Arch Biol Sci. 2026;78(1):101-118 https://doi.org/10.2298/ABS260211005R

Potential genotoxicity of *Salvia pratensis* L. aqueous extracts and their biogenic silver nanoparticles

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Received: February 11, 2026; **Revised**: March 10, 2026; **Accepted**: March 14, 2026; **Published online**: March 18, 2026

Abstract: Biosynthesis of metal nanoparticles using plant extracts is considered an eco-friendly approach with promising biomedical applications; however, data on their safety, particularly genotoxicity, remain limited. This study evaluated the phenolic composition and genotoxic potential of aqueous extracts from the root (SPR) and aerial parts (SPA) of *Salvia pratensis* L. and their corresponding biogenic silver nanoparticles (SPR-AgNPs and SPA-AgNPs). Spectrophotometric methods were used to quantify total phenolic and flavonoid contents, and LC-MS analysis was employed to characterize the phenolic profiles. The genotoxic potential of the extracts and AgNPs was evaluated in human lymphocytes via the comet assay, with treatments applied at four concentrations ranging from 25 to 100 μg/mL, either independently or in combination with hydrogen peroxide (H_2O_2). Both extracts exhibited high phenolic content, with lower flavonoid levels in SPR. LC-MS analysis identified rosmarinic acid as the predominant compound in SPA and salvianolic acid K in SPR. Both extracts and AgNPs increased DNA damage in lymphocytes. In contrast, only the SPA extract lowered H_2O_2-induced DNA damage, demonstrating a pronounced protective effect. These findings indicate potential genotoxic risks associated with *S. pratensis* extracts and their AgNPs, while also highlighting selective protective properties depending on the plant part used.

Keywords: *Salvia pratensis* L., phenolic compounds, LC-MS, genotoxic effect, protective effect

INTRODUCTION

Silver nanoparticles (AgNPs) are among the most widely used nanomaterials and have found potential applications in many fields, especially in biomedicine and biotechnology [1]. The biosynthesis of metal nanoparticles using plant extracts has gained immense attention in recent years due to its simplicity, eco-friendliness, and potential biomedical applications. Numerous studies have focused on synthesizing plant-based nanoparticles and evaluating their biological activities, including antimicrobial, antioxidant, and cytotoxic effects. However, despite this massive research effort, data on the genotoxic potential of these nanomaterials remain scarce, raising concerns about their safety for human health.

The genus *Salvia*, commonly known as sage, is a rich source of bioactive compounds, including flavonoids, phenolic acids, and terpenes, which contribute to a wide range of biological activities [2,3]. Plants of the genus *Salvia* have proven to be excellent substrates for the eco-friendly green synthesis of AgNPs, and to date, different nanoparticles have been synthesized and characterized using extracts from different *Salvia* species [4-6]. Among them, *Salvia pratensis* L. (meadow sage) has attracted attention due to its traditional use in treating abdominal, skin ailments, and ulcers, as well as a substitute for common sage (*S. officinalis*) [7]. To date, methanolic extracts of *Salvia pratensis* have been chemically characterized, with rosmarinic acid, caffeic acid, and salvianolic acids A and B identified as

the dominant compounds [2]. However, the chemical composition of the aqueous extract of *Salvia pratensis* remains unexplored. Recent studies have demonstrated that methanolic extracts of its root possess remarkable antimicrobial activity against Gram-positive bacteria and significant cytotoxic effects against cancer cell lines [2]. Furthermore, biogenic silver nanoparticles synthesized using *S. pratensis* root extracts have shown enhanced antimicrobial and cytotoxic potential, highlighting the plant's suitability for green nanoparticle synthesis [4]. Before any further preclinical or clinical investigations, such as testing for antimicrobial activity against Gram-positive skin pathogens or evaluating cytotoxicity against skin cancer cell lines, a thorough assessment of the genotoxic potential of these extracts and their biogenic silver nanoparticles is essential.

