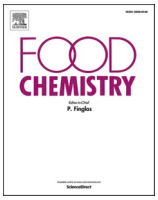
Food Chemistry 463 (2025) 141481

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Unveiling the impact of thermal water in German chamomile infusions: effects on phenolic compounds, antimicrobial and antioxidant properties

Tayse F.F. da Silveira [a,b,*,1], Noemí Echegaray [a,c,1], Rafaela Guimarães [d], André Lemos [d], Tânia C.S.P. Pires [a,b], Isabel C.F.R. Ferreira [a], Maria José Alves [a,b,d], Lillian Barros [a,b]

[a] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[b] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[c] Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia, San Cibrao das Viñas, Avd. Galicia N° 4, 32900 Ourense, Spain
[d] AquaValor - Centro de Valorização e Transferência de Tecnologia da Água – Associação, Rua Doutor Júlio Martins n°1, 5400-342 Chaves, Portugal.

ARTICLE INFO

Keywords:
natural mineral waters
functional beverage
bioactive properties
minerals
chromatography

ABSTRACT

German chamomile (GC) and thermal water (TW) are widely known for their biological properties. This study explored whether combining GC with TW could promote an improvement in the bioactivities of GC infusions compared to using drinking water (DW). DW was tested at 100 °C (GC-100DW) and TW at both 100 °C (GC-100TW) and 60 °C (GC-60TW). The use of TW for preparing infusions was associated with the reduction of the number and concentration of extracted phenolic compounds, with GC-60TW showing the lowest levels. It was also associated with a decrease in the antioxidant activity of the samples, as indicated by lower ORAC values and higher EC50 levels for TBARS. However, GC infusions prepared with TW, particularly GC-100TW, exhibited higher antibacterial and antifungal activities. These findings suggest that while TW's high mineral content affected phenolic extraction and antioxidant potential, it was associated with enhanced antimicrobial activity, partially confirming our hypothesis.

1. Introduction

German chamomile (GC) (*Matricaria recutita* L.) is recognized for its biological properties, traditionally linked to its analgesic, antibacterial, anti-inflammatory, antioxidant, and antispasmodic effects (Asadi, Ghazanfari, & Hatami, 2020; Stanojevic, Marjanovic-Balaban, Kalaba, Stanojevic, & Cvetkovic, 2016). Despite these attributes, the general public often consumes chamomile infusions not specifically for medicinal purposes but rather for their pleasant taste and the growing awareness of the beverage's positive impact on overall well-being, such as reducing the risk of certain diseases, attributed to the plant's rich phytochemical profile, which includes terpenes and phenolic compounds (Catani, Rinaldi, Tullio, Gasperi, & Savini, 2021; de Angelis et al., 2025; Mulinacci, Romani, Pinelli, Vincieri, & Prucher, 2000; Rocha, Moura, & Cunha, 2020; Sousa, Pádua, Gonçalves, Ribeiro, & Leal, 2024).

Thermal waters (TW) are natural mineral waters to which a variety of therapeutic effects have been historically ascribed (Araujo, Sarraguça, Ribeiro, & Coutinho, 2017; Silva et al., 2020), including improvements in skin health, respiratory and musculoskeletal disorders, as well as metabolic-endocrine, gynecological, gastrointestinal, nephron urinary, and circulatory conditions (Araujo et al., 2017; Quattrini, Pampaloni, & Brandi, 2016)). These effects are generally linked to the unique physicochemical composition of TW, which includes dissolved salts, minerals, and trace elements (Araujo et al., 2017; Quattrini et al., 2016; A. Silva et al., 2020).

While TW has primarily been applied in hydrotherapy and cosmetics, there has been a significant rise in its intake in recent years, a practice known as *hidropinia*. Research suggests that TW intake may provide benefits for intestinal disorders (Barnich et al., 2021), cardiovascular diseases (Pérez-Granados, Navas-Carretero, Schoppen, & Vaquero, 2010; Schoppen et al., 2004), as well as improvements in lipid profiles (Zair et al., 2013), blood pressure (Schorr, Distler, & Sharma, 1996), insulin sensitivity (Schoppen et al., 2007), and reductions in oxidative stress markers (Benedetti et al., 2009).

* Corresponding author at Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.
E-mail address: tayse.silveira@ipb.pt (T.F.F. da Silveira).
[1] These authors contributed equally to the manuscript.

https://doi.org/10.1016/j.foodchem.2024.141481
Received 17 June 2024; Received in revised form 27 September 2024; Accepted 28 September 2024
Available online 30 September 2024

In this context, using thermal waters (TW) in developing functional beverages, such as herbal infusions, represents an innovative approach to creating products that combine the beneficial properties of TW and German chamomile (GC), achieving consumers seeking innovative products that emphasize health, local traditions, and authenticity. Given that TW has a chemical composition significantly different from common potable water, it is essential to investigate its effects on the resulting infusion, both in terms of chemical composition and *in vitro* biological properties.

Hence, this research aimed to prepare GC infusions using TW from Chaves, Portugal, as a substitute for potable drinking water (DW) and to evaluate the impact of this substitution on the phenolic compound profile and the bioactivities of the resulting extract, with a particular focus on antioxidant and antimicrobial properties. By demonstrating the potential of TW in infusion production, we introduce a novel approach to the utilization of this abundant natural resource across Europe and the globe, which is paramount to promoting the valorization and local development of regions with thermal springs. Additionally, the naturally high temperature of Chaves TW makes it especially well-suited for infusion preparation and may serve as a model for similar high-temperature thermal sites worldwide. Consequently, our findings offer novel insights that could broaden the applications of TW and guide future exploration and development strategies in this area.

2. Material and methods

2.1. Reagents and samples

LC-MS grade acetonitrile was purchased from Fisher Scientific (Hampton, New Hampshire, USA). Standards of phenolic compounds were obtained from Sigma (St. Louis, Missouri, USA) (*p*-coumaric acid, chlorogenic acid, ferulic acid) or Extrasynthèse (Genay, France) (luteolin-*O*-7-glicoside, quercetin 3-*O*-glucoside, rutin). All other mentioned chemicals were purchased from scientific retailers.

Dried flowers of German chamomile (*Matricaria recutita* L.) were purchased in Chás do Mundo (pack of 250 g), Portugal, and stored under appropriate conditions before being used in the experiments.

Potable water, designated drinking water (DW), was sourced from the tap. Thermal water (TW) was obtained from the thermal spring located in Chaves, Northern Portugal. It was collected in sterile polypropylene flasks at the emerging temperature of 76 °C and brought to the laboratory in appropriate insulated food sample containers. Table 1 shows the physicochemical properties and chemical composition of the used waters.

2.2. Preparation of infusions

GC infusions were prepared according to Guimarães et al. (2013). The sample (1 g of dried flowers) was added to 200 mL of water under three conditions:

- i) boiling DW at 100 °C (GC-100DW);
- ii) boiling TW at 100 °C (GC-100TW);
- iii) TW without additional heating following source collection. This procedure aimed to replicate the infusion preparation process at the thermal place, leveraging the naturally high emerging temperature from the Chaves springs. While the infusion preparation was underway, the recorded temperature of the water was 60 °C (GC-60TW).

The plant material of GC was added to infuse, left to stand at room temperature for 5 min, and subsequently filtered through Whatman No. 4 paper (Maidstone, UK). Three infusions were independently prepared using the procedure mentioned above.

The GC infusions obtained were frozen and lyophilized (VaCo 2, Got 2000, Zirbus Technology, Bad Grund, Germany). Afterward, the lyophilized GC extracts obtained were redissolved in distilled water at the time of analysis and carried out in triplicate.

Table 1
Physicochemical characteristics of the drinking water (DW) and thermal water (TW) obtained from the Chaves spring.

Outlet temperature (°C)	DW	TW
	Room temperature	76
pH (at 20 °C)	6.6	6.8
Conductivity (at 20 °C, µS/cm)	45.0	2270.0
Alkalinity (mg $CaCO_3$/ L)	35.0	1369.0
Total mineralization (mg/ L)	-	2486.0
HCO_3^- (mg/ L)	34.0	1670.0
Cl^- (mg/ L)	< 10.0	38.0
Ca^{2+} (mg/ L)	9.8	19.0
Mg^{2+} (mg/ L)	1.1	4.9
SiO_2 (mg/ L)	-	79.0
NO_3^- (mg/ L)	< 2.0	< 0.3
Na^+ (mg/ L)	2.8	582.0
K^+ (mg/ L)	< 1.0	61.0
Li^+ (mg/ L)	< 0.1	2.4
Fe^{2+} (mg/ L)	< 0.05	0.2
Mn (mg/ L)	< 0.015	0.023
Pb (mg/ L)	< 0.003	< 0.003
Cd (mg/ L)	< 0.0005	< 0.0004
Al (mg/ L)	0.032	< 0.003
Cu (mg/ L)	< 0.020	< 0.002
Cr (mg/ L)	< 0.002	< 0.001
Ni (mg/ L)	< 0.005	< 0.005
Se (mg/ L)	< 0.0005	< 0.0004
As (mg/ L)	< 0.00059	0.049
Zn (mg/ L)	-	< 0.05

