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Bioactivity, biocompatibility and phytochemical assessment of lilac sage, *Salvia verticillata* L. (Lamiaceae) - A plant rich in rosmarinic acid

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ABSTRACT

The plants from genus *Salvia*, as one of the largest genus in Lamiaceae family, are frequently in use for various purposes, as foods, in cosmetic industry, or in traditional and official medicine. *Salvia verticillata* L. (liliac sage) is one of sidelined sage species with potential bioactivity, reported in traditional medicine. The aim of this study was to acquire a phytochemical profile of the methanol extract obtained from *S. verticillata* aerial parts and to evaluate its antioxidant, antimicrobial, and biocompatibility potential. Characteristic compounds of the genus *Salvia*, such as rosmarinic and caffeic acids, along with their derivatives (*e.g.* salvianolic and yunnaneic acids isomers) and flavonoids, have been identified by ultrahigh-performance Orbitrap metabolomic fingerprinting as the main phenolic metabolites in *S. verticillata*. The extract displayed moderate antimicrobial properties and significant antioxidant potential, with the half maximal inhibitory concentration values (IC_{50}) ranging from 33 to 73 μg/mL. Importantly, full biocompatibility of the extract with eukaryotic cell lines was observed up to 72 h. The obtained results revealed the presence of polyphenolic bioactive compounds in *S. verticillata* extract with promising antioxidant potential and significant biocompatibility. In this regard, *S. verticillata* can find new perspectives of application as a food ingredient, in cosmetic and pharmaceutical industries, as it represents a valuable source of compounds with prominent health properties, with a special focus on rosmarinic acid.

1. Introduction

The genus *Salvia,* the largest genus in the Lamiaceae family, consists of over a thousand plant species distributed worldwide (Lu and Yeap Foo, 2002; Walker et al., 2004). The name of the genus was derived from the Latin word "salvare", which means "to heal/to save" (Topçu, 2006). The *Salvia* species are aromatic plants used as food spices and culinary herbs, as tea, in cosmetic industries, and in traditional medicine because of their bioactive properties (Ghorbani and Esmaeilizadeh, 2017). Since ancient times, the most known and most used *Salvia* spp. is a common sage (*Salvia officinalis* L.). This sage is used as a spice in food products, in traditional medicine for different ailments such as hemorrhage, menstrual disorders like dysmenorrhea, against tuberculosis, as well as in the treatment of numerous inflammatory diseases, dyspepsia, diarrhea, age-related cognitive disorders, tremor, excessive sweating, and hyperglycemia (EMA, 2016; Ghorbani and Esmaeilizadeh, 2017; Sharifi-Rad et al., 2018; Topçu, 2006). Besides *S. officinalis*, many other plants from genus *Salvia* are in use due to their biological benefits. For example, roots of red sage (*Salvia miltiorrhiza* Bunge) are highly respected and widely utilized in the treatment of cardiovascular and cerebrovascular diseases (Chen and Chen, 2018; Zhang et al., 2016). Also, chia seeds (*Salvia hispanica* L.) are used worldwide for their multifunctional properties and benefits to human health (Parker et al., 2018). One *Salvia* spp. that is marginalized from the use in modern pharmacological and functional food formulations is *Salvia verticillata* L.

Salvia verticillata L., called "lilac sage" or even "purple rain", is a herbaceous perennial herb with tiny lilac-blue flowers which grow tightly packed in whorls (Forouzin et al., 2015). This plant has been employed in the cheese-making process to obtain specific taste and for the preservation of meat products and cheese (Topçu, 2006). In Serbia, this herb is known by the name "sjeruša" that indicates its use for

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flavoring in cheese production. In traditional Serbian medicine, *S. verticillata* tea, made from its aerial parts, has been used as an expectorant, for disinfection of the oral cavity and as cataplasm for healing wounds (Jarić et al., 2015).

The chemical composition of *Salvia* plants is mostly comprised of several groups of secondary metabolites: terpenes (mono-, di-, and triterpenes), phenolic compounds (flavonoids and phenolic acids), and saccharides (Ghorbani and Esmaeilizadeh, 2017; Lu and Yeap Foo, 2002; Topçu, 2006; Xu et al., 2018). Moreover, a wide range of terpenoid compounds (*e.g.*, tanshinones, camphor, caryophyllene, borneol, α- and β-thujone) and polyphenolics (with characteristic high content of rosmarinic acid, caffeic acid and its metabolites salvianolic and yunnaneic acids) detected in *Salvia* spp. were found to endow a vast array of recorded bioactivities (Ghorbani and Esmaeilizadeh, 2017; Jassbi et al., 2016; Lu and Yeap Foo, 2002; Sharifi-Rad et al., 2018; Topçu, 2006; Wu et al., 2012; Xu et al., 2018).

In this respect, the aim of the presented study was to thoroughly investigate the phytochemical profile of methanol extract obtained from *S. verticillata* aerial parts (herein denoted as SV), to quantify targeted compounds, to evaluate its antioxidant and antimicrobial properties (antibacterial and antifungal), as well as its cytotoxicity on different cancer and immortalized cell lines.

2. Materials and methods

2.1. Chemicals

The reagents and chemicals used in the assays for evaluation of total phenolic compounds, antioxidant and antimicrobial activities were purchased from Sigma Aldrich (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). Nutrient agar (NA), Sabouraud dextrose agar (SDA), Müller–Hinton broth (MHB), and Sabouraud dextrose broth (SDB) were purchased from Torlak Institute of Virology, Vaccines and Sera (Belgrade, Serbia). Standards of 5-*O*-caffeoylquinic acid (chlorogenic acid), caffeic acid, quercetin 3-*O*-rutinoside (rutin), quercetin 3-*O*-rhamnoside (quercitrin), rosmarinic acid, salvianolic acid B, apigenin-7-*O*-glucoside (apigetrin), apigenin, carnosol, and carnosic acid were purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Plant material and extract preparation

The aerial parts of *Salvia verticillata* L. (Lamiaceae) were collected in July 2016 during flowering season in the area of village Prijevor (43°55′19.3″N 20°15′14.5″E), near the Ovčar-Kablar Gorge (Western Serbia) by J. S. Katanić Stanković. A voucher specimen (No. 125/016) was deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac (Kragujevac, Serbia). Taxonomic and botanical identification was confirmed by Dr. Milan S. Stanković. The air-dried *S. verticillata* aerial parts (130 g) were fine powdered and macerated with methanol at the room temperature for 24 h three times (500 mL each). The extract was filtered and the solvent was entirely removed using the rotary evaporator (RV 10 basic, IKA, Staufen, Germany) under low pressure to obtain the dry extract. The final weight of *S. verticillata* dry extract (SV) was 16.8 g. The percentage yield of SV was found to be 12.92% (w/w). The concentrations used in the experiments were based on the dry weight of the extract.

2.3. Determination of phenolic compounds

2.3.1. Total phenolic content (TPC)

The total phenolic content was estimated according to Singleton et al. (1999). Concisely, in 0.5 mL of SV extract (0.5 mg/mL) 2.5 mL of Folin–Ciocalteu reagent (diluted 10-fold) and 2 mL of $NaHCO_3$ (7.5%) were added. The absorbance was measured at 765 nm on UV–Vis double beam spectrophotometer Halo DB-20S (Dynamica GmbH, Dietikon, Switzerland) with temperature control after 15 min of incubation. TPC value was expressed as gallic acid equivalents (mg GA/g dry extract).

2.3.2. Total flavonoids content

The total flavonoid content was determined using the $AlCl_3$-method according to Brighente et al. (2007). The aluminum trichloride solution (0.5 mL, 2% $AlCl_3$) and the same volume of a methanol solution of SV (0.5 mg/mL) were incubated for 1 h at room temperature and the absorbance was measured at 415 nm. The total flavonoid content was expressed as rutin equivalents (mg RU/g extract).

