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LC-MS/MS Characterization, Biological Activity, and Carbonic Anhydrase Inhibitory Potential of Five Medicinal Plant Extracts from Romania and Türkiye

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Abstract

Natural carbonic anhydrase (CA) inhibitors are gaining significant attention for their potential applications in diuretic, antiglaucoma, anti-obesity, and anticancer therapies. This study provides the first comparative evaluation of CA inhibitory potential, antioxidant activity, and LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) phytochemical profiles of hydroalcoholic extracts from *Satureja hortensis*, *Juniperus communis*, *Matricaria chamomilla*, *Hypericum perforatum*, and *Valeriana officinalis* sourced from Romania and T rkiye. Principal Component Analysis (PCA) was employed to correlate secondary metabolites with observed biological activities. While *S. hortensis* and *J. communis* displayed consistent metabolic profiles across both countries, the other species exhibited geographic variation. Correlation analysis indicated that while specific phenolic acids (e.g., gallic, ferulic) supported antioxidant capacity, rosmarinic acid and luteolin were strong contributors to CA inhibition and diuretic potential. Notably, *S. hortensis* emerged as the most promising source; the Turkish sample demonstrated superior efficacy, attributed to high rosmarinic acid content (49.76 µg/mL) and substantial CA inhibition (64.87 µg acetazolamide equivalent/mL). The highest values of polyphenol and flavonoid content were found in *H. perforatum* from T rkiye, with 16.95±0.221 and 9.77±0.17, respectively. These findings underscore the therapeutic potential of *S. hortensis* in managing CA-related disorders.

Keywords: Carbonic anhydrase inhibitor, Rosmarinic acid, Secondary metabolites, LC-MS/MS profiling

Introduction

The demand for innovative and effective drugs and bioactive compounds to combat human disorders such as edema, hypertension, and heart failure is increasingly urgent. Additionally, these compounds have potential applications in acute renal failure, glaucoma, epilepsy, and high-altitude disorders. The plant ecosystem, with its remarkable biodiversity, is home to over 50,000 identified medicinal plant species ^{1,2}. This vast and varied repository of botanical life serves as a treasure trove for the discovery of novel bioactive compounds and pharmaceuticals, offering promising avenues for developing new treatments. For this reason, studying the plants in terms of the effect on these diseases can shed new light on the new treatment and applications.

Diuretics are drugs that increase the urine excretion ³. There are many synthetic substances that have this effect on the human body. Almost all diuretics function initially by hindering sodium reabsorption in various sites within the renal tubules. According to the mechanism of action, they are grouped into five classes: carbonic anhydrase inhibitors (CAIs), loop diuretics, osmotic diuretics, potassium-sparing diuretics, and thiazides⁴. Due to their different mechanism of action, diuretic drugs are used as

standalone medication or in combination with other drugs⁵. In addition to their well-known use in the treatment of edema, hypertension, and heart failure, they can also be used in acute renal failure, glaucoma, epilepsy, and high-altitude disorders. Their long-term use can lead to electrolyte disturbances⁶. For this, diverse cultures across the globe have relied on a wide array of plants with preventive and curative properties, including diuretic ones⁷⁻¹¹. Their widespread use can be attributed to their proven efficacy, affordability, and minimal side effects. Whether used as a standalone treatment or in conjunction with other antihypertensive medications, diuretics play a pivotal role in the management of hypertension¹². Notably, diuretics are often the first-line treatment prescribed for hypertensive patients due to their remarkable effectiveness, potential for synergistic effects when combined with other antihypertensive medications, and their value in managing heart failure¹³.

The diuretic effect, as an in vitro carbonic anhydrase (CA)-inhibitory potential of plants, was correlated with the presence of secondary metabolites, including phytochemicals belonging to the classes of alkaloids, polyphenols, saponins, terpenoids, and glycosides^{6,11,14,15}. Polyphenols are the main group of phytochemicals encountered in plant regna. Their ability to act as carbon anhydrase was attributed to the chemical properties of the main structural unit^{10,11,16-18}. Avicenna, a renowned philosopher and physician, was one of the earliest advocates of using plants for medicinal purposes. In his famous book "The Canon of Medicine"¹⁹, *Hypericum perforatum* L., *Juniperus communis* L., *Matricaria chamomilla* L., *Satureja hortensis* L., and *Valeriana officinalis* L. were mentioned as having diuretic activity. In modern pharmacology, this effect is often correlated with in-vitro CA inhibitory potential.

The literature survey on the pharmacological significance of the studied plants reveals that; *H. perforatum* L., commonly known as St. John's Wort, is one of the most widely used medicinal plants globally. Its medicinal applications encompass a broad spectrum, including the treatment of skin wounds, eczema, burns, digestive system ailments, psychological disorders²⁰ and diuretic effects^{21,22}.

Aerial part of *H. perforatum* contains compounds belonging to different classes, such as anthraquinone derivatives and naphthodianthrone, phloroglucinols, flavonol glycosides, biflavone, phenylpropanes, proanthocyanidins and tannins, and xanones²³⁻²⁵.

J. communis is utilized in traditional medicine for its diuretic properties, as well as its anti-inflammatory, antifungal, analgesic, hepatoprotective, antidiabetic, antihyperlipidemic, antimicrobial, antioxidant, antihypercholesterolemic, antibacterial, anticataleptic, and neuroprotective activities in the context of Parkinson's disease^{26,27}. The juniper berries contain essential oil, flavonoids, tannins, organic acid, terpenic acids, leucoanthocyanidin, bitter compounds, catechin, lignins, resin, wax, and gums²⁸. *M. chamomilla*, often referred to as the "star among medicinal species, is a highly favoured and widely used medicinal plant in folk and traditional medicine for a wide range of therapeutic effects, including soothing, calming, sedative, relaxation, anti-inflammatory, and treatments for indigestion, hay fever, asthma, morning sickness, eczema, and diuretic activity²⁹. Chamomile flowers contain flavonoids, polyphenolic acids, coumarins, volatile oils, terpenes, sterols, and saponins³⁰. *S. hortensis* flowers, leaves, stems, and seeds have been used worldwide to treat gastrointestinal cramps, nausea, diarrhoea, muscle pains, and infectious diseases. The literature survey indicates various pharmacological properties, including antioxidant, anti-inflammatory, analgesic, anti-hypercholesterolemic, and diuretic activity³¹. Aerial parts contain polyphenolic compounds and essential oil³². *V. officinalis* has been traditionally used in Europe and the United States to alleviate mild and moderate insomnia, but antiepileptic, sedative, emmenagogue, spasmolytic, and diuretic effects were also reported³³. The main compounds found in Valeriana root are alkaloids, terpenes, polyphenolic acids and their derivatives, flavones, and valepotriates³⁴. Based on all the literature reviews and the ancient knowledge of Ibn Sina, this study aimed to simultaneously confirm, for the first time, the biological activities and chromatographic analyses (LC-MS/MS) of five medicinal plants reported separately in the literature and found in overlapping geographical regions of Romania and Türkiye.

This study aimed to investigate, for the first time, the simultaneous comparison of the carbonic anhydrase inhibitory potential, the secondary metabolites, phenolic compounds, and antioxidant capacities of hydroalcoholic extracts of five plants (*H. perforatum*, *J. communis*, *M. chamomilla*, *S. hortensis*, and *V. officinalis*) obtained in Romania and Türkiye to find a natural carbonic anhydrase inhibitor for healing in edema, hypertension, and heart failure. The study simultaneously evaluated the secondary metabolites, phenolic compounds, and antioxidant activities of these plants, focusing specifically on estimating their diuretic potential by correlating their phenolic compounds with in vitro carbonic anhydrase activity.

Results and Discussion

Secondary Metabolite LC-MS/MS Profile of Samples

The retention time (t_R) and method performance parameters in terms of limit of detection (LOD), limit of quantification (LOQ), and parameters of the calibration achieved in the LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) method by screening a number of 35 secondary metabolites (phenolic compounds) are summarized in Table 1. According to these results, the LC-MS/MS method demonstrates robust analytical performance across the polyphenolic and phenolic compounds considered in this study. The regression coefficients (R^2 values), mostly higher than 0.995, indicate excellent linear relationships between concentration and signal response, supporting the accurate quantification for all the investigated compounds across the full linear range 0.125 – 4.000 $\mu\text{g/mL}$. LOD and LOQ are low for most compounds, some of them (Epicatechin, Taxifolin, Quercetin) showing ultrasensitive detection (LODs < 0.01 $\mu\text{g/mL}$). The retention times (t_R) from 8.00 to 23.74 minutes show effective chromatographic separation with no indication of co-elution or poor separation, essential for accurate quantitation of structurally similar compounds.

Table 1. The method performance parameters achieved in the LC-MS/MS method for the screened compounds.

