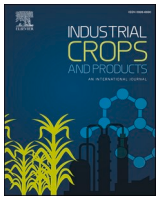
Industrial Crops & Products 189 (2022) 115841

Contents lists available at ScienceDirect

Industrial Crops & Products

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



Meadow sage (*Salvia pratensis* L.): A neglected sage species with valuable phenolic compounds and biological potential

Nikola Srećković [a], Danijela Mišić [b], Uroš Gašić [b], Sanja Lj. Matić [c], Jelena S. Katanić Stanković [c], Nevena R. Mihailović [a], Daria Maria Monti [d], Luigi D'Elia [d], Vladimir Mihailović [a,*]

[a] University of Kragujevac, Faculty of Science, Department of Chemistry, Radoja Domanovića 12, 34000 Kragujevac, Serbia
[b] University of Belgrade, Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, Bulevar despota Stefana 142, 11060 Belgrade, Serbia
[c] University of Kragujevac, Institute for Information Technologies Kragujevac, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia
[d] University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, Department of Chemical Sciences, via Cinthia 4, 80126 Naples, Italy

ARTICLE INFO

Keywords:
Meadow sage
Rosmarinic acid
Antioxidant activity
Antimicrobial activity
Antigenotoxicity
Cytotoxic activity

ABSTRACT

The genus *Salvia* is well-known for its use in food as aromatic and spicy herbs, as well as in the pharmaceutical and fragrance industries. Regardless of their importance, some *Salvia* species have not been thoroughly examined and chemically characterized, including *Salvia pratensis* L. In the present study, the detailed phenolic composition using LC/MS analysis, some bioactivities, and *in vitro* digestion stability of *S. pratensis* aerial part (SPA) and root (SPR) methanol extracts were determined. The results showed that both extracts possess high phenolic content, while SPR was richer in rosmarinic (11065.56 μg/g) and caffeic (509.00 μg/g) acids, as well as salvianolic acid A (519.22 μg/g) and B (291.60 μg/g) amounts quantified by UHPLC-DAD/(-)HESI-MS/MS analysis than SPA. The extracts demonstrated considerable antioxidant activity, particularly radical scavenger activity with IC_{50} values ranging between 24 and 90 μg/mL. The high inhibitory effect of extracts against DNA oxidative damage induced by peroxyl and hydroxyl radicals was noticed. The root extract was the most effective in inhibiting bacterial growth with MIC < 0.156 mg/mL for some G+ bacterial species. SPR extract was substantially more cytotoxic than SPA against all examined cell lines, particularly on cancer cells. The IC_{50} values of SPR for two cancerogenic cell lines A431 and SVT2 were 24.3 and 49.6 μg/mL, respectively. *S. pratensis*, especially its root, possess valuable phenolic compounds and biological properties for potential application as a substitute for some commonly used *Salvia* species.

1. Introduction

By using herbs in a daily diet, mankind was gradually acquainting their medicinal properties. Interest in herbal remedies in the prevention of disease and curative and rehabilitative properties is increaseing. Today, modern medicine uses many compounds isolated from plants as the basis for health care formulations, pharmaceutical drugs, and herbal nutritional supplements (Dzoyem et al., 2013). *Salvia* L., usually known as sage, is the largest genus in the Lamiaceae family, containing over 900 species widespread throughout the world (Lopresti, 2017). The *Salvia* genus contains compounds with highly beneficial effects for human health, which is one of the reasons for use of plants from this genus to treat a variety of ailments since ancient times, excessive sweating, including bronchitis, asthma, digestive, memory problems, angina, circulatory disturbances, mouth, and throat inflammation (Katanić Stanković et al., 2020; Xu et al., 2018; Wu et al., 2012). Essential oils and phenolic compounds of plants belonging to this genus possess a high antimicrobial and antioxidant potential and, therefore, sage extracts are used in food, not only for flavoring but also for preservation purposes (Katanić Stanković et al., 2020). Monoterpenes, triterpenoids, and flavonoids are characteristic compounds found in the aerial part of the *Salvia* species, while diterpenoids and phenolic acids are found in roots (Xu et al., 2018). Numerous *Salvia* species have significant commercial value on the herbal market and, above all, they are of great interest to the food and pharmaceutical industry. However, there are many species of this genus that have not been sufficiently tested and whose potential use has not been exploited. *Salvia pratensis* L. represents a valuable research area for its use as a substitute and/or application in the food

* Corresponding author.
E-mail address: vladimir.mihailovic@pmf.kg.ac.rs (V. Mihailović).

https://doi.org/10.1016/j.indcrop.2022.115841
Received 23 May 2022; Received in revised form 12 October 2022; Accepted 16 October 2022
Available online 27 October 2022

and pharmacy industries.

S. pratensis (meadow sage) is a perennial plant with large blue flowers, which usually grows in Mediterranean meadows. The use of *S. pratensis* in cookery reaches ancient times. The large leaves of *S. pratensis* were considered much more aromatic than cultivated *Salvia officinalis* L. and, for this reason, *S. pratensis* has been usually used for meat cooking. Essentially, meadow sage can be used as a substitute for common sage or as an additive (Vitalini et al., 2009). Moreover, the leaves and flowers of meadow sage have been traditionally used as tea to treat abdominal pains, skin diseases, and ulcers (Sharifi-Rad et al., 2018). Numerous scientific studies have shown that *Salvia* plants are rich in phenolic content. Shojaeifard et al. (2021) identified a number of phenolic compounds in 50 *Salvia* species growing in Iran and Central Asia using LC-UV-ESIMS analyses. They also determined the antioxidant potential of the identified compounds. The most recent study conducted by Avula et al. (2022) confirmed the presence of many phenolic acids (caffeic, rosmarinic, salvianolic, lithospermic, sagernic, tormentic, danshensu, carnosolic, hardwickic acids, *etc.*) and flavonoids (such as luteolin 7-O-β-rutinoside, santin-7-O-glucoside, viscosine, cirsimaritin, genkwanin, salvigenin, and nepetin 7-glucoside) in five different types of *Salvia* species. In general, although the aerial parts of sage species are in extensive use as medicine, there is no information about the bioactive compounds present in *S. pratensis* root and its bioactivities. Also, the data about the phytochemical composition of *S. pratensis* aerial part are rarely found in the literature. Rosmarinic acid was reported as the main compound in *S. pratensis* aerial part ethanolic extract by Šulniūtė et al. (2017). Kucekova et al. (2013) identified a low amount of gallic acid, catechin, and cinnamic acid in the methanol extract of meadow sage flowers. The higher contents of luteolin-7-glycoside and apigenin-7-glycoside were detected in *S. pratensis* methanol extract compared with other *Salvia* species (Coisin et al., 2012). According to Anaya et al. (1989), the aerial part of *S. pratensis*, also, possesses di- and triterpenes *β*-amyrin, germanicol, lupeol, and loranthol. So far, there have been no comprehensive investigations of the chemical composition and bioactivity of *S. pratensis* methanol extracts, especially root extract. In that sense, this research aims to determine the phenolic profile of both *S. pratensis* aerial parts (SPA) and roots (SPR) methanol extracts to evaluate their potential for different applications as sources of valuable phenolic compounds. The study also aims to define the potential application of this under-researched sage species as a substitute for commonly known sage species that have a long tradition of use and are described in the Pharmacopeia. For that purpose, some aspects of the potential application of *S. pratensis* such as antimicrobial activity, antioxidant potential, DNA protective activity, and cytotoxicity on healthy and cancerous cells were examined in this study. One of the aims of this study is also to determine the possible changes in phenolic compounds quantity of *S. pratensis* during the digestion process to better understand the bioaccessibility of biologically active compounds if it would be consumed orally.

2. Materials and methods

2.1. Chemicals

All reagents and standard phenolic compounds utilized in the analysis of total phenolic compounds, antioxidant activity, and antibacterial properties were obtained from Sigma Aldrich (Deisenhofen, Germany) and Alfa Aesar (Karlsruhe, Germany). Sigma–Aldrich (St Louis, MO, USA) also supplied the enzymes utilized in the *in vitro* gastrointestinal simulation assay (alpha-amylase, pepsin, pancreatin, and bile extract). Roth (Karlsruhe, Germany) provided all solvents required in high-performance liquid chromatography (HPLC) analyses as well as deoxyribonucleic acid from herring sperm utilized in antigenotoxic testing. Torlak Institute of Virology, Vaccines, and Sera (Belgrade, Serbia) produced the nutrient medium used for microbiological tests.

2.2. Extracts preparation

S. pratensis was harvested in Central Serbia (44°08′42″ N, 20°54′23″ E) in May 2018, during the flowering season. Prof. Dr. Milan S. Stanković validated the botanical identity (IPNI, 2022) of plant while the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac (Kragujevac, Serbia) assigned a voucher specimen (No. 125/016). The maceration procedure was used to obtain the SPA and SPR extracts. Previously dried and powdered parts of the plant SPA (80.33 g) or SPR (90.19 g) were saturated with the volume of methanol three times as much as the plant weight and left at room temperature for 24 h in a dark place. The procedure was performed three times with the same plant material after filtering using filter paper (Whatman, No. 1). The obtained SPA and SPR extracts were concentrated under a vacuum and temperature below 40 °C (Srećković et al., 2020; Aygun et al., 2022). The dried concentrated *S. pratensis* aerial parts (SPA) and root (SPR) extracts were stored at 4 °C.

2.3. Determination of phenolics in extracts

For the determination of total phenolic content in extracts the method with the Folin-Ciocalteu reagent was used (Singleton et al., 1999). The extract solution (1 mL) mixed with 5 mL of diluted Folin-Ciocalteu reagent (1:10) and 4 mL of $NaHCO_3$ solution (7.5 % w/w in water). The blue color of samples was developed after heating of these mixtures at 45 °C for 15 min. UV–Vis double beam spectrophotometer Halo DB-20S (Dynamica GmbH, Dietikon, Switzerland) was used to measure the absorbance of a sample at 765 nm after applying protocol. The total phenolic content in extracts was calculated using a gallic acid calibration curve and represent equivalents of gallic acid in milligrams per gram of dry extract (mg GAE/g dry extract).

The determination of the total flavonoids (Quettier-Deleu et al., 2000) and flavonols (Yermakov et al., 1987) contents was conducted with $AlCl_3$ solution as a colorimetric reagent. For quantification of the total flavonoids in the extracts, the same volume of 2 % $AlCl_3$ methanolic solution and *S. pratensis* extracts combined. The same procedure was applied for the determination of flavonols content in the extracts with the addition of 3 mL of CH_3COONa aqueous solution (50 mg/mL). The intensity of developed yellow color in the dark place of the sample solutions after 60 min for flavonoids and 150 min for flavonols was measured spectrophotometrically at 415 nm. The content of total flavonoids and flavonols expressed as quercetin (QUE) equivalents per gram of dry extract (mg QUE/g dry extract).

The total phenolic acids quantity in the extracts was estimated using the procedure explained in the Polish Pharmacopeia described by Matkowski et al. (2008). According to this method, 1 mL of *S. pratensis* extracts mixed with 5 mL distilled water and 1 mL of 0.1 M HCl, Arnow reagent (10 % w/v Na_2MoO_4 and 10 % w/v $NaNO_2$), and 1 M NaOH. The absorbance was measured at 490 nm immediately after mixing, and the concentration of phenolic acids in the extract was estimated using a caffeic acid standard curve, with the results expressed in mg caffeic acid per g of dry extracts (mg CAE/g dry extract).

Following the detection of total phenolic components in extracts, total condensed tannins in plant extracts were quantified using the method described by Scalbert et al. (1989). In the mexuture consisted of 2 mL of extract solutions, 1 mL of a 2:5 HCl/H_2O solution, and 1 mL of formaldehyde (37 %) 0.5 mol-equivalent of phloroglucinol was added for each gallic acid aquivalent. The formed precipitate was separated after 24 h, and the Folin–Ciocalteu assay was used to quantify unprecipitated phenols in the filtrate. The amount of condensed tannins (mg GAE/g dry extracts) expressed as the difference between the amount of total phenolics and unprecipitated phenolics.

The method based on changes in anthocyanins color at different pH reported by Giusti and Wrolstad (2001) was employed to determine the concentration of monomeric and total anthocyanins in the extracts. First, the dilution factor (F) was calculated using a KCl buffer pH 1.0,

and the extract solutions were diluted with this buffer to achieve the absorption between 0.4 and 0.7 at 520 nm. Prepared diluted extract samples then used for determination of absorbance at 520 and 700 nm after mixing with buffers that set pH of extract solution to 1.0 and 4.5. The monomeric and total anthocyanins content was calculated in cyanidin-3-glycoside equivalents (mg Cy 3-glc/g dry extract) as described in Srećković et al. (2020).

2.3.1. Chromatographic analysis

Ultra-high performance liquid chromatography (UHPLC) with the linear ion trap-orbitrap mass analyzers (LTQ OrbiTrap XL; Thermo Fisher Scientific, Bremen, Germany) was used for the identification of phenolic compounds. Detailed LC conditions and mass spectrometry settings have been described previously by Katanić Stanković et al. (2020).

Targeted phenolic compounds were quantified using Dionex Ultimate 3000 UHPLC system equipped with a triple-quadrupole mass spectrometer (TSQ Quantum Access Max, Thermo Fisher Scientific, Basel, Switzerland). The mass spectra of analyzed samples were obtained using a mass spectrometer with heated electrospray ionization (HESI) in negative ionization mode. The quantification of identified compounds was achieved using selected reaction monitoring (SRM). More details regarding this method of quantification can be found in a paper by Mišić et al. (2015).

