CHEMIJA
2025. Vol. 36. No. 3. P. 121–131

ISSN 0235-7216 eISSN 2424-4538
DOI: https://doi.org/10.6001/chemija.2025.36.3.2

Elemental composition and bioactivity of morphological parts extracts of winter savory (*Satureja montana* L.)

Dovilė Motiejauskaitė[1],

Renata Žvirdauskienė[1, 2],

Agnė Venckutė[1],

Karolina Barčauskaitė[1]*

[1] *Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, 1 Instituto Avenue, 58344 Akademija, Kėdainiai Distr., Lithuania*

[2] *Department of Food Science and Technology, Kaunas University of Technology, 19A Radvilėnų Road, 50254 Kaunas, Lithuania*

Winter savory (*Satureja montana* L.) is well known to exhibit strong antioxidant and antibacterial properties. Winter savory is rarely found growing wild in Lithuania; however, as a common garden plant, it has been the subject of only a few studies. The goal of this research was to determine the mineral composition of winter savory and evaluate how well seven different solvents compositions – deionised water, 50% methanol (MeOH), 75% MeOH, 50% ethanol (EtOH), 75% EtOH, 2% sodium dodecyl sulfate (SDS) and 2% Triton X-100 – could extract phytochemicals. The results showed that the elemental composition was dominated by calcium (from 4231.23 ± 546.48 mg kg^{-1} in stems to 11494.27 ± 785.01 mg kg^{-1} in leaves) and potassium (from 3626.69 ± 427.98 mg kg^{-1} to 6321.68 ± 241.53 mg kg^{-1}, respectively). It was also determined that the total concentration of phenolic compounds in the plant extracts varied depending on the solvent and morphological part used. Concentrations ranged from 23.91 mg g^{-1} in the stems when ethanol was used to 138.96 mg g^{-1} in the leaves when methanol was used. Additionally, phenolic acids ranged from 6.45 mg g^{-1} in the aqueous extract of flowers to 92.82 mg g^{-1} in the leaf extract obtained with the surfactant Triton X-100. Flavonoid content varied from 9.94 mg g^{-1} in flower extracts obtained with distilled water to 151.39 mg g^{-1} in leaf extracts when the surfactant SDS was used. Winter savory extracts exhibited a high radical scavenging activity by DPPH assays ranging from 13.01 to 85.71%. The strongest antibacterial effect was observed in 2% SDS stem extract against *Bacillus subtilis*. Considering all the analysis performed, we can conclude that SDS is the most effective solvent for winter savory.

Keywords: winter savory, metals, secondary metabolites, antibacterial activity

Abbreviations:
SDS – sodium dodecyl sulfate
MAE – microwave-assisted extraction
ICP-MS – inductively coupled plasma mass spectrometer
TPA – total phenolic acids
QE – equivalent of quercetin
CE – equivalent of catechin

INTRODUCTION

Winter savory (*Satureja montana* L.) is an aromatic plant natively found in the Mediterranean region but increasingly cultivated in other parts of Europe, including Lithuania [1, 2]. It is a perennial subshrub with bushy growth, featuring woody stems at the base, small linear leaves, and delicate flowers in shades of pale pink and white [3]. This

* Corresponding author. Email: karolina.barcauskaite@lammc.lt

plant holds a prominent place in Mediterranean cuisine. It is primarily employed as a seasoning to enhance the flavours of meats and fish, as well as a delightful addition to soups, sausages, etc. [1]. In traditional medicine, it is used to treat various ailments due to its antibacterial, digestive, expectorant, antifungal, laxative and diuretic effects [4]. Nevertheless, this plant shows promise in the field of plant protection due to its insecticidal and antibacterial properties [3, 5].

The presence of bioactive compounds and minerals in plants offers valuable insights into their medicinal and nutritional properties. Winter savory is rich in phenolic compounds and terpenes. Even though the composition may vary depending on the place of growth and ontogeny, carvacrol and thymol, as well as p-cymene and linalool are the most abundant biologically active compounds [3]. It was discovered that the essential oil of *Satureja montana* L. had notable amounts of carvacrol (29.19%), thymol (15.41%) and p-cymene (11.77%) [6]. During another study conducted by Miladi et al. (2013), even higher amounts of carvacrol (53.35%), also γ-terpinene (13.54%) and p-cymene (13.03%) were determined. These compounds are strong antioxidants, countering the harmful impact of free radicals and mitigating oxidative harm within plants. Additionally, they possess qualities like anti-inflammatory, anti-diabetic, cardioprotective, neuroprotective, antitumor and anti-aging properties [7].

As society becomes more aware, people are increasingly choosing products that are both health-conscious and environmentally friendly. This shift is also impacting on the food industry, with natural preservatives, colourings, flavour enhancers, and packaging materials gaining popularity. Winter savory, due to the significant levels of thymol and carvacrol, along with their appealing aroma and easy cultivation, is thus a promising food additive [8]. Miladi et al. (2013) found that *Satureja montana* L. essential oil was effective against many bacteria found in food, although the results showed that it is more effective against Gram-positive bacteria such as *M. luteus* and *L. monocytogenes* than Gram-negative bacteria. However, it exhibits antimicrobial activity against 13 reference strains and 31 strains belonging to *Salmonella* genus [1]. These findings suggest that winter savory can become an excellent component of food additives to protect food against pathogens, but further research is needed to determine the safety of incorporating the plant into food products. Additionally, it was reported that *Satureja montana* L. showed antibacterial and insecticidal properties. Navarro-Rocha et al. (2020) discovered that hydro-distilled essential oils of re-domesticated *Satureja montana* L. using a 100 μg cm^2 dose was effective and could be very promising in the fight against such pests as *S. littoralis*, *L. decemlineata* and *M. persicae* [3].