No	Analyte	t_R (min)	Regression equation*	R^2	LOD ($\mu\text{g} / \text{mL}$)	LOQ ($\mu\text{g} / \text{mL}$)
1	Pyrogallol	8.00	$Y = 233564 \cdot X$	0.9957	0.052	0.173
2	Gallic Acid	9.69	$Y = 51670 + 915373 \cdot X$	0.9995	0.064	0.213
3	Protocatechuic acid	11.77	$Y = 85437.6 + 450443 \cdot X$	0.9999	0.043	0.143
4	Protocatechuic aldehyde	12.64	$Y = 1.08649 \times 10^6 + 3.04363 \times 10^6 \cdot X$	0.9990	0.025	0.082
5	Catechin	12.98	$Y = 2.26111 \times 10^6 + 3.98729 \times 10^7 \cdot X$	0.9994	0.012	0.039
6	Gentisic acid	12.91	$Y = 2.27475 \times 10^6 \cdot X$	0.9992	0.015	0.049
7	Chlorogenic Acid	13.10	$Y = 3.16018 \times 10^6 \cdot X$	0.9994	0.214	0.714
8	Epicatechin	14.38	$Y = 3.44346 \times 10^6 + 9.38182 \times 10^7 \cdot X$	0.9997	0.002	0.006
9	Caffeic acid	14.76	$Y = 678252 + 5.45207 \times 10^6 \cdot X$	0.9973	0.020	0.067
10	Vanillin	15.27	$Y = 145703 + 1.4072 \times 10^6 \cdot X$	0.9996	0.022	0.072
11	Syringic acid	15.77	$Y = 2.11782 \times 10^7 + 3.49822 \times 10^8 \cdot X$	0.9992	0.207	0.691
12	Syringic aldehyde	15.81	$Y = 5.43971 \times 10^6 \cdot X$	0.9993	0.147	0.491
13	Taxifolin	16.27	$Y = 205866 + 4.26033 \times 10^6 \cdot X$	0.9995	0.005	0.015
14	P-Coumaric acid	16.42	$Y = 193184 + 597275 \cdot X$	0.9997	0.042	0.139
15	Ferulic acid	16.77	$Y = 87217.8 + 662426 \cdot X$	0.9998	0.038	0.128
16	Sinapic acid	16.87	$Y = 177743 \cdot X$	0.9931	0.217	0.723
17	Salicylic acid	17.19	$Y = -830750 + 2.85887 \times 10^7 \cdot X$	0.9922	0.030	0.101
18	p-hydroxybenzoic acid	17.28	$Y = 401166 + 9.65023 \times 10^6 \cdot X$	0.9988	0.246	0.819
19	Hesperidin	17.36	$Y = 2.37316 \times 10^6 \cdot X$	0.9973	0.126	0.421
20	Rosmarinic acid	17.41	$Y = 1.59219 \times 10^6 + 3.05473 \times 10^7 \cdot X$	0.9985	0.005	0.015
21	Oleuropein	17.59	$Y = 709204 + 9.92946 \times 10^6 \cdot X$	0.9992	0.010	0.033

22	Luteolin 7-O-glucoside	18.00	$Y = 5.62229e+007 * X$	0.9989	0.021	0.071
23	Rutin	18.03	$Y = 1.80504e+007 + 3.897e+007 * X$	0.9955	0.028	0.092
24	Rezveratrol	18.14	$Y = 6.69365e+006 + 1.21479e+007 * X$	0.9996	0.018	0.059
25	Ellagic Acid	19.16	$Y = 1.3649e+006 + 8.78955e+006 * X$	0.9978	0.094	0.313
26	Cinnamic acid	19.44	$Y = 799109 * X$	0.9917	0.097	0.322
27	Naringenin	19.71	$Y = 1.55843e+008 * X$	0.9966	0.005	0.017
28	Quercetin	20.38	$Y = 2.34328e+006 + 2.05999e+007 * X$	0.9987	0.003	0.010
29	Luteolin	21.05	$Y = 1.26258e+008 * X$	0.9938	0.153	0.511
30	Kaempferol	21.55	$Y = 7.68282e+006 + 1.12344e+008 * X$	0.9987	0.134	0.447
31	Apigenin	22.15	$Y = 5.70565e+006 * X$	0.9992	0.181	0.604
32	Chrysin	22.54	$Y = 1.73526e+008 * X$	0.9982	0.086	0.288
33	Pinocambrin	22.98	$Y = 2.42104e+008 * X$	0.9994	0.124	0.414
34	Galangin	23.43	$Y = 1.22672e+007 * X$	0.9994	0.034	0.113
35	Flavone	23.74	$Y = 1.38711e+007 + 8.46412e+008 * X$	0.9996	0.037	0.124

* Regression equation was obtained for the linearity range 0.125 – 4.000 µg/mL in all cases.

The LC-MS/MS results obtained for the screened secondary metabolite compounds from the hydroalcoholic extracts of *S. hortensis* (Sa); *J. communis* (Ju); *M. chamomilla* (Ma); *H. perforatum* (Hy), and *V. officinalis* (Va) from Romania (R) and Türkiye (T) are presented in Table 2. Chromatograms of the phenolic compounds screened in the analyzed samples are presented in Fig. S1-Fig. S11.

According to the obtained results, Rosmarinic acid, luteolin, caffeic acid, protocatechuic aldehyde, and naringenin were the main phenolic compounds detected in *S. hortensis* (Figure 1). Samples from Türkiye revealed high content in rosmarinic acid and naringenin, while samples from Romania have higher content in luteolin, caffeic acid, protocatechuic acid, and aldehyde, respectively. Quantitative assessment of the phenolic compound content of *S. hortensis* extract revealed a phenolic compound profile consistent with findings reported in previous literature^{35,36}. A comprehensive summary of the characteristic phenolic composition documented for this species is provided below.

Satureja species are noteworthy plants not only for their aromatic and culinary uses but also for their wide range of biological activities. These biological effects are reported to be largely due to high levels of bioactive components such as polyphenolic compounds and volatile terpenoids. Previous studies have demonstrated that regular consumption of polyphenols plays a significant role in reducing the risk of chronic diseases such as diabetes, obesity, cardiovascular disease, colorectal cancer, and various gastrointestinal disorders. Studies on the chemical composition of *S. hortensis* indicate that the plant's therapeutic properties stem primarily from its phenolic compounds and flavonoids. Analyses using liquid chromatography techniques consistently identified key polyphenolic compounds such as caffeic acid, rosmarinic acid, luteolin, naringenin, and apigenin in the plant's extracts. The primary phenolic acids and flavonoids reported in the literature for *S. hortensis* include rosmarinic acid, chlorogenic acid, caffeic acid, apigenin, luteolin, and various glycoside derivatives. Quantitative assessments indicate that rosmarinic acid generally has the highest concentration among these compounds, followed by luteolin, caffeic acid, apigenin, and naringenin^{32,35-37}. Indeed, an HPLC study on *S. hortensis* cultivated in Western Ukraine reported significant levels of RA (21.42 mg/g), while flavone derivatives and caffeic acid were detected in lower but significant amounts³⁶.

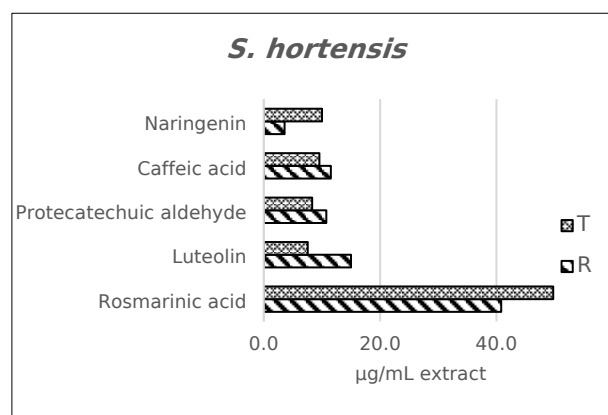


Figure 1. The major phenolic compounds detected in *S. hortensis*

J. communis (Cupressaceae) is the only *Juniperus* species found naturally in both the Northern and Southern Hemispheres, with a widespread distribution. The *Juniperus* genus comprises approximately 68 species and 36 varieties and has held an important place in the traditional medicine of many countries for many years. These species are noted for their wide range of biological activities, including antifungal, antiviral, antimicrobial, anticancer, cytotoxic, and antioxidant properties, and are considered a valuable source of bioactive compounds. *J. communis* holds a significant place in traditional Turkish medicine, particularly in Turkey³⁸. In Turkish folk medicine, juniper berries (juniper cones/berries) are widely used for diuretic, stomachic, and antiseptic purposes. They are also reported to be beneficial in the treatment of rheumatism, gout, angina, and heart and skin diseases. There are studies in the literature demonstrating that *J. communis* berries have antidiabetic and hypoglycemic effects. Important phytochemical groups such as polyphenols, polyphenol esters, and monoterpene hydrocarbons have been identified among the fruit components³⁹. *J. communis* fruits are rich in polyphenols, which contribute to their strong antioxidant capacity, which is widely used in the food, beverage, and pharmaceutical industries. It is known that the free radical scavenging abilities of phenolic compounds in plants are due to their hydroxyl groups. Analytical studies have revealed that phenolic acids and flavonoids are the primary polyphenol classes in *J. communis* fruits. Quantitative data indicate that protocatechuic acid (11.46 ± 0.03 mg/g dry weight) and p-hydroxybenzoic acid (3.12 ± 0.01 mg/g dry weight) are the predominant phenolic acids. Among the flavonoids, catechin (4.47 ± 0.02 mg/g dry weight) is a prominent compound. Catechin (219.01 mg/g dry weight) and rutin (18.08 mg/g dry weight) were reported as the main phenolic compounds in *J. communis* needle methanol extracts. The antioxidant, antimicrobial, and cytotoxic activities of *J. communis* extracts are reported to be closely related to their high phenolic content³⁸⁻⁴¹.

In the present study, the phytochemical profiling of *J. communis* revealed that protocatechuic acid, apigenin, rutin, catechin, luteolin, rosmarinic acid, and protocatechuic aldehyde constituted the major phenolic components detected in the analyzed samples (Figure 2). Notably, protocatechuic acid (12.162 µg/mL) and protocatechuic aldehyde (2.803 µg/mL) were present at substantially higher concentrations in the samples originating from Türkiye, whereas apigenin (8.508 µg/mL), rutin (5.389 µg/mL), catechin (4.940 µg/mL), luteolin (3.026 µg/mL), and rosmarinic acid (1.460 µg/mL) were more abundant in the Romanian samples. These findings highlight a clear geographic variation in the phenolic composition of *J. communis*, underscoring the influence of environmental and regional factors on its phytochemical profile.

The findings regarding the phenolic compound profile obtained in this study reveal that the chemical constituents of *J. communis* can exhibit significant differences depending on geographic origin. These variations detected in the analytical results are consistent with the species' natural diversity and the susceptibility of phytochemical components to environmental conditions. These data, when compared to the broader reported chemical composition and biological properties of *J. communis*, are consistent with the extensive phytochemical and pharmacological findings reported in the literature^{39,40}.

