d ± 0.124	1.097b ± 0.188	1.213bc ± 0.138
EO yield [mL/100 g DW]			0.200a ± 0.001	0.183a ± 0.024	0.150a ± 0.071	0.317bc ± 0.024	0.350bcd ± 0.024	0.350bcd ± 0.023

Note: Data are expressed as means ± standard deviation (n = 3). Means followed by the same letter are not statistically different at p ≤ 0.05 according to Fisher's LSD test. RI calculated = Retention index determined with respect to homologous series of n-alkanes (C9-C23) on a DB-5 column. RI lit. = Retention index from literature – Adams (2007).

Table 8b
Chemical composition of EO of various chamomile samples obtained by hydrodistillation.

Compounds	RI lit.	RI cal.	RAJ			ELTO-BIO s.r.o.		
			Whole flowers	10 mm fraction	4 mm fraction	Whole flowers	10 mm fraction	4 mm fraction
			[rel. %]					
farnesene	1454	1452	3.795a ± 0.049	6.415c ± 0.064	6.935d ± 0.092	10.461 g ± 0.101	17.125i ± 0.091	27.902 l ± 0.130
germacrene D	1480	1482	0.402ab ± 0.031	0.610c ± 0.028	0.542bc ± 0.045	0.989d ± 0.056	0.913d ± 0.033	1.272e ± 0.073
spathulenol	1577	1578	5.524 f ± 0.093	5.005e ± 0.021	4.819e ± 0.157	2.829d ± 0.055	2.451c ± 0.168	1.815b ± 0.148
α-bisabolol oxide B	1656	1655	28.281 f ± 0.140	29.337 h ± 0.131	29.494 h ± 0.122	2.427b ± 0.216	2.217b ± 0.104	1.487a ± 0.053
α-bisabolone oxide A	1684	1682	18.074j ± 0.077	17.257 h ± 0.061	17.447i ± 0.080	0.184a ± 0.020	0.103a ± 0.018	0.084a ± 0.005
α-bisabolol	1685	1685	2.041c ± 0.072	2.380c ± 0.057	2.217c ± 0.052	57.464 h ± 0.049	52.207 g ± 0.222	44.790 f ± 0.029
chamazulene	1730	1730	21.396 g ± 0.051	19.773d ± 0.088	17.752c ± 0.116	20.686e ± 0.107	21.066 f ± 0.136	19.774d ± 0.048
α-bisabolol oxide A	1748	1749	17.525 f ± 0.049	16.961e ± 0.072	17.893 g ± 0.076	0.955ab ± 0.106	0.964ab ± 0.077	0.670a ± 0.043
cis-spiroether	1879	1878	2.380de ± 0.071	1.575b ± 0.036	2.121 cd ± 0.084	2.594e ± 0.178	1.911c ± 0.097	1.434ab ± 0.136
trans-spiroether	1890	1892	0.582a ± 0.025	0.687a ± 0.052	0.781a ± 0.098	1.410c ± 0.099	1.043b ± 0.208	0.771a ± 0.097
EO yield [mL/100 g DW]			0.389 cd ± 0.021	0.167a ± 0.001	0.367bcd ± 0.047	0.333bcd ± 0.047	0.300d ± 0.047	0.400d ± 0.001

Note: Data are expressed as means ± standard deviation (n = 3). Means followed by the same letter are not statistically different at p ≤ 0.05 according to Fisher's LSD test. RI calculated = Retention index determined with respect to homologous series of n-alkanes (C9-C23) on a DB-5 column. RI lit. = Retention index from literature – Adams (2007).