While there are different techniques for obtaining biologically active compounds, traditional methods are frequently utilised due to their simplicity, cost-effectiveness and well-documented higher yield [9]. Traditional methods are considered as those that involve using a range of volatile organic compounds: hydrocarbons (halogenated, aliphatic, or aromatic), esters (ethyl acetate), alcohols (methanol, ethanol), ethers, aldehydes and ketones (acetone) [10, 11]. Research confirms that, regardless of whether an eco-friendly method modification is used or not, organic solvents demonstrate better efficiency than others. For example, natural deep eutectic solvents are about 3 times less efficient than aqueous ethanol solution extracting polyphenols from corn cob [12]. As discussions about environmental concerns grow, the focus is on discovering extraction techniques that are both eco-friendly and effective. This has led to an increasing use of surfactants. Most popular are ionic compounds, such as sodium dodecyl sulphate (SDS) or cetyl trimethylammonium bromide (CTAB), and non-ionic compounds, such as Tritones (X-100 or X-114) [13, 14]. In our previous work, we investigated the influence of different solvents on the biological activity of extracts obtained from hemp inflorescence and found that the extracts produced using Triton X-100 are characterised by the highest amount of phenolic compounds compared to aqueous and methanolic extracts. The amount of compounds was approximately 1.3 times greater than that in the methanolic extract and about 5.8 times greater than that in the aqueous extract [15]. However, Triton 100-X extracts did not perform as well in other analysis, suggesting that surfactants extract some compounds better than others. This is confirmed by a study conducted by Miłek et al. (2019), where they found that Triton 100-X was more effective in extracting luteolin (22.466 ± 2.854 mg g^{-1}) from *Taraxacum officinale* flowers in comparison to the acetone extract (11.867 ± 1.185 mg g^{-1}). However, the amount

of chrysoeriol extracted using Triton 100-X (2.660 ± 0.367 mg g^{-1}) was lower than that obtained with the acetone (11.756 ± 1.520 mg g^{-1}) [13]. Furthermore, scientific research on major minor and trace metals content distribution in morphological parts of winter savory is lacking. Therefore, this paper aims to quantitatively evaluate the elemental composition and to determine the phytochemical, antioxidant and antibacterial properties of winter savory (*Satureja montana* L.) morphological parts using different solvents.

MATERIALS AND METHODS

Chemicals

Nitric (≥65%) acid of analytical grade was obtained from Sigma-Aldrich (Germany). The standard mixture solution of multiple microelements (As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sr, V, Zn) in 2% nitric acid was purchased from CPAchem (Bulgaria). Methanol was obtained from Sigma-Aldrich, France. Aluminium chloride anhydrous (pure for analysis) and sodium nitrite (pure for analysis) were purchased from Chempur, Poland. Caffeic acid ≥98% and rutin ≥97% were obtained from Acros Organics, Germany. Folin–Ciocalteu reagent and sodium carbonate were purchased from Sigma-Aldrich, Germany. Ethyl alcohol was obtained from JSC MV GROUP Production, Lithuania. Sodium hydroxide was purchased from Carl Roth, Germany. DPPH (1,1-Diphenyl-2-picrylhydrazyl Free Radical) was obtained from TCI EUROPE, Belgium. For all dilutions, bidistilled water was obtained using the distillation apparatus Thermo Scientific (USA).

Preparation of plant morphological parts

Three morphological parts (stem, leaves and flowers) of *Satureja montana* L. were analysed. Plants were collected from the field in Kaunas, Lithuania (54.942556, 23.887324). Harvested plants were divided into separate parts, air-dried and ground using a coffee beans mill. The samples were stored in a dark and dry place until analysis.

Sample digestion for inductively coupled plasma mass spectrometry (ICP-MS) analysis

Microwave-assisted extraction (MAE) was carried out using a CEM MARS 6® (Matthews, NC, USA) digestion system equipped with a 100 mL Teflon vessel. Approximately 0.3 g of homogenised sample was accurately weighed into a Teflon vessel and digested using 10 ml concentrated nitric acid. Prior to digestion, the samples were soaked in acid for 30 min at room temperature. Digestion was performed under the following conditions: 200°C temperature, 800 psi pressure, 20 min ramp time, 20 min hold time and 1600 W microwave power. Afterwards, the digested sample was cooled down and thoroughly transferred into a 50 ml volumetric flask and diluted using bidistilled water till the mark. Each sample was prepared in triplicate and the blank sample was included in each digestion run.

Preparation of extracts for spectrophotometric analysis

Seven different solvents were used for the preparation of leaves and stems extracts for spectrophotometric analysis: bidistilled water, 50% methanol, 75% methanol, 50% ethanol, 75% ethanol, 2% SDS and 2% Triton X-100. For the preparation of flower extracts four solvents – bidistilled water, 50% methanol, 50% ethanol and 2% SDS – were used due to the lack of plant resources. The extract was prepared based on the methodology previously described by Barčauskaitė et. al. (2022) with minor corrections [16]. 0.5 g of powdered material was soaked with 10 ml of solvent and treated in an ultrasonic bath (VWR International, Malaysia) at 60°C for 60 min. Thereafter, the extract was filtered through a paper filter (weight 90 g/m^2) into a test tube and stored at +4°C until analysis. The extracts were used to determine total phenolic compounds, total flavonoid and total phenolic acids content, free radical scavenging activity and antibacterial activity.