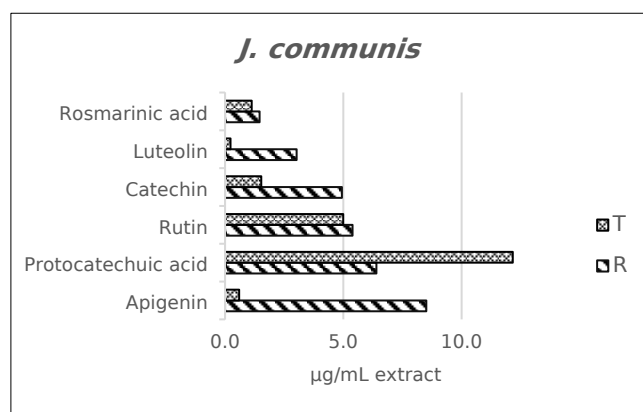


Figure 2. The major phenolic compounds detected in *J. communis*

Although *M. chamomilla* contains over 120 compounds in its overall chemical structure, its aqueous and alcoholic extracts prepared for therapeutic purposes are reportedly largely dominated by hydrophilic phenolic compounds. These phenolic compounds are divided into two main groups: flavonoids and phenolic acids. The literature has extensively demonstrated that both classes contribute significantly to the plant's antioxidant capacity, and that there is a strong positive correlation between total phenolic content and antioxidant activity^{42,43}. The primary flavonoids in *M. chamomilla* are generally found as glycoside derivatives, with apigenin, luteolin, and quercetin, which play a significant role in the plant's pharmacological effects, being among the best-characterized biologically active compounds. Regarding phenolic acid compounds, the plant is reported to be a rich source of cinnamic acid derivatives, in particular. HPLC and LC-MS studies confirmed the presence of key phenolic acids, particularly chlorogenic acid and *p*-coumaric acid, as well as caffeic and ferulic acid⁴²⁻⁴⁵. Furthermore, more complex phenolic acid derivatives such as *cis*- and *trans*-3,5-O-dicaffeoylquinic acid were reported in aqueous extracts. Different morphological parts of the plant (flower and stem), as well as extraction conditions (especially temperature), were observed to lead to significant changes in the amount and distribution of phenolic and flavonoid compounds⁴⁵. For example, the literature details that apigenin is particularly concentrated in white lignified flowers, while luteolin and quercetin derivatives are found at higher levels in tubular flowers.

In our analysis of *M. chamomilla* samples, chlorogenic acid, apigenin, and protocatechuic acid were identified as the predominant phenolic constituents (Figure 3). Notably, chlorogenic acid was present at substantially higher levels in the Romanian samples, exhibiting nearly a four-fold increase (109.455 µg/mL) compared to those from Türkiye. Consistent with our findings, previous studies⁴²⁻⁴⁵ also reported the occurrence of chlorogenic acid, apigenin, luteolin-7-O-glucoside, *p*-coumaric acid in *M. chamomilla* identified as a major component. These similarities suggest that our results align well with the established phytochemical profile of the species.

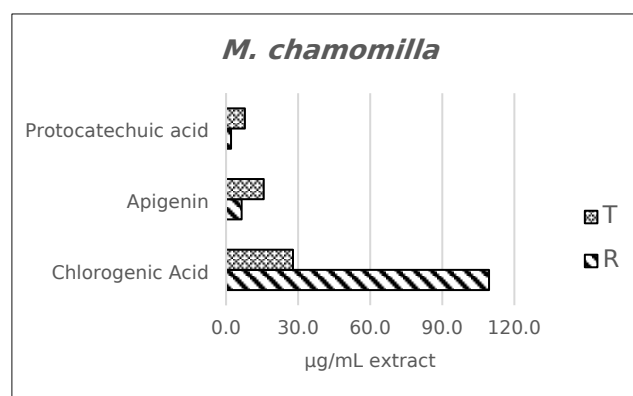


Figure 3. The major phenolic compounds detected in *M. chamomilla*

In this study, the phytochemical profile analysis of *H. perforatum* samples revealed that chlorogenic acid, rutin, quercetin, epicatechin, catechin, rosmarinic acid, and protocatechuic acid were the major phenolic constituents (Figure 4). Among these compounds, rutin was present at substantially higher levels in the samples collected from Romania (52.544 µg/mL), whereas chlorogenic acid, quercetin, epicatechin, catechin, rosmarinic acid, and protocatechuic acid were found at higher concentrations in the samples originating from Türkiye. These findings are in agreement with the data reported in the literature regarding the chemical structure of *H. perforatum* rich in phenolic compounds and flavonoids, and also support that different geographical origins may lead to significant variations in the distribution of phenolic compounds among samples.

The primary components determining the therapeutic value of *H. perforatum* are phenolic substances and flavonoids, which form the basis of the plant's biological activity. Analytical studies on crude extracts of this species generally categorize phenolic compounds as flavonoids (especially quercetin derivatives), phenolic acids, phloroglucinols, and naphthodianthrones (hypericin and pseudohypericin). Indeed, HPLC analyses performed on different *Hypericum* species revealed that chlorogenic acid was the predominant phenolic acid in all samples. Within the flavonoid fraction, various derivatives, including quercetin, isoquercitrin, quercitrin, and the pharmacologically important compound hyperoside, were identified and isolated. Furthermore, the characteristic signals of chlorogenic acid and coumarin-acyl glycosides can be clearly distinguished in crude extract analyses. *H. perforatum* is considered one of the most medicinally important species within the genus, and its traditional use spans the globe. The plant's biological activity has been largely attributed to its phenolic compounds, naphthodianthrones (hypericin and pseudohypericin) and flavonoids (hyperoside and quercetin glycosides). Various extract studies have shown a strong correlation between total phenolic content and antioxidant capacity, and *H. perforatum* extracts have been reported to exhibit high levels of free radical scavenging (antiradical) activity. In addition, a standardized *H. perforatum* extract has been reported to exhibit protective effects against hydrogen peroxide-induced oxidative damage in PC12 cells and to exhibit significant antioxidant properties in rat brain tissue with elevated oxidative status⁴⁶⁻⁴⁹.

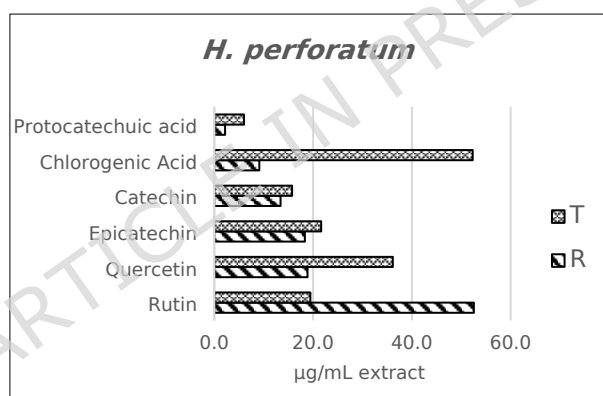


Figure 4. The major phenolic compounds detected in *H. perforatum*

V. officinalis extracts were revealed by the presence of hesperidin with significantly higher values (14 times higher) in samples from Türkiye (22.54 µg/mL) and chlorogenic acid, which was revealed in high quantity also in samples of Turkish origin (111.803 µg/mL, Figure 5). The obtained results are consistent with studies in literature using a high-performance liquid chromatography (HPLC) method^{33,49}.

V. officinalis, belonging to the Caprifoliaceae family, has been used in traditional medicine for centuries to treat sleep and anxiety disorders. The roots and rhizomes of this plant, which is considered a food ingredient with a GRAS rating in the United States, contain over 150 phytochemicals, primarily sesquiterpenes, iridoids, and flavonoids. Studies reported in the literature indicate that these compounds contribute to a wide range of biological effects, including antioxidant, anti-inflammatory, antimicrobial, anxiolytic, antirheumatic, spasmolytic, and neuroprotective activities. HPLC and other phenolic analyses have revealed that *V. officinalis* roots and rhizomes have high phenolic acid contents. Among the primary phenolic acids reported in the literature are chlorogenic acid, gallic acid, caffeic acid, p-coumaric acid, and protocatechuic acid, and the amounts of these compounds vary depending on the cultivation system and extraction method. Chlorogenic acid and p-coumaric acid were found to be higher in conventionally grown roots, while gallic and caffeic acid levels were higher in organically grown roots. Furthermore, protocatechuic acid was the primary phenolic compound in extracts prepared with higher alcohol

concentrations. When examining the flavonoid profile, quercetin, kaempferol, and myricetin were the main compounds frequently identified in the literature, and higher quercetin levels were reported in organically grown roots compared to conventional roots. In general, the high phenolic and flavonoid content of *V. officinalis* roots and rhizomes is emphasized in the literature as the key chemical property supporting the plant's biological activities ⁴⁹⁻⁵¹.