2.3. Analysis of phenolic compounds by LC-DAD-ESI-MS^n

For phenolic compounds analysis, 10 mg of extract of each infusion was reconstituted in 1 mL of distilled water (final concentration 10 mg/ mL), filtered through 0.22 µM nylon filters, and injected into the high-performance liquid chromatography (UHPLC) (Dionex Ultimate 3000 UHPLC, Thermo Scientific, San Jose, CA, USA), as described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). The compounds were chromatographically separated utilizing a Waters Spherisorb S3 ODS-2C18 column (3 µm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) maintained at a constant temperature of 35 °C. The mobile phase consisted of 0.1 % formic acid in water (A) and acetonitrile (B), with a gradient elution as follows: 0 % B for 5 min, 15% to 20% B over 5 min, 20% to 25% B over 10 min, 25% to 35% B over 10 min, and 35% to 50% B over 10 min, all at a flow rate of 0.5 mL/min. Detection was performed using a diode array detector (DAD) at wavelengths of 280 nm, 330 nm, and 370 nm and connected to a Linear Ion Trap LTQ XL mass spectrometer (Thermo Scientific) equipped with an electrospray ionization (ESI) source operating in negative mode. For MS detection, nitrogen was employed as the sheath gas at a pressure of 50 psi; the system operated with a spray voltage of 5 kV, a source temperature of 325 °C, and a capillary voltage of -20 V. The full scan spanned the mass range from *m/z* 100-1800, with a collision energy of 35 (arbitrary units). Phenolic compounds were identified by comparing their retention times, ultraviolet-visible (UV-Vis), and mass spectra with those obtained from standard compounds, when available, or with data provided in the literature. To quantify the phenolic compounds, the following calibration curves of different standard compounds were used: chlorogenic acid ($y = 312503x - 199432$, $R^2 = 0.9999$); *p*-coumaric acid ($y = 466578x + 527324$, $R^2 = 0.9987$); ferulic acid ($y = 633126x - 185462$, $R^2 = 0.9990$); luteolin-*O*-7-glicoside ($y = 13848x - 10349$, $R^2 = 0.9927$); quercetin 3-*O*-glucoside ($y = 34843x - 160173$, $R^2 = 0.9998$); and rutin ($y = 13848x - 10349$; $R^2 = 0.9939$). When the standard of a given compound was not available, the calibration curve of a chemically similar compound was used for quantification. The results are expressed as mg/g of extract.

2.4. Evaluation of antioxidant activity

The lyophilized GC infusions were dissolved in water to a final concentration of 10 mg/mL; the final solution was further diluted to different concentrations to be submitted for antioxidant activity evaluation by *in vitro* assays, namely, oxygen radical absorbance capacity (ORAC) assay, and thiobarbituric acid reactive substances (TBARS) method. These assays were chosen to evaluate the antioxidant activity of the samples due to their intrinsic experimental conditions, which include pH levels close to physiological conditions (pH 7.0), lipid substrates derived from biological tissues (porcine brain), and free radicals commonly found in vivo (peroxyl radicals). This approach aims to provide a more accurate approximation of real-world oxidative stress scenarios (Ghani, Barril, Bedgood, & Prenzler, 2017; Prior, Wu, & Schaich, 2005).

2.4.1. Oxygen radical absorbance capacity (ORAC) assay

The capacity of the extracts to scavenge peroxyl radicals was assessed using ORAC following the protocol indicated by (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). The reaction was performed in 75 mM potassium phosphate buffer (pH 7.4) for a final volume of 200 μL, employing fluorescein as a fluorescent probe. Briefly, 20 μL of the sample was mixed with 120 μL fluorescein (0.4 μg/mL) and 60 μL of radical 2.2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH, 108 mg/mL) in microplates. The mixture was immediately placed in a microplate reader FLUOstar Omega (BMG LABTECH, Ortenberg, Germany), and the fluorescence was recorded every 1 min for a total of 80 min (emission wavelength of 485 nm and excitation wavelength of 520 nm) under controlled temperature at 37 °C. Thermal water was included alongside the samples as a control and exhibited negligible antioxidant activity, consistent with previous findings (Pinto-Ribeiro et al., 2024). Eight calibration solutions, ranging from 244 to 847 μM Trolox standard, were included in each assay. The potassium phosphate buffer was employed as blank. ORAC results were calculated using a regression equation correlating Trolox concentrations with the net area under the curve of fluorescein kinetic decay. The results are expressed as μmol Trolox Equivalent (TE)/g of extract.

2.4.2. Evaluation of the inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

The inhibition of lipid peroxidation was examined employing porcine (*Sus scrofa*) brain homogenates by measuring the reduction in thiobarbituric acid reactive substances (TBARS) as described in Pinela et al. (2012). Briefly, an aliquot of 100 μL of the supernatant obtained after centrifuging (3000 g for 10 min) pig brain tissue homogenized in cold Tris-HCl buffer (20 mM, pH 7.4) was incubated with 200 μL of the different concentrations of extract solutions (ranging between 10-0.078125 mg/mL) in the presence of $FeSO_4$ (10 μM; 100 μL) and ascorbic acid (0.1 mM; 100 μL) at 37 °C for 60 min. The reaction was terminated by adding 500 μL of trichloroacetic acid (TCA, 28% w/v). Subsequently, 380 μL of thiobarbituric acid (TBA, 2% w/v) was added, and then the mixture was heated at 80 °C for 20 min. Following centrifugation at 3000 x g for 10 min to eliminate precipitated proteins, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was gauged based on its absorbance at 532 nm. Thermal water was included alongside the samples as a control and exhibited negligible antioxidant activity, consistent with previous findings (Pinto-Ribeiro et al., 2024). The inhibition ratio (%) was determined using the ensuing formula: [(A - B)/ A] × 100, where A was the absorbance of the control, and C was the absorbance of the solution containing the sample at 532 nm. The outcomes are expressed as the EC_{50} value (the effective concentration of the sample providing 50% antioxidant activity), with Trolox serving as a positive control (EC_{50} = 5.4 μg/mL).

2.5. Evaluation of antimicrobial activity

2.5.1. Antibacterial potential

The microorganisms utilized to screen the antibacterial activities were a comprehensive panel of bacteria, including clinical strains (obtained from patients hospitalized in various departments at the Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal) and common foodborne pathogens (ATCC). The clinical strains comprised five Gram-negative bacteria (*Escherichia coli* (VRU12881), *Klebsiella pneumoniae* (VRI17214), *Morganella morganii* (VRU14272), *Proteus mirabilis* (VRU17684), and *Pseudomonas aeruginosa* (VRU14123)) and three Gram-positive bacteria (*Enterococcus faecalis* (VRU14123), *Listeria monocytogenes* (VRU17684), and methicillin-resistant *Staphylococcus aureus* (MRSA) (VRI17654)). Additionally, the foodborne pathogens included five Gram-negative species (*Enterobacter cloacae* (ATCC 49741), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* (ATCC 13076), and *Yersinia enterocolitica* (ATCC 8610)), as well as three Gram-positive species (*Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19111), and *Staphylococcus aureus* (ATCC 25923)). They were incubated at 37 °C in a suitable fresh medium for 24 h before analysis to maintain the exponential growth phase. The minimum inhibitory concentration (MIC) was determined in all bacteria using a colorimetric assay according to the method described by Pires et al. (2018). First, the lyophilized GC infusion samples were dissolved in 5% (v/ v) dimethyl sulfoxide (DMSO)/ Tryptic Soy Broth (TSB) to achieve a final concentration of 20 mg/mL. Subsequently, the solution acquired was serially diluted to obtain concentration ranges from 0.15 to 10 mg/mL. Then, 100 μL of the first concentration was added to a well (96-well microplate) containing 90 μL of Tryptic Soy Broth (TSB) and diluted until the final concentration was 0.07 mg/mL. After, 10 μL of bacteria inoculum (standardized at 1.5×10^6 colony forming units (CFU)/mL) was added at all wells. The microplates were sealed with a sterile cover, agitated on a plate shaker to mix their contents, and then incubated at 37 °C for 24 h. The MIC of samples was determined by adding 40 μL of 0.2 mg/mL *p*-iodonitrotetrazolium chloride (INT) and incubating at 37 °C for 30 min (Kuete et al., 2011). Viable microorganisms reduced the yellow dye to a pink color. Simultaneously, three negative controls were set up: one containing TSB, another with the sample extract, and the third with medium and antibiotic. A positive control (Ampicillin, Imipenem, Streptomycin, and Vancomycin) was also prepared using MHB and each bacteria inoculum. The MIC was calculated as the sample's lowest concentration (mg/mL), which inhibits the growth of visible bacteria (color change from yellow dye to pink). To determine the minimum bactericidal concentration (MBC), 40 μL of liquid from each well that exhibited no color change was plated on a solid medium and then incubated at 37 °C for 24 h. The lowest concentration that showed no bacterial growth after this subculturing was identified as the MBC (minimum bactericidal concentration), defined as the minimum concentration needed to kill bacteria.

2.5.2. Antifungal potential

Aspergillus brasiliensis (ATCC 16404) and *Aspergillus fumigatus* (ATCC 204305) were utilized for the antifungal bioassays. These organisms were purchased at Frilabo, Porto, Portugal. Specifically, the antifungal activity was assessed using the microdilution method following the protocol described by Heleno et al. (2013) and employing a commercial fungicide (Ketoconazole) as positive control. Both the MIC and the Minimum Fungicidal Concentration (MFC) were determined. The MFC represents the lowest concentration at which no visible growth was observed, indicating a 99.5% reduction in the original inoculum, and is expressed in mg/mL.