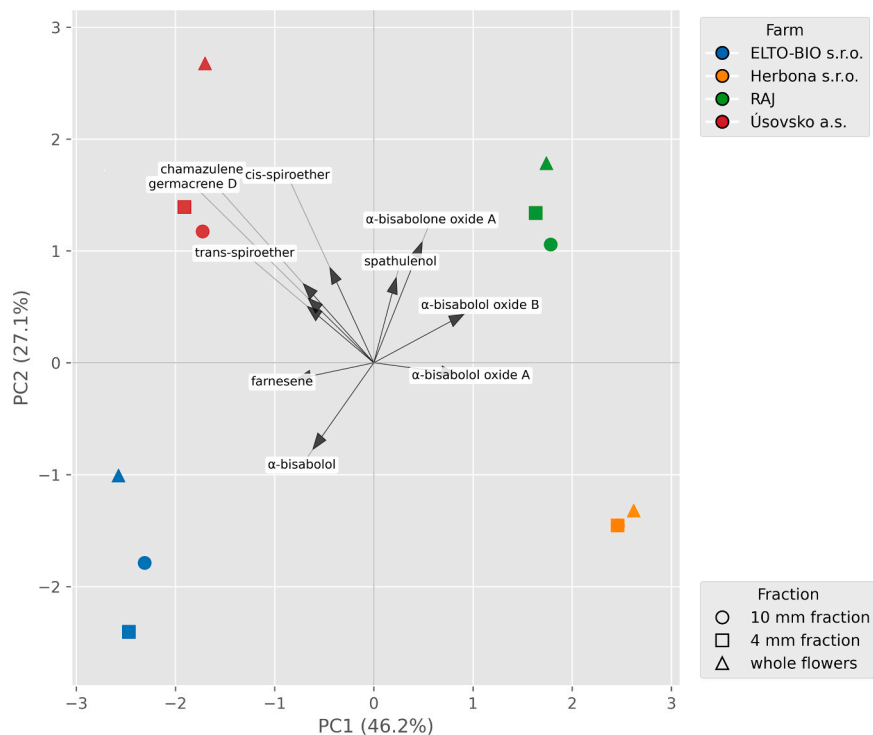


Fig. 4. PCA biplot of EO composition in chamomile samples of different origins.

α-bisabolol oxide B, and spathulenol. Although the plot also includes different fraction types (whole flowers, 10 mm, and 4 mm), these are not clearly separated in PCA space, indicating that the level of fragmentation has a negligible impact compared to the influence of sample origin. Therefore, the primary source of variation in EO composition appears to be the sample origin rather than the processing fraction. These findings were further supported by HCA. The dendrogram (Fig. 5) clearly shows that the samples cluster according to their origin rather than particle size. Fractions of whole flowers, 10 mm, and 4 mm from the same source consistently group together, indicating that the comminution of the chamomile flowers has only a minor impact on the overall EO profile. This finding reinforces the conclusion that the cultivation site and producer exert a stronger influence on chemical variability than fractionation. The results from PCA and HCA are further supported by the heatmap of standardized values (Z-scores), which also shows a consistent clustering of samples by origin rather than by particle

size fraction (Fig. S10, Supplementary material).

4. Discussion

The present study comprehensively assessed the impact of extraction method, particle size, and sample origin on both the antioxidant activity and the detailed phytochemical composition of *Matricaria chamomilla* L. flower aqueous extracts. In addition, the impact of particle size and sample origin on EO composition was also evaluated. Unlike many previous papers have recently described the phytochemical composition of chamomile (Catani et al., 2021; Cvetanović et al., 2018; Harbourn et al., 2009; Hroboňová et al., 2021; Pavlova et al., 2018; Rezaei et al., 2015; Salamon et al., 2023; Stanojevic et al., 2016; Tselika et al., 2021), this work provides applied insights into green extraction strategies for maximizing the recovery of bioactive compounds from this widely used medicinal herb, while also underscore the critical role of

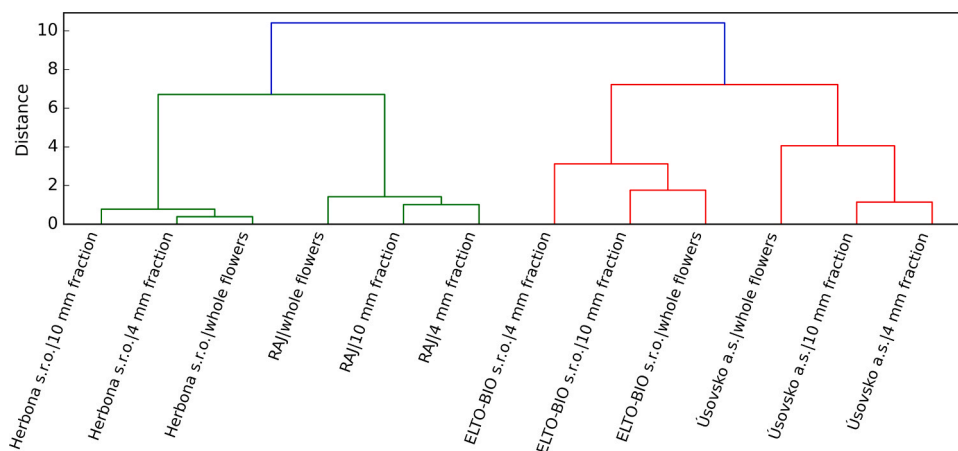


Fig. 5. Cluster dendrogram of chamomile EO composition of different origins.

raw material origin in ensuring the production of high-quality chamomile products.