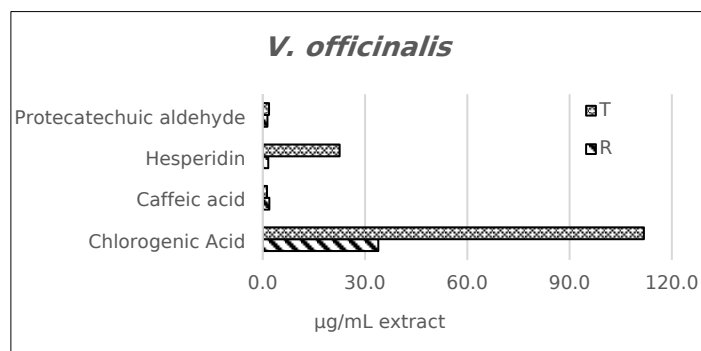


Figure 5. The major phenolic compounds detected in *V. officinalis*

Table 2. Results of phenolic compound content in the studied samples

Compound	Content ($\mu\text{g} / \text{mL extract}$) $\pm$ SD*									
	Sa(R)	Sa(T)	Ju(R)	Ju(T)	Ma(R)	3Ma(T)	Hy(R)	Hy(T)	Va(R)	Va(T)
Gallic Acid	<LOD	<LOD	0.278 $\pm$ 0.013	0.432 $\pm$ 0.019	<LOD	<LOD	<LOD	<LOD	<LOD	0.465 $\pm$ 0.021
Protocatechuic acid	4.458 $\pm$ 0.143	2.314 $\pm$ 0.074	6.398 $\pm$ 0.205	12.162 $\pm$ 0.389	2.081 $\pm$ 0.067	7.843 $\pm$ 0.251	2.182 $\pm$ 0.07	6.01 $\pm$ 0.192	0.562 $\pm$ 0.018	0.871 $\pm$ 0.028
Protocatechuic aldehyde	10.746 $\pm$ 0.247	8.316 $\pm$ 0.191	0.925 $\pm$ 0.021	2.803 $\pm$ 0.064	1.06 $\pm$ 0.024	1.135 $\pm$ 0.026	0.411 $\pm$ 0.009	0.763 $\pm$ 0.018	1.363 $\pm$ 0.031	1.827 $\pm$ 0.042
Sesamol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Catechin	<LOD	<LOD	4.940 $\pm$ 0.183	1.531 $\pm$ 0.057	<LOD	<LOD	13.408 $\pm$ 0.496	15.748 $\pm$ 0.583	<LOD	<LOD
Gentisic acid	<LOD	<LOD	<LOD	<LOD	1.191 $\pm$ 0.035	<LOD	<LOD	<LOD	<LOD	<LOD
Chlorogenic Acid	2.40 $\pm$ 0.091	1.947 $\pm$ 0.074	<LOD	<LOD	109.455 $\pm$ 4.159	27.857 $\pm$ 1.059	9.113 $\pm$ 0.346	52.309 $\pm$ 1.988	33.884 $\pm$ 1.288	111.803 $\pm$ 4.249
Epicatechin	<LOD	<LOD	0.338 $\pm$ 0.007	0.153 $\pm$ 0.003	<LOD	<LOD	18.337 $\pm$ 0.385	21.633 $\pm$ 0.454	0.02 $\pm$ 0	<LOD
Caffeic acid	11.525 $\pm$ 0.23	9.557 $\pm$ 0.191	0.015 $\pm$ 0	<LOD	2.704 $\pm$ 0.054	2.592 $\pm$ 0.052	0.156 $\pm$ 0.003	0.434 $\pm$ 0.009	1.92 $\pm$ 0.038	1.234 $\pm$ 0.025
Vanillin	0.645 $\pm$ 0.027	0.574 $\pm$ 0.024	0.523 $\pm$ 0.022	1.077 $\pm$ 0.045	0.243 $\pm$ 0.01	0.478 $\pm$ 0.02	0.322 $\pm$ 0.014	0.134 $\pm$ 0.006	0.621 $\pm$ 0.026	0.728 $\pm$ 0.031
Syringic acid	0.276 $\pm$ 0.014	<LOD	<LOD	<LOD	0.029 $\pm$ 0.001	0.121 $\pm$ 0.006	0.032 $\pm$ 0.002	0.022 $\pm$ 0.001	0.461 $\pm$ 0.023	0.063 $\pm$ 0.003
Syringic aldehyde	0.177 $\pm$ 0.008	0.184 $\pm$ 0.009	<LOD	<LOD	0.1 $\pm$ 0.005	0.128 $\pm$ 0.006	0.072 $\pm$ 0.003	<LOD	0.049 $\pm$ 0.002	0.065 $\pm$ 0.003
Taxifolin	6.568 $\pm$ 0.197	9.864 $\pm$ 0.296	0.510 $\pm$ 0.015	0.131 $\pm$ 0.004	<LOD	2.524 $\pm$ 0.076	1.083 $\pm$ 0.032	4.318 $\pm$ 0.13	<LOD	0 $\pm$ 0
<i>p</i> -Coumaric acid	<LOD	0.223 $\pm$ 0.005	<LOD	<LOD	<LOD	0.146 $\pm$ 0.003	<LOD	0.08 $\pm$ 0.002	<LOD	<LOD
Ferulic acid	<LOD	0.107 $\pm$ 0.003	<LOD	0.885 $\pm$ 0.022	3.407 $\pm$ 0.085	<LOD	0.041 $\pm$ 0.001	<LOD	0.731 $\pm$ 0.018	0.128 $\pm$ 0.003
<i>p</i> -hydroxybenzoic acid	1.303 $\pm$ 0.029	1.734 $\pm$ 0.038	0.001 $\pm$ 0	0.032 $\pm$ 0.001	0.308 $\pm$ 0.007	0.532 $\pm$ 0.012	0.448 $\pm$ 0.01	0.517 $\pm$ 0.011	0.319 $\pm$ 0.007	0.058 $\pm$ 0.001
Salicylic acid	1.399 $\pm$ 0.067	1.800 $\pm$ 0.086	0.069 $\pm$ 0.003	0.131 $\pm$ 0.006	0.4 $\pm$ 0.019	0.557 $\pm$ 0.027	0.513 $\pm$ 0.025	0.637 $\pm$ 0.031	0.394 $\pm$ 0.019	0.121 $\pm$ 0.006
Hesperidin	<LOD	2.783 $\pm$ 0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.604 $\pm$ 0.029	22.54 $\pm$ 0.406
Rosmarinic acid	40.819 $\pm$ 1.061	49.762 $\pm$ 1.294	1.460 $\pm$ 0.038	1.127 $\pm$ 0.029	0.293 $\pm$ 0.008	0.079 $\pm$ 0.002	0.276 $\pm$ 0.007	7.628 $\pm$ 0.198	0.369 $\pm$ 0.01	0.264 $\pm$ 0.007
Oleuropein	<LOD	<LOD	<LOD	<LOD	<LOD	0.011 $\pm$ 0	0.065 $\pm$ 0.002	<LOD	0.062 $\pm$ 0.002	<LOD
Luteolin 7- <i>O</i> -glucoside	1.289 $\pm$ 0.05	0.168 $\pm$ 0.007	0.747 $\pm$ 0.029	0.354 $\pm$ 0.014	2.225 $\pm$ 0.087	0.293 $\pm$ 0.011	<LOD	<LOD	0.033 $\pm$ 0.001	0.016 $\pm$ 0.001
Rutin	<LOD	<LOD	5.389 $\pm$ 0.075	4.995 $\pm$ 0.07	0.024 $\pm$ 0	0.792 $\pm$ 0.011	52.544 $\pm$ 0.736	19.442 $\pm$ 0.272	0.18 $\pm$ 0.003	<LOD
Ellagic Acid	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.161 $\pm$ 0.008	0.254 $\pm$ 0.013	<LOD	<LOD
Naringenin	3.568 $\pm$ 0.114	10.004 $\pm$ 0.32	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Quercetin	0.004 $\pm$ 0.000	0.108 $\pm$ 0.002	0.144 $\pm$ 0.003	0.005 $\pm$ 0	0.668 $\pm$ 0.015	2.407 $\pm$ 0.055	18.873 $\pm$ 0.434	36.138 $\pm$ 0.831	0.806 $\pm$ 0.019	0.291 $\pm$ 0.007
Luteolin	14.991 $\pm$ 0.585	7.550 $\pm$ 0.294	3.026 $\pm$ 0.118	0.231 $\pm$ 0.009	0.756 $\pm$ 0.029	0.846 $\pm$ 0.033	0.316 $\pm$ 0.012	2.418 $\pm$ 0.094	<LOD	<LOD

Kaempferol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.401 ± 0.016	1.921 ± 0.079	<LOD	<LOD
Apigenin	1.198 ± 0.047	1.854 ± 0.072	8.508 ± 0.332	0.597 ± 0.023	6.49 ± 0.253	15.688 ± 0.612	0.345 ± 0.013	0.294 ± 0.011	0.057 ± 0.002	0.06 ± 0.002

*SD - standard deviation calculated for 3 replicates of sample

Overall, the comparative LC-MS/MS profiling underscores that while the qualitative composition of the selected medicinal plants is generally conserved within species, the quantitative abundance of specific bioactive markers is heavily dependent on their geographic origin. The distinct variations observed such as the dominance of phenolic acids (e.g., rosmarinic and chlorogenic acids) in Turkish samples of *S. hortensis* and *V. officinalis*, versus the flavonoid rich profile of Romanian *J. communis* suggest that local environmental conditions differentially modulate metabolic pathways. These phytochemical discrepancies provide a biochemical rationale for the variations in biological activity; specifically, the superior diuretic potential of Turkish *S. hortensis* is strongly supported by its elevated rosmarinic acid content, whereas the robust antioxidant capacity of Turkish *H. perforatum* correlates with its high levels of quercetin and chlorogenic acid. Consequently, this study highlights the necessity of comprehensive chemical profiling alongside biological testing to standardize herbal preparations and optimize their therapeutic efficacy based on geographic sourcing.

Bioactivity study and phenolic compounds profile

Diuretic potential and antioxidant activity of extracts obtained from *S. hortensis* (Sa), *J. communis* (Ju), *M. chamomilla* (Ma), *H. perforatum* (Hy) and *V. officinalis* (Va) samples collected from Romania and Türkiye were assessed considering both the determined polyphenolic compound profile and total amounts of flavonoids (TFC) and polyphenols (TPC) contained in these extracts. The obtained results presented in Table 5 show that among the analyzed samples, *S. hortensis* (Sa) extracts have the highest carbonic anhydrase inhibitory activity values (% Inhibition = 64.10 µg acetazolamide /mL and % Inhibition = 64.87 µg acetazolamide /mL extract for Sa(R) and Sa(T) samples respectively) and the highest in-vitro CA inhibitory potential (lowest IC₅₀ values, IC₅₀ = 13.78 and IC₅₀ = 11.76 µL extract for Sa(R) and Sa(T) samples, respectively) revealing in both cases a higher diuretic potential for samples from Türkiye (Sa (T)) region. Excepting the *H. perforatum* (Hy) extracts (revealed with the highest antioxidant potential, highest polyphenols and flavonoids content), *S. hortensis* (Sa) samples also show high antioxidant potential (lowest values in the ABTS assay for EC₅₀ parameters, EC₅₀ = 1.28 and EC₅₀ = 1.01 µL extract for Sa(R) and Sa(T) samples, respectively) and a high content in polyphenols and flavonoids (TPC = 4.82 µg gallic acid /mL extract and TPC = 6.98 µg gallic acid /mL extract and TFC = 1.51 µg rutin /mL extract and TFC = 1.57 µg rutin /mL extract for Sa(R) and Sa(T) samples, respectively) with higher values for samples from Türkiye region in all cases.