Mineral composition analysis by inductively coupled plasma mass spectrometry

ICP-MS analysis was performed under helium collision-cell (He-cell) with the kinetic energy discrimination mode to remove polyatomic interferences. Samples were introduced from an autosampler incorporating an ASXpress™ rapid uptake module (Cetac ASX-520, Teledyne Technologies Inc., USA) through a PEEK nebuliser (Burgener Mira Mist, Mississauga, Burgener Research Inc., Canada). Analysed micro (Fe, Zn, Si) and macro (Ca, K, Mg, P) elements, as well as trace metals (Na, Co, Pb), were estimated using an external multi-element seven-point calibration

curve in the range 20–2000 μg l^{-1} (20, 50, 100, 200, 500, 1000, 2000 μg l^{-1}).

SPECTROPHOTOMETRIC MEASUREMENTS

Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu colourimetric method, using rutin as a standard phenolic compound (0.0625–1.9996 mg ml^{-1}) as previously described by Barčauskaitė el al. (2022) [16]. 0.1 ml of extract was mixed with 2.5 ml of bidistilled water, followed by the addition of 0.1 ml Folin–Ciocalteu reagent and 0.5 ml 20% sodium carbonate. The absorbance of the resulting blue solution was measured at 760 nm after 30 min of incubation in the dark and the concentration (mg/g^{-1}) was calculated from the equation of the standard curve: $y = 0.9282x + 0.171$ ($R^2 = 0.9927$).

Total phenolic acids

Total phenolic acids (TPA) were determined using the colourimetric method with an Arnow reagent, using caffeic acid as a standard phenolic acid (0.03125–1.9992 mg ml^{-1}). 0.5 ml of the extract was mixed with 1 ml 0.5M HCl, followed by the addition of 1 ml 8.5% NaOH, 1 ml Arnow reagent and 1.5 ml bidistilled water. The absorbance of the resulting yellow solution was measured at 505 nm and the concentration (mg g^{-1}) was calculated from the equation of the standard curve: $y = 0.2364x + 0.0699$ ($R^2 = 0.9979$).

Total flavonoid content

Total flavonoid content was determined using the spectrophotometric method based on the formation of flavonoid complex with Al^{3+} ions, using rutin as a standard flavonoid (0.0625–1.9996 mg/ml) as previously described by Barčauskaitė et al. (2022) [16]. 0.25 ml of the extract was mixed with 1.35 ml of bidistilled water, followed by the addition of 0.75 ml 5% $NaNO_2$ and 0.15 ml 10% $AlCl_3$. The absorbance of the resulting yellow solution was measured at 510 nm after 30 min of incubation in the dark and concentration (mg g^{-1}) was calculated from the equation of the standard curve: $y = 0.2335x + 0.0675$ ($R^2 = 0.9998$).

Antioxidant activity

The antioxidant activity of the extracts was determined according to the previously used our method by using the 2,2-diphenyl-2-picrylhy-drazyl (DPPH) free radical scavenging activity method [15]. 0.077 ml of the extract was mixed with 3 ml $6 \cdot 10^{-5}$ M DPPH. The absorbance of the resulting faded pink or yellow solution was measured at 515 nm after 15 min of incubation in the dark. DPPH radical scavenging activity (%) was calculated using the formula:

$$\text{DPPH radicals cavenging activity} = \frac{\text{Abs of blank} - \text{Abs of sample}}{\text{Abs of blank}} * 100\%.$$

Antibacterial activity

Antibacterial activity was determined using the agar well diffusion method against seven different bacteria: *Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 13525, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 13932. Each bacterial suspension with a concentration of 1.0×10^6 cfu $\cdot$ ml^{-1} was prepared from an 18 h bacterial culture according to the 0.5 McFarland standard. 100 ml of prepared PCA (Plate count agar, Liofilhem Italy) medium was inoculated with 1 ml of bacterial suspension, mixed well, and 10 ml each of the mixture dispensed into empty petri dishes. After preparing the petri dishes with cultures of different microorganisms, they are left to solidify at room temperature. 40 μl of extract was then transferred to a precut circle well (d = 9 mm) in each plate in triplicate. Plates were incubated at 37°C for 24 h. The antibacterial activity of extracts was determined by measuring diameters of inhibition zones using a calliper. The degree of bacterial susceptibility was evaluated based on the radius of the inhibition zone which was calculated using the formula:

$$\text{Radius} = \frac{\text{diameter of inhibition zone} - \text{diameter of the well}}{2}.$$

Bacterial susceptibility was categorised into four levels based on the size of the inhibition zone: very susceptible when the radius of inhibition zone exceeds 10 mm, susceptible when the zone is between 5 and 10 mm, slightly susceptible between 4 and 5 mm, and when the zone is less than 4 mm, bacteria are considered as resistant.

Statistical analysis

All experiments were performed in triplicate. The results were expressed as mean ± standard deviation (SD). The Microsoft Office Excel software was used to perform data analysis. The statistical analysis was done by one-way ANOVA or t-test where only two variables were analysed. Statistical analysis was conducted using the software Jamovi. The difference was considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Mineral composition analysis by ICP-MS

Samples of morphological parts of winter savory were analysed for 10 elements (Na, Mg, Si, P, K, Ca, Fe, Co, Zn, Pb) categorised as major, minor and trace elements. The concentrations of Na, Co and Pb were below detection limits in all samples. Table 1 represents the composition of major (Ca, K, Mg, P) and minor (Si, Fe, Zn) elements in different *Satureja montana* L. morphological parts. It was found that winter savory is rich in Ca (11494 ± 785 mg kg^{-1}) which was most abundant in the samples of leaves. Similar results were obtained in a previous study by Zeiner et al. (2015), where they determined that Ca levels found in different plant organs are much higher than other elements; the amount varies from 16022 mg kg^{-1} in shoots to 27940 mg kg^{-1} in leaves [17]. We also observed potassium to be the second most abundant element found in winter savory with an average content ranging from 3627 mg kg^{-1} in stems to 6322 mg kg^{-1} in leaves, followed by phosphorus and magnesium.