2.4. Determination of antioxidant activity

2.4.1. Total antioxidant potential

The total antioxidant capacity of the examined SPA and SPR extracts was estimated using the phosphomolybdate test. The phosphomolybdate reagent was prepared as 28 mM Na_3PO_4 and 4 mM $(NH_4)_2MoO_4$ in 0.6 M H_2SO_4 and 3 mL of this reagent was added to 0.3 mL of extracts solution (0.2 mg/mL). After heating at 95 °C during 90 min, the absorbance of the samples was recorded at 695 nm (Prieto et al., 1999). Trolox equivalents (mg Trolox/g extracts) were used to express the level of the total antioxidant capacity of SPA and SPR.

2.4.2. DPPH assay

The same volume of different extract concentrations or the reference antioxidants and DPPH radical methanolic solution (80 μg/mL) were mixed to determine their potential to neutralize DPPH radicals. The samples were incubated for 30 min at room temperature in a dark place and the absorbance of the samples was recorded at 517 nm. (Kumarasamy et al., 2007). The percentage of $DPPH^{\bullet}$ inhibition and IC_{50} values were calculated as described in Srećković et al. (2020).

2.4.3. ABTS assay

Using the method established by Re et al. (1999), the ability of extracts to neutralize the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical-cation was estimated. The radical cation was generated at a dark place using 7 mM ABTS and 2.45 mM potassium persulphate in the same solution 16 h before the experiment. Ethanol (80%) was used to dilute the obtained $ABTS^{\bullet+}$ solution and absorbance at 734 nm set to 0.700. Serial dilutions of extracts (0.2 mL) or reference antioxidants (0.2 mL) and 1.8 mL of $ABTS^{\bullet+}$ solution were mixed and left at room temperature in a dark place. After 30 min the absorbance of experimental samples was measured at 734 nm. The percentage of $ABTS^{\bullet+}$ neutralization and IC_{50} values were estimated.

2.4.4. Ferrous ion chelating activity assay

The chelating assay was used to test the possibility of extracts to chelating ferrous ions (Yau Yan, 2006). The same volume of extract solutions was mixed with water solution of $FeSO_4$ (0.125 mM) and ferrozine (0.3125 mM) solutions. The absorbance of these samples was measured at 562 nm after 10 min of incubation at room temperature. The percent of the chelating ability of extracts and their IC_{50} values were calculated in the same way as described in the previous methods.

2.4.5. Linoleic acid assay

The thiocyanate assay was used to assess the suppression of lipid peroxidation by *S. pratensis* extracts. The method is based on measuring linoleic acid autoxidation inhibition in emulsion at pH 7 for 72 h at a temperature of 37 °C (Hsu et al., 2008). The degree of autoxidation of linoleic acid was measured using ammonium thiocyanate solution as an indicator of oxidation of Fe^{2+} to Fe^{3+} by formatted radicals. The detailed protocol was described in previously published paper by Srećković et al. (2020). The IC_{50} values were calculated as previously described.

2.4.6. Reducing capacity assay

The assay is based on spectrophotometric monitoring of the intensity of the formed Prussian blue complex as a result of the reduction of Fe(III) to Fe(II) by antioxidant compounds using method described by Oyaizu (1986). Using the calibration curve of antioxidant Trolox, the obtained results are presented as mg Trolox/g of extracts.

2.5. Antimicrobial activity

For determination of antimicrobial activity of tested extracts, 11 bacterial (*Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10876), *Bacillus subtilis* (ATCC 6633), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Micrococcus lysodeikticus* (ATCC 4698), *Salmonella typhimurium* (ATCC 14028), *Salmonella enteritidis* (ATCC 13076), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 25922)) and 9 fungal (*Fusarium oxysporum* (FSB 91), *Aspergillus brasiliensis* (ATCC 16404), *Alternaria alternata* (FSB 51), *Doratomyces stemonitis* (FSB 41), *Trichoderma longibrachiatum* (FSB 13), *Trichoderma harzianum* (FSB 12), *Penicillium canescens* (FSB 24), *Penicillium cyclopium* (FSB 23), *Candida albicans* (ATCC 10259)) strains were used. The microorganisms used to determine antimicrobial activity were cultivated 24–48 h before experiments. The microorganisms used in experiments are part of the microorganism collection of the Department of Biology and Ecology, Faculty of Science, Serbia. The ATCC strains are obtained from the Institute of Public Health, Kragujevac, Serbia.

The microdilution technique (Sarker et al., 2007) was utilized to determine the minimum inhibitory concentrations (MICs) of SPA and SPR against selected bacterial and fungal strains. Antibacterial activity of extracts was performed in Müller–Hinton broth, whereas antifungal activity was estimated in the Sabouraud dextrose broth using microtiter plates with 96 wells. The suspensions of the tested strains in sterile normal saline were prepared according to the standard methods (CLSI, 2012; CLSI, 2008a,b). The same protocol for the microdilution method was applied as described in a previously published paper by Srećković et al. (2020). The bacterial growth was checked after 24 h of incubation at 37 °C using the resazurin solution as an indicator for bacterial growth. The MIC values for examined fungi were visually detected 48 h after their growth at 28 °C.

2.6. In vitro antigenotoxic assays

The protection of DNA against hydroxyl and peroxyl radicals was determined according to methods previously described in detail by Srećković et al. (2020). In these experiments, different concentrations of SPA and SPR, as well as phenolic compounds identified in extracts were applied for the protective activity of DNA. Quercetin was used as a reference standard at the concentration of 100 μg/mL. The experiments were performed using herring sperm DNA at physiological conditions (0.9 % NaCl and pH = 7.4). Hydroxyl radical was generated using $FeSO_4$ and H_2O_2 solutions, while peroxyl radical was formed using 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) in DNA samples. The samples of DNA were electrophoresed on agarose gel with the addition of ethidium bromide. The formed DNA bands are then observed

using a UV transilluminator and obtained pictures were evaluated using ImageJ software.

2.7. In vitro gastrointestinal digestion simulation

The *in vitro* gastrointestinal digestion of the *S. pratensis* extracts was performed using standardized static method described by Minekus et al. (2014). To simulate oral, gastric, and intestinal digestion phases, SPA and SPR extracts were mixed with different fluids and enzyme solutions. In addition, control samples without extracts were also prepared. The oral phase of digestion of extracts was simulated using α-amylase solution during 2 min, then samples mixed with gastric fluids containing porcine pepsin solution in an acidic environment for 2 h, and then intestine condition simulated in the presence of pancreatin and bile acids solutions at pH 7 for 2 h as described in previous paper published by Srećković et al. (2020). During *in vitro* digestion process the samples incubated at 37 °C. For further analyses (HPLC analyses, total phenolics determination, and DPPH radical inhibition), the samples of digested extracts were taken after 0, 2 min, 1, 2, 3, and 4 h and frozen. The changes in the quantity of single phenolic compounds in samples during the simulated digestion process were monitored using the same LC/MS method described in the previous section.

2.8. Cytotoxic activity of extracts

For determination of the cytotoxic activity of SPA and SPR, human carcinoma A431, murine BALB/c-3T3, and SVT2 fibroblasts cells were obtained from ATCC, whereas human keratinocytes (HaCaT) were obtained from Innoprot (Berio, Spain). To evaluate the cytotoxicity of the extracts, the method described in the article published by Srećković et al. (2020) was used.

2.9. Statistical analysis

For the determination of phenolic contents in the extracts, their antioxidant activity, and changes during *in vitro* digestion in extracts, three independent experiments were performed for each methodology. The obtained results were presented as the mean of the results from these three experiments ± a standard deviation (SD). Based on the drawn inhibition percentage - extract concentration dependence curve, using OriginPro9 software (Origin Lab, Northampton, MA, USA), the concentration of extracts that removes 50% of the free-radical concentration (IC_{50}) was determined. SPSS version 13.0 for Windows was used to establish statistically significant differences between the means of the group using a one-way analysis of variance at $p < 0.05$. (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Phytochemical composition of S. pratensis methanolic extracts

Results presented in Table 1 showed that SPR possessed a higher total phenolic, tannins, and phenolic acids contents than SPA. The SPA was richer in total flavonoid, flavonols, and anthocyanins content. UHPLC–MS^4 Orbitrap metabolic fingerprinting was used for the identification of phenolic compounds in SPA and SPR. Based on MS^2, MS^3, and MS^4 fragmentation of molecules and mass of the deprotonated molecule $[M-H]^-$, phenolic compounds were detected in SPA and SPR extracts. A total of 67 identified compounds with main MS data are listed in Table 2. In SPR extract, forty phenolic acids and their derivatives were detected, while thirty-six of them were identified in SPA extract. Twenty-one flavonoids and their derivatives were found in SPA, while the presence of flavonoids in SPR was not confirmed by UHPLC–MS^4 Orbitrap metabolic fingerprint. Corresponding chromatograms of SPA and SPR extracts are presented in Fig. 1S (Supplementary material). Rosmarinic and caffeic acids were identified as the main polyphenolic components in SPA and SPR. Also, caffeic acid derivates were identified in *S. pratensis* extracts, such as its monomers (caffeoyl and danshensu isomers, and ferulic acid), dimers (methyl rosmarinate), trimers (lithospermic acid, sagecoumarin, salvianolic acids A and C, and yunnaneic acid E and F), and tetramers (salvianolic acid B). Besides numerous phenolic acids and their derivatives, many flavonoids, flavanols, and their glycosides were successfully identified and quantified only in SPA. However, these compounds were not detected or have been detected in traces in SPR.

Totally 20 phenolic compounds were quantified in SPA, while 18 were quantified in SPR by UHPLC-DAD/(-)HESI-MS/MS analysis. This is the first quantitative polyphenolic characterization of *S. pratensis* root extract. The results of the quantification of phenolic compounds are shown in Table 3. Based on the presented results, SPA extract possessed a higher quantity of total flavonoids, while higher amounts of phenolic acids was quantified in SPR. In SPA, the dominant compound was rosmarinic acid (8.42 mg/g), with caffeic (0.368 mg/g) and *p*-hydroxybenzoic acids (0.175 mg/g) in high quantities. Among flavonoids, luteolin (0.150 mg/g) was quantified in the highest concentration. Contrary, rosmarinic and caffeic acids levels in SPR (11.06 and 0.509 mg/g, respectively) were higher than in SPA. Also, SPR contained high amounts of salvianolic acid A (0.519 mg/g) and salvianolic acid B (0.292 mg/g) in comparison with SPA.

Luteolin, apigenin, quercetin-3-*O*-glucoside, and cirsimaritin were the dominant compounds in SPA among quantified flavonoids. Only minor amounts of flavonoids were detected in SPR extract. Among quantified flavonoids luteolin and apigenin were found in the highest concentrations in both extracts. The concentrations of luteolin in SPA and SPR was 149.85 and 8.58 μg/g of dry extract, respectively. The presence of naringenin and naringin was not confirmed in root extract, while their presence in the aerial part was detected only at trace amounts.

3.2. Antioxidant capacity

The antioxidant activity (Table 4) of *S. pratensis* extracts was determined using different methods based on the ability of compounds in the extract to oxidize or reduce metal ions, scavenge free radicals such as $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, inhibit lipid peroxidation, and chelate metal ions. Both examined extracts showed remarkable antioxidant potential compared with referent antioxidants. The total antioxidant capacity assay showed that SPA and SPR possess a high potential for reduction Mo (VI) to (V). This method showed that 1 g of SPR has the same total antioxidant capacity as 614.80 mg of Trolox, while SPA extract showed

Table 1
The content of different classes of phenolic compounds in the aerial part (SPA) and root (SPR) extracts of *S. pratensis*.

Samples	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QUE/g extract)	Total flavonol content (mg RUE/g extract)	Condensed tannins content (mg GAE/g extract)	Total phenolic acid content (mg CA/g extract)	Monomeric anthocyanins content (mg Cy-3-glc/g extract)	Total anthocyanins content (mg Cy-3-glc/g extract)
SPA	128.94 ± 2.65[a]	68.46 ± 6.73	1.59 ± 0.41	69.68 ± 4.83	3.91 ± 0.61	17.83 ± 2.27	29.92 ± 2.75
SPR	177.85 ± 0.35	33.27 ± 1.66	0.36 ± 0.06	96.36 ± 3.29	37.95 ± 2.99	10.20 ± 0.71	15.40 ± 0.76

[a] Results are mean values ± SD from three measurements; GAE – gallic acid equivalents; QUE – quercetin equivalents; RUE– rutin equivalents; Cy-3-glc – cyanidin-3-O-glucoside equivalents

Table 2
UHPLC–MS^4 Orbitrap metabolic fingerprinting (negative ionization mode) of *Salvia pratensis* L. aerial part and root extracts.