2.3.3. Total flavonols content

Total flavonols content in the extract was evaluated by the method of Yermakov et al. (1987). The SV extract (1 mL, 1 mg/mL) was mixed with $AlCl_3$ (1 mL, 2%) and sodium acetate (3 mL, 50 mg/mL). The absorbance was read after 2.5 h at 440 nm. The content of flavonols was calculated as rutin equivalents and expressed as mg RU/g extract.

2.3.4. Total phenolic acids content

Total phenolic (hydroxycinnamic) acids content was adopted from Polish Pharmacopoeia as reported by Matkowski et al. (2008). Distilled water (5 mL) was added to SV (1 mL, 1 mg/mL). Thereafter, the mixture of HCl (1 mL, 0.1 M), Arnow reagent (1 mL, 10% w/v of sodium molybdate and 10% w/v sodium nitrite) and NaOH (1 mL, 1 M) was added and filled up to 10 mL. The absorbance was read immediately at 490 nm. The result was expressed as caffeic acid equivalents (mg CA/g extract).

2.4. UHPLC/MS-MS orbitrap analysis

Separations of compounds of interest were performed using an ultrahigh-performance liquid chromatography (UHPLC) system consisting of a quaternary Accela 600 pump and Accela autosampler (ThermoFisher Scientific, Bremen, Germany). A Syncronis C18 column (100 × 2.1 mm, 1.7 µm particle size), thermostated at 40°C, was used for compounds separation. The flow rate was set to 300 µL/min and the mobile phase consisted of 0.1% acetic acid in water (A) and acetonitrile (B). The injection volumes were 5 µL and the linear gradient program was previously described (Božunović et al., 2018).

The UHPLC system was coupled to a linear ion trap - orbitrap mass spectrometer (LTQ OrbiTrap MS) equipped with heated electrospray ionization probe (HESI-II, ThermoFisher Scientific, Bremen, Germany) operating in negative ionization mode. Parameters of the ion source were as in the literature (Božunović et al., 2018). The MS spectra were acquired by full-range acquisition covering 100-1000 *m/z*. The resolution was set to 30.000 for full scan analysis. The data-dependent MS/MS events were always performed on the most intense ions detected in the full scan MS. The ions of interest were isolated in the ion trap with an isolation width of 3 ppm and activated with 35% collision energy levels.

Xcalibur software (version 2.1) was used for the instrument control, data acquisition, and analysis. The identification of unknown compounds was done by exact mass search of their deprotonated molecule ($[M-H]^-$) and its MS^4 fragmentation, as well as by literature search of available chromatographic and MS data.

2.5. UHPLC-DAD/(-)HESI-MS/MS analysis

Targeted phenolic compounds were quantified using Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Bremen, Germany) configured with a triple-quadrupole mass spectrometer (TSQ Quantum Access Max, Thermo Fisher Scientific, Basel, Switzerland) with a heated electrospray ionization (HESI) source. Phenolics in the methanol extract of *S. verticillata* were chromatographically separated on Hypersil gold C18 column (50 × 2.1 mm) with 1.9 µm particle size (Thermo Fisher Scientific, USA), thermostated at 30°C. The mobile

phase, applied with a flow rate of 0.4 mL/min, consisted of 0.02% acetic acid in water (A) and LC–MS grade acetonitrile (B). Elution was performed according to Mišić et al. (2015), while the settings of the mass spectrometer were as described in Boroja et al. (2018): vaporizer temperature 350°C, spray voltage 3510 V, sheet gas (N_2) pressure 28 AU, ion sweep gas pressure 0 AU and auxiliary gas pressure at 4 AU, capillary temperature at 270°C, capillary offset 35 V, and skimmer offset 0 V. Targeted compounds were quantified in a selected reaction monitoring (SRM) mode of the mass spectrometer operated in the negative ionization mode. Collision-induced fragmentation was achieved using argon as the collision gas and collision energy was set to 30 eV.

Compounds were quantified by the external standard quantification procedure. Salvianolic acid C, rosmarinic acid hexoside, and methyl-rosmarinate were quantified relatively, based on the calibration curve of rosmarinic acid, while dicaffeoylquinic acid was quantified using the calibration curve of caffeic acid. Stock standard solutions were prepared by dissolving 1 mg of a pure compound in 1 mL methanol, and working standard solution was further prepared by mixing the stock solutions of pure compounds in methanol to obtain the concentration of 100 µg/mL. Calibration levels in the concentration range from 20 to 0.002 µg/mL were obtained by diluting the working solution with methanol. Determination of the limit of detection (LOD), limit of quantitation (LOQ), linearity, repeatability, and sensitivity of the developed UHPLC-DAD/(-)HESI-MS/MS method was performed. The LOD and LOQ were determined as peak-to-peak values by the signal-to-noise ratios (S/N), with S/N > 3 for LOD and S/N > 10 for LOQ. Five replicates of each calibration level were run for LOD and LOQ testing. Regressions of calibration curves showed good linearity with correlation coefficients between $r = 0.990$ and 0.999, $p <$ 0.001. The amounts of targeted compounds in the sample were expressed as µg per 100 mg of dry extract (µg/100 mg d.e.).

2.6. Antimicrobial activity

2.6.1. Tested microorganisms

The bacterial and fungal cultures (ATCC cultures and the clinically isolated strains) were used to evaluate the antimicrobial activity of *S. verticillata* extract. Sixteen microorganisms were tested, eight bacterial and eight fungal strains. In particular, the bacterial strain tested were: *Micrococcus lysodeikticus* ATCC 4698, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70063, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus cereus* ATCC 10876, *Bacillus mycoides* FSB 1, and *Azobacter chroococcum* FSB 14; and the eight fungal strains were: *Candida albicans* ATCC 10259, *Aspergillus brasiliensis* ATCC 16404, *Fusarium oxysporum* FSB 91, *Alternaria alternata* FSB 51, *Aureobasidium pillulans* FSB 61, *Trichoderma harzianum* FSB 12, *Penicillium canescens* FSB 24, and *Doratomyces stemonitis* FSB 41. All microbial strains were obtained from the Institute of Public Health Kragujevac, University of Kragujevac, Serbia and Laboratory for Microbiology, Department of Biology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia. The bacteria and fungi cultures were stored at 4°C and subcultured once a month. Bacterial strains were cultured overnight at 37°C in nutrient agar (NA) and fungi were cultured on Sabouraud dextrose agar (SDA) and potato glucose agar (PDA) at 28°C for 3 days.

2.6.2. Antibacterial activity tests

The minimum inhibitory concentration (MIC) of SV extract against tested microorganisms was determined based on the microdilution method in 96 multi-well microtiter plates (Sarker et al., 2007), with some modifications. All tests were performed in Müller–Hinton broth (MHB). Briefly, a fresh overnight culture of bacteria was suspended in sterile 0.85% saline (8.5 g/L NaCl) and adjusted by the colorimeter to a concentration of 5×10^6 CFU/mL (colony-forming units per milliliter) (CLSI, 2012). Different solvent dilutions of plant extract were dissolved in 5% dimethyl sulfoxide (DMSO) in sterile water and added to over the wells containing 50 µL of MHB; then, 10 µL of resazurin indicator solution (6.25 mg/mL in sterile distilled water) and 30 µL of MHB were added to each well. Finally, 10 µL of bacterial colony suspension was added to all the wells. The final bacterial concentration of in each well was 5×10^5 CFU/mL (CLSI, 2012). For each strain, the growth conditions and the sterility of the medium were checked. Standard antibiotic chloramphenicol was used to control the sensitivity of the tested bacteria. The microplates were incubated for 24 h at 37°C. Any color change of the indicator from purple to pink or colorless was recorded as positive. The lowest concentration that produced a significant inhibition of the growth of the bacteria in comparison with the positive control was identified as the MIC.