Among the minor elements, the highest concentration was that of silicon (108.48 ± 2.73 mg kg^{-1}), which was predominantly found in the leaves. To our knowledge, we found no analysis conducted to assess the content of all metals in *Satureja montana* L. plants. According to our analysis, Si is the dominant minor element in winter savory plants, followed by iron and zinc. Nevertheless, a study conducted previously confirms that Fe is found in about 3 to 4 times more than Zn, depending on the part of the plant [17]. This research showed that winter savory grown in Lithuania contains about 6.45 times more Fe than Zn, reaching 94.04 ± 9.33 mg kg^{-1} in the dry weight of leaves. Iron is an essential micronutrient for plants, playing a crucial role in various morphological parts and physiological processes. Its significance can be observed throughout a plant's life cycle, from seed germination to overall growth and development. Moreover, Fe is an indispensable element for various metalloproteins that participate in critical biochemical pathways and processes within plants. These metalloproteins enable plants to perform essential functions, such as energy production, nitrogen assimilation, photosynthesis, chlorophyll synthesis, respiration, uptake mechanisms, oxygen transport, DNA replication, and protection against oxidative stress. Without adequate Fe, these processes can be compromised, leading to various physiological and growth issues in plants [18].

Among all the samples, the highest concentrations of elements were found in leaves while the smallest concentrations were observed in stems. Gupta et al. (2019) state in their study that metals usually end up in the leaves or fruits of

Table 1. **The composition of major (Ca, K, Mg, P) and minor (Si, Fe, Zn) elements in different *Satureja montana* L. morphological parts**

Leaves		Concentration, mg kg^{-1}		
		Steams	Flowers	
Macro elements	Ca	$11494^{a**} \pm 785$*	$4231^{b} \pm 546$	$5199^{b} \pm 531$
	K	$6322^{a} \pm 242$	$3627^{b} \pm 428$	$3959^{b} \pm 259$
	Mg	$2699^{a} \pm 157$	$1368^{b} \pm 85$	$792^{c} \pm 103$
	P	$1592^{a} \pm 85$	$843^{c} \pm 58$	$1251^{b} \pm 86$
Micro elements	Si	$108.48^{a} \pm 2.73$	ND	$67.81^{b} \pm 4.41$
	Fe	$94.04^{a} \pm 9.33$	$13.53^{b} \pm 0.94$	$86.57^{a} \pm 9.13$
	Zn	14.57 ± 1.88	ND	3.77 ± 0.92

Note: * Standard deviation; ** Values in the same row with different letters are statistically different at a significance level of $p < 0.05$; ND, not detected.

the plant [19]. Although when it comes to trace metals, this trend is frightening, because large amounts of them become toxic to both plants and humans. However, after analysing winter savory, we note that the concentrations of these metals are low, and such dangerous metals as cobalt were not even detected.

Total polyphenol content

The impact of various solvents (dH_2O, 50 and 75% MeOH, 50 and 75% EtOH, 2% SDS, 2% Triton X-100) on the total polyphenol content extracted from winter savory leaves, stems and inflorescences are illustrated in Fig. 1. 50% MeOH is the most effective solvent for all morphological parts of the plant. The highest amount was found in the leaves and reached 138.96 mg g^{-1}. However, slightly higher polyphenol contents in stems and flowers were found in extracts obtained using 2% SDS (42.63 and 81.76 mg g^{-1}, respectively) compared with 50% MeOH extracts (34.52 and 62.90 mg g^{-1}, respectively). Conversely, extraction with distilled water (dH_2O) proved to be the least effective for extracting polyphenolic compounds from all the morphological parts of winter savory. Our results are in line with Turkmen et al. (2006) findings that distilled water as a solvent has the lowest efficiency, and as the concentration of organic solvents increases, the efficiency decreases. Additionally, the most effective concentration for methanol, ethanol and acetone was found to be 50% [20]. These findings can be explained by the polarity of the solvent and the fact that water has the ability to help extractable compounds flow across plant tissues through diffusion [21].

Total phenolic acids content

Figure 2 shows the effect of different solvents on the total phenolic acids yield in winter savory leaves, stems and flowers. Extraction with 2% Triton X-100 yields better than other solvents regarding TPA in leaves and steam extracts, with an average value of 92.82 and 18.88 mg g^{-1}, respectively. However, out of the four flower extracts examined (dH_2O, 50% MeOH, 50% EtOH and 2% SDS), 50% MeOH showed the best result regarding the TPA