No	Compound names	t_R, min	Molecular formula, $[M–H]^-$	Calculated mass, $[M–H]^-$	Exact mass, $[M–H]^-$	Δ mDa	MS^2 Fragments, (% Base Peak)	MS^3 Fragments, (% Base Peak)	MS^4 Fragments, (% Base Peak)	Presence	
										SPA	SPR
Phenolic acids											
1	Dihydroxybenzoyl hexoside 1[c]	2.25	$C_{13}H_{15}O_9^-$	315.07216	315.06927	2.88	108(7), 109(12), 151(7), 152(43), **153**(100), 163(7), 165(11)	**109**(100)	**81**(100)	–	+
2	Gallic acid[a]	3.86	$C_7H_5O_5^-$	169.01425	169.01237	1.88	69(10), 84(4), 123(17), 124(11), **125**(100), 126(12), 150(4)	71(14), 79(30), **81**(100), 83(51), 97(78), 98(16), 107(15)	ND	+	–
3	Galloyl hexoside[d]	4.90	$C_{13}H_{15}O_{10}^-$	331.06707	331.06390	3.17	125(30), 150(10), 167(18), 168(98), 169(37), **313**(100), 314(17)	125(76), 137(22), 150(54), **151**(100), 165(22), 193(58)	95(35), 107 (47), **123**(100), 141(3)	–	+
4	Danshensu hexoside[c]	5.57	$C_{15}H_{19}O_{10}^-$	359.09837	359.09482	3.55	135(3), 179(14), **197**(100), 198(6)	73(17), 153(3), **179**(100)	107(3), **135** (100)	+	+
5	Vanillic acid[a]	5.72	$C_8H_7O_4^-$	167.03498	167.03321	1.77	69(14), 121(3), **123**(100), 124(6), 137(3), 139(7), 149(6)	77(34), 79(42), 81(18), 93(8), **95**(100), 105(23), 108(9)	ND	+	+
6	Dihydroxybenzoyl hexoside 2[c]	5.77	$C_{13}H_{15}O_9^-$	315.07216	315.06960	2.56	109(11), 152(11), **153**(100), 154(6), 285(20), 287(5), 298(4)	**109**(100)	65(46), **81** (100)	+	+
7	3,4-dihydroxyphenethyl alcohol 4-*O*-hexoside[c]	5.88	$C_{14}H_{19}O_8^-$	315.10854	315.10434	4.20	123(5), **153**(100)	**123**(100)	77(22), 79(5), 81(14), 93(13), **95**(100), 105 (37), 108(13)	+	+
8	Decaffeoylverbascoside[e]	6.13	$C_{20}H_{29}O_{12}^-$	461.16645	461.16094	5.51	135(67), 153(96), 161(20), 167(30), 297(19), **315**(100), 413(25)	101(5), 113(4), 119(4), 131(4), **135**(100), 153(11)	ND	+	+
9	Protocatechuic acid[a]	6.16	$C_7H_5O_4^-$	153.01933	153.01826	1.07	108(3), **109**(100), 123(27), 154(16)	80(13), **81**(100), 82(12)	ND	+	+
10	Caffeoyl dihexoside 1[c]	6.17	$C_{21}H_{27}O_{14}^-$	503.14063	503.13667	3.96	**161**(100), 179(50), 221(12), 251(21), 281(32), 323(12), 341(13)	**133**(100)	ND	+	+
11	Hydroxybenzoyl hexoside[f]	6.18	$C_{13}H_{15}O_8^-$	299.07724	299.07450	2.74	**137**(100), 138(7)	65(6), 70(18), 81(11), 92(14), **93**(100), 108(18), 109(80)	ND	+	+
12	Caffeoyl dihexoside 2[c]	6.51	$C_{21}H_{27}O_{14}^-$	503.14063	503.13805	2.58	161(29), 179(57), 221(32), 251(59), **281**(100), 323(54), 341(56)	135(7), **179**(100), 221(31)	**135**(100)	+	+
13	Caffeoyl hexoside 1[c]	6.63	$C_{15}H_{17}O_9^-$	341.08781	341.08478	3.02	135(7), **179**(100), 180(8), 181(21), 221(3), 251(9), 281(13)	**135**(100)	ND	+	+
14	Sinapyl aldehyde[g]	6.94	$C_{11}H_{11}O_4^-$	207.06628	207.06434	1.94	115(9), 143(5), 159(6), 161(21), 163(5), **192**(100), 193(12)	**177**(100)	77(11), 105(4), 121(20), **149** (100)	+	+
15	Caffeoyl threonic acid 1 [h]	7.05	$C_{13}H_{13}O_8^-$	297.06159	297.05923	2.36	**135**(100), 179(23)	59(4), 71(5), **75**(100), 89(77), 117(95)	ND	+	+
16	*p*-Hydroxybenzoic acid[a]	7.13	$C_7H_5O_3^-$	137.02442	137.02354	0.88	92(30), **93**(100), 108(27), 109(94), 137(42)	**66**(100), 182(78)	ND	+	+
17	Gentisic acid[a]	7.27	$C_7H_5O_4^-$	153.01933	153.01821	1.12	107(8), 108(8), **109**(100), 110(4), 123(5), 125(5)	65(68), 66(6), 67(14), **81**(100), 83(35), 91(8), 123(10)	ND	+	+
18	Caffeoyl glycerol[i]	7.35	$C_{12}H_{13}O_6^-$	253.07176	253.07058	1.18	**135**(100), 136(7), 161(55), 162(4), 179(38), 180(3)	79(7), **107**(100), 117(19), 135(56)	ND	+	+
19	Caffeic acid[a]	7.53	$C_9H_7O_4^-$	179.03498	179.03360	1.38	**135**(100)	75(8), 79(18), 91(21), 93(11), **107**(100), 117(15), 135(30)	ND	+	+
20	Caffeoyl threonic acid 2 [h]	7.60	$C_{13}H_{13}O_8^-$	297.06159	297.05982	1.77	**135**(100), 163(3), 179(21)	59(5), 73(3), 75(98), **89**(100), 117(88)	**59**(100)	+	–
21	Salvianic acid C[c]	7.60	$C_{18}H_{17}O_9^-$	377.08781	377.08439	3.41	197(3), 273(22), 289(5), 317(7), **359**(100), 360(7)	133(5), **161**(100), 179(21), 197(22), 223(8)	**133**(100)	+	–
22	Ferulic acid[a]	7.61	$C_{10}H_9O_4^-$	193.05063	193.04922	1.41	111(37), **134**(100), 135(9), 147(15), 149(34), 173(5), 178(4)	**106**(100), 107(11), 134(14)	ND	+	+
23	Caffeoyl hexoside 2[c]	7.76	$C_{15}H_{17}O_9^-$	341.08781	341.08509	2.71	**179**(100), 180(9), 193(5), 195(6), 223(7), 295(7), 323(3)	75(17), 81(9), 87(5), 99(31), 135(16), **143**(100), 161(45)	71(14), **81** (100), 99(29), 125(22)	+	–
24	Verbascoside[j]	8.04	$C_{29}H_{35}O_{15}^-$	623.19814	623.19496	3.18	**461**(100), 462(15)	134(3), 135(57), 143(7), 161(13), 297(14), **315**(100)	113(4), 119 (13), 125(5), 131(3), **135** (100), 143(5)	–	+
25	Yunnaneic acid F[k]	8.09	$C_{29}H_{25}O_{14}^-$	597.12498	597.12358	1.40	197(18), 267(21), **311**(100), 312(15), 329(39), 355(10), 491(20)	249(4), 251(19), 252(3), 265(7), **267**(100), 268(7), 269(3)	223(38), 224 (21), 239(52), 246(22), 249 (78), 250(62), **252**(100)	+	+
26	Salviaflaside[c]	8.17	$C_{24}H_{25}O_{13}^-$	521.13007	521.12553	4.54	161(3), 323(3) **359**(100), 360(8)	133(5), 135(3), **161**(100), 179(21), 197(31), 223(7)	**133**(100)	+	+

(*continued on next page*)

Table 2 (*continued*)

No	Compound names	t_R, min	Molecular formula, $[M–H]^-$	Calculated mass, $[M–H]^-$	Exact mass, $[M–H]^-$	Δ mDa	MS^2 Fragments, (% Base Peak)	MS^3 Fragments, (% Base Peak)	MS^4 Fragments, (% Base Peak)	Presence	
										SPA	SPR
27	Caffeoyl hexoside 3[c]	8.25	$C_{15}H_{17}O_9^-$	341.08781	341.08382	3.98	135(9), 145(4), **179**(100), 180(8), 295(9), 296(3), 297(5)	**135**(100)	**107**(100)	–	+
28	Verbascoside isomer[j]	8.32	$C_{29}H_{35}O_{15}^-$	623.19814	623.19588	2.26	**461**(100), 462(20)	135(58), 136(3), 143(5), 161(10), 297(19), **315**(100), 316(3)	101(3), 119(3), **135**(100), 136 (5)	–	+
29	Feruloyl threonic acid[l]	8.37	$C_{14}H_{15}O_8^-$	311.07724	311.07473	2.51	135(3), **193**(100)	**134**(100), 149(18)	**106**(100), 134 (7)	+	–
30	Caffeoyl-dihydroxybenzoyl hexoside[c]	8.49	$C_{22}H_{21}O_{12}^-$	477.10385	477.10018	3.67	153(4), 161(4), **315**(100), 316(11), 323(8), 433(21), 434(4)	108(10), 109(12), 152(44), **153**(100), 163(10), 165(13), 225(8)	**109**(100)	+	+
31	Lithospermic acid[b]	8.67	$C_{27}H_{21}O_{12}^-$	537.10385	537.10003	3.82	355(15), **359**(100), 360(15), 373(30), 491(14), 519(14), 519(29)	161(12), 179(17), **197**(100)	73(19), 153(4), **179**(100)	–	+
32	Rosmarinic acid[a]	9.02	$C_{18}H_{15}O_8^-$	359.07724	359.07323	4.01	133(3, **161**(100), 162(5), 179(13), 197(12), 223(5)	**133**(100)	**77**(100), 105 (46)	+	+
33	Danshensu[b]	9.04	$C_9H_9O_5^-$	197.04555	197.04394	1.61	73(19), 123(9), 151(48), 153(11), 167(7), **179**(100), 180(19)	91(3), 107(3), **135**(100)	**107**(100)	+	+
34	Salvianolic acid C 1[c]	9.65	$C_{26}H_{19}O_{10}^-$	491.09837	491.09361	4.76	295(3), **311**(100), 312(11)	**267**(100), 268(7)	197(21), 211 (23), 221(17), **239**(100), 249 (46), 267(35)	–	+
35	Sagecoumarin[m]	9.70	$C_{27}H_{19}O_{12}^-$	535.08820	535.08391	4.29	161(15), 177(13), 179(14), 311(16), **329**(100), 330(13), 491(48)	283(4), **311**(100), 312(6)	133(17), 147 (16), 221(7), 239(43), 249 (36), 267(27), **283**(100)	+	–
36	Salvianolic acid C 2[c]	9.79	$C_{26}H_{19}O_{10}^-$	491.09837	491.09468	3.70	161(5), 179(3), 311(8), 313(3), 357(5), **359**(100)	133(5), **161**(100), 179(20), 197(23), 223(7)	**133**(100)	–	+
37	Clinopodic acid A[n]	9.82	$C_{18}H_{15}O_7^-$	343.08233	343.07916	3.17	135(15), 145(14), **161**(100), 162(10), 179(26), 197(14), 325(11)	**133**(100)	**77**(100), 105 (98)	+	+
38	Salvianolic acid A[c]	9.96	$C_{26}H_{21}O_{10}^-$	493.11402	493.10963	4.39	**359**(100), 360(4)	133(3), **161**(100), 179(12), 197(13), 223(5)	**133**(100)	+	–
39	Yunnaneic acid E[c]	10.18	$C_{26}H_{21}O_{11}^-$	509.10894	509.10602	2.91	179(11), 311(31), 313(13), 329(34), **359**(100), 447(53), 491(9)	133(5), **161**(100), 179(19), 197(22), 223(7)	**133**(100)	+	+
40	Salvianolic acid F 1[b]	10.24	$C_{17}H_{13}O_6^-$	313.07176	313.06905	2.71	**161**(100), 178(21), 267(74), 268(17), 283(26), 297(20), 300(23)	**133**(100)	**79**(100)	–	+
41	Methyl lithospermate 1[b]	10.39	$C_{28}H_{23}O_{12}^-$	551.11950	551.11596	3.54	161(11), 357(32), 358(11), 359(70), 360(13), **519**(100), 520(30)	161(3), **339**(100)	133(8), **161** (100), 177(15), 179(7), 295(3), 311(3)	+	+
42	Methyl rosmarinate[c]	10.61	$C_{19}H_{17}O_8^-$	373.09289	373.08972	3.17	135(40), **179**(100), 180(3), 287(3), 313(30), 331(6), 331(28)	**135**(100)	106(57), **107** (100), 135(17)	+	+
43	Salvianolic acid F 2[b]	11.09	$C_{17}H_{13}O_6^-$	313.07176	313.06870	3.06	**161**(100), 162(4)	**133**(100)	89(7), **105** (100), 107(20), 133(5), 140(8)	+	+
44	Methyl lithospermate 2[b]	11.16	$C_{28}H_{23}O_{12}^-$	551.11950	551.11763	1.87	359(33), **389**(100), 390(13), 461(6), 505(11), 519(29), 520(6)	123(15), **151**(100), 179(10), 193(9), 195(4), 197(62), 357(32)	79(3), 81(4), 107(9), 108(3), 109(3), **123** (100), 133(4)	+	+
45	Salvianolic acid C 3[c]	11.23	$C_{26}H_{19}O_{10}^-$	491.09837	491.09555	2.82	179(14), 267(21), 293(11), **311**(100), 312(10), 329(19), 447(11)	174(4), 202(4), 223(4), 249(3), 267(42), **283**(100), 293(20)	173(40), **174** (100), 239(30), 255(14), 255 (79), 265(13), 268(20)	–	+
46	Salpalaestinin[n]	11.47	$C_{28}H_{25}O_{12}^-$	553.13515	553.13407	1.08	177(43), 341(29), 343(53), 373(87), **477**(100), 489(31), 521(56)	267(25), 311(41), 312(16), 313(54), **343**(100), 433(16), 459(35)	133(12), 135 (23), 177(24), 178(9), **179** (100), 267(22), 311(10)	+	+

(*continued on next page*)

Table 2 (*continued*)