2.6. Statistical analysis

All the assays were carried out in triplicate using three distinct infusion samples. The results are presented as mean values with

associated standard deviations (SD), and the standard error of the mean (SEM) was calculated for all three GC infusions. The impact of infusion conditions, specifically the use of TW at different temperatures, on the bioactive properties and the concentration of extracted phenolic compounds in GC infusions, were examined using a one-way analysis of variance (ANOVA). Significance levels are indicated as $p < 0.05$, $p < 0.01$, and $p < 0.001$. Subsequently, Tukey's statistically significant difference post hoc test was conducted at a significance level of 0.05. These statistical analyses were performed using SPSS v. 25.0 software (IBM SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Phenolic compounds by LC-DAD-ESI-MS^n

Thirty-nine distinct phenolic compounds were tentatively identified based on their chromatographic behavior, UV-Vis spectra, and fragmentation pattern compared with literature data (Table 2) (Caleja et al., 2015; Guimarães et al., 2013). Specifically, 10 hydroxycinnamic acids and derivatives were identified (peaks 1-4, 7, 9, 10, 16, 18, and 28). Moreover, 14 flavonol derivatives (glycosylated, acetylated) and 12 flavone derivatives (alone, glycosylated, and/or acetylated) were also found in this study (peaks 5, 6, 8, 11-14ab, 17b, 19, 21, 24, 26, and 27; and peaks 15b, 20, 22, 23, 25, 29, 30, 31bc, and 32-34, respectively). All of the compounds identified in this study have previously been reported by other authors, including different parts of the GC plant (Caleja et al., 2015; Cvetanović et al., 2019; Guimarães et al., 2013; Mailänder et al., 2022; Šibul, Orčić, Berežni, Anačkov, & Mimica-Dukić, 2020; Tsivelika, Irakli, Mavromatis, Chatzopoulou, & Karioti, 2021). However, unlike other studies, phenolic compounds such as hydroxybenzoic acids (ellagic, *p*-hydroxybenzoic, and protocatechuic acid) (Caleja et al., 2015; Cvetanović et al., 2019) and other flavonols such as morin (Šibul et al., 2020) and kaempferol (Dai et al., 2023; Nováková, Vildová, Mateus, Gonalves, & Solich, 2010; Šibul et al., 2020) were not detected in any of the GC infusions investigated. These absences could be due to the different extraction methodologies, and the diversity in extraction conditions (solvent, extraction time, temperature, etc.) has been shown to result in different extracted compounds (Cvetanović et al., 2019; Mailänder et al., 2022). Furthermore, using other parts of GC (flower heads, leafy flowering stems, and roots) and even different sources of

Table 2
Retention time (Rt), wavelengths of maximum absorption in the UV-Vis region (λ_{max}), mass spectral data, and tentative identification of the phenolic compounds of German Chamomile extracts prepared with drinking water and thermal water at different temperature (60 °C and 100 °C).

Peak number	Rt (min)	λ_{max} (nm)	[M−H]-	MS^2	MS^3	MS^4	Tentative identification
1	6.78	324	353	173, 179, 191, 135			4-*O*-caffeoylquinic acid
2	7.30	297, 326	353				Chlorogenic acid
3	8.78	288, 304	355	193, 149			Ferulic acid hexoside
4	10.51	-	713	550	370, 388, 532, 325, 300, 193	193, 149	Ferulic acid derivative
5	11.36	-	639	477, 314, 315, 163			Isorhamnetin-*O*-dihexoside
6	12.02	-	669	507	345, 387, 329	330, 331	Syringetin-*O*-dihexoside
7	13.74	325	367	191, 173, 193			3-*p*-Coumaroylquinic acid
8	15.02	297, 327	479	317			Quercetagin-*O*-hexoside
9	15.02	296, 320	367	173			4-Feruloylquinic acid
10	15.34	298, 319	355	193	149		Ferulic acid hexoside
11	15.87	-	681	477, 519	314, 315, 357, 445		Isorhamnetin-*O*-acetyldihexoside
12	17.98	347	609	301	151, 179		Rutin
13	18.22	255, 330	593	285			Luteolin-*O*-rutinoside
14a	19.01	257,361	493	331	316, 317		Patuletin-O-hexoside
14b	19.01	-	681	315, 621, 477, 501	300		Isorhamnetin-*O*-acetyldihexoside
15a	19.27	-	477	315, 316, 433	153		Unknown 1
15b	19.27	-	447	285			Luteolin-*O*-hexoside
16	19.35	327	515	353	173, 179, 191		3,4-Dicaffeoylquinic acid
17a	20.19	275, 350	711	531, 549	369, 433, 532	271	Unknown 2
17b	20.19	-	331	316, 317			Patuletin
18	20.45	284, 323	193	149			Ferulic acid
19	20.52	-	505	301, 463	179, 151		Quercetin-*O*-acetylhexoside
20	21.81	330	577	269			Apigenin-*O*-rutinoside
21	22.72	255, 361	505	301			Quercetin-*O*-acetylhexoside
22	23.17	239, 267sh, 339,	607	299, 300	284		Chrysoeriol-*O*-rutinoside
23	23.51	267, 284sh, 330	431	269			Apigenin-*O*-hexoside
24	23.88	260, 273, 348	535	331	316		Patuletin-*O*-acetylhexoside
25	24.13	255, 265sh, 347	489	285			Luteolin-*O*-acetylhexoside
26	24.93	330	507	345, 387	330	302, 303, 287	Syringetin-*O*-hexoside
27	26.24	254, 264sh, 295, 347	519	315, 357	300		Isorhamnetin-*O*-acetylhexoside
28	26.51	321	517	323, 341, 355, 281, 251, 221, 193, 179, 161			Caffeoyl-ferulic acid hexoside
29	27.30	267, 330	473	268, 269, 413			Apigenin-*O*-acetylhexoside
30	28.43	267, 337	473	268, 269, 414			Apigenin-*O*-acetylhexoside
31a	29.36	252, 267sh, 344	503	488, 299	283, 313, 255	255	Unknown 3
31b	29.36	252, 267, 339	299	284, 285			Chrysoeriol
31c	29.36	252, 269sh, 338	531	285, 471	241		Luteolin-*O*-diacetylhexoside
32	33.04	267, 336	515	269, 455,431			Apigenin-*O*-diacetylhexoside
33	33.70	267, 336	515	269, 455, 432			Apigenin-*O*-diacetylhexoside
34	36.04	267, 337	515	269, 455, 433			Apigenin-*O*-diacetylhexoside

GC-100DW: German chamomile infusion with boiling (100 °C) drinking water; **GC-60TW**: German chamomile infusion with thermal water at 60 °C; **GC-100TW:** German chamomile infusion with boiling (100 °C) thermal water.

plant material could magnify the differences in extracted compounds (Piri et al., 2019; Tsivelika et al., 2021).

The quantification of the detected phenolic compounds is presented in Table 3. Ferulic acid hexoside, chlorogenic acid, and apigenin-*O*-acetylhexoside were the primary compounds in GC-100DW, agreeing with previous findings (Avula et al., 2014; de Angelis et al., 2025). However, depending on the infusion conditions employed, variable numbers and concentrations of individual compounds were recorded (Figure 1). Specifically, 39 compounds were found in GC-100DW, while in infusions made with TW, these were reduced to 25 and 27 for GC-60TW and GC-100TW, respectively.

Regarding hydroxycinnamic acid derivatives, out of 10 compounds identified in GC-100DW, only five were identified in GC-60TW, and six were identified in GC-100TW. Similarly, the diversity of determined flavonols in GC-100DW was reduced from 14 compounds to 8 for GC-60TW and 9 for GC-100TW. In contrast, the flavones identified in GC-100DW (12 distinct flavones) were reduced to 11 for both TW infusions. Additionally, two of the detected compounds, which have yet to be tentatively identified, only one (namely, unknown compound 3) was detected in TW infusions.

The concentrations of all compounds detected in the three infusions were ($p < 0.01$) affected using TW (***Table 3***), significantly reducing them ($p < 0.01$), except for 4-feruloylquinic acid, luteolin-*O*-hexoside, and apigenin-*O*-hexoside, which were found in significantly higher ($p < 0.01$) concentrations in GC-100TW. In particular, the concentrations of the predominant phenolic acids chlorogenic acid, and ferulic acid hexoside were significantly impacted. Ferulic acid hexoside levels were reduced by more than 40-fold in GC-60TW and GC-100TW compared to GC-100DW, while chlorogenic acid was undetectable in these samples. The primary flavonoid, apigenin-*O*-acetylhexoside, also showed a notable decrease, with levels approximately 60% and 20% lower in GC-60TW and GC-100TW, respectively, compared to GC-100DW ($p < 0.05$). Furthermore, several flavonoids, including patuletin derivatives, luteolin-*O*-acetylhexoside, rutin, and quercetin-*O*-acetylhexoside, were no longer detectable in GC-60TW and GC-100TW. These results underscore the significant impact of TW on the extraction efficiency of bioactive compounds during infusion preparation. Consequently, the total levels of phenolic compounds were much higher in GC-100DW (29.32 mg/g) than in GC-60TW and GC-100TW (6.51 and 9.40 mg/g, respectively).

The decrease in the extraction yield of phenolic compounds with TW

Table 3
Quantification of phenolic compounds in German Chamomile extracts prepared with drinking water and thermal water at different temperature (60 °C and 100 °C). The results are presented as mean ± standard deviation (mg/g extract).