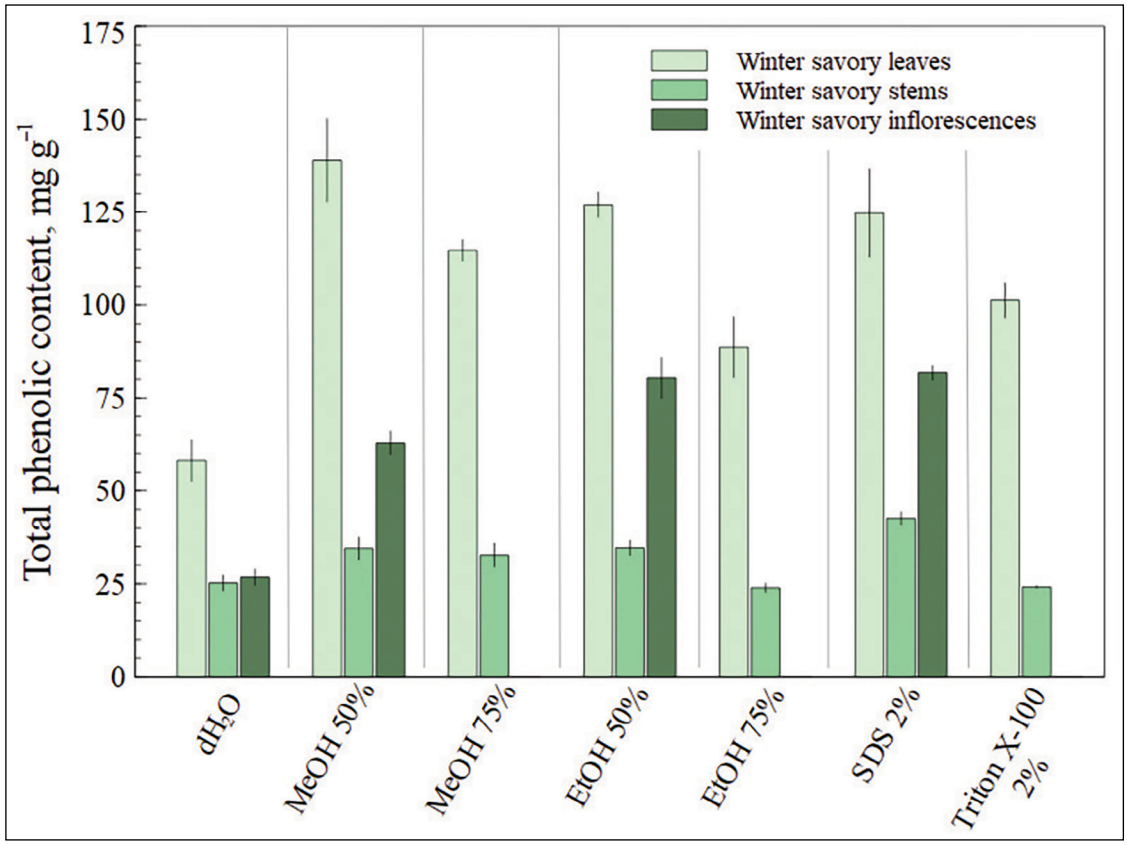

Fig. 1. Amount of total polyphenolic compounds content extracted from winter savory (*Satureja montana* L.) using different polarity solvents

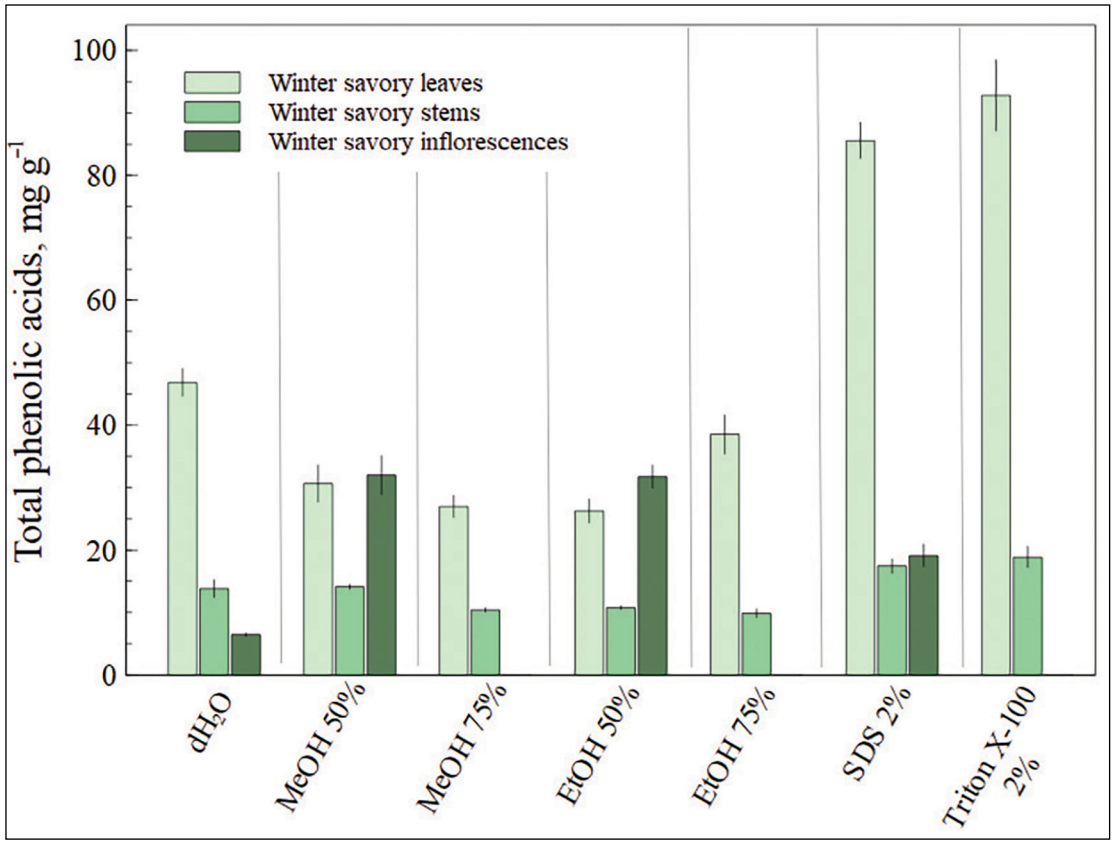

Fig. 2. Amount of total phenolic acids content extracted from winter savory (*Satureja montana* L.) using different polarity solvents

content, which reached 31.98 mg g^{-1}. The literature mainly describes the qualitative composition of phenolic acids of winter savory; however, Ćetković et al. (2007) reported that the total phenolic acid content in the ethyl acetate extract was 47.59 μg g^{-1}, whereas in the n-butanol extract, it was 96.70 μg g^{-1} [22]. Comparing the results indicates that the solvents used in this study are significantly more effective at extracting phenolic acids from *Satureja montana* L., yielding approximately 333 times more than reported in a previous study.