Although several studies have examined the biological properties of *S. pratensis* extracts, including antioxidant [8,9], cytotoxic [2,8], antimicrobial, and DNA-protective effects [2], no data exist on the genotoxicity of its aqueous extracts or their biosynthesized silver nanoparticles. To our knowledge, no prior study has comprehensively examined the phenolic composition or the genotoxic and DNA-protective properties of aqueous extracts from the roots (SPR) and aerial parts (SPA) of *S. pratensis*, nor evaluated the AgNPs derived from their environmentally friendly synthesis. No previous study has evaluated the genotoxic potential of *Salvia pratensis* extracts or their corresponding AgNPs in human peripheral lymphocytes. The use of lymphocytes is particularly relevant, as they represent a primary target for circulating xenobiotics and serve as a sensitive indicator of systemic genotoxic stress. Therefore, the observed effects in this study provide important insights into the potential risks associated with human exposure to these extracts and nanoparticles.

MATERIALS AND METHODS

Ethics statement

The study was approved by the Ethics Committee of the Clinical Centre of Kragujevac (01/18/4927). Written informed consent was obtained from all the patients according to the guidelines of the World Medical Association (Declaration of Helsinki).

Preparation of *S. pratensis* extracts

The aerial parts and roots of *S. pratensis* were collected in May 2018 in the village of Veliko Krčmare, located in central Serbia. Identification of the plant material was performed at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia (voucher No. 129/018). Powdered, dried SPR and SPA (10 g each) were separately soaked in 100 mL of boiling deionized water and sonicated for 1 h in an ultrasonic bath. The resulting extracts were filtered using Macherey-Nagel 85/70 mm filter paper. After filtration, the aqueous extract was concentrated using a rotary vacuum evaporator at 45°C under reduced pressure. The obtained dry extracts, 2.36 g of SPA extract and 1.06 g of SPR, were stored at 4°C for further use.

Preparation and characterization of silver nanoparticles

The AgNPs used in this study were synthesized following the procedure previously described [4]. Two types of nanoparticles, SPR-AgNPs and SPA-AgNPs, were produced via green synthesis using the root and aerial parts of *S. pratensis* aqueous extracts, respectively. Briefly, the synthesis conditions were optimized by evaluating the effects of temperature, pH, and the concentrations of $AgNO_3$ and plant extracts on nanoparticle yield and size. This optimization was monitored by recording UV-Vis spectra in the range of 800-300 nm. A large batch of nanoparticles was prepared under optimized conditions for further characterization. Following synthesis, the AgNPs suspensions were purified by two sequential centrifugation steps at 12,000 rpm for 10 min, with intermediate washing in demineralized water to remove unreacted materials. The resulting nanoparticles were dried at 40°C in a hot air oven and stored at 4°C until further analysis.

The AgNPs were characterized by UV-Vis spectroscopy (Halo DB-20S, Dynamica GmbH, Switzerland), X-ray powder diffraction (XRPD, PHILIPS PW 1710 diffractometer) for crystalline structure determination, and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS; JEOL JSM IT 300LV with Oxford Instruments X-max EDS detector) for morphological and elemental analysis. Particle size distribution in aqueous suspension was determined

by dynamic light scattering (DLS, Mastersizer 2000, Malvern Panalytical). To evaluate the role of phenolic compounds in nanoparticle formation and stabilization, Fourier transform infrared spectroscopy (FTIR) spectra of both nanoparticles and plant extracts were recorded using the KBr pellet method.

The results of XRPD analysis confirmed the crystalline nature of SPR-AgNPs and SPA-AgNPs, with diffraction peaks corresponding to the nanocrystalline form of silver. SEM analysis revealed larger agglomerates composed of spherical nanoparticles, formed during the drying process for sample preparation. According to EDS results, silver was the major constituent of SPR-AgNPs and SPA-AgNPs, accompanied by smaller amounts of carbon and oxygen, likely originating from phenolic compounds involved in nanoparticle synthesis. Their nanoscale dimensions were confirmed using DLS analysis. The majority of SPA-AgNPs and SPR-AgNPs had diameters below 80 nm. According to FTIR results, the presence of bands corresponding to phenolic, aromatic, C=O, ester, amide, and C-O-C (flavonoid) functional groups in the AgNP spectra at positions similar to those in the extract spectra indicates their role in the stabilization of the formed AgNPs. The particle size distribution for both types ranged predominantly from 35 to 80 nm, supporting their classification as nanoparticles [4].