Peak	Rt (min)	Tentatively identified compound	GC-100DW	GC-60TW	GC-100TW	SEM	Sig.
1	6.78	4-Caffeoylquinic acid	0.97 ± 0.01	n.d.	n.d.	0.205	-
2	7.30	Chlorogenic acid	4.75 ± 0.11	n.d.	n.d.	1.002	-
3	8.78	Ferulic acid hexoside I	5.32 ± 0.11 [b]	0.08 ± 0.00 [a]	0.13 ± 0.00 [a]	1.099	***
4	10.51	Ferulic acid derivative	0.09 ± 0.00 [b]	n.d.	0.07 ± 0.00 [a]	0.017	***
5	11.36	Isorhamnetin-*O*-dihexoside	0.52 ± 0.00 [c]	0.47 ± 0.00 [a]	0.50 ± 0.00 [b]	0.010	***
6	12.02	Syringetin-*O*-dihexoside	0.50 ± 0.00 [c]	0.46 ± 0.00 [a]	0.47 ± 0.00 [b]	0.006	***
7	13.74	3-*p*-Coumaroylquinic acid	0.07 ± 0.00	n.d.	n.d.	0.015	-
8	15.02	Quercetagin-*O*-hexoside	0.56 ± 0.00	n.d	n.d	-	-
9	15.02	4-Feruloylquinic acid	0.09 ± 0.00	n.d	n.d	-	-
10	15.34	Ferulic acid hexoside II	4.91 ± 0.09 [b]	0.06 ± 0.00 [a]	0.12 ± 0.01 [a]	1.017	***
11	15.87	Isorhamnetin-*O*-acetyldihexoside	0.51 ± 0.00 [c]	0.47 ± 0.00 [a]	0.49 ± 0.00 [b]	0.007	***
12	17.98	Rutin	0.14 ± 0.01	n.d.	n.d.	0.029	-
13	18.22	Luteolin-*O*-rutinoside	0.20 ± 0.01 [c]	0.09 ± 0.00 [a]	0.15 ± 0.00 [b]	0.020	***
14a	19.01	Patuletin-O-hexoside[1]	0.63 ± 0.01	n.d.	n.d.	0.132	-
14b	19.01	Isorhamnetin-*O*-acetyldihexoside [1]	0.63 ± 0.01	n.d.	n.d.	0.132	-
15a	19.27	Unknown 1 [2]	0.20 ± 0.01	n.d.	n.d.	0.042	-
15b	19.27	Luteolin-*O*-hexoside [2]	0.20 ± 0.01 [a]	0.18 ± 0.01 [a]	0.63 ± 0.06 [b]	0.092	**
16	19.35	3,4-Dicaffeoylquinic acid	0.44 ± 0.05 [c]	0.10 ± 0.01 [a]	0.27 ± 0.02 [b]	0.063	**
17a	20.19	Unknown 2[3]	0.66 ± 0.02	n.d.	n.d.	0.139	-
17b	20.19	Patuletin[3]	0.66 ± 0.02	n.d.	n.d.	0.139	-
18	20.45	Ferulic acid	0.09 ± 0.00	n.d.	n.d.	0.018	-
19	20.52	Quercetin-*O*-acetylhexoside	0.49 ± 0.00 [c]	0.46 ± 0.00 [a]	0.47 ± 0.00 [b]	0.006	***
20	21.81	Apigenin-*O*-rutinoside	0.23 ± 0.01 [c]	0.11 ± 0.00 [a]	0.14 ± 0.01 [b]	0.022	***
21	22.72	Quercetin-*O*-acetylhexoside	0.55 ± 0.01	n.d.	n.d.	0.116	-
22	23.17	Chrysoeriol-*O*-rutinoside	0.16 ± 0.00 [c]	0.09 ± 0.00 [a]	0.14 ± 0.00 [b]	0.014	***
23	23.51	Apigenin-*O*-hexoside	0.31 ± 0.00 [a]	0.52 ± 0.01 [b]	1.16 ± 0.01 [c]	0.162	***
24	23.88	Patuletin-*O*-acetylhexoside	0.56 ± 0.02	n.d.	n.d.	0.117	-
25	24.13	Luteolin-*O*-acetylhexoside	0.14 ± 0.00	n.d.	n.d.	0.030	-
26	24.93	Syringetin-*O*-hexoside	0.74 ± 0.00 [c]	0.48 ± 0.00 [a]	0.56 ± 0.00 [b]	0.050	***
27	26.24	Isorhamnetin-*O*-acetylhexoside	0.63 ± 0.00 [b]	0.47 ± 0.00 [a]	0.47 ± 0.00 [a]	0.033	***
28	26.51	Caffeoyl-ferulic acid hexoside	0.31 ± 0.00 [c]	0.04 ± 0.00 [a]	0.08 ± 0.01 [b]	0.052	***
29	27.30	Apigenin-*O*-acetylhexoside	0.19 ± 0.01 [c]	0.10 ± 0.00 [a]	0.14 ± 0.00 [b]	0.017	***
30	28.43	Apigenin-*O*-acetylhexoside	1.96 ± 0.03 [c]	0.67 ± 0.02 [a]	1.57 ± 0.02 [b]	0.242	***
31a	29.36	Unknown 3 [4]	0.48 ± 0.01 [c]	0.11 ± 0.00 [a]	0.22 ± 0.00 [b]	0.070	***
31b	29.36	Chrysoeriol [4]	0.48 ± 0.01 [c]	0.11 ± 0.00 [a]	0.22 ± 0.00 [b]	0.070	***
31c	29.36	Luteolin-*O*-diacetylhexoside [4]	0.48 ± 0.01 [c]	0.11 ± 0.00 [a]	0.22 ± 0.00 [b]	0.088	***
32	33.04	Apigenin-*O*-diacetylhexoside	0.75 ± 0.01 [b]	0.11 ± 0.01 [a]	0.12 ± 0.00 [a]	0.133	***
33	33.70	Apigenin-*O*-diacetylhexoside	0.77 ± 0.01 [b]	0.17 ± 0.01 [a]	0.18 ± 0.01 [a]	0.125	***
34	36.04	Apigenin-*O*-diacetylhexoside	0.42 ± 0.00 [b]	0.11 ± 0.00 [a]	0.11 ± 0.01 [a]	0.064	***
		Total phenolic acids	17.04 ± 0.03 [c]	1.1 ± 0.04 [a]	1.97 ± 0.02 [b]	3.273	***
		Total flavonoid	12.27 ± 0.08 [c]	5.41 ± 0.01 [a]	7.44 ± 0.04 [b]	1.288	***
		Total phenolic compounds	29.32 ± 0.05 [c]	6.51 ± 0.05 [a]	9.40 ± 0.06 [b]	4.535	***

In each line, different letters ([a-c]) indicate significant ($p < 0.05$) differences between samples by a Tukey HSD test. **Sig:** Significance, (* ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$); n.s.: no significant difference); **GC-100DW:** German chamomile infusion with boiling (100 °C) drinking water; **GC-60TW**: German chamomile infusion with thermal water at 60 °C; **GC-100TW:** German chamomile infusion with boiling (100 °C) thermal water; **SEM:** Standard error of the mean **n.d.:** Not detected; **tr.:** Traces. [1] The concentration displayed for patuletin-*O*-hexoside and isorhamnetin-*O*-acetyldihexoside is the sum of both in GC-100DW; [2] The concentration displayed for unknown 1 and luteolin-*O*-hexoside is the sum of both in GC-100DW; [3] The concentration displayed for unknown 2 and patuletin is the sum of both in GC-100DW; [4] The concentration displayed for unknown 3, chrysoeriol, and luteolin-*O*-diacetylhexoside is the sum of them in GC-100DW, GC-60TW, and GC-100TW.