2.6.3. Antifungal activity tests

The fungal colonies were washed from the surface of agar plates with sterile 0.85% saline and the inoculum suspension was adjusted to a concentration of 5×10^4 CFU/mL according to NCCLS recommendation (NCCLS, 2002a,b). Identically, the 2-fold serial microdilution method was used for determination of MIC. The test was performed on Sabouraud dextrose broth (SDB). The SV extract (50 µL) was dissolved in sterile water to obtain a concentration of 40 mg/mL, added into the first row of the plate and double dilutions were made in all the other rows that were filled with 50 µL of SDB. Thereafter, 10 µL of SDB was added in all wells followed by addition of fungal inoculum suspension. For each strain, the growth conditions and the sterility of the medium were checked. Nystatin was used as the control against the tested fungi. Plates were placed in an incubator at 28°C for 48 h. The lowest concentrations without visible growth of fungi were defined as MICs.

2.7. Antioxidant activity

2.7.1. Total antioxidant capacity

The total antioxidant capacity of SV (Prieto et al., 1999) was monitored by the formation of a green phosphate/Mo (V) complex at acid pH. In 0.3 mL of extract solution (0.5 mg/mL) was added 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then, the mixtures were incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm. The total antioxidant capacity is expressed as ascorbic acid equivalents (mg AA/g).

2.7.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging activity

To determine scavenging DPPH radical activity different concentrations of SV in methanol (2 mL, eight double dilutions from 2 mg/mL) were mixed with the same volume of DPPH solution (80 µg/mL) (Kumarasamy et al., 2007). After 30 min of incubation at room temperature, the absorbance was measured at 517 nm. Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were used as reference standards. The DPPH free-radical scavenging activity (%) was calculated with the following equation [Eq. 1]:

$$((1))\ \%\ radical\ scavenging\ activity = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the DPPH radical in methanol and A_{sample} is the absorbance of the samples. The IC_{50} value, which is the concentration of the test material that reduces 50% of the free-radical concentration, was calculated as µg/mL through a sigmoidal dose-response curve.

2.7.3. 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS) radical-cation scavenging activity

The ABTS radical cation scavenging activity was estimated by the method described by Re et al. (1999). The radical cation ($ABTS^{\cdot+}$) was generated by reacting 7 mM stock solution of ABTS (2,2′-azinobis-(3-

ethylbenzothiazoline-6-sulfonic acid) diammonium salt) with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 16 h before use. The $ABTS^{\cdot+}$ solution was diluted with 5 mM phosphate-buffered saline (pH 7.4) to rich the absorbance of 0.70 ± 0.02 at 734 nm. After 30 min from the addition of 100 µL of sample to 900 µL of $ABTS^{\cdot+}$ solution, the absorbance was measured at 734 nm. Two compounds, AA and BHT, were used as reference antioxidants. A control sample was prepared to contain the same volume without test compounds or reference antioxidants. The percent of $ABTS^{\cdot+}$ scavenging activity of the samples was calculated using a previous equation [Eq. 1] and IC_{50} values were expressed as previous using a sigmoidal dose-response curve.

2.7.4. Nitric oxide radical scavenging activity

Nitric oxide radical ($NO^{\cdot}$) scavenging capacity was measured using the Griess reaction, according to the method described by Green et al. (1982). The samples (0.5 mL of SV or reference antioxidants AA and BHT) at different concentrations were mixed with the same volume of 5 mM sodium nitroprusside dissolved in 0.01 M phosphate-buffered saline (NaCl 0.138 M; KCl 0.0027 M, pH 7.4). Incubation was performed at 25°C for 2.5 h, and after that 1 mL of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid, and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride) was added to the mixture. After an additional incubation at 25°C for 30 min, the absorbance of the solution was measured at 546 nm. The results of nitric oxide radical scavenging activity were expressed as IC_{50}.

2.7.5. Determination of the inhibitory activity toward lipid peroxidation

The thiocyanate method (Hsu et al., 2008) was used to determine the antioxidant activity of SV extract in an oil-in-water emulsion. The extract or reference compounds (0.5 mL; SV, AA, and BHT) were added to linoleic acid emulsion (2.5 mL; 0.2804 g linoleic acid, 0.2804 g Tween-80 as an emulsifier in 50 mL 40 mM phosphate buffer, pH 7.0) and the mixture was homogenized. The final volume was adjusted to 5 mL with phosphate buffer (40 mM, pH 7.0). After incubation at 37°C in the dark for 72 h, a 0.1 mL aliquot of the reaction solution was mixed with methanol (4.7 mL, 75%), $FeSO_4$ (0.1 mL, 20 mM), and ammonium thiocyanate (0.1 mL, 30%). The absorbance of this mixture was measured at 500 nm, after 3 min of stirring. Inhibition percent of linoleic acid peroxidation was calculated using the following equation [Eq. 2]:

$$\%\ inhibition = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the samples. The IC_{50} values were calculated as µg/mL through a sigmoidal dose-response curve.

2.7.6. Measurement of ferrous ion chelating ability

The ferrous ion chelating activity of SV extract was measured by the decrease in absorbance at 562 nm of the iron (II)-ferrozine complex (Yan et al., 2006). Iron (II) sulfate (1 mL, 0.125 mM $FeSO_4$) was added to 1 mL sample (with different dilutions), followed by ferrozine (1 mL, 0.3125 mM). After 10 min the absorbances of the mixtures were measured. AA and BHT were used as standards. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the equation [Eq. 3]:

$$\%\ chelating\ effect\ [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the samples. The IC_{50} values were calculated as previous using a sigmoidal dose-response curve.

2.8. Biocompatibility analysis

Murine BALBC-3T3 (fibroblasts), human A431 (epidermoid carcinoma), HepG2 (hepatic carcinoma), and LoVo (colorectal adenocarcinoma) cells were obtained from ATCC, whereas HaCaT (primary epidermal keratinocyte) cells were from AddexBio (San Diego, CA, USA). All cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St Louis, MO, USA), supplemented with 10% fetal bovine serum (HyClone), 2 mM L-glutamine and antibiotics, all from Sigma-Aldrich, under a 5% CO_2 humidified atmosphere at 37 °C. For toxicity experiments, cells were seeded in 96-well plates at a density of 2.5×10^3 cells per well. Twenty-four hours after seeding, increasing concentrations of SV extract were added to the cells (5–50 µg/mL). Cell viability was assessed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after 48–72 h, as described by Petruk et al. (2016).

2.9. Statistical analysis

The data are expressed as the mean ± standard deviation (SD). The IC_{50} for *in vitro* antioxidant potential was calculated using nonlinear regression analysis from the sigmoidal dose-response inhibition curve using OriginPro 8 Software. For statistical analyses of the data, the analysis of variance (ANOVA) was applied and the group means were compared with the least significant difference test (LSD). The results were considered statistically significant at $p < 0.05$.

3. Results

3.1. Phytochemical composition of S. verticillata extract

The spectrophotometrically measured contents of total phenolic compounds, along with total flavonoids, flavonols, and phenolic acids in SV extract are shown in Table 1. Data indicated a high content in total phenolics in aerial parts of *S. verticillata*, with flavonoids (244.4 mg QU/g d.e.) and phenolic acids (41.9 mg CA/g d.e.) as the most abundant phytochemicals.

The qualitative evaluation of phenolic compounds in methanol extract of *S. verticillata* aerial parts was performed using high resolution mass spectrometry (HRMS) in combination with MS^4 fragmentation. The obtained UHPLC-MS^4 Orbitrap metabolic fingerprinting data of SV extract resulted in the detection of totally 29 phenolic compounds (Table 2) and the corresponding base peak chromatogram is depicted in Fig. 1. The identified compounds can be divided into two structurally different groups: 1) phenolic acids and their derivatives (21 compounds) and 2) flavonoids and their derivatives (8 compounds). Among all identified compounds, eight were confirmed using available standards, while the others were identified using HRMS technique (exact mass search of their deprotonated molecule $[M–H]^-$) and MS^2, MS^3, and MS^4 fragmentation behavior, as well as the comparison with the available literature. The peak numbers, retention times (t_R, min), compound names, molecular formulas, calculated and exact masses ($[M–H]^-$, *m/z*), mean mass accuracy errors (ppm), as well as major MS^2, MS^3 and MS^4 fragment ions of phenolics founds in SV extracts are summarized in Table 2.