Carbonic anhydrase is a ubiquitous zinc-containing metalloenzyme that plays a crucial role in numerous physiological processes due to its remarkable ability to reversibly convert carbon dioxide (CO₂) into bicarbonate ions (HCO₃⁻). It is among the fastest enzymes identified, capable of hydrating and the enzyme's catalytic efficiency is typically constrained by the rate at which its substrates diffuse. It is often clustered along cellular membranes or localized in extracellular spaces, enhancing its capability to facilitate the rapid diffusion of both carbon dioxide and protons (H⁺) within cells. By promoting the movement of protons, carbonic anhydrase effectively mitigates intracellular pH gradients, aiding in the maintenance of a consistent and optimal pH level throughout the cell. The applications of carbonic anhydrase are diverse and far-reaching, encompassing diseases such as diabetes, epilepsy, Alzheimer's disease, and cardiovascular disorders. Consequently, the inhibitory effects of various analyzed plant extracts on carbonic anhydrase enzyme activity were assessed as a bioactivity experiment that is commonly utilized to reveal of the potential activity of plants ^{1,2}, highlighting the potential for therapeutic targeting of this essential enzyme.

The diuretic potential and antioxidant activity of extracts derived from various botanical sources, including *S. hortensis* (summer savory), *J. communis* (European juniper), *M. chamomilla* (chamomile), *H. perforatum* (St. John's wort), and *V. officinalis* (valerian), were rigorously evaluated. These samples were collected from a range of regions in Romania and Türkiye, each contributing distinct environmental factors that may influence the phytochemical profiles of the plants. The study thoroughly analyzed the specific polyphenolic compound profiles in these extracts, highlighting the diversity and concentrations of these bioactive constituents. Additionally, it quantified the total flavonoid content (TFC) and total polyphenol content (TPC), offering insights into the overall antioxidant capacity and potential therapeutic applications of these herbal extracts. The findings presented in Table 5 reveal that among the analyzed samples, *S. hortensis* (Savory) demonstrated exceptional carbonic anhydrase inhibitory activity. Specifically, the % Inhibition values reached 64.10 µg acetazolamide/mL for the Romanian sample (Sa(R)) and 64.87 µg acetazolamide/mL for the Turkish sample (Sa(T)). Furthermore, these extracts exhibited the highest in-vitro CA inhibitory potential (diuretic activity), as indicated by the lowest IC₅₀ values recorded for the Sa(R) extract. This data underscores a notably superior diuretic potential in the Savory samples sourced from Türkiye. In contrast, while extracts of *H. perforatum* (St. John's Wort) displayed the highest antioxidant potential along with significant concentrations of polyphenols and flavonoids, the *S. hortensis* (Savory) samples also exhibited remarkable antioxidant

capabilities. This was especially evident from the lowest values observed in the ABTS assay for EC_{50} parameters, further highlighting the impressive phytochemical profile of these extracts.

Studies investigating the carbonic anhydrase (CA) inhibitory potential of plant derived extracts and fractions in the literature frequently report moderate to high levels of enzyme inhibition. In this context, the CA inhibition percentages of different extracts and fractions obtained from *Rondeletia odorata* roots have been reported to range from 51.60% to 76.50%. Specifically, the ethyl acetate fraction exhibited the highest inhibition at $76.50\% \pm 0.67$, followed by the ethanol extract ($72.59\% \pm 1.39$) and the n-butanol fraction ($68.75\% \pm 1.69$). n-Hexane ($56.64\% \pm 0.67$) and the aqueous fraction ($51.60\% \pm 1.13$) showed lower but biologically significant CA inhibitory activities. Compared to the strong inhibition of 95.51 ± 1.29 by the standard inhibitor acetazolamide, it is emphasized that the fractions with medium polarity, in particular, present a promising natural inhibitor profile, and this efficacy may largely be due to phenolic and flavonoid components⁵².

The in-vitro CA inhibitory results obtained in this study largely coincide with the relevant literature data⁵² and reveal that naturally occurring samples have significant biological potential. The CA inhibition exhibited by samples Sa(R) and Sa(T) at rates of $64.10 \pm 1.25\%$ and $64.87 \pm 1.23\%$, respectively, is comparable to that reported in the literature for *R. odorata* ethanol extract and n-butanol fraction. Similarly, the inhibition values observed in samples Ju(R) ($56.02 \pm 1.18\%$) and Hy(T) ($58.80 \pm 2.38\%$) support the idea that plant extracts may have moderate-to-high CA inhibitory potential. Although lower inhibition rates (32.06–43.14%) were detected in samples coded Ma and Va, these results still demonstrate that enzyme activity can be significantly suppressed. Compared to the strong inhibitory effect of standard acetazolamide, these findings indicate significant biological potential for the development of natural compounds that could be alternatives to synthetic CA inhibitors, and this study makes a unique contribution to the literature.

As expected, a significant correlation (correlation coefficient, $r = -0.99$) was observed between the parameters related to the diuretic potential of samples (carbonic anhydrase inhibitory activity - % inhibition vs. IC_{50} parameters, inverse correlation) (Table 3). The carbonic anhydrase inhibitory activity of these samples is strongly correlated with rosmarinic acid, luteolin, taxifolin, and protocatechuic aldehyde content. Also, in these samples, the polyphenols content (TPC) is strongly correlated with the presence of flavonoids ($r = 0.94$), where quercetin, ellagic acid, epicatechin, and catechin are revealed with the highest contribution.

Table 3. The correlation coefficients between the biological activity and compounds from the phenolic profile of the analyzed all extracts

Determined compounds	Inhibition / in-vitro CA inhibitory potential (diuretic activity)	IC_{50}	EC_{50} / Antioxidant activity	TFC	TPC
Protocatechuic acid	0.17	-0.13	0.35	0.05	-0.15
Protocatechuic aldehyde	0.68*	-	-0.22	-0.19	-0.04
Catechin	0.12	-0.09	-0.38	0.85*	0.75*
Chlorogenic Acid	-0.54	0.52	-0.07	0.05	0.01
Epicatechin	0.06	-0.02	-0.42	0.88*	0.82*
Caffeic acid	0.56	-0.54	-0.37	-0.16	0.01
Vanillin	0.06	-0.08	0.71	-	-
Syringic acid	-0.05	0.05	0.28	0.69*	-0.64*
Syringic aldehyde	0.15	-0.13	-0.47	-0.25	-0.05
				-0.25	-0.09

Taxifolin	0.72*	- 0.68*	-0.56	0.27	0.42
P-Coumaric acid	0.26	-0.24	-0.40	0.20	0.33
Ferulic acid	-0.30	0.21	0.27	-0.22	-0.32
p_hydroxybenzoic acid	0.60	-0.58	-0.52	0.12	0.31
Salicylic acid	0.62	-0.60	-0.51	0.14	0.33
Rosmarinic acid	0.76*	- 0.74*	-0.41	0.01	0.18
Luteolin 7-O-glucoside	0.05	-0.07	-0.08	-0.24	-0.38
Rutin	-0.14	0.16	-0.30	0.49	0.43
Ellagic Acid	0.12	-0.08	-0.42	0.94 *	0.88*
Naringenin	0.63	- 0.64*	-0.34	-0.08	0.11
Quercetin	0.12	-0.07	-0.42	0.96 *	0.90*
Luteolin	0.74*	- 0.69*	-0.42	0.02	0.10
Apigenin	-0.34	0.38	-0.17	-0.18	-0.32
Inhibition activity	1.00	- 0.99*	-0.21	0.28	0.33
IC50	-0.99*	1.00	0.13	-0.22	-0.29
EC50	-0.21	0.13	1.00	-0.53	-0.52
TFC	0.28	-0.22	-0.53	1.00	0.94*
TPC	0.33	-0.29	-0.52	0.94 *	1.00

* correlations are significant at $p < 0.05$.

Principal component analysis (PCA)

The PCA analysis was applied to the obtained results to better understand how different secondary metabolites and bioactive compounds contribute to various biological activities such as antioxidant activity and diuretic effects. PC1 accounts for the largest variance (71.59%) of data indicating that it represents the dominant source of variability among samples. This axis can therefore be interpreted as reflecting differences in metabolite abundance associated with biological activity. PC2 explains an additional, smaller but still meaningful percentage of variance (26.92%). When combined with PC1, the cumulative variance explained by the first two components is 98.51%. Such a high cumulative variance implies that the major trends in metabolite differences across samples can be effectively visualized in two dimensions. By PCA score plot representation (PC1 and PC2) samples group according to similarities in their metabolomic composition, forming clusters that correlate with their biological activity (Figure 6a). *S. hortensis* (Sa(R) and Sa(T)) and *H. perforatum* (Hy(R) and Hy(T)) samples significantly differ from the rest of the samples, being differently located related to principal components PC1 and PC2. Also for these plants, some differences can be revealed between samples from Romania and Türkiye regions (Figure 6a). *M. chamomilla* (Ma(R) and Ma(T)), *J. communis* (Ju(R) and Ju(T)) and *V. officinalis* (Va(R) and Va(T)) samples are located together in the upper right corner, thus representing a group of plants with some similarity in the composition and biological activity profile regardless of the country of origin.

Projection of the variables along the factor plane (PC1 x PC2, PCA loading plot) was used to visualize how the original variables and different biological activities (inhibition, diuretic, and antioxidant activity) are correlated with each other and how they are related to the principal components (PC1 and PC2). The projection along PC1 essentially ranks samples by their antioxidant effectiveness (EC₅₀ parameters) and also diuretic activity (IC₅₀ parameters) revealing distinct groups (clusters) with high and low antioxidant activity while the projection along PC2 stratifies the samples by their enzyme inhibition potential (Figure 6b). In practical terms, PC1 essentially represents the axis along which samples are ordered by the abundance of radical-scavenging

constituents while PC2 appears to capture the aspect related to carbonic anhydrase (CA) inhibitory activity. Inspection of the metabolite loadings (Figure 6b) suggests that a distinct set of compounds including rosmarinic acid, salicylic acid, *p*-hydroxybenzoic and caffeic acids, protocatechuic aldehyde, naringenin and taxifolin align with carbonic anhydrase inhibitory activity. Ferulic acid, apigenin, chlorogenic acid and protocatechuic acid seems to align with both antioxidant and diuretic activity (EC_{50} and IC_{50} parameters). Intriguingly, many flavonoid and phenolics known for antioxidant effects are associated with carbonic anhydrase inhibition / diuretic activity. This aspect suggest a dual functionality of polyphenols that can serving as antioxidants and as CA inhibitors. So, samples rich in these bioactive metabolites tend to excel in both activities. Ferulic acid, chlorogenic acid, protocatechuic acid and apigenin are clustered together with antioxidant activity related parameters (EC_{50}) and located in a similar direction (positive loadings of PC1 and PC2, Figure 6b) indicating a substantial contribution of these compound (longer vectors mean stronger contributions) for antioxidant potential of *M. chamomilla* (Ma), *J. communis* (Ju) and *V. officinalis* (Va) samples. On the other hand, diuretic potential (inhibition of carbonic anhydrase activity) is correlated more with the compounds located along the negative PC1 loadings, revealing the rosmarinic, *p*-hydroxybenzoic, caffeic, and salicylic acids content associate strongly with the diuretic potential of samples.