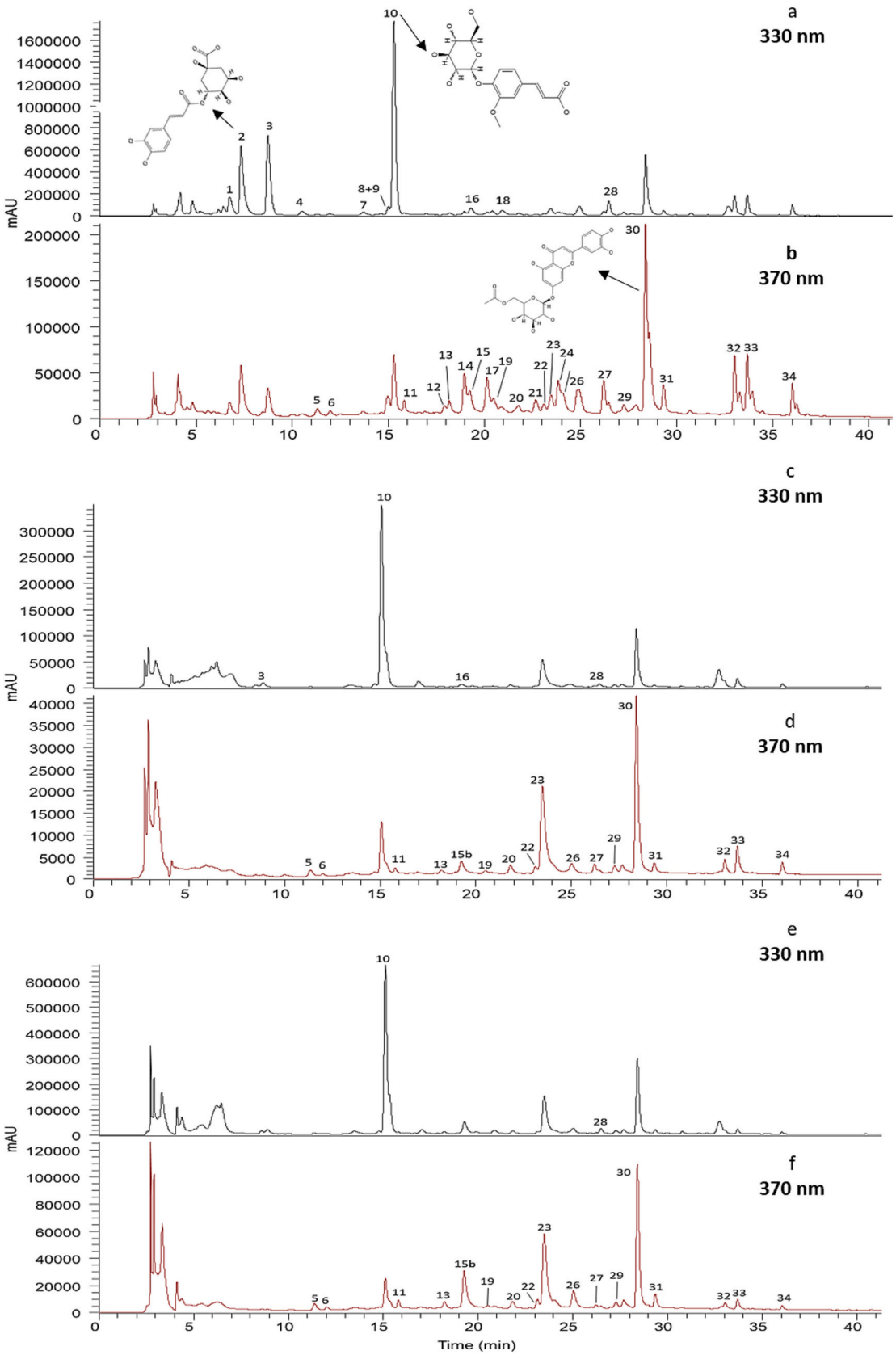


Fig.1. Representative chromatograms of the German chamomile infusions prepared with drinking water (GC-100DW, a and b); thermal water at 60 °C (GC-60TW, c, and d); thermal water at 100 °C (GC-100TW, e, and f); and proposed chemical structures of the predominant compounds in the samples. For peak identities, see Table 2.

could be attributed to the high presence of salts, minerals, and metals in this type of water compared to DW (Table 1). These results are consistent with those obtained by Wyrostek and Kowalski (2021), who observed how highly mineralized waters decreased the extracted phenolic compounds in various plant matrices, including GC, as well as *Camellia sinensis* L., *Lavandula angustifolia* L., *Mentha piperita* L., and *Salvia officinalis* L. The presence of Ca^{2+} and Fe^{2+} ions in the water may be one of the factors that contributed to the decrease in the extraction of phenolic compounds because these ions can react with the pectin found in the cell walls of plants, such as GC. According to Mierczyńska, Cybulska, Sołowiej, and Zdunek (2015), this interaction forms a type of film with high viscosity and gelling properties, which could hinder phenolic compounds from the cell from releasing into the infusion water. Thus, the higher content of Ca^{2+} and Fe^{2+} ions present in the TW of Chaves (19 *vs.* 9.8 mg/L and 0.17 *vs.* <0.05 mg/L, respectively) could hinder the extraction of phenolic compounds from the GC.

In addition to Ca^{2+} and Fe^{2+} ions, the effectiveness of extraction of phenolic compounds can be adversely impacted by other ions like Mg^{2+}, salts, and minerals. It has been suggested that these solutes can interact with water molecules through various electrostatic interactions, which could change their capacity to solubilize the phenolic compounds present in GC. As a result, the binding sites between water and the functional groups of phenolic compounds (such as hydroxyl groups) may be affected, thus limiting the solubility of these bioactive compounds and reducing their extraction compared to waters with lower solute content, such as DW. In a study developed by Xu et al. (2017), the authors also observed that the extraction yield of catechins in various tea infusions (green, oolong, and black) was affected when different matrices of water were used (namely, mineral water, mountain spring water, purified water, and tap water) since the concentration of solutes present in these matrices ranges. Once again, the higher mineral content in the water negatively impacted the extraction of phenolic compounds, in this case, catechins.

On the other hand, the temperature at which TW was used barely affected the profile of extracted phenolic compounds, as, except for the ferulic acid derivative and rutin, which were only found in GC-100TW, the GC-60TW and GC-100TW infusions extracted the same phenolic compounds (Table 3). However, the quantity obtained of these compounds was indeed influenced by the temperature. Specifically, GC-100TW reported higher concentrations for all detected compounds, significantly differing from the other samples ($p < 0.001$) in most compounds. These results are consistent with the literature, where generally, mild temperatures (below 80 °C) tend to result in lower extraction of phenolic compounds from GC (Cvetanović et al., 2019; Harbourne, Jacquier, & O'Riordan, 2009).

3.2. Antioxidant activity

Over the past years, there has been an effort of the scientific community to evaluate antioxidant activity using biologically relevant methods. This includes using cell-based assays, reaction media and substrates or free radicals physiologically significant (Granato, 2023). Based on this, we assessed the antioxidant activity of GC extracts using ORAC and TBARS assays. The results are shown in Figure 2. All samples demonstrated antioxidant activity through these methods. The TBARS assay evaluates the antioxidant activity of compounds or extracts by measuring their ability to counteract the oxidation of a lipid substrate (porcine brain homogenates) (Ghani et al., 2017). On the other hand, the ORAC assay assesses the inhibition of oxidation induced by peroxyl radicals and is primarily a hydrogen atom transfer-based method (Prior et al., 2005). Although the TBARS assay also involves the neutralization of peroxyl radicals, it may include other radicals involved in lipid oxidation, such as alkyl and alkoxyl radicals, indicating that GC extracts may counteract lipid oxidation through multiple pathways. Lipid oxidation in biological membranes (e.g., unsaturated fatty acids, phospholipids) is associated with the development of chronic diseases, such as atherosclerosis, highlighting an essential mechanism by which antioxidants may exert protective effects in the body.

GC-100DW exhibited significantly higher antioxidant activity compared to GC-60TW and GC-100TW in both antioxidant tests as it obtained the highest ($p < 0.001$) values in the ORAC assay (1246.34 vs. 372.25 and 924.45 μmol TE/g, respectively) and lower ($p < 0.001$) EC_{50} value (0.05 μg/mL vs. 0.48 μg/mL for GC-60TW and 0.49 μg/mL for GC-100TW). These findings suggest a superior ability of GC-100DW to neutralize lipid oxidation-related free radicals.

Phenolic compounds are well known for their ability to neutralize radicals by donating electrons or hydrogen atoms (Prior et al., 2005). Therefore, the reduction in antioxidant activity observed in the TW-based samples may be associated with the decrease in the total concentration of phenolic compounds (Table 3). Moreover, the number of phenolic compounds extracted varied across different infusions, decreasing from 39 compounds in GC-100DW to 25 in GC-60TW and 27 in GC-100TW (Table 3). Flavonoids, such as patuletin and its derivatives (peaks 17, 19, 24), rutin, and luteolin-O-acetylhexoside, were no longer detectable in GC-60TW and GC-100TW. Additionally, the concentrations of the predominant compounds, chlorogenic acid and ferulic acid hexoside isomers, were significantly reduced in the TW-based infusions. These alterations likely contributed to the observed decrease in

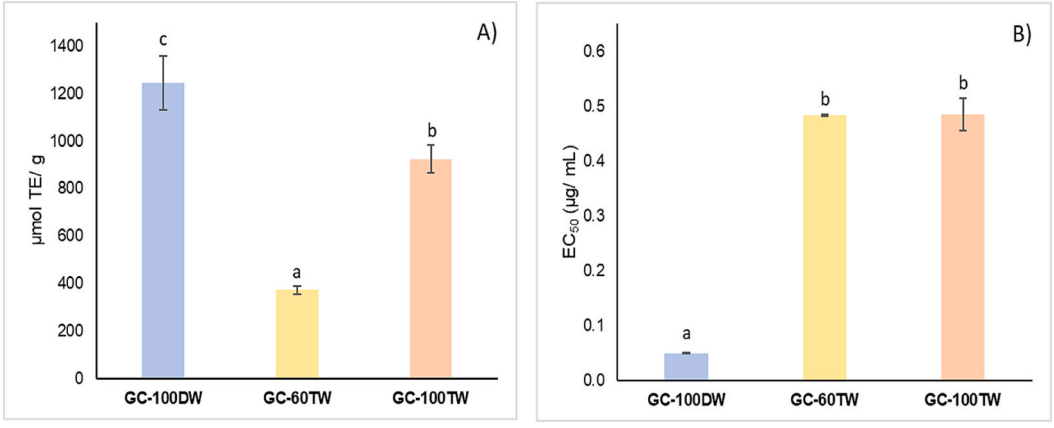


Fig.2. Antioxidant activity (A) ORAC assay, and B) TBARS EC_{50} value) evaluated in German Chamomile extracts prepared with drinking water and thermal water at different temperature (60 °C and 100 °C) (mean ± standard error). Different letters ($^{a\text{-}c}$) indicate significant ($p < 0.05$) differences between samples by a Tukey HSD test. Influence by infusion water: *** ($p < 0.001$). **ORAC:** Oxygen radical absorbance capacity; **TE:** Trolox equivalents; **TBARS:** Thiobarbituric acid reactive substances; EC50 values correspond to the sample concentration achieving 50% of antioxidant activity. **GC-100DW**: German chamomile infusion with boiling (100 °C) drinking water; **GC-60TW:** German chamomile infusion with thermal water at 60 °C; **GC-100TW**: German chamomile infusion with boiling (100 °C) thermal water.

antioxidant activity, underscoring the critical role of these phenolic compounds in the antioxidant potential measured by the ORAC and TBARS assays.