Table 1
Total phenolic compounds, flavonoids, flavonols, and phenolic acids in *S. verticillata* aerial part extract (SV).

Extract	Total phenolic compounds (mg GA/g d.e.)	Total flavonoids (mg QU/g d.e.)	Total flavonols (mg RU/g d.e.)	Total phenolic acids (mg CA/g d.e.)
SV	175.6 ± 16.3	244.4 ± 4.7	16.9 ± 0.8	41.9 ± 5.4

GA – gallic acid equivalents, QU – quercetin equivalents, RU – rutin equivalents, CA – caffeic acid equivalents, d.e. – dry weight of the extract.

Table 2
UHPLC–MS^4 Orbitrap metabolic fingerprinting (negative ionization mode) of *Salvia verticillata* L. aerial part extract.

No[a]	t_R, min	Compound name	Molecular formula, $[M-H]^-$	Calculated mass, $[M-H]^-$	Exact mass, $[M-H]^-$	Δ ppm	MS^2 Fragments, (% Base Peak)	MS^3 Fragments, (% Base Peak)	MS^4 Fragments, (% Base Peak)
	Phenolic acids								
1[a]	3.90	Danshensu	$C_9H_9O_5^-$	197.04555	197.04539	0.81	**179**c(100), 151(15), 135(10)	**135**(100)	107(100), 79(60)
2	5.16	Caffeic acid hexoside	$C_{15}H_{17}O_9^-$	341.08781	341.08707	2.17	**179**(100), 135(10)	**135**(100)	91(100)
3	5.20	5-*O*-Caffeoylquinic acid[b]	$C_{16}H_{17}O_9^-$	353.08781	353.08719	1.76	**191**(100), 179(5)	173(75), **127**(100), 111(40), 93(60), 85(90)	109(40), 99(50), 85(100)
4	5.28	Coumaric acid hexoside	$C_{15}H_{17}O_8^-$	325.09289	325.09241	1.48	**163**(100), 119(10)	**91**(100)	–
5	5.75	Caffeic acid[b]	$C_9H_7O_4^-$	179.03498	179.03491	0.39	**135**(100)	135(60), 117(15), **107**(100), 91(55), 79(15)	–
6	5.85	Salvianolic acid C	$C_{18}H_{17}O_9^-$	377.08781	377.08789	−0.21	**359**(100)	223(10), 197(20), 179(15), **161**(100), 133(10)	133(100)
7	6.10	Yunnaneic acid E isomer 1	$C_{27}H_{23}O_{14}^-$	571.10933	571.10876	1.00	553(60), **527**(100), 509(80), 483(40), 439(90)	347(40), 329(50), **285**(100), 259(20)	–
8	6.28	Yunnaneic acid E isomer 2	$C_{27}H_{23}O_{14}^-$	571.10933	571.10895	0.67	553(30), **527**(100), 509(30), 329(20), 285(20)	**509**(100), 347(10), 329(20), 331(30), 285(70)	–
9	6.38	Rosmarinic acid hexoside isomer 1	$C_{24}H_{25}O_{13}^-$	521.13006	521.12970	0.69	**359**(100)	223(10), 197(20), 179(15), **161**(100), 133(10)	133(100)
10	6.70	Rosmarinic acid hexoside isomer 2	$C_{24}H_{25}O_{13}^-$	521.13006	521.12976	0.58	**359**(100)	223(10), 197(20), 179(15), **161**(100), 133(10)	133(100)
11	6.88	Methyl yunnaneate E	$C_{28}H_{25}O_{14}^-$	585.12498	585.12439	1.01	**553**(100), 541(10), 509(20)	535(20), **509**(100), 355(40), 311(60), 267(30)	465(10), 329(20), 311(100), 285(10), 267(30)
12	7.02	Sagerinic acid isomer 1	$C_{36}H_{31}O_{16}^-$	719.16176	719.16119	0.79	673(15), 539(30), 521(20), **359**(100), 341(25)	223(15), 197(20), 179(20), **161**(100), 133(5)	143(10), 133(100)
13	7.04	Methylsalvianolic acid C	$C_{19}H_{19}O_9^-$	391.10346	391.10324	0.56	**359**(100)	223(10), 197(20), 179(15), **161**(100), 133(10)	133(100)
14	7.12	Dicaffeoylquinic acid	$C_{25}H_{23}O_{12}^-$	515.11950	515.11957	−0.14	**353**(100), 335(5), 191(5)	**191**(100), 179(20), 135(10)	173(70), 171(30), 127(100), 111(50), 85(90)
15	7.20	Salvianolic acid B isomer 1	$C_{36}H_{29}O_{16}^-$	717.14611	717.14545	0.92	**555**(100), 519(10), 357(30), 313(20), 295(20)	357(40), 331(5), **313**(100), 269(5)	295(100), 269(30), 203(30), 191(20), 175(10)
16	7.50	Sagerinic acid isomer 2	$C_{36}H_{31}O_{16}^-$	719.16176	719.16339	−2.27	**359**(100)	223(5), 197(15), 179(10), **161**(100), 133(5)	161(5), 133(100)
17	7.50	Hydroxyrosmarinic acid	$C_{18}H_{15}O_9^-$	375.07216	375.07196	0.53	**179**(100)	**135**(100)	–
18	7.50	Rosmarinic acid[b]	$C_{18}H_{15}O_8^-$	359.07724	359.07736	−0.33	223(10), 197(30), 179(40), **161**(100), 133(10)	**133**(100)	105(100)
19	7.80	Salvianolic acid B isomer 2	$C_{36}H_{29}O_{16}^-$	717.14611	717.14539	1.00	537(5), **519**(100), 321(10)	339(20), **321**(100), 295(5), 279(5), 185(5)	303(10), 293(30), 279(100), 277(60), 249(5)
20	8.02	Methylcaffeate	$C_{10}H_9O_4^-$	193.05063	193.05045	0.93	178(100), 161(40), **147**(100), 134(20)	**129**(100), 119(10), 103(10), 85(10)	–
21	8.92	Methylrosmarinate	$C_{17}H_{17}O_8^-$	373.09289	373.09268	0.56	261(10), **179**(100), 135(50)	**135**(100)	–
	Flavonoids								
22	6.44	Quercetin 3-*O*-rutinoside[b]	$C_{27}H_{29}O_{16}^-$	609.14611	609.14594	0.28	343(5), **301**(100), 300(30), 271(10), 255(5)	273(25), 257(20), **179**(100), 151(75)	151(100)
23	6.73	Luteolin 7-*O*-glucoside[b]	$C_{21}H_{19}O_{11}^-$	447.09329	447.09454	−2.80	**285**(100)	257(30), **241**(100), 217(75), 199(85), 175(95)	241(5), 226(15), 213(30), 197(100)
24	6.75	Luteolin 7-*O*-hexuronide	$C_{21}H_{17}O_{12}^-$	461.07200	461.07275	−1.63	**285**(100), 175(5)	267(70), 257(20), 241(90), 213(70), **175**(100)	–
25	7.22	Quercetin 3-*O*-rhamnoside[b]	$C_{21}H_{19}O_{11}^-$	447.09329	447.09290	0.87	**301**(100), 300(30)	283(30), 273(20), 257(10), **179**(100), 151(90)	151(100)
26	7.25	Apigenin 7-*O*-glucoside[b]	$C_{21}H_{19}O_{10}^-$	431.09837	431.09772	1.51	311(5), **269**(100)	269(30), **255**(100), 197(45), 183(30), 151(20)	210(10), 197(100), 181(50), 169(40)
27	7.31	Apigenin 7-*O*-hexuronide	$C_{21}H_{17}O_{11}^-$	445.07763	445.07803	−0.90	**269**(100), 175(15)	**225**(100), 201(20), 183(15), 151(15), 149(40)	–
28	9.51	Apigenin[b]	$C_{15}H_9O_5^-$	269.04554	269.04544	0.37	269(60), **225**(10), 201(30), 151(70), 149(50)	210(10), 197(50), 196(20), 183(40), **181**(100)	–