No	Compound names	t_R, min	Molecular formula, $[M–H]^-$	Calculated mass, $[M–H]^-$	Exact mass, $[M–H]^-$	Δ mDa	MS^2 Fragments, (% Base Peak)	MS^3 Fragments, (% Base Peak)	MS^4 Fragments, (% Base Peak)	Presence SPA	Presence SPR
Flavonoids											
47	Luteolin 7-*O*-glucoside[a]	7.85	$C_{21}H_{19}O_{11}^-$	447.09329	447.09024	3.04	**285**(100), 286(8)	151(27), 175(73), 199(69), 213(24), 217(62), **241**(100), 243(49)	185(16), 197 (99), 198(87), **199**(100), 213 (25), 213(47), 226(25)	+	–
48	Quercetin 3-*O*-(6''-rhamnosyl) glucoside (Rutin)[a]	7.95	$C_{27}H_{29}O_{16}^-$	609.14611	609.14498	1.13	255(4), 271(8), 299(3), 300(42), **301**(100), 302(12), 343(6)	151(82), **179**(100), 255(8), 257(13), 271(9), 272(14), 273(17)	**151**(100)	+	–
49	Luteolin 7-*O*-(6''-pentosyl) hexoside[o]	8.05	$C_{26}H_{27}O_{15}^-$	579.13554	579.13412	1.42	**285**(100), 286(8)	151(41), 175(93), 199(88), 213(26), 217(74), **241**(100), 243(68)	185(16), 196 (15), 197(63), **198**(100), 199 (60), 213(56), 226(15)	+	–
50	Quercetin 3-*O*-glucoside[a]	8.21	$C_{21}H_{19}O_{12}^-$	463.08820	463.08444	3.76	299(3), 300(18), **301**(100), 302(5)	107(5), 151(79), **179**(100), 229(5), 257(11), 273(15), 283(5)	**151**(100)	+	–
51	Luteolin 7-*O*-acetyl hexoside 1 [m]	8.28	$C_{23}H_{21}O_{12}^-$	489.10385	489.09944	4.41	**285**(100), 286(11), 323(10), 327(4)	149(25), 175(60), 197(24), 199(49), 217(41), **241**(100), 243(61)	173(21), 185 (80), 197(77), **198**(100), 199 (47), 213(75), 214(31)	+	–
52	Luteolin 7-*O*-hexuronide[f]	8.37	$C_{21}H_{17}O_{12}^-$	461.07255	461.06739	5.16	**285**(100), 286(8), 299(4), 301(11), 323(7), 341(3), 357(5)	151(36), 175(82), 199(88), 201(26), 217(62), **241**(100), 243(66)	179(9), 197 (87), 198(79), 199(32), **213** (100), 223(22), 241(10)	+	–
53	Luteolin 7-*O*-hexoside isomer[f]	8.39	$C_{21}H_{19}O_{11}^-$	447.09329	447.08982	3.47	281(20), **285**(100), 286(11)	151(49), 175(96), **199**(100), 201(31), 217(73), 241(91), 243(61)	143(18), 153 (5), 155(19), 157(5), **171** (100), 181(5), 182(10)	+	–
54	Quercetin 3-*O*-acetyl hexoside[i]	8.42	$C_{23}H_{21}O_{13}^-$	505.09876	505.09465	4.11	179(3), 300(57), **301**(100), 302(8), 343(3), 445(3), 463(20)	151(86), **179**(100), 255(22), 271(34), 272(12), 273(17), 283(14)	**151**(100)	+	–
55	Luteolin 7-*O*-[6''-(3-hydroxy-3-methylglutaryl) hexoside[p]	8.79	$C_{27}H_{27}O_{15}^-$	591.13554	591.13064	4.90	**447**(100), 448(18), 489(76), 490(14), 529(40), 530(10)	**285**(100), 286(4)	151(43), **175** (100), 199(92), 201(30), 217 (82), 241(95), 243(65)	+	–
56	Luteolin 7-*O*-acetyl hexoside 2 [m]	8.81	$C_{23}H_{21}O_{12}^-$	489.10385	489.10048	3.37	**285**(100), 286(7)	151(42), 175(98), 199(92), 217(74), **241**(100), 243(64), 257(27)	185(14), **197** (100), 198(80), 199(82), 212 (8), 213(53), 226(14)	+	–
57	Apigenin 7-*O*-hexuronide[f]	8.96	$C_{21}H_{17}O_{11}^-$	445.07763	445.07313	4.50	175(11), **269**(100), 270(15), 285(24)	149(44), 151(28), 183(20), 201(39), **225**(100), 227(26), 269(25)	168(15), 169 (19), **181**(100), 183(11), 196 (18), 197(29), 210(35)	+	–
58	Genkwanin[a]	9.85	$C_{16}H_{11}O_5^-$	283.06120	283.05956	1.64	197(5), 211(5), 237(4), 239(32), **241**(100), 242(7), 255(12)	**197**(100), 198(8)	**141**(100), 151 (48), 153(24), 169(20), 179 (33), 180(30), 197(13)	+	–

(*continued on next page*)

Table 2 (*continued*)

No	Compound names	t_R, min	Molecular formula, $[M–H]^-$	Calculated mass, $[M–H]^-$	Exact mass, $[M–H]^-$	Δ mDa	MS^2 Fragments, (% Base Peak)	MS^3 Fragments, (% Base Peak)	MS^4 Fragments, (% Base Peak)	Presence	
										SPA	SPR
59	Luteolin[a]	10.40	$C_{15}H_9O_6^-$	285.04046	285.03843	2.03	151(39), 175(91), 199(79), 217(65), **241**(100), 243(62), 285(56)	185(12), **197**(100), 198(82), 199(70), 213(44), 214(8), 226(14)	151(5), 165 (10), **169**(100), 179(7), 182(7), 197(14), 329 (3)	+	–
60	Apigenin[a]	11.30	$C_{15}H_9O_5^-$	269.04555	269.04395	1.60	149(48), 151(25), 201(27), **225**(100), 227(18), 269(35), 270(30)	157(9), 169(23), 180(27), **181**(100), 183(51), 196(24), 197(48)	122(18), **141** (100), 152(5), 153(20), 166 (7), 180(8), 181(12)	+	–
61	Luteolin 7-*O*-(6''-*p*-coumaroyl) acetylhexoside[r]	11.40	$C_{32}H_{27}O_{14}^-$	635.14063	635.13686	3.77	**285**(100), 286(19), 489(19), 490(6), 575(20), 576(8), 577(3)	151(45), 175(74), **199**(100), 213(39), 217(80), 241(99), 243(62)	143(33), **171** (100), 181(12)	+	–
62	Chrysoeriol[a]	11.55	$C_{16}H_{11}O_6^-$	299.05611	299.05356	2.55	**284**(100), 285(4)	**256**(100), 284(7)	188(29), 200 (18), 211(26), 212(19), 214 (13), **227**(100), 228(35)	+	–
63	Chrysin[a]	12.31	$C_{15}H_9O_4^-$	253.05063	253.04870	1.93	157(9), **181**(100), 182(16), 209(22), 225(9), 253(27)	**153**(100), 155(11)	122(36), **127** (100), 134(37), 171(46), 238 (14)	+	–
64	Hispidulin[a]	12.50	$C_{16}H_{11}O_6^-$	299.05611	299.05332	2.79	**284**(100), 285(9)	211(6), 227(7), 255(7), 256(75), 257(8), **284**(100), 285(23)	183(25), 188 (27), 200(37), 212(49), **227** (100), 228(49), 239(31)	+	–
65	Cirsimaritin[a]	12.73	$C_{17}H_{13}O_6^-$	313.07176	313.06819	3.57	283(5), **298**(100), 299(10)	225(3), 269(30), 270(5), **283**(100)	163(8), 211(5), 227(12), 239 (6), **255**(100)	+	–
66	Eupatorin[q]	13.11	$C_{18}H_{15}O_7^-$	343.08233	343.07865	3.68	313(6), **328**(100), 329(13)	282(15), 299(7), 309(15), 310(4), 312(3), **313**(100)	**285**(100)	+	–
67	Acacetin[a]	13.66	$C_{16}H_{11}O_5^-$	283.06120	283.05923	1.96	**268**(100), 269(11)	172(5), 200(5), 212(5), 239(19), 240(45), **268**(100), 269(24)	172(46), 196 (93), 210(21), **211**(100), 212 (45), 240(26)	+	–

[a] Confirmed using standards. Other compounds were mainly confirmed through the available literature related to *Salvia* or other plant species from the Lamiaceae family. The following references are listed in the Supplementary material: [b]Miao et al., 2016; [c]Shen et al., 2018; [d]Xu et al., 2017; [e]Geng et al., 2014; [f]Uysal et al., 2021; [g]Wang et al., 2009; [h]Krzyzanowska-Kowalczyk et al., 2018; [i]Zhang et al., 2015; [j]Grzegorczyk-Karolak et al., 2019; [k]Chen et al., 2011; [l]Piasecka et al., 2022; [m]Martins et al., 2015; [n]Al-Qudah et al., 2014; [o]Li et al., 2022; [p]Zhumakanova et al., 2021; [q]Shojaeifard et al., 2021. [r]The compound was not found in the literature but was proven by exact mass and fragmentation.

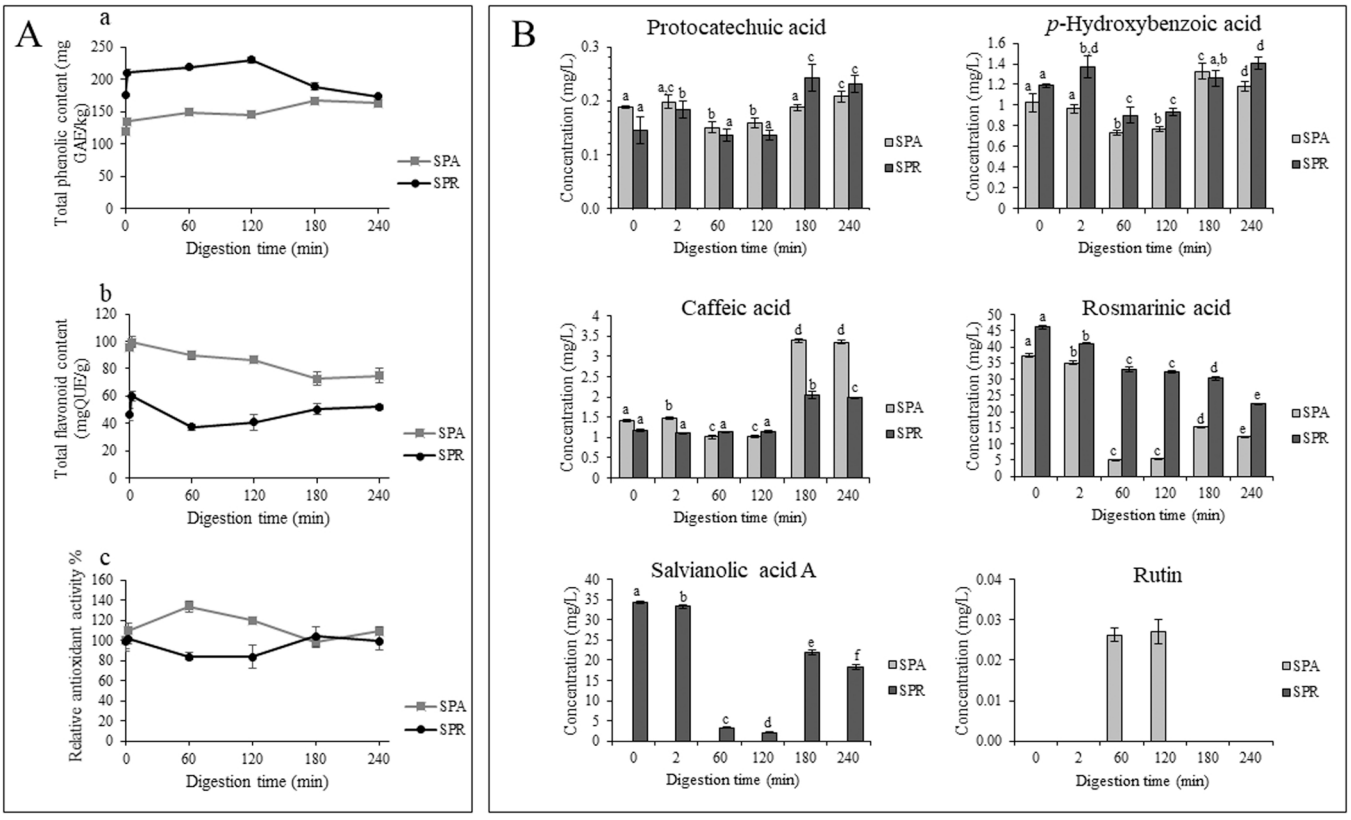


Fig. 1. Influence of *in vitro* simulated gastrointestinal digestion on the bioavailability of total phenolic and flavonoids content, as well as antioxidant activity of *S. pratensis* aerial part (SPA) and root (SPR) using DPPH radicals (A). The changes in identified phenolic compounds concentration in *S. pratensis* extracts during *in vitro* simulated digestion (B).

Table 3
Quantification of phenolic compounds in *S. pratensis* aerial part (SPA) and root (SPR) methanolic extracts using triple quadrupole mass spectrometer.

No.	Compounds	SPA (μg/g)	SPR (μg/g)
1	Quinic acid	39.14	36.05
2	Protocatechuic acid	46.51	36.78
3	*p*-Hydroxybenzoic acid	175.23	150.49
4	5-*O*-Caffeoylquinic acid	28.37	10.48
5	Gentisic acid	37.11	21.01
6	Caffeic acid	367.84	509.00
7	Rutin	26.73	2.87
8	Quercetin 3-*O*-glucoside	66.90	2.23
9	Ferulic acid	n.d.	35.79
10	Naringin	27.87	n.d.
11	Kaempferol 3-*O*-glucoside	27.87	6.97
12	Rosmarinic acid	8420.28	11,065.56
13	Salvianolic acid B	13.86	291.60
14	Salvianolic acid A	57.16	519.22
15	Eriodictyol	7.00	n.d.
16	Luteolin	149.85	8.58
17	Apigenin	69.26	6.62
18	Naringenin	5.08	n.d.
19	Hispidulin	7.50	3.90
20	Cirsimaritin	43.33	5.38
21	Kaempferide	5.53	4.58

n.d. – not detected

a slightly lower total antioxidant potential (447.70 mg Trolox/g of d.e.). Similar results were obtained for reducing capacity of SPA and SPR. Also, the higher radical scavenging activity of SPR was observed in DPPH and ABTS methods (IC_{50} 24.31 μg/mL for DPPH and 56.55 μg/mL for ABTS, respectively). IC_{50} values obtained for SPR were approximately two times lower than that obtained for SPA in both methods. Ascorbic acid showed a significantly higher potential to neutralize $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ compared with SPA and SPR, while the root extract showed a higher potential to neutralize $DPPH^{\bullet}$ in comparison with BHT.