The decrease in antioxidant capacity using TW infusions observed in this study aligns with the results obtained by Wyrostek and Kowalski (2021), who observed that the preparation of GC infusions and other plant matrices infusions with high mineral content waters consistently decreased antioxidant capacity compared to deionized water. Likewise, Danrong, Yuqiong, and Dejiang (2009) noted a comparable trend in preparing infusions from various types of tea, attributing the reduction in antioxidant capacity to the increased solute content in the water.

Overall, the data suggest that using TW to prepare GC infusions may adversely affect the extraction of bioactive compounds and reduce *in vitro* antioxidant activity. However, further research is required to determine whether this observed reduction translates into diminished biological activity *in vivo*. In this context, it is crucial to assess the bioaccessibility and bioavailability of GC infusions prepared with DW or TW and to evaluate the biological activity of these fractions and their metabolites.

3.3. Antimicrobial activity

Due to their importance to public health, a panel of eight clinical and eight foodborne pathogens (Table 4) was used to test the antibacterial activity of GC extracts. GC-100TW extract was associated with a higher antibacterial action than GC-100DW and CG-60TW extracts, evidenced by their lower MIC values. The CG-100TW extract showed MIC values $\leq$ 10 mg/mL for every tested bacterial strain except *Enterococcus faecalis*. Conversely, GC-60TW samples exhibited MIC values greater than 10 mg/mL for *Morganella morganii*, *Proteus mirabilis*, MRSA, *Bacillus cereus,* and *Enterococcus faecalis*. GC-60TW generally demonstrated lower MIC values for all microorganisms than GC-100DW extracts. Regarding MBC, only GC-100TW extract showed values $\leq$ 10 mg/mL for the microorganisms *Morganella morganii*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Yersinia enterocolitica*, suggesting that only infusions with boiled TW showed any apparent bactericidal capacity against the indicated bacteria at the tested concentrations.

The results demonstrated higher overall effectiveness of all the extracts against the two fungi evaluated (Table 4), consistent with previous research indicating that aqueous extracts of chamomile may be more effective against molds and yeast than bacteria (Al-Ismail & Talal Aburjai, 2003; Petronilho, Maraschin, Coimbra, & Rocha, 2012; Romero et al., 2005). Furthermore, aligning with the findings for antibacterial properties, the antifungal activity was enhanced in infusions prepared with thermal water (TW). Specifically, the GC-100TW extract exhibited the lowest minimum inhibitory concentration (MIC) values, ranging from 0.6 to 2.5 mg/mL for *Aspergillus brasiliensis* and *Aspergillus fumigatus*, respectively, and the lowest minimum fungicidal concentration (MFC) value (10 mg/mL for both fungi).

The antimicrobial properties of chamomile have traditionally been attributed to its essential oil, primarily due to its terpene content (Roby, Sarhan, Selim, & Khalel, 2013; N. C. C. Silva, Barbosa, Seito, & Fernandes Junior, 2012). However, crude chamomile extracts have also demonstrated both antibacterial and antifungal activities against *S. aureus*, *B. cereus*, *P. aeruginosa*, *Aspergillus flavus*, and *Candida albicans*, as reported by (Roby et al., 2013; A. Silva et al., 2020; Solidônio et al., 2015). Phenolic compounds are recognized as key contributors to the antimicrobial properties of chamomile crude extracts (Roby et al., 2013; A. Silva et al., 2020; Takó et al., 2020). Nevertheless, our data revealed that samples with the highest number and concentration of phenolic compounds (GC-100DW, Tables 2 and 3) were less effective against the studied microorganisms compared to samples prepared with TW. This suggests that components other than phenolics may contribute to the enhanced antimicrobial activity observed in TW samples.

One possible explanation for this observation is that TW may facilitate the extraction of specific compounds, not identified in this study, that exhibit strong antimicrobial activity. Additionally, TW itself has been reported to possess antimicrobial properties. For example, (Giampaoli et al., 2013) documented the bactericidal capacity of Italian TW against *S. aureus*, *E. coli*, and *E. faecalis*. Similarly, (Oliveira et al., 2020) found significant antimicrobial activity of TW against *S. aureus*, *E. coli*, *C. albicans*, and skin-associated strains such as *S. epidermidis* and *Cutibacterium acnes*. Research has demonstrated that the chemical composition of TW is intrinsically linked to its antimicrobial properties, with sulfur compounds and the ionic composition of TW being primarily responsible for this bioactivity (Giampaoli et al., 2013; Oliveira et al., 2020). Consequently, it is suggested that the elements in TW significantly contributed to the observed bioactivity, potentially surpassing the role of secondary metabolites typically associated with the antimicrobial activity of chamomile extracts. Supporting this hypothesis, previous studies (Pinto-Ribeiro et al., 2024) have shown that TW from Chaves is rich in sulfur, known for its antimicrobial properties. Indeed, earlier screening studies on TW alone (data not shown) revealed significant antimicrobial activity against the microorganisms tested in this study.

On the other hand, it is worth noting that none of the GC infusions surpassed the antimicrobial effect of the antibiotics used as positive controls, irrespective of the water utilized in the infusion process (except for GC-100TW, which obtained lower MIC and MBC values than Ampicillin for the clinical bacteria strains *Morganella morganii* and *Pseudomonas aeruginosa*). These findings contrast with those described previously in the literature, where GC extracts were found to have similar (Alkuraishy, Al-Gareeb, Albuhadilly, & Alwindy, 2015) or even superior (Caleja et al., 2015; Mahdavi, Ghorat, Nasrollahzadeh, Hosseyni-Tabar, & Rezaei-Seresht, 2020) antimicrobial activities compared to various commonly used antibiotics. These variations may be explained by the fact that water (DW or TW), rather than ethanol or other organic solvents, was used in the infusions prepared in this work. According to earlier research, chamomile extracts with different solvents exhibit different levels of antibacterial activity (Petronilho et al., 2012 ref). Additionally, more potent infusions, such as decoctions, may account for the differences in the obtained antimicrobial values (Caleja et al., 2015).

4. Conclusions

The impact of TW on the preparation of GC infusions was investigated for the first time, focusing on the concentration of extracted phenolic compounds and the bioactive properties of GC infusions. The results revealed a notable alteration in the phenolic profile of GC infusions when TW was used, resulting in a decrease in the number of phenols detected and a reduction in their concentration compared to conventional DW. This underscores the influence of TW on phenolic extraction from GC, particularly pronounced at lower temperatures (60 °C vs. 100 °C). This decrease in the extraction of phenolic compounds due to the use of TW was accompanied by a worsening in the antioxidant properties of GC infusions. Despite these observed adverse effects, TW demonstrated a beneficial role in enhancing the antimicrobial activity of GC. Infusions prepared with TW exhibited significantly increased antimicrobial activity against various pathogenic bacteria and fungi, especially when prepared at 100 °C.

The results of this study underline the importance of considering the type of water used in the preparation of GC infusions, as it can significantly influence their bioactive properties. Furthermore, more research is necessary to understand the underlying mechanisms driving these observed effects and explore other plant matrices and approaches to enhance the bioactivity effects of GC infusions with thermal waters. A limitation of this study is that only a single batch of GC flowers (250 g) was used. Although independent replicates were conducted, future research should include testing multiple batches of the plant to ensure broader validity of the results. Finally, studies on the bioaccessibility and bioavailability of these infusions are essential to determine whether the observed reduction in phenolic compounds and antioxidant activity

Table 4
Antimicrobial and antifungal activity (mg/ mL) evaluated in German Chamomile extracts prepared with drinking water and thermal water at different temperature (60 ºC and 100 ºC).

	GC-100DW		GC-60TW		GC-100TW		Ampicillin		Imipenem		Methicillin		Vancomycin		Streptomycin		Ketoconazole	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative clinical bacteria																		
Escherichia coli	>10	>10	10	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Klebsiella pneumoniae	>10	>10	10	>10	10	>10	10	>10	<0.0078	<0.0078	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Morganella morganii	10	>10	>10	>10	5	10	>10	>10	<0.0078	<0.0078	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Proteus mirabilis	5	>10	>10	>10	5	>10	<015	<0.15	<0.0078	<0.0078	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Pseudomonas aeruginosa	>10	>10	10	>10	10	10	>10	>10	0.5	1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Gram-positive clinical bacteria																		
Enterococcus faecalis	>10	>10	>10	>10	>10	>10	<0.15	<0.15	n.t.	n.t.	n.t.	n.t.	<0.0078	<0.0078	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	>10	>10	10	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
MRSA	10	>10	>10	>10	5	>10	<0.15	<0.15	n.t.	n.t.	n.t.	n.t.	0.25	0.5	n.t.	n.t.	n.t.	n.t.
Gram-negative foodborne bacteria																		
Enterobacter cloacae	>10	>10	5	>10	10	10	0.15	0.15	n.t.	n.t	n.t.	n.t	n.t.	n.t	0.007	0.007	n.t.	n.t.
Escherichia coli	>10	>10	10	>10	10	>10	0.15	0.15	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.01	0.01	n.t.	n.t.
Pseudomonas aeruginosa	>10	>10	10	>10	10	10	0.63	0.63	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.06	0.06	n.t.	n.t.
Salmonella enterica	>10	>10	10	>10	10	10	0.15	0.15	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.007	0.007	n.t.	n.t.
Yersinia enterocolitica	10	>10	5	>10	5	10	0.15	0.15	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.007	0.007	n.t.	n.t.
Gram-positive foodborne bacteria																		
Bacillus cereus	10	>10	>10	>10	10	>10	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.007	0.007	n.t.	n.t.
Listeria monocytogenes	10	>10	10	>10	10	>10	0.15	0.15	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.007	0.007	n.t.	n.t.
Staphylococcus aureus	5	>10	10	>10	5	>10	0.15	0.15	n.t.	n.t.	0.007	0.007	n.t.	n.t.	0.007	0.007	n.t.	n.t.
Fungi	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	5	>10	0.6	10	0.6	10	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.06	0.125
Aspergillus fumigatus	10	>10	5	>10	2.5	10	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.5	1

GC-100DW: German chamomile infusion with boiling (100 ºC) drinking water; **GC-60TW**: German chamomile infusion with thermal water at 60 ºC; **GC-100TW:** German chamomile infusion with boiling (100 ºC) thermal water; **MIC:** Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration; **n.t.:** Not tested; **MRSA:** Methicillin-resistant *Staphylococcus aureus*; **MFC:** Minimal fungicide concentration.

translates to *in vivo* conditions.