(*continued on next page*)

Table 2 (*continued*)

No[a]	t_R, min	Compound name	Molecular formula, $[M–H]^-$	Calculated mass, $[M–H]^-$	Exact mass, $[M–H]^-$	Δ ppm	MS^2 Fragments, (% Base Peak)	MS^3 Fragments, (% Base Peak)	MS^4 Fragments, (% Base Peak)
29	10.92	Cirsimaritin	$C_{17}H_{13}O_6^-$	313.07176	313.07141	1.12	**298**(100), 283(5)	**283**(100), 269(30), 150(10)	255(100), 239(5), 227(10), 211(5), 163(5)

[a] Peak number of compounds corresponding to Fig. S1. [b]Confirmed using available standards, all the other compounds were identified based on MS data. [c]Peaks that were further fragmentated in MS^3 and MS^4 experiment are marked **bold** in table.

The examination of mass spectra of phenolic acids and their derivatives revealed various caffeic acid derivatives, which were expected (Li et al., 2015). More specifically, from phenolic acids group, only one identified compound is not a caffeic acid derivative and it is a coumaric acid hexoside (compound **4**). This compound eluting at 5.28 min and showing ($[M–H]^-$) at *m/z* 325, gave MS^2 base peak at *m/z* 163 (mass of deprotonated coumaric acid) and secondary MS^2 peak at 119 *m/z* which was obtained by further loss of CO_2 group (44 Da). As for the other phenolic acid derivatives, they all showed specific fragments of residues of caffeic acid. For example, compound **14**(dicaffeoylqiunic acid, eluting at 7.12 min and showing $[M–H]^-$ at *m/z* 515) showed MS^2 base peak at *m/z* 353 (loss of caffeoyl moiety) and MS^3 base peak at *m/z* 191 which corresponds to deprotonated quinic acid (Table 2).

Eight compounds from the group of flavonoids (quercetin 3-*O*-rutinoside, luteolin 7-*O*-glucoside, quercetin 3-*O*-rhamnoside, apigenin 7-*O*-glucoside, and apigenin) were identified in the SV extract and presence of five of them was confirmed by comparison with appropriate standards. Luteolin 7-*O*-hexuronide (compound **24**) and apigenin 7-*O*-hexuronide (compound **27**) were identified by a specific fragment at 175 *m/z*, which corresponds to deprotonated hexuronic acid. Presence of luteolin and apigenin as aglycones was confirmed by specific MS^3 fragmentation in both cases. These two hexuronyl derivatives were already identified in some *Salvia* species (Šulniūtė et al., 2017). Cirsimaritin (compound **29**), known to be present in *S. verticillata* (Ulubelen and Topcu, 1984) was visible as pseudomolecular ion $[M–H]^-$ at *m/z* 313 and was eluted at 10.92 min. It produced the MS^2 base peak at *m/z* 298 (generated by the loss of CH_3 group – 15 Da) and MS^3 base peak at *m/z* 269 (loss of second CH_3 group). The MS^4 base peak resulting from a further loss of CO group (28 Da) was identified at *m/z* 255. Proposed fragmentation pathway of this compound is depicted in **Fig. S1**.

UHPLC-DAD/(-)HESI-MS/MS analysis was targeted towards totally 14 compounds belonging to the phenolics (12 compounds) and phenolic diterpenes (2 compounds). The quantitative data of targeted phenolic compounds in SV extract (expressed in μg per 100 mg of dry extract) are presented in Table 3. The most abundant compound in SV was rosmarinic acid (235 mg/g d.e.), along with salvianolic acid C (1.1 mg/g d.e.). Their concentration in the extract was approximately more than a dozen times higher than those of the other quantified polyphenolics. Also, a high concentration of several rosmarinic acid derivatives, *i.e.*, rosmarinic acid hexoside and methylrosmarinate (3.6 and 1.07 mg/g d.e., respectively) was found in SV extract. Salvianolic acid B was present in SV in much lower amount (470 μg/g d.e.) compared with salvianolic acid C. The aerial parts of *S. verticillata* had moderate amounts of caffeic acid and its' derivatives with quinic acid. Flavonoids (quercetin, rutin, quercitrin, apigenin, and apigenin-7-*O*-glucoside) and characteristic diterpenes (carnosol and carnosic acid) were also present in SV in lower concentrations.

3.2. Biocompatibility and cytotoxicity of S. verticillata extract

The biocompatibility of *S. verticillata* aerial part extract was first assessed by using a cell survival assay. SV extract was tested on a panel of eukaryotic cell lines, from immortalized murine BalbC-3T3 fibroblasts and human normal HaCaT keratinocytes to human cancer cells: epidermoid carcinoma (A431), liver cancer (HepG2), and colon carcinoma (LoVo). Cells were treated for 48 and 72 h with an increasing amount of SV extract (from 5 to 50 μg/mL) and cell viability was assessed by the MTT assay. As shown in Fig. 2, no toxicity of SV extract was observed on any cell line analyzed after 48 h incubation. After 72 h incubation, the extract did not affect cell viability on immortalized cells, however a very slight, but significant, decrease in the viability of LoVo cancer cells was found after 72 h incubation only at the highest concentration used. These results indicate that SV extract is full biocompatible with eukaryotic cells.

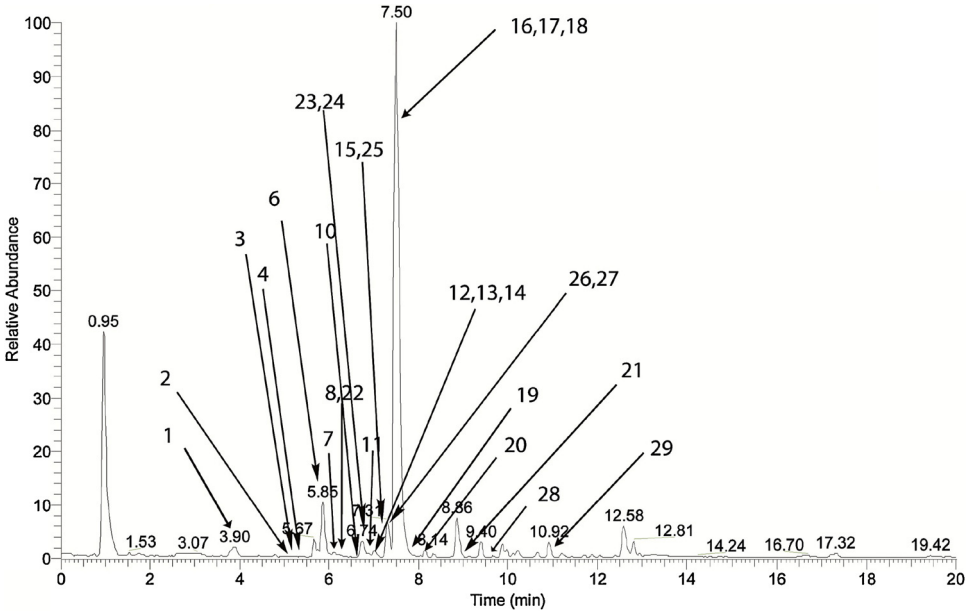

Fig. 1. UHPLC-MS^4 Orbitrap chromatogram (negative ionization mode) of *S. verticillata* aerial part extract. Peak numbers are as indicated in Table 2.