Total flavonoid content

The total amount of flavonoid content is summarised in Fig. 3. The results of the flavonoid content analysis indicated that sodium dodecyl sulfate (SDS) is a better extraction solvent than other used solvents for flavonoid extraction from *Satureja montana* L. leaves. Notably, we extracted nearly twice as many flavonoids from winter savory leaves (151.39 mg g^{-1}) compared to flowers (77.25 mg g^{-1}) using 50% ethanol. Milek et al. (2019) explained this phenomenon by the fact that leaves could have been drier and finer. The flowers may have retained more moisture, which likely made them less prone to breaking down during grinding. This could have impacted solvent penetration, resulting in lower flavonoid recovery. Interestingly, it was found that 75% ethanol was the least effective solvent for extracting flavonoids from the leaves and stems [13].

The total flavonoid concentrations identified in this study when using alcohol-based solvents were slightly lower than those reported in previous studies. Zeljković et al. (2015) reported that the methanolic and chloroform extracts of *Satureja montana* L. showed that the total flavonoid content reached 75.61 ± 3.519 mg QE g^{-1} [23] which was about 1.2 times higher than the results obtained in our study. Furthermore, another study reported that the total flavonoid content varied from 38.3 ± 8.1 to 67.0 ± 12.7 mg CE g^{-1} with the geographical location, playing a factor in the content [24]. However, it should be considered that both research groups analysed the whole plant and not its individual parts.

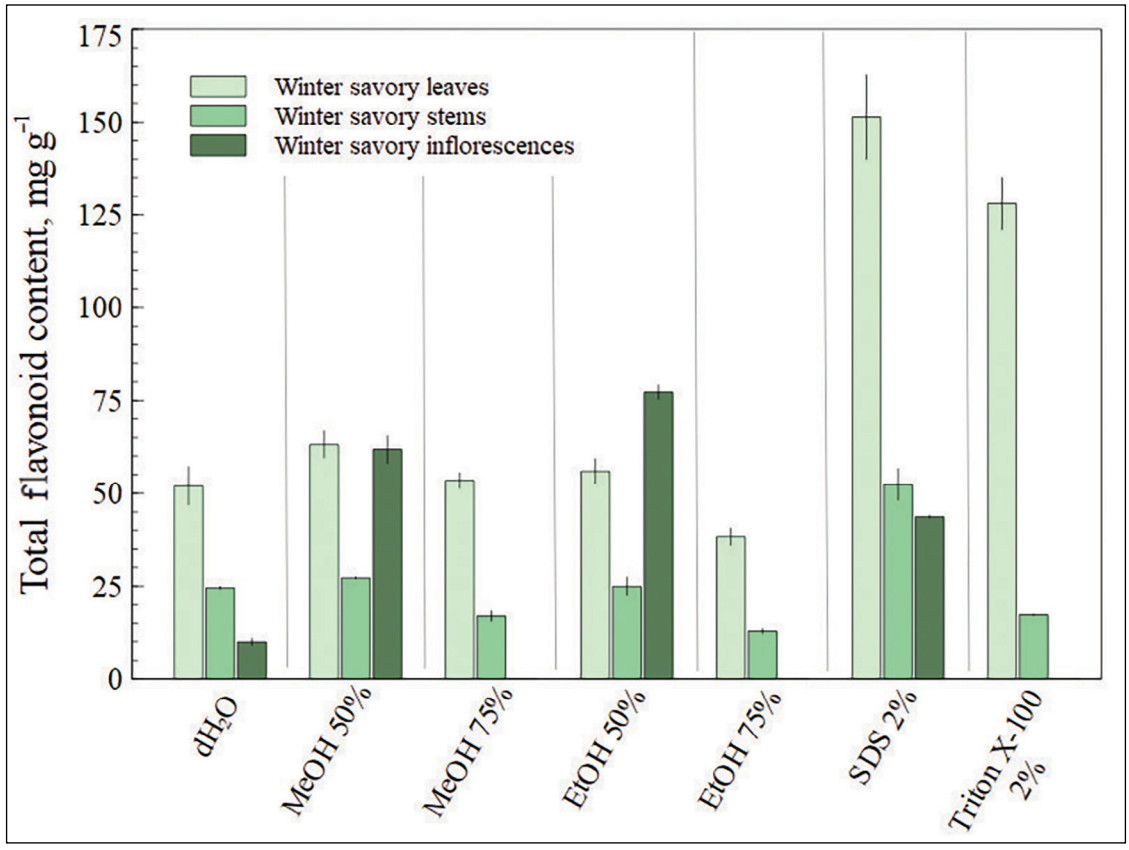

Fig. 3. Amount of total flavonoids content extracted from winter savory (*Satureja montana* L.) using different polarity solvents

Antioxidant activity

The antioxidant properties were evaluated using DPPH assay, and the corresponding results are presented in Fig. 4. It was established that to choose the most effective solvent, it is important to take into account what morphological part of the plant is used. For instance, for leaves, the most efficient solvent was 75% methanol followed by 50% methanol and 50% ethanol (84.95, 84.74 and 84.63%, respectively), for stems, 2% SDS (36.31%) and for flowers, 50% methanol and 50% ethanol (85.73 and 85.71%, respectively). Compared to the findings of Hajdari et al. (2016), our extracts showed an improved DPPH radical scavenging capacity, whereas their methanolic extracts reached only 51.8% activity [24]. Such differences in results could be caused by various factors, such as different concentrations of solvent used, the location of plant growth, and preparation techniques of the analysed sample.