The determination of total phenolic and flavonoid content in aqueous *S. pratensis* extracts

The total phenolic content in the aqueous extracts of *S. pratensis* was determined using the Folin-Ciocalteu method [10]. The SPR and SPA extracts were diluted with water to a concentration of 0.1 mg/mL. To 0.25 mL of each extract, 1.25 mL of Folin-Ciocalteu reagent (previously diluted ten-fold with water) and 1 mL of 7.5% aqueous $NaHCO_3$ solution were added. The reaction mixture was incubated at 45°C for 15 min, and the absorbance was measured at 765 nm. A standard curve was constructed using known concentrations of gallic acid, and the results were reported as mg of gallic acid equivalents (mg GAE) per g of dry extract.

The total flavonoid content was determined as described [11]. Equal volumes (1 mL) of the extracts (0.5 mg/mL for SPA and 1 mg/mL for SPR) and 2% $AlCl_3$ solution in methanol were mixed and allowed to stand in the dark. After 1 h, the absorbance was measured at 415 nm. The total flavonoid content in the extract was expressed as quercetin equivalents (mg QUE) per g of dry extract. The results for both methods are presented as the average value from three independent measurements, along with the standard deviation.

LC-MS untargeted analysis of *S. pratensis* extracts

Chemical analysis of the extracts was conducted using LC/MS (Thermo Scientific™ Vanquish™ Core HPLC system) coupled to the Orbitrap Exploris 120 mass spectrometer (San Jose, CA, USA). All LC/MS parameters are detailed in Stojković et al. [12]. The obtained MS data were processed and analyzed using R Studio software (version 2023.09.1, build 494). Identification of bioactive compounds was conducted based on their chromatographic behavior and HRMS/MS2 data, with comparisons made to standard compounds, when available, and literature data for tentative identification. Data acquisition was carried out using Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA).

Measurement of DNA damage

Blood sampling

Whole blood was obtained by venipuncture into sterile heparinized BD Vacutainer® tubes from three healthy donors aged 25 to 28 years. The donors had not been exposed to ionizing radiation, mutagenic agents, organic solvents, pesticides, or other harmful chemicals in the six months prior to blood collection. They also had no history of malignancy, chronic disease, genetic disorders, or infectious illnesses.

Comet assay

The comet assay was performed according to [13] with some modifications [14]. Lymphocytes were isolated from whole peripheral blood at room temperature using Histopague-1077 density gradient medium (Sigma Aldrich, St. Louis, MO, USA), centrifuged for 15 min at 1900 × *g*, washed twice in RPMI medium (by 10 min centrifugation at 1800 × *g*), and resuspended in phosphate-buffered saline (PBS). Then the cells were resuspended in PBS solution and incubated for 30

min at 37°C with 25, 50, 75, and 100 µg/mL of plant extracts or AgNPs separately and in combination with H_2O_2. At the same time, both negative control (untreated lymphocytes) and positive control (H_2O_2 alone, final concentration of 10 µg/mL) were set up. Cell viability, determined by Trypan blue exclusion, exceeded 85%, with a cell density of 1×10^5 cells/mL obtained from the homogenized suspension. A 100-µL lymphocyte suspension (~10,000 cells) was mixed with 100 µL 1% low melting-point agarose (dissolved in PBS at 37°C in a final concentration of 0.01 g/mL), layered onto the slide (per two drops of 90 µL), and immediately covered by a coverslip. The slides were left for a few s on ice to allow agarose to solidify and then immersed in freshly prepared cold (4°C) lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% dimethyl sulfoxide, pH 10) for 2 h. The slides were kept in an electrophoresis buffer solution (10 M NaOH, 200 mM EDTA, pH>13) for 30 min at 4°C. Electrophoresis was performed for 30 min at 25 V (0.7 V/cm) and 300 mA. All experiments were conducted in the dark to avoid photo-induced DNA damage. Slides were washed in neutralizing Tris-HCl buffer (0.4 M Tris, pH 7.5) and stained with 50 µL of ethidium bromide (final concentration of 20 µg/mL). A total of 100 randomly selected cells (50 cells from each of two replicate gels) was analyzed visually from each subject using a fluorescence microscope (Nikon E50i) at 400 × magnification. Each cell was classified by visual scoring into five classes, from 0 (undamaged class cells) to 4 (the most severely damaged) [15]. The level of DNA damage, expressed as the Genetic Damage Index (GDI), was calculated using the formula of Pitarque et al. [16]:

$$GDI = \frac{Class1 + 2 \times Class2 + 3 \times Class3 + 4 \times Class4}{Class0+Class1+Class2+Class3+Class4}$$

Statistical analysis

Statistical analyses were performed using the SPSS software package (IBM SPSS Statistics 20). The results are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to compare treatments with their respective controls, while comparisons between plant extract or nanoparticle treatments were performed using Student's *t*-test. The relationship between the tested concentrations and the GDI was determined using Pearson's correlation coefficient. For all comparisons, $P<0.05$ indicated statistical significance. The heatmap of the LC-MS data was prepared using the Morpheus software (https://software.broadinstitute.org/morpheus (accessed 14 April 2025)).

RESULTS

Polyphenol profile of S. pratensis aqueous extracts

Analysis of the total phenolic and flavonoid contents revealed significant differences in total phenolic and flavonoid contents between the SPR and SPA extracts (Table 1). The SPA extract exhibited a higher total phenolic content (239.66 mg GAE/g) than the SPR extract (195.06 mg GAE/g). Similarly, the SPA extract contained a higher amount of total flavonoids (87.68 mg QUE/g) than the SPR (26.65 mg QUE/g). The difference in flavonoid content between SPR and SPA was even more pronounced. These results suggest that although both extracts contain relatively high levels of phenolic compounds, the SPR extract has considerably lower flavonoid content.

Table 1. Total phenolic and flavonoid contents in *S. pratensis* root (SPR) and aerial part (SPA) aqueous extracts

Samples	*Total phenolic content (mg GAE[a]/g extracts)*	*Total flavonoid content (mg QUE[b]/g extracts)*
SPA	239.66±2.02	87.68±2.46
SPR	195.06±6.47	26.65±0.53

[a] GAE – gallic acid equivalent. [b] QUE – quercetin equivalent

Phenolic compounds in SPR and SPA extracts were identified by LC-MS Orbitrap metabolic fingerprinting, based on high-resolution MS^2 fragmentation data and the m/z values of the deprotonated ions $[M-H]^-$. The LC-MS data are summarized in Supplementary Table S1. A heatmap (Fig. 1) was created to visualize the quantitative LC-MS data based on the peak areas (Supplementary Table S1). A total of 51 phenolic acids were identified in the analyzed extracts, including 9 hydroxybenzoic acid derivatives and 42 hydroxycinnamic acid derivatives (Fig. 1). Comparing the peak areas, hydroxycinnamic acids were the most abundant class