Contrary to the results of all applied antioxidant methods in which the SPR extract showed better antioxidant properties, the SPA extract had a higher potential in the prevention of lipid peroxidation. As shown in Table 4, both SPA and SPR showed a lower antioxidant effect, with IC_{50} values of 395.73 and 435.27 μg/mL, respectively, towards inhibition of lipid peroxidation, compared with standard antioxidant BHT (IC_{50} 25.62 μg/mL). However, ascorbic acid in concentrations up to 100 μg/mL had no effects on the inhibition of lipid peroxidation.

The metal chelating activity assays were also used to evaluate the antioxidant potential of extracts considering the importance of free iron anions in the formation of free radicals in biological systems. However, SPA and SPR in concentrations up to 4 mg/mL chelated less than 50 % of Fe^{2+} in this method.

The antioxidant activity of some commercially available phenolic compounds quantified in extracts (Table 4) by a triple-quadrupole mass spectrometer was assessed using a DPPH method. Caffeic acid (IC_{50} 2.54 μg/mL), rosmarinic acid (IC_{50} 2.60 μg/mL), luteolin (IC_{50} 2.89 μg/mL), and rutin (IC_{50} 16.22 μg/mL) showed strong antioxidant potential and these compounds may be the most important compounds responsible for antioxidant potential of SPA and SPR. However, apigenin and naringenin inhibited less than 50 % of DPPH radicals at the maximum applied concentration ($IC_{50} > 100$ μg/mL).

3.3. Antimicrobial potential

Table 5 shows the antimicrobial potential of both methanolic extracts of *S. pratensis*. By comparing the antibacterial activity of extracts, SPR displayed the prominent antibacterial potential against most of the G+ bacteria. The root extract was effective in inhibiting the growth of most G+ bacteria (*S. aureus*, *S. epidermidis*, *B. subtilis*, *B. cereus*, and

Table 4
Antioxidative activity of methanolic extracts of *S. pratensis* aerial parts (SPA) and roots (SPR).

Extracts and standards	IC_{50} values (μg/mL)			Metal chelating ability	Total antioxidant activity (mg Trolox/g extract)	Reducing capacity (mg Trolox/g extract)
	DPPH scavenging activity	$ABTS^{+}$ scavenging activity	Inhibition of lipid peroxidation			
SPA	50.17 ± 2.98	90.65 ± 11.87	395.73 ± 2.66	> 4000	447.70 ± 25.86	466.70 ± 10.80
SPR	24.31 ± 2.13	56.55 ± 4.91	435.27 ± 29.59	> 4000	614.80 ± 42.43	529.83 ± 2.70
Ascorbic acid	8.17 ± 0.37	18.79 ± 0.29	>100	–	–	–
BHT	71.68 ± 2.84	21.29 ± 1.98	25.62 ± 3.83	–	–	–
EDTA	–	–	–	22.78 ± 1.14	–	–
Rutin	16.22 ± 2.73	–	–	> 1000	–	–
Caffeic acid	2.54 ± 0.10	–	–	–	–	–
Rosmarinic acid	2.60 ± 0.37	–	–	–	–	–
Luteolin	2.89 ± 0.01	–	–	–	–	–
Apigenin	>100	–	–	–	–	–
Naringenin	>100	–	–	–	–	–

DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); BHT, butylated hydroxytoluene; RU, rutin; EDTA, ethylenediaminetetraacetic acid; -, not tested.

Table 5
Antibacterial and antifungal activity of *S. pratensis* aerial part (SPA) and root (SPR) methanolic extracts.

Bacterial species			MIC*			
			SPA	SPR	Erythromycin	Nystatin
E. faecalis	*G* +	ATCC 29212	10	<0.156	20	–
B. cereus	*G* +	ATCC 10876	1.25	< 0.156	0.625	–
B. subtilis	*G* +	ATCC 6633	5	<0.156	0.625	–
S. epidermidis	*G* +	ATCC 12228	20	< 0.156	0.625	–
S. aureus	*G* +	ATCC 25923	5	< 0.156	< 0.3125	–
M. lysodeikticus	G +	ATCC 4698	10	20	10	
S. typhimurium	*G* -	ATCC 14028	5	< 0.156	1.25	–
S. enteritidis	*G* -	ATCC 13076	20	> 20	20	–
K. pneumoniae	*G* -	ATCC 70063	20	> 20	< 0.3125	–
P. aeruginosa	*G* -	ATCC 10145	20	20	< 40	–
E. coli	*G* -	ATCC 25922	10	> 20	2.5	–
Fungal species						
C. albicans		ATCC 10259	20	20	–	1.25
F. oxysporom		FSB 91	0.3125	5	–	5
A. brasiliensis		ATCC 16404	10	10	–	2.5
A. alternata		FSB 51	10	20	–	1.25
D. stemonitis		FSB 41	10	20	–	1.25
T. longibrachiatum		FSB 13	10	10	–	20
T. harzianum		FSB 12	5	2.5	–	2.5
P. canescens		FSB 24	2.5	2.5	–	5
P. cyclopium		FSB 23	1.25	5	–	1.25

*MIC values for extracts are expressed in mg/mL, while for erythromycin and nystatin are in μg/mL

E. faecalis) and *S. typhimurium* as G- bacteria with MIC value below the lowest applied concentration (MIC < 0.156 mg/mL). However, it has been noticed that SPR had no significant activity against other tested bacteria. On the other hand, SPA showed considerably lower antibacterial properties with the lowest MIC of 1.25 mg/mL for *B. cereus*. The commercial antibiotic erythromycin exerted antimicrobial activity with MIC values in the range from < 0.3125–20 μg/mL, except for *P. aeruginosa* (> 40 μg/mL).

The present investigation also included, for the first time, the antifungal activity of SPA and SPR (Table 5). The extracts showed lower antifungal activities compared with their antibacterial potential. Similar antifungal efficacy of SPA and SPR was observed against most of the examined fungal strains with a slightly higher antifungal potential of SPA against *F. oxysporom* (MIC 0.3125 mg/mL), *A. alternata* (MIC 10 mg/mL), *D. stemonitis* (MIC 10 mg/mL), and *P. cyclopium* (MIC 1.25 mg/mL) than SPR. The root extract had moderate antifungal activities with MIC values between 2.5 and 20 mg/mL. Nystatin was used as a reference antimycotic with MIC values from 1.25 to 20 μg/mL.

3.4. Effects of gastrointestinal digestion on phytochemicals

Fig. 1A shows changes in total phenols and flavonoids concentrations as well as relative antioxidant activity during digestion time. HPLC/DAD analysis was used to track changes in the concentration of major phenolic components in extracts during digestion (Fig. 1B). As it is shown in Fig. 1A, the total phenolic contents (TPC) of SPA and SPR slightly increased during the two first phases of digestion (until 60 min). In the next one hour, the TPC of SPA continued to increase, while its content in SPR stagnated. In accordance with the increase of TPC, the DPPH activity of SPA increased in the first hour of the digestion, surprisingly, the antioxidant activity of SPR decreased (Fig. 1A) in the same period of digestion. During the intestinal phase of digestion, the TPC in SPA gradually decreased until the end of digestion (240 min), while it increased in SPR between 120 and 180 min of digestion and then remained unchanged until the end. Meanwhile, the antioxidant activity of SPR continued to behave following with the change in TPC to the end of the digestion, while the same trend was not observed for SPA. The total flavonoid content (TFC) increased modestly after the end of the oral phase compared to the initial flavonoid level (Fig. 1A). Then, it rapidly decreased during the gastric phase. Interestingly, TFC continued to fall until the end of digestion in SPA, while the increase of TFC in SPR was observed.

The concentrations of selected phenolic compounds (protocatechuic, *p*-hydroxybenzoic, caffeic, rosmarinic acids, salvianolic acid A, and

rutin) in extracts during *in vitro* digestion were also monitored using UHPLC/(−)HESI−MS^2 analysis. Fig. 1B depictures variation in concentrations of these phenolics in extracts during *in vitro* digestion process in simulated salivary, gastric, and intestinal fluids. The concentration of protocatechuic acid was increased in extracts in the oral phase, while it decreased slightly in the gastric phase. However, its concentration in the intestinal phase was similar or with a slight increase compared with the initial concentration or during the oral phase of digestion. A similar trend was observed for *p*-hydroxybenzoic acid in both SPA and SPR. Rosmarinic acid and salvianolic acid A did not show high stability in simulated gastro-intestinal conditions in SPA and SPR. The highest decrease in concentration of rosmarinic acid in extract was noticed after 60 min of digestion, during the gastric phase. Similar effects were also observed for salvianolic acid A detected in SPR during simulated digestion experiments. A significant rise in the concentration of caffeic acid in both extracts was observed during the intestinal phase of digestion, which might suggest its release from various derivatives. Rutin was detected in measurable quantity in digestion solution only during the gastric phase of digestion.

3.5. The DNA-protective ability of the S. pratensis extracts

The effect of SPA and SPR, as well as their main phenolic constituents, to inhibit hydroxyl and peroxyl radicals-induced DNA damage is shown in the Fig. 2. The results obtained for SPA (Fig. 2 A) and SPR (Fig. 2B) demonstrated that extracts applied in concentrations up to 400 μg/mL (lanes 4, 5, 6, 7, 8) effectively inhibited DNA damage induced by hydroxyl radicals, whereby obtained results are comparable with DNA damage protection provided by the quercetin as standard (lane 3). Also, the increase in the concentration of the SPR (Fig. 2B, lanes 4, 5, 6, 7, 8) caused the reduction of its ability to protect DNA from hydroxyl radicals' damage. However, significantly higher DNA protection at all applied concentrations of SPR extract was noticed in comparison with quercetin (line 3) as a standard. It is evident from Fig. 2D, E (lanes 4, 5, 6, 7, 8) that the SPA and SPR extracts inhibited peroxyl radicals in a concentration-dependent manner, and the results were comparable with quercetin.

Phenolic compounds identified in methanolic extracts of *S. pratensis* also had a high potential to protect DNA against peroxyl radicals (Fig. 2F, lanes 3, 4). The rosmarinic and caffeic acids were less effective in reducing hydroxyl and peroxyl radicals-induced DNA damage at applied concentration (100 μg/mL) than investigated extracts (Fig. 2C, F). Interestingly, caffeic acid showed slightly higher DNA protection against hydroxyl radicals-induced damage than rosmarinic acid (Fig. 2C, lanes 3 and 4).

3.6. Biocompatibility and cytotoxicity of S. pratensis extracts

The cytotoxicity of SPA and SPR was studied on two eukaryotic cell lines (immortalized human normal HaCaT keratinocytes and murine BALB/c-3T3 fibroblasts), and two cancerogenic cells (human epidermoid carcinoma A431 and murine transformed SVT2 fibroblasts) by the MTT reduction assay. Based on the cell viability, SPR showed cytotoxic effects on all tested cell lines within a dose-dependent manner (Fig. 3B, D), whereas SPA showed weaker cytotoxicity against all the examined cell lines (Fig. 3A, C), and no IC_{50} values were determined for the SPA extracts up to 200 μg/mL. The IC_{50} values show that the SPR extract was more toxic on cells, and in particular on cancer cells. The IC_{50} values of SPR for two cancerogenic cell lines A431 and SVT2 were 24.3 and 49.6 μg/mL, respectively.

4. Discussion

The presence of secondary metabolites in plants is often explained by

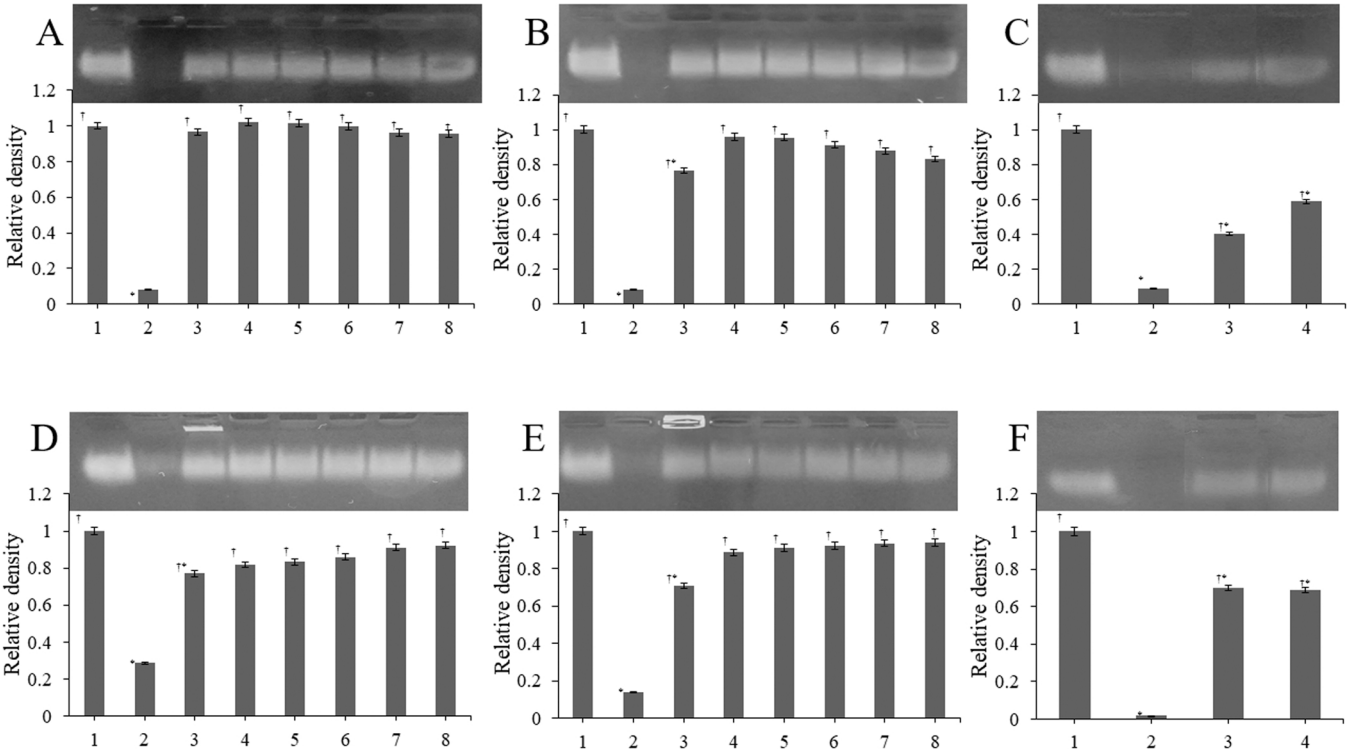


Fig. 2. DNA protective effect of the methanolic extracts of *S. pratensis* aerial parts (A), roots (B), and phenolic compounds detected in extracts (C) against hydroxyl radicals. DNA protective effect of the methanol extracts of *S. pratensis* aerial parts (D), roots (E), and phenolic compounds detected in extracts (F) against peroxyl radicals. Lane 1 – DNA from herring sperm (negative control); lane 2 – DNA damage control (positive control); lane 3 (A, B, D, E) – quercetin (100 μg/mL, standard); lanes 4–8 (A, B, D, E) – extracts in concentration of 25, 50, 100, 200, and 400 μg/mL, respectively. Phenolic compounds detected in the extracts (C, F): lane 3 – rosmarinic acid and lane 4 – caffeic acid.