Among the tested extraction techniques, PHWE consistently outperformed cold-brew, hot-water, and UAE methods in terms of individual phenolic compounds and coumarins content, as well as TPC and antioxidant activity (DPPH radical assay). This observation is consistent with previous findings of [Cvetanović et al. \(2015\)](#), who reported high efficiency of subcritical water extraction compared to Soxhlet, MAE, and UAE of biologically active compounds from chamomile. This efficiency is likely attributed to the enhanced mass transfer and improved solubility of target compounds under subcritical water conditions, since elevated temperature and pressure promote the disruption of plant matrices and improve recovery of biologically active compounds ([Adeyo et al., 2023](#); [dos Santos et al., 2025](#)). Temperature optimization revealed that 100 °C provided the best compromise between extraction efficiency and the preservation of extract quality. Although higher temperatures (above 120 °C) yielded the maximum of total sum of individual target compounds, they also resulted in extract browning and undesirable odors, indicating potential degradation, consistent with earlier studies ([Antony and Farid, 2022](#); [Cvetanović et al., 2019](#); [Temerdashev et al., 2023](#)).

In comparison, both hot-water and cold-brew extractions were markedly less effective. Hot-water extraction (80 °C, 15 min) recovered significantly more target compounds than cold-brew extraction (25 °C, 20 min), confirming that temperature accelerates diffusion and solubilization of thermally stable compounds. For instance, [Foschi et al. \(2024\)](#) optimized cold water extraction of bioactive compounds from chamomile and reported that time, temperature, and material amount all materially impact the polyphenolic profile and antioxidant potential of its water extracts. Similar findings were described by [Sotiropoulou et al. \(2020\)](#), who examined the effect of the extraction temperature on the bioactivity of chamomile infusions at various temperatures (25, 80, and 100 °C). The highest TPC and antioxidant activity of chamomile infusion were achieved at 80 °C. This is in good accordance with our results.

UAE methods showed intermediate performance, with the ultrasonic bath being slightly superior to probe homogenization. However, UAE did not reach the efficiency of PHWE or conventional hot-water extraction. This may be related to the ultrasonic conditions applied, including power (particularly for the probe) and extraction time, as well as to the fact that ultrasound-induced cavitation under the applied conditions may be less effective in disrupting plant tissues and promoting mass transfer. In addition, temperature control during UAE minimized thermal effects that can otherwise enhance mass transfer during hot-water extraction. These findings highlight the importance of method optimization with respect to matrix structure and target compounds.

These findings were supported by the SEM analysis, which clearly demonstrated the structural integrity of chamomile flowers in dependence on the applied extraction technique. Gentle methods such as cold-brew extraction result in only minor alterations of the cellular architecture, preserving much of the original morphology. However, more intensive treatments, including conventional hot-water, UAE, and particularly PHWE, induce substantial tissue disintegration and increased porosity. These morphological disruptions are likely to facilitate higher extraction yields by enhancing solvent access to intracellular compounds but may also alter the composition and stability of the extracted phytochemicals. Therefore, the choice of extraction method must consider both efficiency and the preservation of desirable bioactive constituents, as reflected by the distinct microstructural outcomes observed for each method ([Confortin et al., 2019](#); [Tepe and Doyuk, 2023](#)).