3.3. Bioactivity of S. verticillata extract

To explore the possibility to use SV extract as a source of the bioactive molecule(s), the extract was tested for its antibacterial and antifungal effects, as well as for its antioxidant activity *in vitro*. The antibacterial potential of SV, reported in Table 4, clearly indicated that this extract was very active on *B. cereus*, as it was able to inhibit the growth of this strain at a concentration of 1.25 mg/mL. On the other hand, the extract was less active on the other bacterial species tested (as indicated by higher MIC values). As for antifungal activity, the lowest MIC value was found on *P. canescens* (5 mg/mL), then *C. albicans* and *F. oxysporum* (10 and 20 mg/mL, respectively), while the extract had no antifungal potential on other treated fungal species (MIC > 20 mg/mL). Compared with the positive control used, *i.e.*, the commercial antibiotic chloramphenicol and the antimycotic nystatin, SV extract had moderate activity.

Then, the antioxidant activity of SV extract was evaluated, by using six different *in vitro* assays. The results of the experiments are shown in Table 5. SV extract exerted a very good antioxidant activity, in some cases comparable with the reference antioxidants used (ascorbic acid and BHT). The total antioxidant capacity of 1 g SV was in the range of 255 mg of ascorbic acid activity. The scavenging potential of SV towards DPPH radicals (IC_{50} 33.04 μg/mL) was much lower than ascorbic acid, but comparable with that of BHT (IC_{50} 26.11 μg/mL, $p < 0.05$). The $ABTS^{\cdot+}$ scavenging results indicated that SV extract had a pronounced activity (IC_{50} 67.01 μg/mL), but around three and two times lower, compared with ascorbic acid and BHT (15.43 and 37.18 μg/mL, respectively). NO radical scavenging potential of SV was comparable with ascorbic acid activity (IC_{50} 73.12 and 50.56 μg/mL, respectively), given that BHT was not active at the highest concentration tested. BHT was able to markedly inhibit lipid peroxidation in linoleic acid emulsion (IC_{50} value 3.82 μg/mL), while SV had moderate activity (IC_{50} 58.07 μg/mL) and ascorbic acid had no effects at the highest concentration applied. All tested samples did not exert metal chelating effects.

4. Discussion

In very few previous studies addressing the phytochemical characterization of *S. verticillata* extracts, caffeic acid and its derivatives were the main identified compounds (Fig. 3), with rosmarinic acid being the most abundant bioactive metabolite (Öztürk et al., 2011; Šulniūtė et al., 2017; Tepe et al., 2007), according to our results. Caffeic acid has an essential role in the biosynthesis of secondary metabolites of

Table 3
UHPLC/(−)HESI–MS/MS quantitative data of targeted phenolic compounds in *S. verticillata* aerial part methanol extract. Concentration is presented as μg per 100 mg of dry extract [μg/100 mg d.e.]. Values are means of three replicates ± SD.

No.	Rt (min)	Compounds	[M–H]$^-$	Diagnostic MS^2 fragments [M–H]$^-$	Concentration [μg/100 mg d.e.]
1	1.80	5-*O*-Caffeoylquinic acid[s]	353	127; 191	145.303 ± 22.461
2	2.45	Caffeic acid[s]	179	134; 135	9.539 ± 2.054
3	3.17	Salvianolic acid C	377	161; 359	1115.486 ± 19.941
4	3.92	Quercetin 3-*O*-rutinoside[s]	609	301; 179	0.262 ± 0.031
5	4.14	Rosmarinic acid hexoside	521	161; 359	359.519 ± 20.830
6	4.38	Dicaffeoylquinic acid	515	135; 191	35.981 ± 1.062
7	4.55	Quercetin 3-*O*-rhamnoside[s]	447	201; 300	0.013 ± 0.007
8	4.55	Apigenin-7-*O*-glucoside[s]	431	269; 311	7.964 ± 0.274
9	4.58	Rosmarinic acid[s]	359	133; 161	23458.624 ± 521.508
10	5.10	Salvianolic acid B[s]	717	321; 519	46.980 ± 1.055
11	5.72	Methylrosmarinate	373	135; 179	107.425 ± 2.690
12	5.88	Apigenin[s]	269	117; 149	0.676 ± 0.032
13	7.70	Carnosol[s]	329	201; 285	0.257 ± 0.002
14	9.86	Carnosic acid[s]	331	244; 287	0.169 ± 0.011

[s] Identified according to the standards.

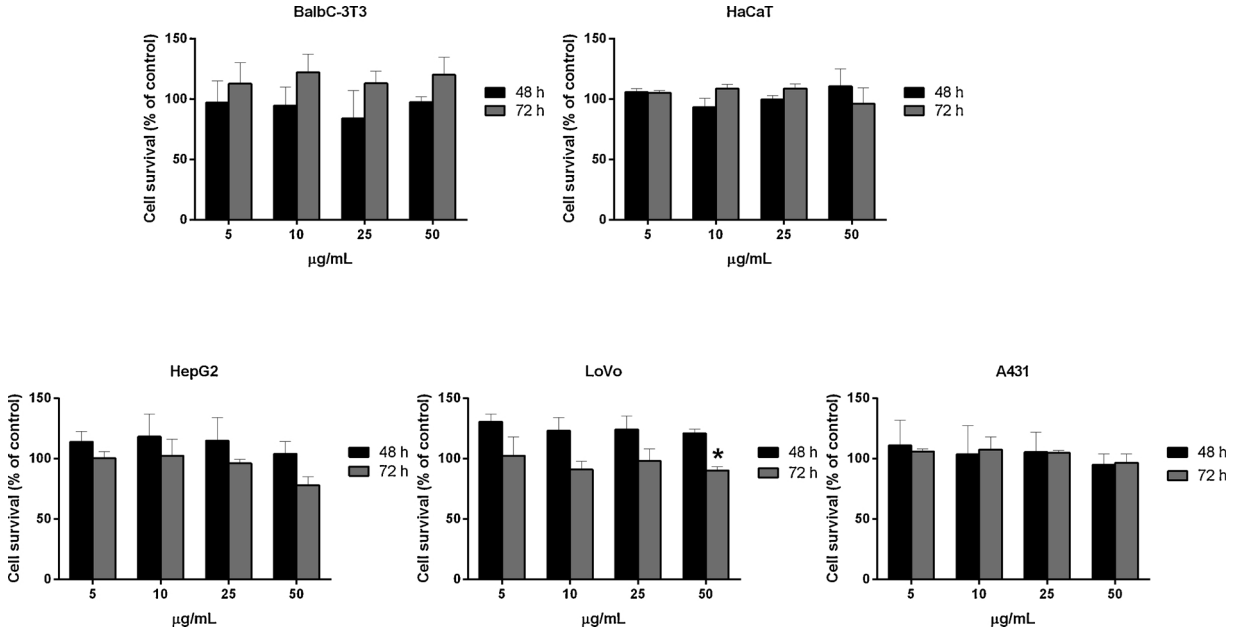

Fig. 2. Biocompatibility of the *S. verticillata* extract (SV) on BalbC-3T3, HaCaT, A431, HepG2, and LoVo cell lines. Dose- and time-response curves of cells after 48 and 72 h of incubation in the presence of 5, 10, 25, and 50 µg/mL of the extract. Values are given as means ± SD (n ≥ 3). * indicated $p < 0.05$ with respect to untreated cells.

Table 4
Antibacterial and antifungal activity of *S. verticillata* methanolic extract.