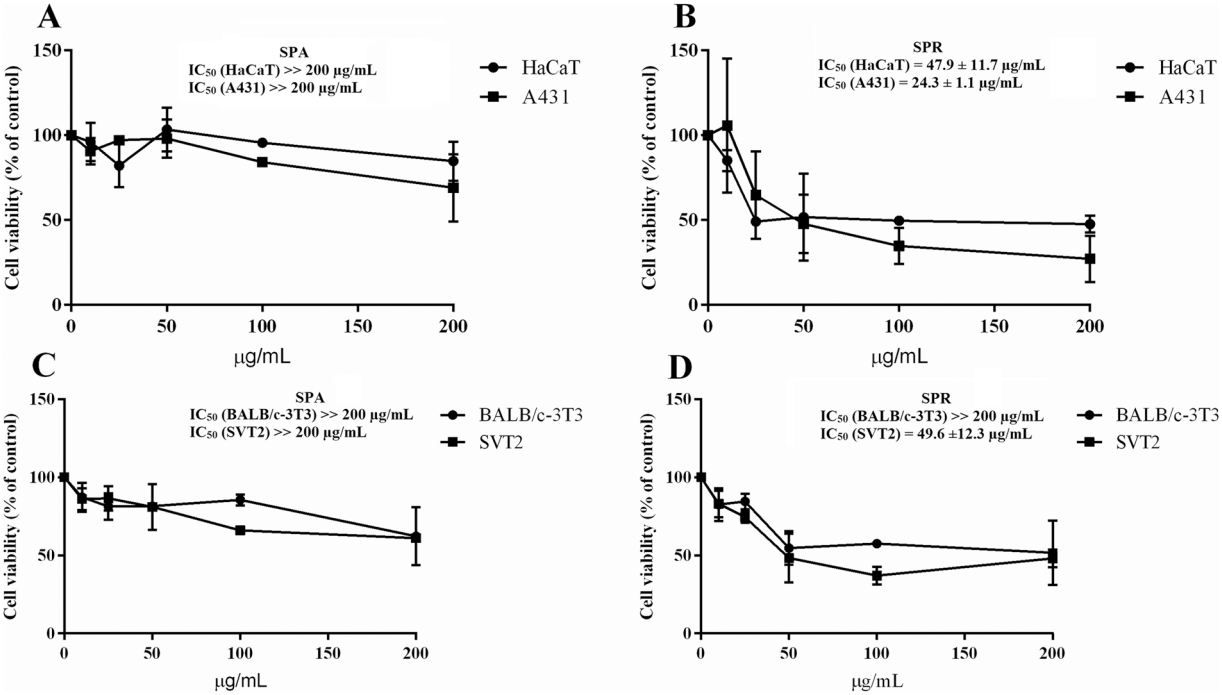


Fig. 3. Effect of SPA and SPR extracts on the viability of immortalized and cancer cells. Dose-response curves and IC_{50} values of HaCaT and A431 (A,B) and BALB/c-3T3 and SVT2 (C,D) cells after 48 h incubation with increasing concentrations of SPA (A,C) or SPR extracts (B,D) (10–200 µg/mL). Cell viability was assessed by the MTT assay and cell survival expressed as the percentage of viable cells in the presence of the extract under test, with respect to control cells grown in the absence of the extract. Data shown are means ± S.D. of three independent experiments.

the fact that plants mainly synthesize them as part of their immune answer to bacteria and fungi infections, herbivores, and environmental conditions (Longaray Delamare et al., 2007; Chamkhi et al., 2021). Most of the plants with a high potential for the synthesis of secondary metabolites with multidirectional biological activity come from the Lamiaceae family. Analyzed *S. pratensis* extracts had high phenolic content, root extract was particularly reach in total phenolics, phenolic acids, and condensed tannins (Table 1). Previous studies confirmed that *S. pratensis* and other examined *Salvia* species also contained high amounts of phenolic compounds. The methanolic extracts of *S. pratensis* leaves and flowers from Bulgaria were slightly richer in total phenolics (Tzanova et al., 2019) compared with SPA in this study. The aerial part of *Salvia verticillata* L. contained a higher amount of total phenolic compounds as reported by Katanić Stanković et al. (2020), compared with results obtained for SPA, but the total phenolic content in this extract is comparable with their content in SPR. However, it has been observed that SPR possessed higher total phenolic content (177.85 mg GAE/g extract) than root extracts of some other examined sage species such as *Salvia fruticosa* Mill. with total phenolic content of 80 mg GAE/g of extract (Boukhary et al., 2016) and *Salvia viridris* L. with 102.03 mg GAE/g of extract (Zengin et al., 2019).

Rosmarinic and caffeic acids, as well as caffeic acid derivates, are marked as the main polyphenolic compounds identified in SPA and SPR using UHPLC–MS^4 technic. The presence of caffeic and rosmarinic acids was previously confirmed by Šulniūtė et al. (2017) in the ethanolic extract of *S. pratensis* aerial part. A recent investigation of 27 Iranian wild *Salvia* species, reported by Fotovvat et al. (2018), showed that the aerial parts of most *Salvia* species were richer in phenolic acids (rosmarinic acid, salvionolic acid A and B, carnosic and caffeic acids) in comparison with their root extracts, while the results for SPA and SPR showed the opposite trend. Salvianolic acids, especially salvianolic acid A and B identified in SPA and SPR, have been found to possess strong antioxidant potential due to their polyphenolic structure as well as excellent cardioprotective activity (Ho and Hong, 2011; Sun et al., 2009; Hitl et al., 2021; Heydari and Chamani, 2022). *S. pratensis* aerial part contained lower caffeic and rosmarinic acid amounts compared with their content in the ethanolic aerial part extract of *S. pratensis* as well as some *Salvia* spp. from Lithuania (Šulniūtė et al., 2017) and hydromethanolic extract of *Salvia cadmica* Boiss. (Piątczak et al., 2021).

Many previously published studies about phenolic analyses of various *Salvia* species confirmed the presence of lithospermic acid in some of them (Xu et al., 2018) and its presence is also confirmed in SPR. Clindopolic acid A detected in SPA and SPR has also been confirmed in *Salvia palaestina* Benth. by Al-Qudah et al. (2014). Based on our knowledge, sagecoumarin (caffeic acid trimer) was detected for the first time in SPA and SPR in a sage species other than *S. officinalis* (Lu and Yeap Foo, 2002). Also, the detection of verbascoside and its derivatives (de-caffeoyl verbascoside and verbascoside isomer), compounds characteristic for *Verbascum* L. species, are detected in *S. pratensis* for the first time. Verbascoside was previously detected only in *S. viridis* among *Salvia* species (Grzegorczyk-Karolak and Kiss, 2018).

Flavonoids, flavanols, and their glycosides were identified and quantified predominantly in SPA, which is in accordance with previously reported research on the chemical composition of *S. cadmica* root extract (Piątczak et al., 2021). It is known that the content of flavonoids is highest in the leaves of sage plants, whereby luteolin and its derivatives are the most common flavonoids identified in SPA. Luteolin and apigenin are well-known flavonoid compounds found in aerial parts of various *Salvia* species (Lu and Yeap Foo, 2002). Šulniūtė et al. (2017) reported the high concentration of luteolin-7-*O*-β-D-glucuronide (2838 µg/g) in pressurized ethanolic extract of *S. pratensis* aerial part. Miski et al. (1983) reported that cirsimaritin, isolated from *S. palaestina,* possesses high anti-microbial activity against several bacteria strains.

Both *S. pratensis* extract showed high antioxidant potential in comparison with well-known referent antioxidant that were used. Their highest antioxidant potential is manifested towards neutralization of free radicals (DPPH· and $ABTS^{+}$). The higher antioxidant potrential revealed SPR in $ABTS^{\bullet+}$, $DPPH^{\bullet}$, total antioxidant activity, and reducing capacity in comparison with SPA. These findings correlate with the higher content of polyphenolic compounds in root extract in comparison

with the aerial part. The methanolic extract of *S. pratensis* aerial part (2.5 mg/mL) in the previous study showed the potential to inhibit 93% DPPH radical (Miliauskas et al., 2004). However, López et al. (2007) failed to detect the antioxidant activity of the methanol leaves extract of *S. pratensis* using the DPPH method. According to Tzanova et al. (2019), *S. pratensis* flowers extract possessed higher DPPH radical scavenging activity (79.1 mmol Trolox/kg d.e.) compared with leaves extract (40.8 mmol Trolox/kg d.e.).

The thiocyanate assay was used to evaluate the antioxidant potential of *S. pratensis* extracts and the prevention of linoleic acid peroxidation. This method may provide useful information about the ability of plant extracts for food preservation as inhibitors of the oxidation process in foods. *S. pratensis* showed low potential to inhibit peroxidation of linoleic acid in oil-in-water emulsion. The antioxidant potential of SPA and SPR obtained in the oil-in-water system is not in relation to their total phenolic content (Table 1), suggesting that all quantified phenolic compounds do not have the possibility to act as inhibitors of lipid peroxidation in emulsions. The higher antioxidant potential of SPA in this method may be a consequence of higher flavonoid content in SPA compared with SPR which possesses lower polarity and higher solubility in the nonpolar lipid layer. Also, SPA and SPR did not show the possibility to chelate Fe^{2+}, which has an important role in the generation of oxidative stress in aerobic organisms. Zhao et al. (2006) reported that *Salvia miltiorrhiza* Bunge ethanolic extract also did not show any chelating properties (3.5 mg/mL), as well as the aerial part extract of *S. verticillata* (Katanić Stanković et al., 2020). In another study, the ethyl-acetate extracts of several *Salvia* species showed better chelation capacity in relation to ethanolic extracts (Orhan et al., 2013).

The results for DPPH scavenging activity of some phenolic compounds identified in SPA and SPR (Table 4) indicate that exhibited antioxidant potential of *S. pratensis* extracts may be in correlation with concentrations of the strongest antioxidants, caffeic and rosmarinic acid, in extracts. Overall results for antioxidant activity obtained in this investigation demonstrated the comprehensive antioxidant potential of the aerial part and the root of *S. pratensis* methanolic extracts by different methods for the first time. Moreover, the antioxidant potential of *S. pratensis* root was reported for the first time in the current study. In addition to common sage (*S. officinalis*), as the most representative plants of the genus *Salvia*, many other *Salvia* species, such as *Salvia sclarea* L., *S. verticillata*, *Salvia nemorosa* L., and *S. cadmica*, are known for a high content of polyphenols and excellent antioxidant activity (Lu and Yeap Foo, 2002, Sharifi-Rad et al., 2018).

The antimicrobial potential of extracts and essential oils of *Salvia* species has been reported so far (Longaray Delamare et al., 2007; Annemer et al., 2022; Ghavam et al., 2020). Kamatou et al. (2007) isolated and identified oleanolic acid and its isomer ursolic acid, carnosol, and 7-O-methylepirosmanol from *Salvia chamelaeagnea* K. Bergius as compounds with notable antimicrobial properties against *S. aureus*. According to the obtained results, SPR showed better antibacterial activity against *E. faecalis* and *S. epedermidis*, with MIC $<$ 0.156 mg/mL than root extract of *S. cadmica* (MIC 1.25 mg/mL for *E. faecalis*; 0.625 mg/mL for *S. epedemidis*) in study published by Piątczak et al. (2021). The antimicrobial potential of *S. pratensis* aerial part in research published so far, also demonstrated its the low antimicrobial activity (Veličković et al., 2002). The antimicrobial activity of *S. pratensis* root methanolic extract was demonstrated for the first time in this research. One of the main groups of compounds from plants responsible for their antimicrobial effects is phenolic compounds (Sukhadiya et al., 2021). Rosmarinic acid, as the dominant compound in both extracts, is well-known for its different biological activities including good antimicrobial activity. However, Piątczak et al. (2021) showed that rosmarinic acid possesses meager antimicrobial activity in comparison with the aerial part and root extracts of *S. cadmica*. Thus, it is possible that the antibacterial properties of the SPR may be a consequence of the synergism of several compounds.