CRediT authorship contribution statement

Tayse F.F. da Silveira: Writing – review & editing, Validation, Formal analysis, Data curation, Conceptualization. **Noemí Echegaray:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Rafaela Guimarães:** Writing – review & editing, Formal analysis, Conceptualization. **André Lemos:** Formal analysis. **Tânia C.S. P. Pires:** Writing – review & editing, Formal analysis, Data curation. **Isabel C.F.R. Ferreira:** Writing – review & editing, Resources, Methodology. **Maria José Alves:** Resources, Project administration, Funding acquisition. **Lillian Barros:** Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to the Fundação "La Caixa" and to FCT for the financial support through Project "Aquae Vitae - Água Termal como fonte de vida e Saúde", Promove Mobilizador "O futuro do Interior," call 2020. National funds al so supported this work through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020). L. Barros thanks FCT through the institutional scientific employment program–contract for her contract (CEEC-INST, DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002). T. F.F. da Silveira, thanks to Fundação BPI La Caixa, within the project titled "AquaeVitae", for her research contract. N. Echegaray acknowledges Axencia Galega de Innovación (GAIN) for granting a postdoctoral scholarship (grant number IN606B-2022/006). R. Guimarães and A. Lemos acknowledge national public funding through "Investimento RE-C05-i02 – Missão Interface N.° 01/C05-i02/2022", a project supported under the PRR (www.recuperarportugal.gov.pt), and financed by the European Union/Next Generation EU.

References

Al-Ismail, K., & Talal Aburjai, T. A. (2003). *A study of the effect of water and alcohol extracts of some plants as antioxidants and antimicrobial on long-term storage of anhydrous butter fat*.

Alkuraishy, H., Al-Gareeb, A., Albuhadilly, A., & Alwindy, S. (2015). In vitro assessment of the antibacterial activity of *Matricaria chamomile* alcoholic extract against pathogenic bacterial strains. *British Microbiology Research Journal, 7*(2), 55–61. https://doi.org/10.9734/bmrj/2015/16263

de Angelis, M., Amicucci, C., Banchelli, M., D'Andrea, C., Gori, A., Agati, G., Brunetti, C., & Matteini, P. (2025). Rapid determination of phenolic composition in chamomile (Matricaria recutita L.) using surface-enhanced Raman spectroscopy. *Food Chemistry, 463*, Article 141084.

Araujo, A. R. T. S., Sarraguça, M. C., Ribeiro, M. P., & Coutinho, P. (2017). Physicochemical fingerprinting of thermal waters of Beira Interior region of Portugal. *Environmental Geochemistry and Health, 39*(3), 483–496. https://doi.org/10.1007/s10653-016-9829-x

Asadi, Z., Ghazanfari, T., & Hatami, H. (2020). Anti-inflammatory effects of *Matricaria chamomilla* extracts on BALB/c mice macrophages and lymphocytes. *Iranian Journal of Allergy, Asthma and Immunology, 19*, 63–73. https://doi.org/10.18502/ijaai.v19i(s1.r1).2862

Avula, B., Wang, Y.-H., Wang, M., Avonto, C., Zhao, J., Smillie, T. J., … Khan, I. A. (2014). Quantitative determination of phenolic compounds by UHPLC-UV–MS and use of partial least-square discriminant analysis to differentiate chemo-types of Chamomile/Chrysanthemum flower heads. *Journal of Pharmaceutical and Biomedical Analysis, 88*, 278–288.

Barnich, N., Rodrigues, M., Sauvanet, P., Chevarin, C., Denis, S., Le Goff, O., … Alric, M. (2021). Beneficial effects of natural mineral waters on intestinal inflammation and the mucosa-associated microbiota. *International Journal of Molecular Sciences, 22*(9), 1–14. https://doi.org/10.3390/ijms22094336

Benedetti, S., Benvenuti, F., Nappi, G., Fortunati, N. A., Marino, L., Aureli, T., … Canestrari, F. (2009). Antioxidative effects of sulfurous mineral water: protection against lipid and protein oxidation. *European Journal of Clinical Nutrition, 63*(1), 106–112. https://doi.org/10.1038/sj.ejcn.1602892

Bessada, S. M. F., Barreira, J. C. M., Barros, L., Ferreira, I. C. F. R., & Oliveira, M. B. P. P. (2016). Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.f.: An underexploited and highly disseminated species. *Industrial Crops and Products, 89*, 45–51. https://doi.org/10.1016/j.indcrop.2016.04.065

Caleja, C., Barros, L., Antonio, A. L., Ciric, A., Barreira, J. C. M., Sokovic, M., … Ferreira, I. C. F. R. (2015). Development of a functional dairy food: Exploring bioactive and preservation effects of chamomile (*Matricaria recutita* L.). *Journal of Functional Foods, 16*, 114–124. https://doi.org/10.1016/j.jff.2015.04.033

Catani, M. V., Rinaldi, F., Tullio, V., Gasperi, V., & Savini, I. (2021). Comparative analysis of phenolic composition of six commercially available chamomile (*Matricaria chamomilla* L.) extracts: Potential biological implications. *International Journal of Molecular Sciences, 22*(19), 10601. https://doi.org/10.3390/ijms221910601

Cvetanović, A., Švarc-Gajić, J., Zeković, Z., Jerković, J., Zengin, G., Gašić, U., Tešić, Ž., Mašković, P., Soares, C., Fatima Barroso, M., Delerue-Matos, C., & Đurović, S. (2019). The influence of the extraction temperature on polyphenolic profiles and bioactivity of chamomile (*Matricaria chamomilla* L.) subcritical water extracts. *Food Chemistry, 271*, 328–337. https://doi.org/10.1016/j.foodchem.2018.07.154

Dai, Y. L., Li, Y., Wang, Q., Niu, F. J., Li, K. W., Wang, Y. Y., … Gao, L. N. (2023). Chamomile: A review of its traditional uses, chemical constituents, pharmacological activities and quality control studies. *Molecules, 28*(1), 133. https://doi.org/10.3390/molecules28010133

Danrong, Z., Yuqiong, C., & Dejiang, N. (2009). Effect of water quality on the nutritional components and antioxidant activity of green tea extracts. *Food Chemistry, 113*(1), 110–114. https://doi.org/10.1016/j.foodchem.2008.07.033

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-Fluorescein) assay. *Journal of Agricultural and Food Chemistry, 52*(1), 48–54. https://doi.org/10.1021/jf0305231

Ghani, M. A., Barril, C., Bedgood, D. R., & Prenzler, P. D. (2017). Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. *Food Chemistry, 230*, 195–207.

Giampaoli, S., Valeriani, F., Gianfranceschi, G., Vitali, M., Delfini, M., Festa, M. R., … Romano Spica, V. (2013). Hydrogen sulfide in thermal spring waters and its action on bacteria of human origin. *Microchemical Journal, 108*, 210–214.

Granato, D. (2023). Next-generation analytical platforms for antioxidant capacity assessment: The urge for realistic and physiologically relevant methods. *Biomedicine & Pharmacotherapy, 165*, Article 115155.

Guimarães, R., Barros, L., Dueñas, M., Calhelha, R. C., Carvalho, A. M., Santos-Buelga, C., … Ferreira, I. C. F. R. (2013). Infusion and decoction of wild German chamomile: Bioactivity and characterization of organic acids and phenolic compounds. *Food Chemistry, 136*(2), 947–954. https://doi.org/10.1016/j.foodchem.2012.09.007

Harbourne, N., Jacquier, J. C., & O'Riordan, D. (2009). Optimisation of the extraction and processing conditions of chamomile (*Matricaria chamomilla* L.) for incorporation into a beverage. *Food Chemistry, 115*(1), 15–19. https://doi.org/10.1016/j.foodchem.2008.11.044

Heleno, S. A., Ferreira, I. C. F. R., Esteves, A. P., Ćirić, A., Glamočlija, J., Martins, A., … Queiroz, M. J. R. P. (2013). Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food and Chemical Toxicology, 58*, 95–100. https://doi.org/10.1016/j.fct.2013.04.025

Kuete, V., Ango, P. Y., Fotso, G. W., Kapche, G. D. W. F., Dzoyem, J. P., Wouking, A. G., … Abegaz, B. M. (2011). Antimicrobial activities of the methanol extract and compounds from *Artocarpus communis* (Moraceae). *BMC Complementary and Alternative Medicine, 11*, 6. https://doi.org/10.1186/1472-6882-11-42