Antibacterial activity

After evaluating the antibacterial activity of winter savory (*Satureja montana* L.) leaves and stem extracts using the agar diffusion method, it was observed that the winter savory grown in Lithuania does not have such strong antibacterial properties as reported in the literature from other parts of Europe [25, 26]. Extracts of winter savory grown in Serbia, obtained using an alcoholic solvent (n-Butanol), were effective in inhibiting *Staphylococcus aureus* and *Bacillus cereus*. Other results showed the effectiveness of ester ethyl acetate as a solvent with outstanding antimicrobial activities against four bacterial strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Sarcina lutea* and *Bacillus cereus* [25]. However, due to the different types of solvents used in our study, specifically alcohols and surfactants, direct comparisons with these results are not possible. In our research, the most effective solvents for obtaining the highest antibacterial activity from leaves and stems were surfactants: 2% SDS and 2% Triton X-100 (Table 2). Leaves extracts obtained using 2% SDS demonstrated a strong antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus*. Moreover, extracts obtained using 2% Triton X-100 were effective against

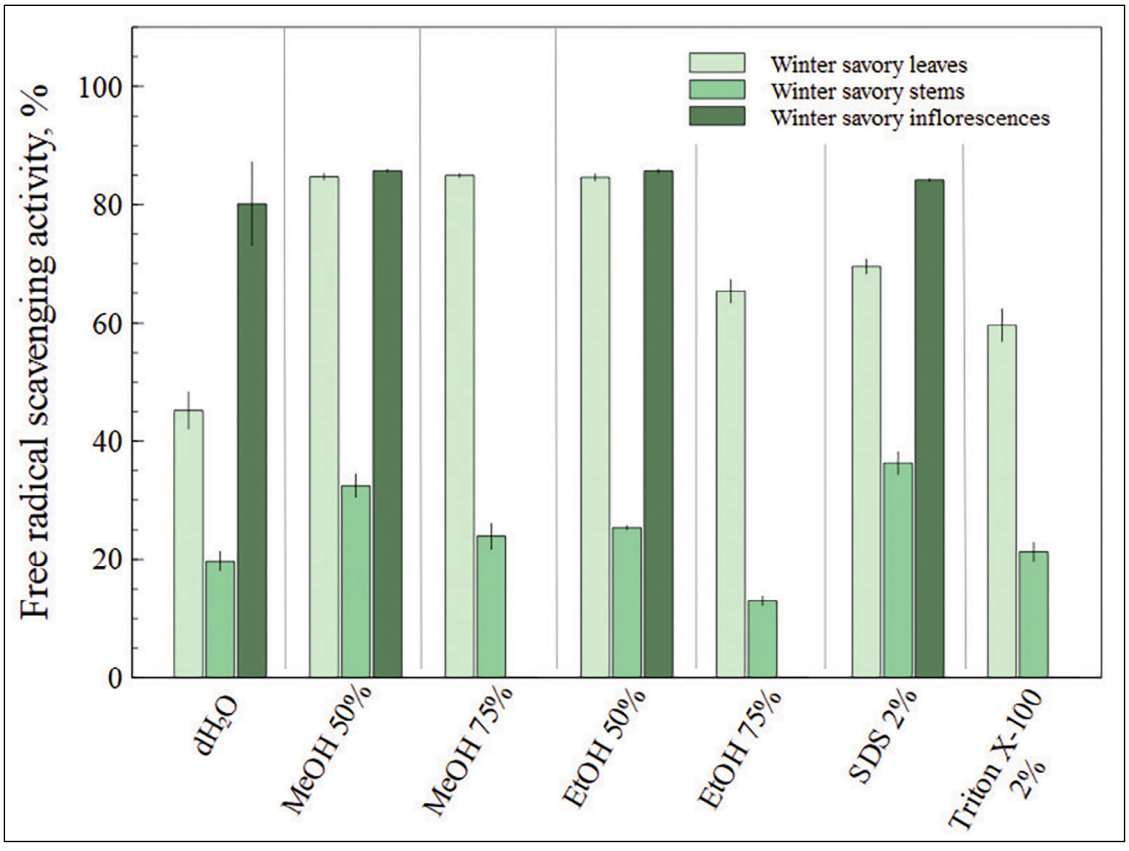

Fig. 4. Free radical scavenging activity of winter savory (*Satureja montana* L.) extracts extracted using different polarity solvents

Table 2. **Antimicrobial activity of different *Satureja montana* L. morfological parts extracts**

Morphological part	Solvent	Microorganism				
		E. coli	***B. subtilis***	***P. aeuruginosa***	***S. typhimurium***	***S. aureus***
Leaves	dH_2O	–	–	–	–	–
	MeOH 50%	–	–	–	–	–
	MeOH 75%	–	–	–	–	–
	EtOH 50%	–	–	–	–	–
	EtOH 75%	–	–	–	–	–
	SDS 2%	–	–	–	–	++
	Triton X-100 2%	–	–	++	–	+
Stem	dH_2O	–	–	–	–	–
	MeOH 50%	–	–	–	–	–
	MeOH 75%	–	–	–	–	–
	EtOH 50%	–	–	–	–	–
	EtOH 75%	–	–	–	–	–
	SDS 2%	–	+++	–	–	++
	Triton X-100 2%	–	–	++	–	++

Note: +, weak antimicrobial activity detected; ++, strong antimicrobial activity detected; +++, very strong antimicrobial activity detected; –, no antimicrobial activity detected.