Bacterial species		MIC*	
		S. verticillata	Chloramphenicol
Micrococcus lysodeikticus	ATCC 4698	10	1.25
Enterococcus faecalis	ATCC 29212	20	10
Escherichia coli	ATCC 25922	20	2.5
Klebsiella pneumoniae	ATCC 70063	20	2.5
Pseudomonas aeruginosa	ATCC 10145	20	40
Bacillus cereus	ATCC 10876	1.25	2.5
Bacillus mycoides	FSB 1	10	10
Azobacter chroococcum	FSB 14	10	10
Fungal species		**MIC**	
		S. verticillata	Nystatin
Candida albicans	ATCC 10259	10	5
Aspergillus brasiliensis	ATCC 16404	> 20	40
Fusarium oxysporum	FSB 91	20	20
Alternaria alternata	FSB 51	> 20	40
Aureobasidium pillulans	FSB 61	> 20	> 40
Trichoderma harzianum	FSB 12	> 20	> 40
Penicillium canescens	FSB 24	5	10
Doratomyces stemonitis	FSB 41	> 20	> 40

* MIC - minimum inhibitory concentration values given as mg/mL for plant extracts and as µg/mL for antibiotic (Chloramphenicol) and antimycotic (Nystatin).

the Lamiaceae family and exists principally in its dimeric form: rosmarinic acid (Lu and Yeap Foo, 2002). Nevertheless, many other derivatives of caffeic acid were detected in our study on *S. verticillata* monomers, such as danshensu and dimers like salvianolic acid B (or lithospermic acid B). Other salvianolic acids dimers (G, F), normally present in *Salvia* plants, were not detected in our study. Usually, caffeic acid trimers, generally represent the largest group of metabolites in the genus *Salvia* (Lu and Yeap Foo, 2002); accordingly, in SV extract a quite high contents of salvianolic acid C (most likely obtained by conversion of unstable salvianolic acid A) and yunnaneic acid E (from oxidation of yunnaneic acid C) were detected. A representative of tetramers derived from caffeic acid, sagerinic acid, was also found in aerial parts of *S. verticillata*. Besides phenolic acids derived from caffeic acid, the SV extract was rich in other polyphenolic compounds, like flavones, flavonols and their glycosides, which are also widely distributed constituents in genus *Salvia* (Lu and Yeap Foo, 2002; Wu et al., 2012). In the analyzed SV extract, apigenin and 7-*O*-derivatives of apigenin and luteolin were detected, along with 3-*O*-derivatives of quercetin (rutin and quercitrin) and cirismaritin (6- hydroxyapigenin-6,7-dimethyl ether), which are all typical flavonoids of *Salvia* plants (Lu and Yeap Foo, 2002). However, the amount of analyzed flavonoids was much lower compared with phenolic acids. Similar results on the phytochemical composition of *S. verticillata* were recently reported by Šulniūtė et al. (2017). Also, Zengin et al. (2018) showed that *S. verticillata* subsp. *amasiaca* contained most of those compounds, claiming

Table 5
Antioxidant activity of *S. verticillata* methanolic extract.

Sample and standards	Total antioxidant activity (mg AA/g)	IC_{50} values* (µg/mL)				
		DPPH radical scavenging activity	ABTS radical-cation scavenging activity	NO radical scavenging activity	Inhibitory activity toward lipid peroxidation	Metal chelating activity
S. verticillata	254.55 ± 17.75	33.04 ± 5.83[a]	67.01 ± 13.62[a]	73.12 ± 19.04	58.07 ± 9.72	> 4000
Ascorbic acid	–	5.69 ± 0.82[b]	15.43 ± 2.65[b]	50.56 ± 20.69	> 100	> 100
BHT	–	26.11 ± 2.58[a]	37.18 ± 4.92[c]	> 1000	3.82 ± 0.42	> 100

* IC_{50} values were determined by nonlinear regression analysis. Results are mean values ± SD from three independent experiments; –, not tested; AA – ascorbic acid equivalents; DPPH – 2,2 – diphenyl – 1 – picrylhydrazyl; ABTS – 2,2′ – azino – bis(3 – ethylbenzothiazoline – 6 – sulphonic acid); NO – nitric oxide; BHT – butylated hydroxytoluene. Means in the same column with different letters as superscripts are significantly different at $p < 0.05$.

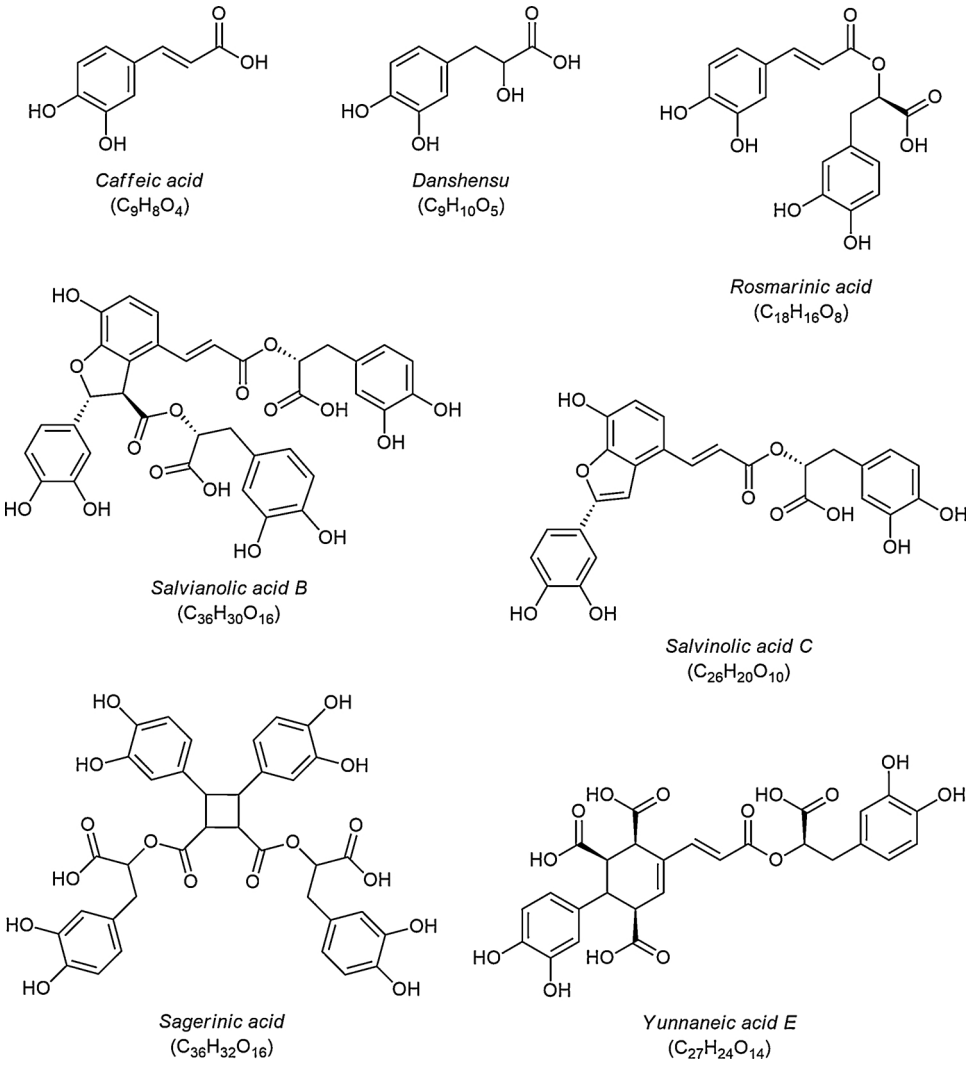

Fig. 3. Chemical structures of caffeic acid and its derivatives (mono-, di-, tri-, and tetramers) identified in the *S. verticillata* aerial part extract (SV).

that the amount of rosmarinic acid in SV (67 mg/g) is higher than the usual amounts found in other *Salvia* species. However, in this study, the concentration of rosmarinic acid in SV was much higher (*ca.* 235 mg/g), consistently with the results of Tepe et al. (2007) who quantified similar levels of rosmarinic acid in methanol extracts of both subspecies of *S. verticillata* (subsp. *verticillata* and subsp. *amasiaca*). Besides polyphenolic compounds, characteristic *Salvia* diterpenes, including carnosic acid and carnosol (phenolic diterpene), were detected and quantified in this study. Carnosic acid was also detected in this plant by Šulniūtė et al. (2017), but with no traces of carnosol. Generally, the aerial parts of *Salvia* spp. contain flavonoids, triterpenoids, and monoterpenes, mainly in the leaves and flowers, while the roots are rich in diterpenoids (Topçu, 2006). Moreover, Matkowski et al. (2008) reported that the levels of total phenolic content (TPC) in *S. verticillata* leaf and root extracts are quite similar, while Šulniūtė et al. (2016) showed that ethanolic extract of *S. verticillata* aerial parts was considerably richer in total phenolics than water extract. Our results also confirmed high TPC in SV extract, where flavonoids and phenolic acids were mostly present.