The digestive system, as a complex biochemical mechanism, has a role to provide the body with water, electrolytes, and nutrients in bioavailable forms. The processes inside the digestive system often may change the structure of the active compounds and their biological activities at the same time (Wojtunik-Kulesza et al., 2020). Thus, the determination of the impact of *in vitro* digestion on the phenolic composition and antioxidant activity of *S. pratensis* extracts is important for evaluating the bioavailability of bioactive compounds from these extracts. The results showed that concentration of phenolic compounds form *S. pratensis* extracts available for absorption in digestive tract, as well as their antioxidant potential, changes during *in vitro* digestion process. The changes in TPC, TFC, and antioxidant activity of SPA and SPR during simulated digestion were not pronounced, while the changes in concentration of individual phenolic compounds in the extracts were significantly noticeable. Protocatechuic and *p*-hydroxybenzoic acids showed significant stability during digestion protocol suggesting that they possess high bioaccessibility for further absorption. Also, there is the possibility that the increased content of these two phenolic acids in extracts during the digestion process is due to their liberation from the complex phenolic compounds such as their glycosides. Rosmarinic acid and salvianolic acid A did not show high stability in simulated gastro-intestinal conditions in SPA and SPR. The detection of a significantly lower concentration of rosmarinic acid in extract after 60 min of digestion, especially during the gastric phase, may be caused by its hydrolysis in an acidic environment or structural changes associated with the presence of different enzymes, changes in pH, and addition of chemical compounds for simulation of gastrointestinal conditions. Similar effects were also observed for salvianolic acid A detected in SPR during simulated digestion experiments. A significant rise in the concentration of caffeic acid in both extracts was observed during the intestinal phase of digestion, which might suggest its release from various derivatives. The changes in concentrations in rosmarinic and caffeic acids can be linked considering that rosmarinic acid is a caffeic acid ester with 3, 4-dihydroxyphenyl lactic acid. The increase in caffeic acid and decrease in rosmarinic acid concentrations may indicate that some amount of rosmarinic acid in extracts is decomposed to give caffeic acid as a product. Also, a significantly higher decrease in rosmarinic acid concentration and increase in caffeic acid concentration in the intestinal phase of digestion was observed for SPA than for SPR, suggesting the correlation between these changes in concentration. This result supports the hypothesis that rosmarinic acid can be hydrolyzed during digestion with caffeic acid as one of the products. Zorić et al. (2016) reported that the stability of the rosmarinic acid, when examined alone in solution, was notably higher than its stability in Lamiaceae plant extracts after gastrointestinal digestion. They confirmed that acid medium (pH=2.5) significantly decreases the concentration of rosmarinic acid ($\geq$ 50 %). During digestion process rutin was quantified in digestion solution only during the gastric phase. This behavior of rutin during digestion can be explained by the better stability of flavonoids in an acidic environment than in alkali media as is in intestinal degradation (Chen et al., 2020). Obtained results demonstrated low stability of the main phenolic compounds, rosmarinic acid and salvianolic acid A as hydroxycinnamic acid derivatives, in studied extracts during gastrointestinal digestion *in vitro*, but hydroxybenzoic and hydroxycinnamic acids are quite stable under digestion conditions. Thus, such bioaccessibility of these compounds identified in extracts suggests that the bioactivity of extracts determined *in vitro* may be changed in the human body after their oral application.

The effect of SPA and SPR on DNA damage induced by hydroxyl and peroxyl radicals has been also determined. Oxidative stress is one of the causes of DNA damage leading to a variety of modifications in DNA. Hydroxyl and peroxyl radicals are major biologically relevant free radicals responsible for oxidative DNA damage (Perron et al., 2008). Both *S. pratensis* extracts and main phenolic compounds identified in extracts showed high protection of free radical-induced DNA damage. Obtained results for SPA and SPR are comparable with DNA protective properties of quercetin molecule used as a standard compound. Genotoxicity and/or antigenotoxicity have been investigated for extracts from various

Salvia species. Also, the methanolic extract of *S. officinalis* showed a protective effect against H_2O_2-induced DNA damage in human lymphocytes (Ewadh et al., 2013). Yurtseven et al. (2008) indicated that *S. officinalis* extract could significantly decrease the DNA damage induced by antibiotic flavomycin in partridges (*Alectoris chukar*). According to Mathew and Thoppil (2012), *S. officinalis* extract does not produce a genotoxic effect in Swiss albino mice using micronuclei and comet assay. The antioxidant activity of phenolic compounds, detected in the extracts, could be involved in the prevention of DNA damage. Rosmarinic acid reduces the frequency of micronuclei and protects the DNA from damage induced by doxorubicin. Also, it has been confirmed that caffeic acid possesses inhibition ability against hydroxyl radical-induced DNA damage (Furtado et al., 2010).

The cytotoxicity assay on immortalized eukaryotic and cancerogenic cell lines for SPA and SPR showed biocompatibility of SPA with all examined cells in concentrations up to 200 μg/mL, while SPR showed higher cytotoxic potential with lower IC_{50} values for cancerogenic cells (A431 and SVT2). These results indicate that the aerial part of *S. pratensis* has a higher level of biocompatibility than the root, which thus should be considered with more caution when *S. pratensis* root is selected for different applications. Previous studies showed that methanolic extract of *S. verticillata* is fully biocompatible on normal cells (Katanić Stanković et al., 2020), while the hydro-methanolic extracts of *S. cadmica* showed weak cytotoxic activity against murine L929 fibroblasts (Piątczak et al., 2021). Previously, *Salvia* species have been reported as a source of powerful anticancer compounds (Lu and Yeap Foo, 2002). High concentrations of rosmarinic and salvianolic acids in the extracts, especially in the root, might be responsible for the cytotoxic activity against cancer cells (Piątczak et al., 2021). Caffeic acid-treated HeLa and ME-180 cancer cells showed enhanced apoptotic and morphological alterations (Kanimozhi and Prasad, 2015). Also, various studies confirmed the high cytotoxic activity of salvianolic acids A and B against breast, lung, liver, and squamous cell carcinoma (Ma et al., 2019). All these molecules identified in *S. pratensis* may be responsible for its recorded cytotoxic potential.

5. Conclusions

The obtained results showed that rosmarinic acid was the dominant phenolic compound in *S. pratensis*, with significant amounts of caffeic acid and salvianolic acid A and B, especially in the root extract. The root extract showed more pronounced biological properties in comparison with the aerial part extract and all these findings represent the first report about the chemical composition and potential application of *S. pratensis* root extract. Rosmarinic acid and salvianolic acid A, as hydroxycinnamic acid derivatives, showed low bioaccessibility during the gastrointestinal digestion of extracts *in vitro*, but hydroxybenzoic and hydroxycinnamic acids are quite stable under digestion conditions. Also, *S. pratensis* aerial part extract may be considered a non-cytotoxic plant food when applied in concentrations that showed strong antioxidant and DNA-protective effects, while the root extract had a more pronounced cytotoxic potential in those concentrations. Accordingly, meadow sage may be considered as a valuable ingredient for functional foods and nutraceuticals, as well as a good alternative for the use of the common sage in culinary and medicinal uses. The obtained results open the possibility for further research of *S. pratensis* root application as a nutraceutical, considering its high bioactivity potential and valuable phytochemical composition. Further research activities, also, may be focused on the definition of the safe dose of *S. pratensis* root extract for application considering its cytotoxic effects, as well as on research dealing with the identification of cytotoxic compounds in that extract.

CRediT authorship contribution statement

Conceptualization: Nikola Z. Srećković, Vladimir B. Mihailović; **Methodology:** Nikola Z. Srećković, Vladimir B. Mihailović, Danijela Mišić, Daria Maria Monti, Sanja Lj. Matić; **Formal analysis and investigation:** Nikola Z. Srećković, Nevena R. Mihailović, Luigi D'Elia, Uroš Gašić, Jelena S. Katanić Stanković; **Writing - original draft preparation:** Nikola Z. Srećković, Vladimir B. Mihailović; **Writing - review and editing:** Jelena S. Katanić Stanković, Daria Maria Monti; Nevena R. Mihailović; **Supervision:** Vladimir B. Mihailović;.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreements No. 451–03-68/2022–14/200122, No. 451–03-68/2022–14/ 200007, and 451–03-68/2022–14/200378).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2022.115841.

References

Al-Qudah, M.A., Al-Jaber, H.I., Abu Zarga, M.H., Abu Orabi, S.T., 2014. Flavonoid and phenolic compounds from *Salvia palaestina* L. growing wild in Jordan and their antioxidant activities. Phytochem 99, 115–120. https://doi.org/10.1016/j.phytochem.2014.01.001.

Anaya, J., Caballero, M.C., Grande, M., Navarro, J.J., Tapia, I., Almeida, J.F., 1989. A lupeol derivative from *Salvia pratensis*. Phytochem 28, 2206–2208. https://doi.org/10.1016/S0031-9422(00)97947-0.

Annemer, S., Farah, A., Stambouli, H., Assouguem, A., Almutairi, M.H., Sayed, A.A., Peluso, I., Bouayoun, T., Nouh, N.A.T., Lalami, A.E.O., Ez Zoubi, Y., 2022. Chemometric investigation and antimicrobial activity of *Salvia rosmarinus* Spenn essential oils. Molecules 27 (9), 2914. https://doi.org/10.3390/molecules27092914.

Avula, B., Bae, J.-Y., Chittiboyina, A.G., Wang, Y.-H., Wang, M., Srivedavyasasri, R., Ali, Z., Li, J., Wu, C., Khan, I.A., 2022. Comparative analysis of five *Salvia* species using LC-DAD-QToF. J. Pharm. Biomed. Anal. 209, 114520 https://doi.org/10.1016/j.jpba.2021.114520.

Aygun, R.B., Zengin, G., Yıldıztugay, E., Jugreet, S., Yılmaz, M.A., Mahomoodally, F.M., 2022. Chemical characterization, anti-oxidant, and anti-enzymatic properties of extracts from two *Silene* species: a focus on different plant parts and extraction methods. Process Biochem. 116, 206–213. https://doi.org/10.1016/j.procbio.2022.03.016.

Boukhary, R., Raafat, K., Ghoneim, A.I., Aboul-Ela, M., El-Lakany, A., 2016. Anti-inflammatory and antioxidant activities of *Salvia fruticosa*: an HPLC determination of phenolic contents. Evid. -Based Complement. Altern. Med. 2016, 7178105 https://doi.org/10.1155/2016/7178105.

Chamkhi, I., Benali, T., Aanniz, T., Menyiy, N.E., Guaouguaou, F.E., Omari, N.E., Shazly, M.E., Zengin, G., Bouyahyak, A., 2021. Plant-microbial interaction: the mechanism and the application of microbial elicitor induced secondary metabolites biosynthesis in medicinal plants. Plant Physiol. Biochem. 167, 269–295. https://doi.org/10.1016/j.plaphy.2021.08.001.

Chen, J., Kou, T., Fan, Y., Niu, Y., 2020. Antioxidant activity and stability of the flavonoids from *Lycium barbarum* leaves during gastrointestinal digestion. *Vitr.* Int. J. Food Eng. 16 (7), 20190315. https://doi.org/10.1515/IJFE-2019-0315.

Clinical and Laboratory Standards Institute, 2008a. third ed. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts (Approved Standard. Document M27. CLSI) M27-A3 Clinical and Laboratory Standards Institute, Wayne, PA.

Clinical and Laboratory Standards Institute, 2008b. second ed. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi (Approved Standard. Document M38. CLSI) M38-A2 Clinical and Laboratory Standards Institute, Wayne, PA.

Clinical and Laboratory Standards Institute (ninth ed) (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically (Approved Standard. Document M07. CLSI), M07-A9, Clinical and Laboratory Standards Institute, Wayne, PA.

Coisin, M., Necula, R., Grigoraş, V., Gille, E., Rosenhech, E., Zamfirache, M.M., 2012. Phytochemical evaluation of some *Salvia* species from Romanian flora. Al. I. Cuza" Iaşi s. II a. Biol. Veg. 58, 35–44. ISSN: 1223-6578.

Dzoyem, J.P., Tshikalange, E., Kuete, V., 2013. Medicinal plants market and industry in Africa. In: Medicinal Plant Research in Africa: Pharmacology and Chemistry. Elsevier, pp. 859–890. https://doi.org/10.1016/B978–0-12–405927-6.00024–2.

Ewadh, M., Zaidan, H., Haleem, Z., Al-Terehi, M., Saadi, A., 2013. Protective activity of some antioxidant plant against hydrogen peroxidase genotoxic effects on human lymphocyte. Adv. Life Sci. Technol. 13, 34–44. ISSN 2224-7181.

Fotovvat, M., Radjabian, T., Saboora, A., 2018. HPLC fingerprint of important phenolic compounds in some *Salvia* L. species from Iran. Rec. Nat. Prod. 13 (1), 37–49. https://doi.org/10.25135/rnp.72.18.02.228.

Furtado, R.A., De Araújo, F.R.R., Resende, F.A., Cunha, W.R., Tavares, D.C., 2010. Protective effect of rosmarinic acid on V79 cells evaluated by the micronucleus and comet assays. J. Appl. Toxicol. 30, 254–259. https://doi.org/10.1002/jat.1491.

Ghavam, M., Manca, M.L., Manconi, M., Bacchetta, G., 2020. Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of *Salvia hydrangea* DC. ex Benth. Sci. Rep. 10, 15647. https://doi.org/10.1038/s41598-020-73193-y.

Giusti, M.M., Wrolstad, R.E., 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. Curr. Protoc. Food Anal. Chem. F1.2.1–F1.2.13.. https://doi.org/10.1002/0471142913.faf0102s00.

Grzegorczyk-Karolak, I., Kiss, A.K., 2018. Determination of the phenolic profile and antioxidant properties of *Salvia viridis* L. Shoots: A comparison of aqueous and hydroethanolic extracts. Molecules 23. https://doi.org/10.3390/molecules23061468.