The influence of particle size was significant across all methods, with smaller particle fractions (4 mm) generally resulting in higher recovery of most target compounds, especially chlorogenic acid, as well as TPC and antioxidant activity of aqueous extracts. This trend is consistent with established diffusion principles and previous studies showing enhanced solvent penetration with finer plant material ([Catani et al., 2021](#); [Molnar et al., 2017](#); [Pereira et al., 2018](#)). Conversely, apig-7-glc displayed an inverse trend, with the highest concentrations found in whole flowers, suggesting that this flavonoid glycoside is particularly susceptible to structural instability under mechanical and thermal stress, as increased extraction severity and fragmentation of plant material may promote its degradation. Interestingly, this pattern was not accompanied by a corresponding increase in apigenin, which implies that hydrolysis is not the prevailing mechanism and indicates a more complex interplay of degradation, structural transformation, extraction efficiency, and possibly matrix interactions. This observation aligns with previous studies ([Antony and Farid, 2022](#); [Catani et al., 2021](#)), underscoring the importance of optimizing particle size not only to maximize yield but also to preserve the integrity of specific phytochemicals.

Significant chemical variation was observed between chamomile samples from different sources. While the ELTO-BIO s.r.o. sample exhibited the highest overall levels of total sum of phenolic compounds and coumarins, TPC, and antioxidant activity, as well as a distinct profile rich in umbelliferone, herniarin, and kaempferol, the RAJ sample consistently showed the lowest values across all chemical parameters. PCA and HCA confirmed that both the sample origin and particle size of the sample contributed significantly to variation in phenolic compounds and coumarins composition, whereas EO composition was driven almost exclusively by sample origin, with negligible influence from sample fractionation. The ELTO-BIO s.r.o. sample demonstrated a distinct profile with α -bisabolol dominance (up to 57.46%), while the Herbona s.r.o., Úsovsko a.s., and RAJ samples showed higher levels of α -bisabolol

oxides, α -bisabolone oxide A, and chamazulene. These variations are consistent with previous studies and known genotype- and environment-driven differences in chamomile chemotypes (Aqeel et al., 2023; Bagheri et al., 2020; Franke and Schilcher, 2005; Jeshni et al., 2017).

With regard to well-documented pharmacological activities of individual chamomile constituents (Akram et al., 2024; El Joumaa and Borjac, 2022), the compositional differences observed among samples are not merely chemical but may translate into substantial differences in therapeutic efficiency. Therefore, careful selection of both raw material and extraction parameters is essential for the development of chamomile-based formulations with predictable and reproducible biological activity. This further underscores the necessity of controlled and standardized cultivation conditions to ensure consistent phytochemical composition and reliable bioactivity of the chamomile plant matrix, as well as effective monitoring and quality control systems for undesirable contaminants (Wawroszová et al., 2025). Future research should focus on establishing robust correlations between cultivation parameters, post-harvest processing, and extraction methodology on the phytochemical profile of chamomile extracts or EOs, with the goal of ensuring both efficiency and safety of chamomile-based products.

5. Conclusion

This study presents a comprehensive evaluation of *Matricaria chamomilla* L. flower extraction strategies, distinguishing itself from previous works by three key factors: the extraction method, the particle size of the raw material, and its geographical origin. Our work provides applied insights into green extraction strategies, highlighting the superior efficiency of PHWE while simultaneously revealing critical and contradictory dependencies of the main bioactive compound extraction, such as chlorogenic acid and apig-7-glc on temperature and plant fragmentation. Specifically, we found that while the maximum total sum of individual target compounds via the PHWE method was achieved at 160 °C, maintaining extraction at 100 °C proved optimal for preserving organoleptic properties and preventing thermal degradation, representing a practical balance between efficiency and extract quality. In addition to extraction optimization, we emphasize the crucial role of raw material origin, as we demonstrated by significant phytochemical variability and distinct antioxidant activity among samples. We also confirmed that while the phytochemical composition of aqueous chamomile extracts is influenced by particle size, the chemical profile of EOs is resistant to this factor.

The obtained results offer a practical and innovative perspective for the food and pharmaceutical industries, underscoring the critical role of raw material provenance in achieving consistent phytochemical profiles of chamomile-based products, especially its EOs. These findings can also form the basis for developing protocols that maximize the yield of bioactive compounds while ensuring the high quality of the final chamomile-derived products.

CRedit authorship contribution statement

Barbora Kudláčková: Writing – original draft, review & editing, Methodology, Investigation, Formal analysis. **Pavel Karásek:** Writing – original draft, Supervision, Methodology. **Petr Misák:** Writing – original draft, Visualization, Data curation. **Helena Pluháčková:** Writing – original draft, review & editing, Resources, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of Competing Interest

The 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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jarmap.2026.100702.

Data availability

Data supporting the findings are available in the article or from the corresponding author upon request.

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