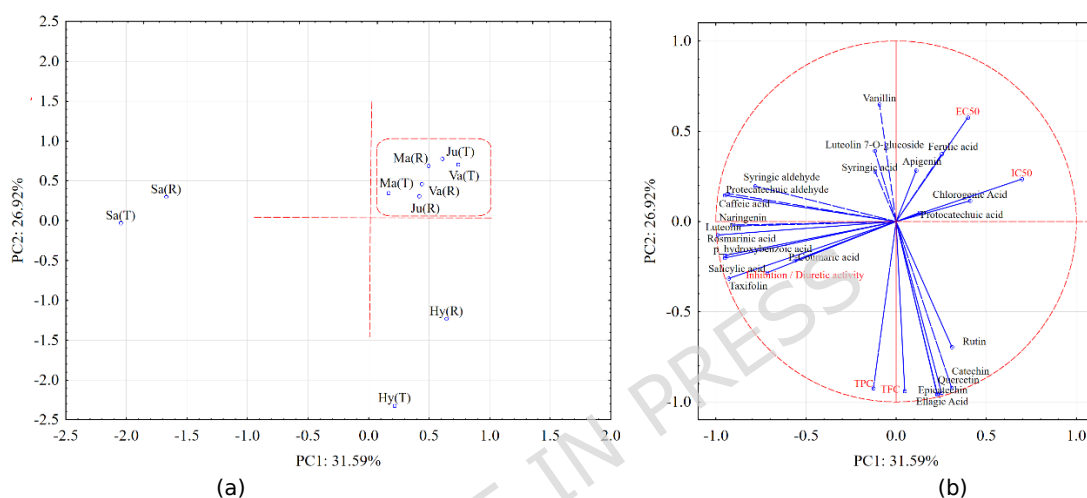


Figure 6. The PCA score plot of the experimental data illustrating the similarity/differentiation of the analyzed samples according to their geographic origin (a), and the corresponding PCA loading plot of the first two PCs compare the analysed parameters (b).

The above insights guide further analysis by examination of factor loadings (correlation coefficients between the original variables and the principal components) obtained by varimax rotation of the principal components. According to the obtained results (Table 4), PC1 shows very high loadings for compounds such as rosmarinic acid (+0.99), *p*-hydroxybenzoic acid (+0.97), salicylic acid (+0.97), taxifolin (+0.96), caffeic acid (+0.93), protocatechuic aldehyde (+0.90), naringenin (+0.90), and luteolin (+0.87). These loadings indicate that variations in the levels of these specific metabolites are highly correlated with PC1. In practical terms, samples with a high PC1 score are those containing elevated amounts of these compounds, whereas samples with low (or negative) PC1 scores have lower levels of them. Notably, rosmarinic acid stands out as the single highest contributor to PC1 (+0.99 loading), marking it as a key differentiator among the samples. This is significant because rosmarinic acid is a well-known bioactive phenolic acting as powerful antioxidant and, according to above discussed results, also as a potent inhibitor of carbonic anhydrase enzyme. Caffeic acid, which is biosynthetically related to rosmarinic acid (rosmarinic acid is an ester of caffeic acid and 3,4- dihydroxyphenyllactic acid)⁵³, also loads strongly on PC1 (+0.93). The strong presence of these compounds on PC1 suggests that PC1 captures phytochemical profile rich in certain phenolic acids like rosmarinic, caffeic, *p*-hydroxybenzoic acid, salicylic acid, protocatechuic aldehyde, naringenin and luteolin.

The second principal component is predominantly defined by flavonoid and polyphenol content. In contrast to PC1, PC2 shows its highest loadings for flavonoid glycosides and polyphenolic indices. Key contributors to PC2 (high PC2 loadings) include quercetin (+0.98), ellagic acid (+0.98), epicatechin (+0.97) and catechin (+0.94), all of which have substantial positive correlations with PC2. In addition, the measures of phenolics (total flavonoid content (TFC) and total phenolic content (TPC)) are very strongly correlated with PC2 (high loadings, 0.94 and +0.90 respectively). This indicates that PC2 essentially represents the overall polyphenol- richness of the samples (particularly flavonoid-richness), separating samples by their total

content of flavonoids and related polyphenols. Samples with high PC2 scores are those with generally higher TPC/TFC and higher concentrations of flavanol compounds (catechin, epicatechin) and flavonols (quercetin, ellagic acid). By contrast, PC2 has low and negative loadings for some simpler phenolics like vanillin (-0.72), suggesting those are more abundant in samples with lower PC2 scores.

Table 4. Factor loadings (varimax normalized coefficients related to the PCs correlation with the determined parameters) obtained for the analysed samples. (Marked loadings indicate a significant correlation)

Determined compounds	PC1	PC2	PC3
Protocatechuic acid	-0.18	-0.12	0.71
Protocatechuic aldehyde	0.90	-0.28	0.05
Catechin	-0.18	0.94	0.19
Chlorogenic Acid	-0.38	0.03	-0.74
Epicatechin	-0.12	0.97	0.08
Caffeic acid	0.93	-0.25	-0.17
Vanillin	-0.03	-0.72	0.51
Syringic acid	0.09	-0.26	-0.21
Syringic aldehyde	0.77	-0.27	-0.35
Taxifolin	0.96	0.17	0.14
P-Coumaric acid	0.57	0.09	0.22
Ferulic acid	-0.26	-0.26	-0.66
p_hydroxybenzoic acid	0.97	0.06	-0.04
Salicylic acid	0.97	0.08	-0.04
Rosmarinic acid	0.99	-0.07	0.06
Luteolin 7-O-glucoside	0.10	-0.33	-0.61
Rutin	-0.21	0.72	0.12
Ellagic Acid	-0.09	0.98	0.08
Naringenin	0.90	-0.12	0.10
Quercetin	-0.09	0.98	0.08
Luteolin	0.87	-0.09	-0.00
Apigenin	-0.16	-0.29	-0.02
Inhibition activity	0.73	0.15	0.34
in-vitro CA inhibitory potential (diuretic activity)	-0.71	-0.11	-0.31
Antioxidant activity	-0.49	-0.55	0.30
TFC	0.09	0.94	0.03
TPC	0.26	0.90	0.01

The developed PCA integrated analysis suggests that by looking at a sample's position on the PCA score plot representation, one can predict its relative bioactivity profile. Sample located with high PC1 scores can be expected to be characterized by elevated levels of radical-scavengers, whereas a high score for PC2 is characterized by the presence of specific CA-binding phytochemicals. This aspect can be used to easier reveal the variation in radical-scavengers and CA inhibitors phytochemicals along the samples from different geographical provenience (Romania and Türkiye). Based on this PCA approach, differences between samples from Romania and Türkiye were revealed related to both antioxidant and diuretic profile of the investigated samples. These differences can be due to the atmospheric circulation, seasonal variations in solar radiation, planetary and geographical factors, including the angle of sunlight incidence, and the distribution of land, seas, and oceans, all contribute to the Earth's principal climatic conditions. Due to these factors, climate affects the vegetation. Given the close relationship between climate and vegetation, many climate researchers focus on this interplay. The ecological conditions, soil composition, and distribution of vegetation in any geographical area are largely shaped by the prevailing climatic conditions. Consequently, the climate type in a given area plays a crucial role in determining ecological conditions and the geographical distribution of

plant life. Key global factors affecting vegetation distribution, secondary metabolite composition, pharmacological effects, and plant characteristics include climate, harvest time, and harvest conditions. Findings from five different plant species collected from two distinct regions, Romania and Türkiye, further emphasized these relationships.

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Table 5. Diuretic potential, antioxidant activity, total flavonoids content (TFC), and total polyphenols content (TPC) determined for *S. hortensis* (Sa), *J. communis* (Ju), *M. chamomilla* (Ma), *H. perforatum* (Hy), and *V. officinalis* (Va) samples.

Sample No.	Sample code	in-vitro CA inhibitory potential (diuretic activity)		Antioxidant activity	TFC	TPC
		% Inhibition (μg acetazolamide /mL) $\pm$ SD	IC50 (μL extract) $\pm$ SD	EC50 (ABTS assay, μL extract) $\pm$ SD	Total flavonoids (μg rutin /mL extract) $\pm$ SD	Total polyphenols (μg gallic acid /mL extract) $\pm$ SD
1	Sa(R)	64.10 $\pm$ 1.25	13.78 $\pm$ 1.62	1.28 $\pm$ 0.03	1.51 $\pm$ 0.01	4.82 $\pm$ 0.06
2	Sa(T)	64.87 $\pm$ 1.23	11.76 $\pm$ 1.58	1.01 $\pm$ 0.04	1.57 $\pm$ 0.01	6.98 $\pm$ 0.05
3	Ju(R)	56.02 $\pm$ 1.18	16.65 $\pm$ 2.35	3.64 $\pm$ 0.05	0.93 $\pm$ 0.01	1.71 $\pm$ 0.02
4	Ju(T)	49.77 $\pm$ 2.05	18.85 $\pm$ 2.57	12.21 $\pm$ 0.11	-	0.59 $\pm$ 0.01
5	Ma(R)	38.44 $\pm$ 1.11	23.77 $\pm$ 1.12	3.69 $\pm$ 0.08	1.24 $\pm$ 0.02	2.43 $\pm$ 0.07
6	Ma(T)	32.06 $\pm$ 2.03	28.93 $\pm$ 1.19	2.55 $\pm$ 0.03	1.44 $\pm$ 0.01	3.76 $\pm$ 0.07
7	Hy(R)	38.37 $\pm$ 1.73	24.95 $\pm$ 2.06	1.10 $\pm$ 0.02	3.53 $\pm$ 0.01	7.46 $\pm$ 0.12
8	Hy(T)	58.80 $\pm$ 2.38	15.94 $\pm$ 2.35	0.50 $\pm$ 0.02	9.77 $\pm$ 0.17	16.95 $\pm$ 0.221
9	Va(R)	43.14 $\pm$ 1.07	21.65 $\pm$ 1.09	9.60 $\pm$ 0.10	0.20 $\pm$ 0.01	5.05 $\pm$ 0.08
10	Va(T)	34.46 $\pm$ 2.35	26.79 $\pm$ 1.36	3.78 $\pm$ 0.03	0.42 $\pm$ 0.010	3.01 $\pm$ 0.02

*(R) - sample from Romania region; (T) - sample from Turke region; SD - standard deviation calculated for 3 replicates of sample

Conclusions

The findings of this research confirmed both Avicenna's ancient knowledge and literature data, and for the first time, simultaneously confirm the biological activities and chromatographic analysis with LC-MS/MS of 5 medicinal plants reported separately in the literature and that coincide in the geographies of Romania and Türkiye through comparison. For this reason, the in-vitro CA inhibitory potential (diuretic activity) of hydroalcoholic extracts from *S. hortensis*, *J. communis*, *M. chamomilla*, *H. perforatum*, and *V. officinalis*, was assessed based on carbonic anhydrase activity study, antioxidant activity determinations, and polyphenols and flavonoids screening analysis in this research.