Heydari, H.R., Chamani, E., 2022. High-frequency plant regeneration and genetic fidelity assessment of regenerants by molecular and biochemical markers in Woodland Sage (*Salvia nemorosa* L.). Ind. Crops Prod. 187, 115404 https://doi.org/10.1016/j.indcrop.2022.115404.

Hitl, M., Kladar, N., Gavarić, N., Božin, B., 2021. Rosmarinic acid–human pharmacokinetics and health benefits. Planta Med. 87 (04), 273–282. https://doi.org/10.1055/a-1301-8648.

Ho, J.H.C., Hong, C.Y., 2011. Salvianolic acids: small compounds with multiple mechanisms for cardiovascular protection. J. Biomed. Sci. 18 (1), 30. https://doi.org/10.1186/1423-0127-18-30.

Hsu, C.K., Chiang, B.H., Chen, Y.S., Yang, J.H., Liu, C.L., 2008. Improving the antioxidant activity of buckwheat (*Fagopyrum tataricm* Gaertn) sprout with trace element water. Food Chem. 108, 633–641. https://doi.org/10.1016/j.foodchem.2007.11.028.

IPNI, 2022. International Plant Names Index. Published on the Internet https://www.ipni.org/n/457007–1, The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Botanic Gardens. [Retrieved 05 October 2022].

Kamatou, G.P.P., Van Vuuren, S.F., Van Heerden, F.R., Seaman, T., Viljoen, A.M., 2007. Antibacterial and antimycobacterial activities of South African *Salvia* species and isolated compounds from *S. chamelaeagnea*. S. Afr. J. Bot. 73, 552–557. https://doi.org/10.1016/j.sajb.2007.05.001.

Kanimozhi, G., Prasad, N.R., 2015. Anticancer effect of caffeic acid on human cervical cancer cells. Coffee in Health and Disease Prevention. Elsevier Inc., pp. 655–661. https://doi.org/10.1016/B978–0-12–409517-5.00073–5

Katanić Stanković, J.S., Srećković, N., Mišić, D., Gašić, U., Imbimbo, P., Monti, D.M., Mihailović, V., 2020. Bioactivity, biocompatibility and phytochemical assessment of lilac sage, *Salvia verticillata* L. (Lamiaceae) – a plant rich in rosmarinic acid. Ind. Crop Prod. 143, 111932 https://doi.org/10.1016/j.indcrop.2019.111932.

Kucekova, Z., Mlcek, J., Humpolicek, P., Rop, O., 2013. Edible flowers - antioxidant activity and impact on cell viability. Cent. Eur. J. Biol. 8, 1023–1031. https://doi.org/10.2478/s11535-013-0212-y.

Kumarasamy, Y., Byres, M., Cox, P.J., Jaspars, M., Nahar, L., Sarker, S.D., 2007. Screening seeds of some Scottish plants for free radical scavenging activity. Phytother. Res. 21, 615.

Longaray Delamare, A.P., Moschen-Pistorello, I.T., Artico, L., Atti-Serafini, L., Echeverrigaray, S., 2007. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem. 100, 603–608. https://doi.org/10.1016/j.foodchem.2005.09.078.

López, V., Akerreta, S., Casanova, E., María García-Mina, J., Yolanda Cavero, R., Isabel Calvo, M., 2007. *In vitro* antioxidant and anti-rhizopus activities of Lamiaceae herbal extracts. Plant Foods Hum. Nutr. 62, 151–155. https://doi.org/10.1007/s11130-007-0056-6.

Lopresti, A.L., 2017. *Salvia* (Sage): a review of its potential cognitive-enhancing and protective effects. Drugs R. D. 17, 53–64. https://doi.org/10.1007/s40268-016-0157-5.

Lu, Y., Yeap Foo, L., 2002. Polyphenolics of *Salvia* – a review. Phytochem 59 (2), 117–140. https://doi.org/10.1016/S0031-9422(01)00415-0.

Ma, L., Tang, L., Yi, Q., 2019. Salvianolic acids: potential source of natural drugs for the treatment of fibrosis disease and cancer. Front. Pharmacol. 10, 97. https://doi.org/10.3389/fphar.2019.00097.

Mathew, J., Thoppil, J.E., 2012. Genotoxicity of methyl parathion and antimutagenic activity of *Salvia officinalis* L. (sage) extracts in Swiss albino mice. Asian J. Pharm. Clin. Res. 5, 164–170.

Matkowski, A., Zielińska, S., Oszmiański, J., Lamer-Zarawska,, E., 2008. Antioxidant activity of extracts from leaves and roots of *Salvia miltiorrhiza* Bunge, *S. przewalskii* Maxim., and *S. verticillata* L. Bioresour. Technol. 99, 7892–7896. https://doi.org/10.1016/j.biortech.2008.02.013.

Miliauskas, G., Venskutonis, P.R., Van Beek, T.A., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 85, 231–237. https://doi.org/10.1016/j.foodchem.2003.05.007.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrì, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, L.S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., Mcclements, D.J., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S.J., Weitschies, W., Brodkorb, A., Doutor, R., Jorge, I.P., 2014. A standardised static in vitro digestion method suitable for food–an international consensus. Food Funct. 5, 1113. https://doi.org/10.1039/c3fo60702j.

Mišić, D., Šiler, B., Gašić, U., Avramov, S., Živković, S., Živković, J.N., Milutinović, M., Tešić, Ž., 2015. Simultaneous UHPLC/DAD/(+/-)HESI-MS/MS analysis of phenolic acids and nepetalactones in methanol extracts of *Nepeta* species: a possible application in chemotaxonomic studies. Phytochem. Anal. 26, 72–85. https://doi.org/10.1002/PCA.2538.

Miski, M., Ulubelen, A., Johansson, C., Mabry, T.J., 1983. Antibacterial activity studies of flavonoids from *Salvia palaestina*. J. Nat. Prod. 46, 874–875. https://doi.org/10.1021/np50030a007.

Orhan, I.E., Senol, F.S., Ercetin, T., Kahraman, A., Celep, F., Akaydin, G., Sener, B., Dogan, M., 2013. Assessment of anticholinesterase and antioxidant properties of selected sage (*Salvia*) species with their total phenol and flavonoid contents. Ind. Crop. Prod. 41, 21–30. https://doi.org/10.1016/j.indcrop.2012.04.002.

Oyaizu, M., 1986. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet. 44, 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307.

Perron, N.R., Hodges, J.N., Jenkins, M., Brumaghim, J.L., 2008. Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. Inorg. Chem. 47, 6153–6161. https://doi.org/10.1021/ic7022727.

Piątczak, E., Owczarek, A., Lisiecki, P., Gonciarz, W., Kozłowska, W., Szemraj, M., Chmiela, M., Kiss, A.K., Olszewska, M.A., Grzegorczyk-Karolak, I., 2021. Identification and quantification of phenolic compounds in *Salvia cadmica* Boiss. and their biological potential. Ind. Crop Prod. 160, 113113 https://doi.org/10.1016/j.indcrop.2020.113113.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341. https://doi.org/10.1006/abio.1999.4019.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.C., Bailleul, F., Trotin, F., 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacol. 72, 35–42. https://doi.org/10.1016/S0378-8741(00)00196-3.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Sarker, S.D., Nahar, L., Kumarasamy, Y., 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods 42, 321–324. https://doi.org/10.1016/j.ymeth.2007.01.006.

Scalbert, A., Monties, B., Janin, G., 1989. Tannins in wood: comparison of different estimation methods. J. Agric. Food Chem. 37, 1324–1329. https://doi.org/10.1021/jf00089a026.

Sharifi-Rad, M., Ozcelik, B., Altın, G., Daşkaya-Dikmen, C., Martorell, M., Ramírez-Alarcón, K., Alarcón-Zapata, P., Morais-Braga, M.F.B., Carneiro, J.N.P., Alves Borges Leal, A.L., Coutinho, H.D.M., Gyawali, R., Tahergorabi, R., Ibrahim, S.A., Sahrifi-Rad, R., Sharopov, F., Salehi, B., del Mar Contreras, M., Segura-Carretero, A., Sen, S., Acharya, K., Sharifi-Rad, J., 2018. *Salvia* spp. plants-from farm to food applications and phytopharmacotherapy. Trends Food Sci. Technol. 80, 242–263. https://doi.org/10.1016/j.tifs.2018.08.008.

Shojaeifard, Z., Hemmatinejad, B., Jassbi, A.R., 2021. Chemometrics-based LC-UV-ESIMS analyses of 50 *Salvia* species for detecting their antioxidant constituents. J. Pharm. Biomed. Anal. 193, 113745 https://doi.org/10.1016/j.jpba.2020.113745.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. Methods Enzymol. 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

Srećković, N., Katanić Stanković, J.S., Matić, S., Mihailović, N.R., Imbimbo, P., Monti, D. M., Mihailović, V., 2020. *Lythrum salicaria* L. (Lythraceae) as a promising source of phenolic compounds in the modulation of oxidative stress: Comparison between aerial parts and root extracts. Ind. Crop Prod. 155. https://doi.org/10.1016/j.indcrop.2020.112781.

Sukhadiya, M.L., Thakur, N.S., Patel, V.R., Gunaga, R.P., Kharadi, V.B., Tyagi, K.K., Singh, S., 2021. Provenance variations in proximate principles, mineral matter, total phenols and phytochemicals of *Melia dubia* drupes: an unexplored alternate livestock feed stock. J. For. Res. 32, 119–131. https://doi.org/10.1007/s11676-019-01080-y.

Šulniūtė, V., Pukalskas, A., Venskutonis, P.R., 2017. Phytochemical composition of fractions isolated from ten *Salvia* species by supercritical carbon dioxide and pressurized liquid extraction methods. Food Chem. 224, 37–47. https://doi.org/10.1016/j.foodchem.2016.12.047.

Sun, Y., Zhu, H., Wang, J., Liu, Z., Bi, J., 2009. Isolation and purification of salvianolic acid A and salvianolic acid B from *Salvia miltiorrhiza* by high-speed counter-current chromatography and comparison of their antioxidant activity. J. Chromatogr. B 877 (8–9), 733–737. https://doi.org/10.1016/j.jchromb.2009.02.013.

Tzanova, M.T., Gerdzhikova, M.A., Grozeva, N.H., Terzieva, S.R., 2019. Antioxidant activity and total phenolic content of five Salvia species from Bulgaria. Bulg. Chem. Commun. 51, 90–94.

Veličković, D.T., Randjelović, N.V., Ristić, M.S., Šmelcerović, A.A., Veličković, A.S., 2002. Chemical composition and antimicrobial action of the ethanol extracts of *Salvia pratensis* L., *Salvia glutinosa* L. and *Salvia aethiopis* L. J. Serb. Chem. Soc. 67 (10), 639–646. https://doi.org/10.2298/JSC0210639V.

Vitalini, S., Tomè, F., Fico, G., 2009. Traditional uses of medicinal plants in Valvestino (Italy). J. Ethnopharmacol. 121, 106–116. https://doi.org/10.1016/j.jep.2008.10.005.

Wojtunik-Kulesza, K., Oniszczuk, A., Oniszczuk, T., Combrzyński, M., Nowakowska, D., Matwijczuk, A., 2020. Influence of *in vitro* digestion on composition, bioaccessibility and antioxidant activity of food polyphenols – a non-systematic review. Nutrients 12, 1401. https://doi.org/10.3390/nu12051401.

Wu, Y.B., Ni, Z.Y., Shi, Q.W., Dong, M., Kiyota, H., Gu, Y.C., Cong, B., 2012. Constituents from *Salvia* species and their biological activities. Chem. Rev. 112 (11), 5967–6026. https://doi.org/10.1021/cr200058f.

Xu, J., Wei, K., Zhang, G., Lei, L., Yang, D., Wang, W., Han, Q., Xia, Y., Bi, Y., Yang, M., Li, M., 2018. Ethnopharmacology, phytochemistry, and pharmacology of Chinese *Salvia* species: a review. J. Ethnopharmacol. 225, 18–30. https://doi.org/10.1016/J.JEP.2018.06.029.

Yau Yan, L., 2006. Antioxidant properties of guava fruit: comparison with some local fruits. Sunway Acad. J. 3, 9–20.

Yermakov, A.I., Arasimov, V.V., Yarosh, N.P., 1987. Methods of Biochemical Analysis of Plants. John Wiley and Sons, New York.

Yurtseven, S., Çetin, M., Şengül, T., Sögüt, B., 2008. Effect of sage extract (*Salvia officinalis*) on growth performance, blood parameters, oxidative stress and DNA damage in partridges. S. Afr. J. Anim. Sci. 38 (2), 145–152.

Zengin, G., Mahomoodally, F., Picot-Allain, C., Diuzheva, A., Jekő, J., Cziáky, Z., Cvetanović, A., Aktumsek, A., Zeković, Z., Rengasamy, K.R.R., 2019. Metabolomic profile of *Salvia viridis* L. root extracts using HPLC–MS/MS technique and their pharmacological properties: a comparative study. Ind. Crop Prod. 131, 266–280. https://doi.org/10.1016/j.indcrop.2019.01.060.

Zhao, G.R., Xiang, Z.J., Ye, T.X., Yuan, Y.J., Guo, Z.X., 2006. Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem. 99, 767–774. https://doi.org/10.1016/j.foodchem.2005.09.002.

Zorić, Z., Markić, J., Pedisić, S., Bučevic-Popović, V., Generalic-Mekinić, I., Grebenar, K., Kulišić-Bilušic, T., 2016. Stability of rosmarinic acid in aqueous extracts from different Lamiaceae species after *in vitro* digestion with human gastrointestinal enzymes. Food Technol. Biotechnol. 54, 97–102. https://doi.org/10.17113/ftb.54.01.16.4033.