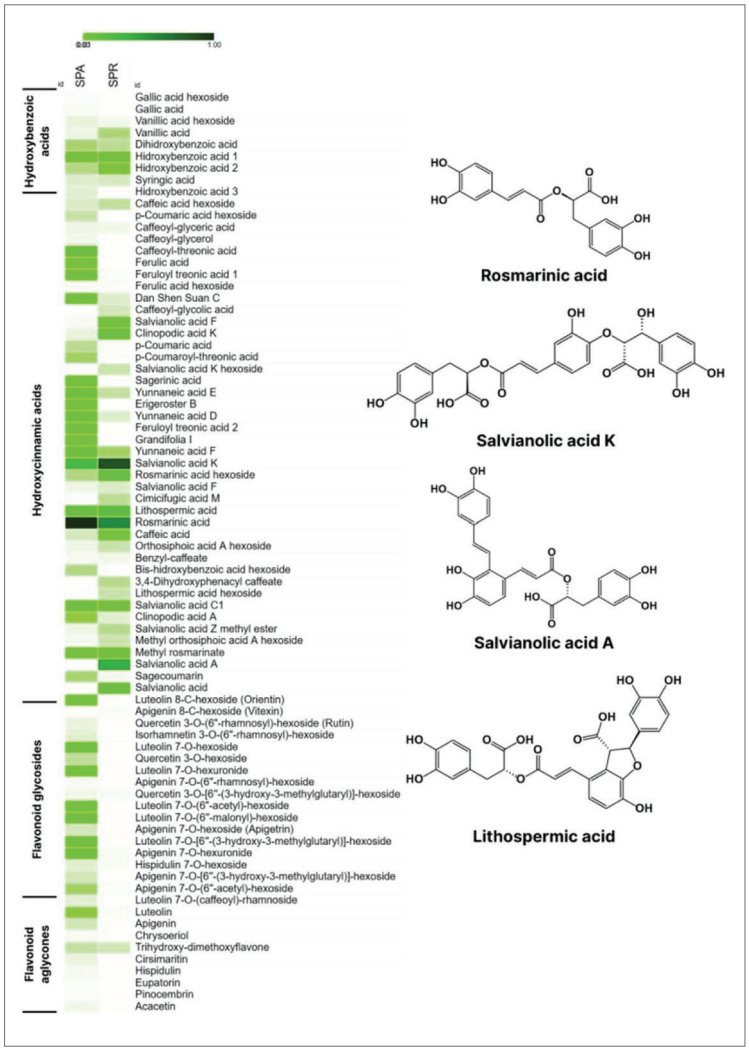

Fig. 1. Heatmap of scaled LC-MS quantitative data and chemical structures of the main compounds identified in *S. pratensis* root (SPR) and aerial part (SPA) aqueous extracts. Color intensity reflects peak area values, with dark green indicating the highest values and white indicating undetected compounds, as shown in the color scale.

of phenolic compounds in both extracts. Among these, rosmarinic acid, salvianolic acid K, and lithospermic acid were identified as the phenolic compounds with the highest content in both extracts. Salvianolic acid C1, caffeoyl-threonic acid, and yunnaneic acid E and D were also identified in high amounts in the SPA. A slightly smaller number of hydroxycinnamic acid derivatives were identified in the SPR. Apart from the three main compounds mentioned above, the dominant hydroxycinnamic acids in the SPR were clinopodic acid K, rosmarinic acid hexoside, salvianolic acid, and salvianolic acid A. Based on peak area intensities, most hydroxycinnamic acids were present in higher amounts in the SPR extract, except for rosmarinic acid, which was more abundant in the SPA.

In addition to the numerous phenolic acids and their derivatives, a total of 27 flavonoids, flavanols, and their glycosides were successfully identified (Supplementary Table S1). Among these, 18 flavonoid glycosides were detected in the SPA, whereas only 6 were found in the SPR extract (Fig. 1). The most abundant flavonoid forms in SPA were luteolin and apigenin glycosides, along with quercetin 3-O-(6'-rhamnosyl)-hexoside, isorhamnetin 3-O-(6'-rhamnosyl)-hexoside, and hispidulin 7-O-hexoside. Nine aglycone forms of flavonoids were identified, with luteolin, apigenin, and trihydroxy-dimethoxyflavone being the most abundant in the SPA. In contrast, most aglycones were only present in trace amounts in the SPR, except for trihydroxy-dimethoxyflavone, which was detected in significantly higher concentrations.