In conclusion, this study demonstrates a significant correlation between the geographical origin of medicinal plants and their distinct phytochemical profiles, which in turn dictates their biological efficacy. Our comparative analysis identified Turkish *S. hortensis* as the most prominent source of rosmarinic acid, while Turkish *H. perforatum* was distinguished by its superior concentrations of chlorogenic acid, epicatechin, and quercetin, alongside the highest total phenolic and flavonoid content.

The research establishes a clear functional link between these chemical markers and enzymatic activity. Specifically, the high rosmarinic acid content in *S. hortensis* predominantly in samples from Türkiye followed by those from Romania was found to be the primary driver behind its potent carbonic anhydrase (CA) inhibitory activity. This suggests that rosmarinic acid serves as a pivotal metabolite for the plant's therapeutic diuretic potential. Conversely, the significant, yet lower, inhibitory activity observed in Turkish *H. perforatum* suggests that compounds like chlorogenic acid and epicatechin contribute to CA inhibition through a secondary or synergistic mechanism.

Geographical provenance was revealed to be a decisive factor in metabolite accumulation. While Turkish samples of *S. hortensis* and *J. communis* favored the production of rosmarinic and protocatechuic acids, Romanian *M. chamomilla* showed a specialized accumulation of chlorogenic acid. These regional variations, further validated by Principal Component Analysis (PCA), confirm that *S. hortensis* and *H. perforatum* possess unique biochemical signatures that set them apart from other species.

Ultimately, these findings highlight Turkish *S. hortensis* as a high potential candidate for the development of natural carbonic anhydrase inhibitors. This study not only supports the traditional use of these species but also emphasizes the necessity of considering geographical origin and specific phenolic fingerprints when standardizing plant-based diuretic therapies for future clinical applications.

Overall, for the first time, the findings in this study highlighted a correlation between the results of secondary metabolites (rosmarinic acid and luteolin) by LC-MS/MS and the inhibition of the carbonic anhydrase enzyme, indicating the therapeutic diuretic potential of *S. hortensis* in Türkiye, which supports further exploration of its application for carbonic anhydrase-related disorders. Recent research investigating plants utilized in traditional medicine has demonstrated that luteolin and rosmarinic acid exhibit significant diuretic activity, which is

accompanied by a potassium-sparing effect. This characteristic highlights the potential of luteolin and rosmarinic acid in promoting electrolyte balance and suggests their relevance in the context of health and wellness initiatives.

Materials and methods

Plant materials and sample preparation

The study was carried out on plant material from five medicinal plants belonging to different families (Table 6). The samples were acquired from specialized stores in Romania (R) and Türkiye (T) and assumed by the producers as certified materials in concordance with the Romanian and Turkish Pharmacopoeias regulations. A specimen/sample of each plant material used in this study is kept at the Chemistry Department of the Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Cluj-Napoca, Romania. According to the Herbarium Catalogue of the Kew Garden, the herbarium numbers are: Davis, P.H. [13324] Türkiye K002126186 for *H. perforatum*, Davis, P.H.; Hedge, I. [30359] Türkiye K000089242 for *J. communis*, Forbes [389] Türkiye K002338127 for *M. chamomilla*, Duman, H. [10681] Türkiye K001767073 for *S. hortensis* and Scheutz, N.I. [s.n.] Sweden Valeriana baltica K000762767 for *V. officinalis*.

All experimental research involving plant materials was conducted in compliance with relevant institutional, national, and international guidelines and legislation. The plant samples used in this study were commercially obtained from specialized herbal stores in Romania and Türkiye and were provided as certified materials in accordance with the Romanian and Turkish Pharmacopoeias. No wild plant species were collected directly from natural habitats, and therefore no specific collection permits were required.

For plant materials sourced from Türkiye, cultivation, harvesting, processing, and labeling practices complied with national regulations, including the Turkish Food Codex and relevant guidelines of the Ministry of Agriculture and Forestry. For plant materials obtained in Romania, compliance with European Union regulations on herbal products, phytosanitary standards, and labeling requirements integrated into national legislation under the Ministry of Agriculture and Rural Development (MADR) was ensured. All samples were handled, stored, and documented according to institutional research practices.

Table 6. Studied plant samples and their botanical and common names

No.	Common name	Binomial name	Family	Part of plant	Sample code*
1	Summer savory	<i>Satureja hortensis</i> L.	Lamiaceae	Herba	Sa(R); Sa(T)
2	Common juniper	<i>Juniperus communis</i> L.	Cupressaceae	Fructus	Ju(R); Ju(T)
3	Wild chamomile	<i>Matricaria chamomilla</i> L.	Asteraceae	Flos	Ma(R); Ma(T)
4	St. John's wort	<i>Hypericum perforatum</i> L.	Hypericaceae	Herba	Hy(R); Hy(T)
5	Valerian	<i>Valeriana officinalis</i> L.	Caprifoliaceae	Radix	Va(R); Va(T)

*R – samples from Romania region; T – samples from Türkiye region

In order to perform the experiment, 10 grams of plant material were finely ground using an herb grinder. Subsequently, the powdered samples underwent further processing using Retsch MM400 ball mill (Retsch, Haan, Germany). In the cold maceration process⁵⁴, 4 grams of the milled samples were accurately weighed and placed into glass tubes. These tubes were then filled with a 40 mL solvent mixture of ethanol and water (70:30 v/v). The filled glass tubes were sealed and left undisturbed for ten days, with daily mixing. After the ten-day period, the extracted samples were filtered using a 0.45 µm syringe filter. The resulting filtrates were used for HPLC-MS/MS analysis and spectrophotometric determinations of total flavonoids content, total polyphenols content, antioxidant activity, and in-vitro diuretic properties, respectively.

Chromatographic analysis

A number of 35 polyphenols were screened via LC-MS/MS using a Dionex Ultimate 3000 model Thermo Scientific UHPLC system interfaced with a tandem mass spectrometry (TSQ Quantum Access Max) apparatus. The names of the analyzed polyphenols and the LC-MS/MS conditions are presented in Table 7. The method linearity was evaluated for all of the screened compounds.

Table 7. LC-MS/MS conditions for polyphenols analysis. Parent ion (m/z): Molecular ions of the standard compounds, MS/MS(CE): MRM fragments for the related molecular ions (CE refers to related collision energies of the fragment ions).

No	Analyte	Parent ion (m/z)	MS/MS (CE)	Ionization Mode
1	Pyrogallol	124.9	69.3 (20)-79.3 (23)	-
2	Gallic Acid	169.7	80.5 (25)-126.2 (16)	-
3	Protocatechuic acid	155.0	65.4 (22)-93.2 (13)	+
4	Protocatechuic aldehyde	136.9	92.3 (25)-108.2 (25)	-
5	Catechin	289.2	203.9 (22) - 245.7 (17)	-
6	Gentisic acid	153.7	109.5 (21)	-
7	Chlorogenic Acid	353.4	86.50 (43)	-
8	Epicatechin	291.5	192.1 (21)	-
9	Caffeic acid	179.7	123.3 (15) - 139.3 (16)	-
10	Vanillin	150.9	135.2 (27) - 136.2 (18)	-
11	Syringic acid	183.07	92.3 (23)-136.1 (16)	-
12	Syringic aldehyde	180.88	77.3 (23)-123.2 (13)	-
13	Taxifolin	303.0	151.1 (24)-166.1 (16)	-
14	P-Coumaric acid	163.9	126.2 (23)-285.5 (15)	-
15	Ferulic acid	195.0	94.3 (33) - 120.2 (17)	-
16	Sinapic acid	223.0	89.4 (30)- 177.4 (7)	+
17	Salicylic acid	137.1	149.1 (22)-164.1 (17)	-
18	p_hydroxybenzoic acid	137.9	65.5 (35)-93.3 (18)	-
19	Hesperidin	608.8	66.6 (38) - 94.6 (17)	-
20	Rosmarinic acid	359.2	164.0 (60)-301.3 (32)	-
21	Oleuropein	539.1	134.3 (44) - 162.2 (20)	-
22	Luteolin 7-O-glucoside	446.9	275.8 (22) - 377.5 (16)	-
23	Rutin	609.37	284.0 (45)-285.0 (40)	-
24	Resveratrol	228.9	300.6 (38) - 301.7 (34)	-
25	Ellagic Acid	301.7	107.2 (22) - 135.1 (14)	+
26	Cinnamic acid	147.8	284.8(30)-174.2 (34)	-
27	Naringenin	273.0	78.4 (22)-104.7 (13)	-
28	Quercetin	301.0	147.1 (20) - 153.0 (24)	+
29	Luteolin	287.0	152.1 (23) - 179.9 (20)	-
			135.1 (32)- 153.0 (31)	+