Salvia species are well-known for their broad spectrum of biological activities. One of the most prominent properties of the Lamiaceae family and, in particular, *Salvia* plants, is the antimicrobial activity. Besides *S. officinalis* (Gericke et al., 2018; Ghorbani and Esmaeilizadeh, 2017), many other members of this genus showed remarkable antimicrobial potential, for instance *S. potentillifolia*, *S. verbenaca*, *S. chorassanica*, *S. veneris*, *etc.*, as it was recently very well summarized by Sharifi-Rad et al. (2018). The same authors reported that *Salvia* spp. showed significant bactericidal and bacteriostatic activities against both Gram-positive and Gram-negative bacteria, mostly *Bacillus*, *Klebsiella*, *Pseudomonas*, and *Salmonella* species, as well as microorganisms from *Candida* and *Aspergillus* spp. Therefore, *Salvia* antimicrobial properties are directed, to the highest point, against food spoilage and foodborne pathogens, in order to be used as a natural preservative in food applications (Sharifi-Rad et al., 2018; Tajkarimi et al., 2010). The antimicrobial potential of *S. verticillata* was not tested previously, so the results of this study showed, for the first time, the antimicrobial properties of *S. verticillata* extract, which were consistent with previously published data on *Salvias*' effects. SV showed antibacterial activity towards all tested bacterial strains, whereby the effects on Gram-positive strain *B. cereus* was particularly noteworthy, followed by *B. mycoides*, *M. lysodeikticus*, and Gram-negative *A. chroococcum*. The antifungal activity of SV was less notable, except for *P. canescens* and *C. albicans*. This potential is ascribed to the chemical composition of the extracts, mainly to the phenolic compounds presented in *Salvia* species. First and foremost compound, presented in high content in SV, is certainly rosmarinic acid, endowed with high antimicrobial potential towards a wide range of microbes, with minimal inhibitory concentration values (MIC) starting from 0.01 mg/mL (Abedini et al., 2013; Amoah et al., 2016). Also, many other phenolics detected in SV, *e.g.* phenolic acids, apigenin, luteolin, and quercetin derivatives (Cushnie and Lamb, 2005),

and diterpenoids like carnosol and carnosic acid (Ghorbani and Esmaeilizadeh, 2017), exert antibacterial and antifungal potential due to the high presence of −OH groups which play an important role in the antimicrobial activity (trough the interaction with the cell membrane, delocalization of electrons, binding the active site of enzymes, and leading to the microorganisms growth restriction) (Gyawali and Ibrahim, 2014). Although the extracts of *Salvia* species generally have good antimicrobial properties, *Salvia* essential oils are mostly used due to the high content of volatile antimicrobial compounds like α-thujene, α- and β-pinene, myrcene, limonene, *etc.*, which were also identified in *S. verticillata* essential oil (Giuliani et al., 2018; Pitarokili et al., 2006; Rzepa et al., 2009). As SV extract had moderate antimicrobial activity, perhaps the more justified use of SV in this sense would be in the form of essential oil.

Besides antimicrobial activity, phenolic compounds are recognized for their prominent antioxidant effects. Their hydrogen atom donating potential render them exceptionally efficacious antioxidants, with different mechanisms of action counteracting oxidative stress in materials and organisms (Brewer, 2011). They can interfere with the process of free radical generation in phases of initiation or propagation, and sometimes even as metal chelators, they have the ability to activate antioxidant enzymes and modification of prooxidant properties of low molecular antioxidants (Procházková et al., 2011; Shahidi and Ambigaipalan, 2015). Many of them are in use in the food industry as food additives for preventing lipid oxidation and therefore extending the shelf-life of food products without any influence on the food nutritional quality and sensory properties (Shahidi and Ambigaipalan, 2015). Regarding Lamiaceae plants, numerous members of this family and one of their main phenolic compounds, rosmarinic acid, are used in industrial applications for cosmetic and food products, because of significant antioxidant activity (Trivellini et al., 2016). Since the tested *S. verticillata* aerial part extract contains a high content of phenolic compounds, known to act as antioxidants, it is not surprising that SV extract exerts good total antioxidant capacity and scavenging potential towards different free radicals. Šulniūtė et al. (2016) reported good $ABTS^{\cdot+}$ scavenging activity and high ORAC values of different *S. verticillata* aerial part extracts, while Matkowski et al. (2008) showed that the root extract had better $ABTS^{\cdot+}$ scavenging activity than its leaves, but lower reducing power and $DPPH^{\cdot}$ antioxidant potential. Moreover, in a study from Tepe et al. (2007), it was shown that both *S. verticillata* subspecies (subsp. *verticillata* and *amasiaca*) exerted similar free radical-scavenging capacities and the inhibition ratio of linoleic acid oxidation, comparable to the effects of rosmarinic acid. Several other studies described the antioxidant activity of *S. verticillata* subsp. *amasiaca* against free radicals, as well as chelating and reducing potential (Orhan et al., 2007, 2013; Zengin et al., 2018). Thus, the traditional use of *S. verticillata* in preparing cheese and meat products is supported by these results, since its antioxidant capacity helps to prevent food quality deterioration. Like many other members of the Lamiaceae family (rosemary, sage, basil, oregano, marjoram, savory, and thyme), rich in rosmarinic acid and other proven antioxidant compounds (Brewer, 2011), *S. verticillata* extract may also be included in the food industry for different purposes. In that sense, the biocompatibility of the extract is very important for its implementation in the human diet and incorporation in human-used products.

Taking into account the content of diverse phenolic compounds with proven bioactivity, it is surprising that there is no literature data related to the influence of *S. verticillata* extract on eukaryotic cell lines. Our results showed, for the first time, that SV was not toxic towards murine immortalized fibroblasts (BalbC-3T3) and human immortalized epidermal keratinocytes (HaCaT) in all tested concentrations. Moreover, there were no significant cytotoxic effects on three cancer cell lines (A431, HepG2, and LoVo), except at the highest concentration of SV. Zengin et al. (2018) confirmed the absence of antiproliferative activity of *S. verticillata* subsp. *amasiaca* on human alveolar lung epithelial carcinoma (A549) and human breast adenocarcinoma (MCF-7), as well as its influence on inhibition of some key enzymes (cholinesterases, tyrosinase, glucosidase, amylase, lipase, and elastase) in different illnesses of modern age, such as Alzheimer's disease, diabetes mellitus, and obesity.

5. Conclusion

The results showed that a methanol extract from *S. verticillata* aerial parts is quite rich in phenolic compounds, particularly rosmarinic acid and its derivatives. The extract exerted good antimicrobial activity against some of the selected bacteria and fungi species. Also, SV extract exhibited prominent antioxidant properties and antiradical potential, comparable to referent antioxidants. Its action on normal and cancer cell lines indicates high biocompatibility and absence of cytotoxicity. The observed results suggested that *S. verticillata* should not be considered just like a weed plant, but rather as a rich source of compounds with prominent bioactive properties and as a potential additive in food or cosmetic industries, with further exploration of concrete possibilities of use and extract standardization.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2019.111932.

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