Mahdavi, B., Ghorat, F., Nasrollahzadeh, M. S., Hosseyni-Tabar, M., & Rezaei-Seresht, H. (2020). Chemical composition, antioxidant, antibacterial, cytotoxicity, and hemolyses activity of essential oils from flower of *Matricaria chamomilla* var Chamomilla. *Anti-Infective Agents, 18*(3), 224–232. https://doi.org/10.2174/2211352517666190528114041

Mailänder, L. K., Lorenz, P., Bitterling, H., Stintzing, F. C., Daniels, R., & Kammerer, D. R. (2022). Phytochemical characterization of chamomile (*Matricaria recutita* L.) roots and evaluation of their antioxidant and antibacterial potential. *Molecules, 27*(23), 8508. https://doi.org/10.3390/molecules27238508

Mierczyńska, J., Cybulska, J., Sołowiej, B., & Zdunek, A. (2015). Effect of Ca^{2+}, $^{Fe2+}$ and $^{Mg2+}$ on rheological properties of new food matrix made of modified cell wall polysaccharides from apple. *Carbohydrate Polymers, 133*, 547–555. https://doi.org/10.1016/j.carbpol.2015.07.046

Mulinacci, N., Romani, A., Pinelli, P., Vincieri, F. F., & Prucher, D. (2000). Characterization of *Matricaria recutita* L. flower extracts by HPLC-MS and HPLC-DAD analysis. *Chromatographia, 51*(5–6), 301–307. https://doi.org/10.1007/bf02490607

Nováková, L., Vildová, A., Mateus, J. P., Gonalves, T., & Solich, P. (2010). Development and application of UHPLC-MS/MS method for the determination of phenolic compounds in Chamomile flowers and Chamomile tea extracts. *Talanta, 82*(4), 1271–1280. https://doi.org/10.1016/j.talanta.2010.06.057

Oliveira, A. S., Vaz, C. V., Silva, A., Ferreira, S. S., Correia, S., Ferreira, R., … Palmeira-de-Oliveira, A. (2020). Chemical signature and antimicrobial activity of Central

Portuguese Natural Mineral Waters against selected skin pathogens. *Environmental Geochemistry and Health, 42*(7), 2039–2057. https://doi.org/10.1007/s10653-019-00473-6

Pérez-Granados, A. M., Navas-Carretero, S., Schoppen, S., & Vaquero, M. P. (2010). Reduction in cardiovascular risk by sodium-bicarbonated mineral water in moderately hypercholesterolemic young adults. *Journal of Nutritional Biochemistry, 21*(10), 948–953. https://doi.org/10.1016/j.jnutbio.2009.07.010

Petronilho, S., Maraschin, M., Coimbra, M. A., & Rocha, S. M. (2012). In vitro and in vivo studies of natural products: A challenge for their valuation. The case study of chamomile (*Matricaria recutita* L.). *Industrial Crops and Products, 40*(1), 1–12. https://doi.org/10.1016/j.indcrop.2012.02.041

Pinela, J., Barros, L., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. F. R. (2012). Antioxidant activity, ascorbic acid, phenolic compounds and sugars of wild and commercial *Tuberaria lignosa* samples: Effects of drying and oral preparation methods. *Food Chemistry, 135*(3), 1028–1035. https://doi.org/10.1016/j.foodchem.2012.05.038

Pinto-Ribeiro, I., Castro, C., Rocha, P. E., Carvalho, M. J., Pintado, A., Mendes, A., … Madureira, A. R. (2024). Chaves Thermal Spring Water Impact on Skin Health: Potential Cosmetic Application. *Applied Sciences, 14*(17), 7911.

Pires, T. C. S. P., Dias, M. I., Barros, L., Alves, M. J., Oliveira, M. B. P. P., Santos-Buelga, C., & Ferreira, I. C. F. R. (2018). Antioxidant and antimicrobial properties of dried Portuguese apple variety (*Malus domestica* Borkh. cv Bravo de Esmolfe). *Food Chemistry, 240*, 701–706. https://doi.org/10.1016/j.foodchem.2017.08.010

Piri, E., Sourestani, M. M., Khaleghi, E., Mottaghipisheh, J., Zomborszki, Z. P., Hohmann, J., & Csupor, D. (2019). Chemo-diversity and antiradical potential of twelve *Matricaria chamomilla* L. populations from Iran: Proof of ecological effects. *Molecules, 24*(7), 1315. https://doi.org/10.3390/molecules24071315

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290–4302.

Quattrini, S., Pampaloni, B., & Brandi, M. L. (2016). Natural mineral waters: chemical characteristics and health effects. *Clin Cases Miner Bone Metab, 13*(3), 173–180.

Roby, M. H. H., Sarhan, M. A., Selim, K. A.-H., & Khalel, K. I. (2013). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare L.) and chamomile (Matricaria chamomilla L.). *Industrial Crops and Products, 44*, 437–445.

Rocha, C., Moura, A. P., & Cunha, L. M. (2020). Consumers' associations with herbal infusions and home preparation practices. *Food Quality and Preference, 86*, Article 104006.

Romero, C. D., Chopin, S. F., Buck, G., Martinez, E., Garcia, M., & Bixby, L. (2005). Antibacterial properties of common herbal remedies of the southwest. *Journal of Ethnopharmacology, 99*(2), 253–257.

Schoppen, S., Pérez-Granados, A. M., Carbajal, Á., Oubiña, P., Sánchez-Muniz, F. J., Gómez-Gerique, J. A., & Vaquero, M. P. (2004). A Sodium-rich carbonated mineral water reduces cardiovascular risk in postmenopausal women. *Journal of Nutrition, 134*(5), 1058–1063. https://doi.org/10.1093/jn/134.5.1058

Schoppen, S., Sánchez-Muniz, F. J., Pérez-Granados, M., Gómez-Gerique, J. A., Sarriá, B., Navas-Carretero, S., & Pilar Vaquero, M. (2007). Does bicarbonated mineral water rich in sodium change insulin sensitivity of postmenopausal women? *Nutricion Hospitalaria, 22*(5), 538–544.

Schorr, U., Distler, A., & Sharma, A. M. (1996). Effect of sodium chloride- and sodium bicarbonate-rich mineral water on blood pressure and metabolic parameters in elderly normotensive individuals: a randomized double-blind crossover trial. *Journal of Hypertension, 14*(1), 131–135.

Šibul, F., Orčić, D., Berežni, S., Anačkov, G., & Mimica-Dukić, N. (2020). HPLC-MS/MS profiling of wild-growing scentless chamomile. *Acta Chromatographica, 32*(2), 86–94. https://doi.org/10.1556/1326.2019.00546

Silva, A., Oliveira, A. S., Vaz, C. V., Correia, S., Ferreira, R., Breitenfeld, L., … Cruz, M. T. (2020). Anti-inflammatory potential of Portuguese thermal waters. *Scientific Reports, 10*(1), 22313.

Silva, N. C. C., Barbosa, L., Seito, L. N., & Fernandes Junior, A. (2012). Antimicrobial activity and phytochemical analysis of crude extracts and essential oils from medicinal plants. *Natural Product Research, 26*(16), 1510–1514.

Solidônio, E., Burle, Y., Silva, P., Vicalvi, M., De Souza, I., Silva, G., Senna, K., & Colaço, W. (2015). Evaluation of antimicrobial activity of extracts of chamomile (Matricaria recutita L.) obtained from irradiated and non-irradiated samples. *The battle against microbial pathogens: Basic science, technological advancements and educational programmes, 1*, 151–156.

Sousa, A. C., Pádua, I., Gonçalves, V. M. F., Ribeiro, C., & Leal, S. (2024). Exploring tea and herbal infusions consumption patterns and behaviours: The case of Portuguese consumers. *Heliyon, 10*(7), Article e28779.

Stanojevic, L. P., Marjanovic-Balaban, Z. R., Kalaba, V. D., Stanojevic, J. S., & Cvetkovic, D. J. (2016). Chemical Composition, antioxidant and antimicrobial activity of chamomile flowers essential oil (*Matricaria chamomilla* L.). *Journal of Essential Oil Bearing Plants, 19*(8), 2017–2028. https://doi.org/10.1080/0972060X.2016.1224689

Takó, M., Kerekes, E. B., Zambrano, C., Kotogán, A., Papp, T., Krisch, J., & Vágvölgyi, C. (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. *Antioxidants, 9*(2), 165. https://doi.org/10.3390/antiox9020165

Tsivelika, N., Irakli, M., Mavromatis, A., Chatzopoulou, P., & Karioti, A. (2021). Phenolic profile by HPLC-PDA-MS of Greek chamomile populations and commercial varieties and their antioxidant activity. *Foods, 10*, 2345.

Wyrostek, J., & Kowalski, R. (2021). The effect of water mineralization on the extraction of active compounds from selected herbs and on the antioxidant properties of the obtained brews. *Foods, 10*(6), 1–16. https://doi.org/10.3390/foods10061227

Xu, Y. Q., Zou, C., Gao, Y., Chen, J. X., Wang, F., Chen, G. S., & Yin, J. F. (2017). Effect of the type of brewing water on the chemical composition, sensory quality and antioxidant capacity of Chinese teas. *Food Chemistry, 236*, 142–151. https://doi.org/10.1016/j.foodchem.2016.11.110

Zair, Y., Kasbi-Chadli, F., Housez, B., Pichelin, M., Cazaubiel, M., Raoux, F., & Ouguerram, K. (2013). Effect of a high bicarbonate mineral water on fasting and postprandial lipemia in moderately hypercholesterolemic subjects: a pilot study. *Lipids in Health and Disease, 12*, 105. https://doi.org/10.1186/1476-511X-12-105