30	Kaempferol	286.9	153.0 (33) - 165.0 (28)	+
31	Apigenin	268.9	117.1(40)- 149.1 (27)	-
32	Chrysin	254.97	103.2 (33)- 153.1 (30)	+
33	Pinocambirin	257.1	153.11 (23)-103.3 (34)	+
34	Galangin	268.9	169.1 (29)-171.1 (31)	-
35	Flavone	222.9	77.3 (35)-121.2 (26)	+

Chromatographic separation was achieved using a C18 reversed-phase Inertsil ODS HYPERSIL analytical column (250 mm × 4.6 mm, 5 μm)^{55,56}, under following parameters: flow rate - 0.7 mL/min.; column temperature - 30 °C; injection volume - 20 μL; running time - 34 min; mobile phase composition - (A) formic acid 0.1 % and (B) methanol; gradient elution according to following program: A (100%) - B (0%) at 0 min; A(100%) - B (0%) 1 min; A (5%) - B (95%) at 22 min; A (5%) - B (95%) maintain for 3 min; A (0%) - B (100%) maintain for 3 min. The gradient elution program was specifically optimized to achieve the best possible resolution and peak capacity for the complex mixture of secondary metabolites present in the plant extracts. By starting with a highly aqueous mobile phase (100% A) and gradually increasing the organic modifier (Methanol) to 100%, we ensured the effective separation of a broad spectrum of compounds, ranging from highly polar phenolic acids to more lipophilic flavonoids. This 34-minute program, as previously utilized and validated in studies^{55,56}, provides a balance between adequate separation of co-eluting peaks and an efficient total run time. The gradient was optimized for the separation of diverse metabolites. A formal matrix effect assessment was not performed; however, validated protocols^{55,56} were followed to ensure data consistency. The MS/MS conditions were: capillary temperature - 300 °C; vaporizer temperature - 350 °C; sheat gas pressure - 30 Arb.; auxiliary gas pressure was 13 Arb.; spray voltage (positive polarity) - 4000 V; spray voltage (negative polarity) - 2500 V, and discharge current - 4 μA. LC-MS/MS data obtained for the quantitation of the phenolic compound were analyzed using Xcalibur 2.2 SP1 version software (URL link: <https://www.thermofisher.com/order/catalog/product/OPTON-30965>).

Total flavonoid content

Total flavonoid content (TFC) was determined by using the spectrophotometric AlCl₃ method⁵⁷. Volume of 0.5 mL hydro-alcoholic extract diluted 1:10 (v/v) with ethanol-water (70:30, v/v) was mixed with 2.5 mL AlCl₃ solution (100 g/L) and 1.5 mL CH₃COONa solution (25g/L) and brought up with methanol to 10 mL (volumetric flask). After 15 min, the absorbance was measured at 430 nm. The reference solution was prepared in the same manner but using water (4 mL) instead of aluminum chloride and sodium acetate solutions. The TFCs results were reported as rutin equivalents (μg rutin/mL extract) calculated based on the linear calibration curve ($y = 0.0258x \pm 0.0088$; $R^2 = 0.9995$) obtained using a solution of known concentration of rutin and plotted in the range of 1 - 50 μg/mL.

Total polyphenol content

Total polyphenol content (TPC) was determined by the Folin-Ciocalteu spectrophotometric method^{55,58}. Volumes between 100-1000 μL of hydroalcoholic extract diluted (1:10) with ethanol-water (70:30, v/v) were mixed with 0.5 mL of Folin-Ciocalteu reagent and brought to 25 mL (volumetric flask) with Na₂CO₃ solution (16%). Absorbance was measured at 715 nm after 3 min. The reference solution was prepared the same way as the sample, but without the reagent. TPCs' results were reported as gallic acid equivalents of (μg gallic acid /mL extract) calculated based on the linear calibration curve ($y = 0.0979x \pm 0.0135$; $R^2 = 0.9990$) obtained using a solution of known concentration of gallic acid and plotted in the range of 0.234 - 4.672 μg/mL.

Antioxidant activity assay

The antioxidant potential of the hydroalcoholic extracts of *S. hortensis* (Sa(R);Sa(T)), *H. perforatum* (Hy(R); Hy(T)), *J. communis* (Ju(R); Ju(T)), *M. chamomilla* Ma(R); Ma(T)), and *V. officinalis* (Va(R); Va(T)) was determined by spectrophotometric assay using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺⁺) radical. In this assay, the ABTS⁺⁺ stock solution was prepared according to the Ozcan protocol⁵⁹. For this, equal volumes of ABTS solution of 7 mmol/L concentration and solution of 2.45 mmol/L potassium persulfate were mixed and allowed to stay in dark conditions at room temperature for 16 hours before use. The ABTS⁺⁺ working solution was obtained by diluting the stock solution with water until an absorbance of 0.7 ± 0.05 was reached. Aliquot of 4 mL of this solution was mixed with different volumes of diluted 1:10 (v/v) hydroalcoholic extract and then brought to a final volume of 5 mL. After a reaction time of 60 minutes in dark conditions, the absorbance was measured at $\lambda=752$ nm. The percentage of consumed radicals was determined using the equation:

$$\text{Consumed ABTS}^{++} (\%) = [(A_0 - A_f)/A_0] \times 100$$

where A_0 is the absorbance of the ABTS⁺⁺ solution (without the addition of the extract); A_f is the final absorbance of the ABTS⁺⁺ solution after 60 minutes of reaction with the added extract. The antioxidant capacity was expressed as efficient concentration parameters (EC_{50})⁶⁰, parameter, denoting the volume of extract (in μL) required to diminish the initial concentration of the ABTS⁺⁺ solution by 50%. In all cases, the EC_{50} parameter was determined based on the calibration curve obtained from the representation of the percentage of consumed ABTS⁺⁺ vs. the added volume of the extract and plotted in the range of 0.10 – 20 μL (added volume). The EC_{50} values mean inverse correlation with the antioxidant capacity of the sample, with lower EC_{50} values unequivocally indicating heightened antioxidant activity within the extract.

In-vitro CA inhibitory potential (diuretic activity) assay

The methodology used was based on the research conducted by Armstrong et al.⁶¹, with some modifications⁶². A substrate stock solution (S) of 3 mM *p*-nitrophenyl acetate was prepared by dissolving the appropriate amount in 10 mL of acetone-water (3:9.7, v/v). The carbonic anhydrase enzyme (human erythrocyte carbonic anhydrases) stock solution (E) was prepared by dissolving 2.71 mg of enzyme in 2 mL phosphate buffer at pH 7.0. To check the reaction between the substrate and enzyme, a substrate sample (S1) was prepared by mixing 0.5 mL substrate (S), 0.65 mL of phosphate buffer (pH 7.0), and 1.02 mL deionized water. The spectra were acquired within the wavelength range of 200-500 nm against a blank sample (a mixture of 0.33 mL of acetone and 9.7 mL of deionized water). Next, a mixture of substrate S solution (0.5 mL), enzyme solution E (20 μL), phosphate buffer (0.65 mL), and deionized water (1.0 mL) was prepared. The spectra were recorded in the range 200 nm – 500 nm. These two spectra were compared in terms of the differences.

The absorbance of samples obtained by 0.5 mL of the substrate solution (S), 0.65 mL of phosphate buffer, 20 μL enzyme solution (E), and 1 mL of hydro-alcoholic plant extract at different dilution ratios was measured at 400 nm against the blank sample. The inhibition activity, expressed in acetazolamide equivalents, was determined from the samples' absorbance using an acetazolamide standard inhibitor calibration curve in the range of 1–76 $\mu\text{g/mL}$ by the equation of $y = 0,0019x + 0,4972$. The results were standardized according to the acetazolamide range. $R^2 = 0,9978$. In-vitro CA inhibitory potential (diuretic activity) was determined according to Yung-Chi and Prusoff⁶², based on IC_{50} (50 % inhibition coefficient) of each plant's extract on carbonic anhydrase enzyme:

$$\% \text{ Inhibition} = [(A_0 - A_{\text{Sample}})/A_0] \times 100$$

where A_0 is the absorbance value for a sample consisting of 0.5 mL substrate solution S, 0.65 mL phosphate buffer, 20 μL of enzyme solution E, and 1 mL water, and A_S is the absorbance of a sample containing the inhibitor (1 mL diluted plant extract). Each determination was performed in triplicate. IC_{50} was expressed as the volume of plant extract that corresponds to a 50% inhibition of the carbonic anhydrase enzyme. The diuretic activity was calculated using efficient concentration parameters (IC_{50}), determined by linear regression of the relationship between the percentage of inhibition and the volume of added extract, as described in reference⁶².

Multivariate analysis of data

Principal component analysis (PCA) was applied for sample classification and revealed the relationships between determined parameters (phenolic compounds, total flavonoids, total polyphenols) and the antioxidant potential and in-vitro CA inhibitory potential (diuretic activity) of the analyzed extracts⁶³. In this approach, the score plot of the first two principal components (PC1 vs. PC2) represents a map of the classification of the analyzed samples. Samples located close to each other have similar profiles, whereas those far from each other are dissimilar.

Projection of the variables on the factor plane (loadings values representation) was used in order to interpret the meaning of the scores and reveal the correlations among the analyzed parameters and biological activities. In this approach, the representation is a circle where the horizontal axis represents principal component 1 (PC1) and the vertical axis represents principal component 2 (PC2). Inside the circle, we have arrows of various lengths pointing in particular directions. The length of the arrows represents how much that variable explains the variance of the data, while the angle between variables provides an indication of how well the variables are correlated. A slight angle indicates the two variables are positively correlated. An angle of 90 degrees indicates no correlation, while an angle of 180 degrees indicates a negative correlation of the variables in the factorial plane determined from all the analyzed characteristics.

Loadings obtained by the factor analysis approach with the varimax procedure application were used to reveal the contribution of determined variables to classification of the samples. For the PCA analysis and correlation coefficients determinations, Statistica 12.0 software package (StatSoft. Inc., 1984-2014) was used.

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Author contribution statement

Eda Bükür and Dorina Casoni: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing original draft preparation, writing review and editing, supervision.

Simona Codruța Aurora Cobzac: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing.

İbrahim Bilici, Ömer Kayır, and Hacer Doğan: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing original draft preparation, writing review and editing.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Disclosure statement

All authors have read and approved the final manuscript. There are no conflicts of interest for any author of this paper.

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