Gram-negative bacteria (*Pseudomonas aeruginosa*). Interestingly, extracts obtained from *Satureja montana* L. stem showed better antibacterial activity, as it had a very strong effect against *Bacillus subtilis*. This effect may be due to the high concentration of antimicrobial compounds in the roots. From the results, we opine that surfactants are effective solvents for achieving antibacterial activity. This can be due to the fact that these compounds are well known for their ability to create chemical and physical connections with both hydrophilic and lipophilic compounds resulting in an increase in extraction yield [14]. The literature mostly describes the antibacterial activity of essential oils; however, Gomes et al. (2020) analysed decoctions of *Satureja montana* L. The obtained results showed that decoctions are promising in the fight against pathogens, as it was effective against Gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus dysgalactiae* and against Gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). However, the decoction showed no effectiveness against *E. coli* [27], which aligns with our study, where none of the tested extracts proved effective. Additionally, Ćetković et al. (2007a) observed the same results, indicating that activity against bacteria depends on the used solvent and *E. coli* is resistant to all extracts tested [25].

CONCLUSIONS

This study was conducted to determine the effects of various solvents on the biologically active compound recovery from the different morphological parts of winter savory (*Satureja montana* L.) It is quite evident that in choosing the most optimal solvent, it is necessary to take into account two important factors: 1) what properties we are trying to gain from the extract; 2) what morphological part of the plant we have. Among all the factors considered, 2% SDS stands out as the most effective solvent, even though a few extracts from various plant parts did not yield optimal results. In the majority of cases, 2% SDS gave the best results regarding phytochemical composition, antioxidant and antimicrobial activity. The total amount of polyphenolic compounds ranged approximately from 42.63 mg g^{-1} in stems to 124.76 mg g^{-1} in leaves, that of polyphenolic acids ranged from 17.41 to 85.59 mg g^{-1}, and the total amount of flavonoids from 43.76 to 151.38 mg g^{-1}, respectively. Using 2% SDS, the highest radical activity was observed in the flower and stem extracts, 84.21 and 36.31%, respectively. On the contrary, extracts obtained from the leaves were not as effective, so it is recommended to use 50 and 75% methanol for leaf extracts (the activity reached 84.72 and 54.95%, respectively) or 75% ethanol (activity reached 84.63%), where a high radical activity is the primary goal. Regarding the antibacterial activity using 2% SDS, the stem extract demonstrated better performance, which significantly inhibited the growth of *B. subtilis* and *S. aureus*. The obtained results further revealed that the dominant elements in winter savory are calcium and potassium, with the highest amount in winter savory (*Satureja montana* L.) leaves, and no heavy metals were detected.

Received 28 July 2025
Accepted 3 September 2025

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Dovilė Motiejauskaitė, Renata Žvirdauskienė, Agnė Venckutė, Karolina Barčauskaitė

KALNINIO DAŠIO (*SATUREJA MONTANA* L.) MORFOLOGINIŲ DALIŲ ELEMENTINĖ SUDĖTIS IR JŲ EKSTRAKTŲ BIOLOGINIS AKTYVUMAS

S a n t r a u k a

Kalninis dašis (*Satureja Montana* L.) yra augalas, pasižymintis ryškiomis antioksidacinėmis ir antibakterinėmis savybėmis. Lietuvoje šis augalas natūraliai aptinkamas retai, dažniau jis auginamas kaip įprastas sodo augalas. Šio tyrimo tikslas buvo nustatyti kalninio dašio mineralinę sudėtį ir įvertinti, kaip septynios skirtingos tirpiklių kombinacijos – dejonizuotas vanduo, 50 % metanolis (MeOH), 75 % MeOH, 50 % etanolis (EtOH), 75 % EtOH, 2 % natrio dodecilsulfatas (SDS) ir 2 % Triton X-100 – gali išgauti biologiškai aktyvias fitochemines medžiagas. Tyrimo rezultatai parodė, kad elementinėje sudėtyje vyravo kalcis (nuo 4 231,23 ± 546,48 mg kg^{-1} stiebuose iki 11 494,27 ± 785,01 mg kg^{-1} lapuose) ir kalis (atitinkamai nuo 3 626,69 ± 427,98 mg kg^{-1} iki 6 321,68 ± 241,53 mg kg^{-1}). Taip pat nustatyta, kad bendra fenolinių junginių koncentracija augalų ekstraktuose kito priklausomai nuo naudojamo tirpiklio ir morfologinės augalo dalies. Šios koncentracijos svyravo nuo 23,91 mg g^{-1} RE stiebuose, kai buvo naudojamas etanolis, iki 138,96 mg g^{-1} RE lapuose, kai buvo naudojamas metanolis. Be to, nustatyta, kad fenolinių rūgščių kiekis svyravo nuo 6,45 mg g^{-1} vandeniniame žiedų ekstrakte iki 92,82 mg g^{-1} lapų ekstrakte, gautame naudojant paviršinio aktyvumo medžiagą Triton X-100. Bendras flavonoidų kiekis svyravo nuo 9,94 mg g^{-1} RE žiedų ekstraktuose, gautuose su distiliuotu vandeniu, iki 151,39 mg g^{-1} RE lapų ekstraktuose, kai buvo naudojama paviršinio aktyvumo medžiaga SDS. Kalninių dašių ekstraktai pasižymėjo dideliu laisvojo DPPH· radikalo surišimu – nuo 13,01 % iki 85,71 %. Stipriausias antibakterinis poveikis buvo nustatytas 2 % SDS stiebų ekstrakte prieš *Bacillus subtilis*. Apibendrinant gautus rezultatus galima daryti išvadą, kad SDS yra veiksmingiausias tirpiklis kalninio dašio ekstraktams.