Measurement of DNA damage in human lymphocytes induced with *S. pratensis* extracts

Both SPR and SPA extracts significantly increased GDI in lymphocytes compared to the negative control (0.24±0.01), rising to 0.66±0.03 and 0.50±0.01 at the highest concentrations, respectively, with the exception of the lowest SPA concentration (25 µg/mL) (Fig. 2a). Analysis of the comet class distribution showed that undamaged cells (class 0) were most represented in the negative control (81.67±2.08) and at 25 µg/mL of both extracts (76.67±3.21 for SPR and 81.67±1.15 for SPA). The percentage of total damage cells (class 4) was highest at a concentration of 100 µg/mL (5.67±2.08 for SPR and 1.67±2.89 for the SPA extract) (Fig. 3a). A strong positive correlation was observed between the concentrations of the extracts and the GDI (Pearson's correlation coefficient: r=0.976, P=0.000 for the SPR; r=0.964, P=0.000 for the SPA extract).

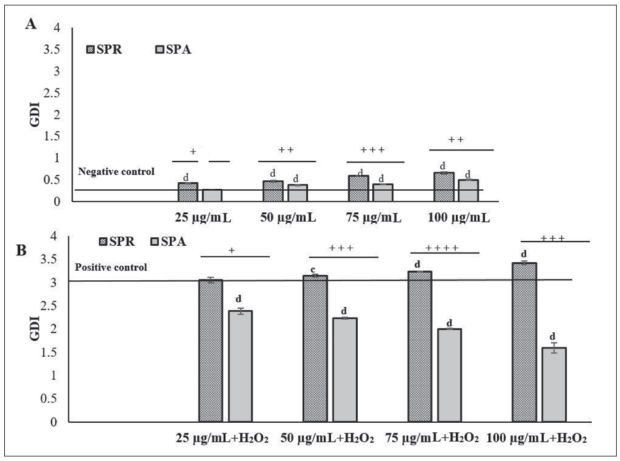

Fig. 2. DNA damage levels in human lymphocytes following treatment with aqueous *S. pratensis* root (SPR) and aerial part (SPA) extracts alone (**A**) or in combination with H_2O_2 (**B**). Significant differences relative to the negative control (A) or positive control (B) were determined by ANOVA (cP<0.0001; dP<0.0005); significant differences between SPR and SPA extracts were determined by Student's t-test ($^{+}$P<0.05; $^{++}$P<0.01; $^{+++}$P<0.001; $^{++++}$P<0.0005).

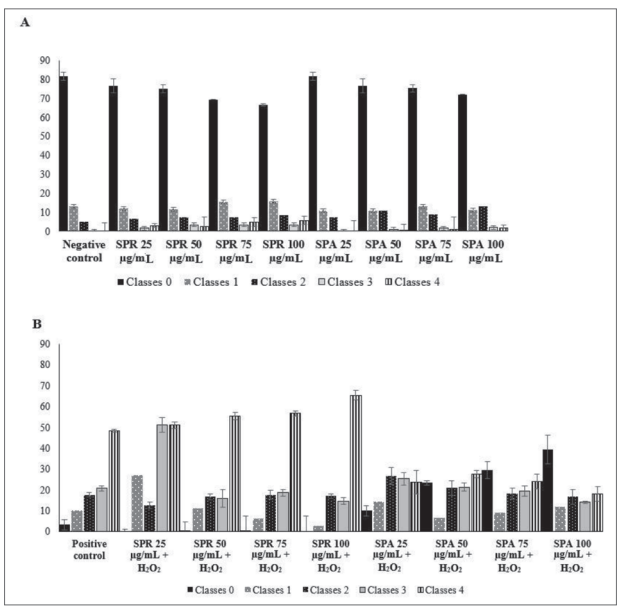

Fig.3. Distribution of comet classes in human lymphocytes induced by aqueous extracts of *S. pratensis* root and aerial parts (**A**); H_2O_2 and *S. pratensis* extracts in combination (**B**).

Although both extracts increased GDI, the SPR extract produced significantly higher values than the SPA extract at all tested concentrations (Student's t-test: t=10.783, P<0.05 for 25 µg/mL; t=13.000, P<0.05 for 50 µg/mL; t=28.000, P<0.001 for 75 µg/mL; t=10.470, P<0.05 for 100 µg/mL).

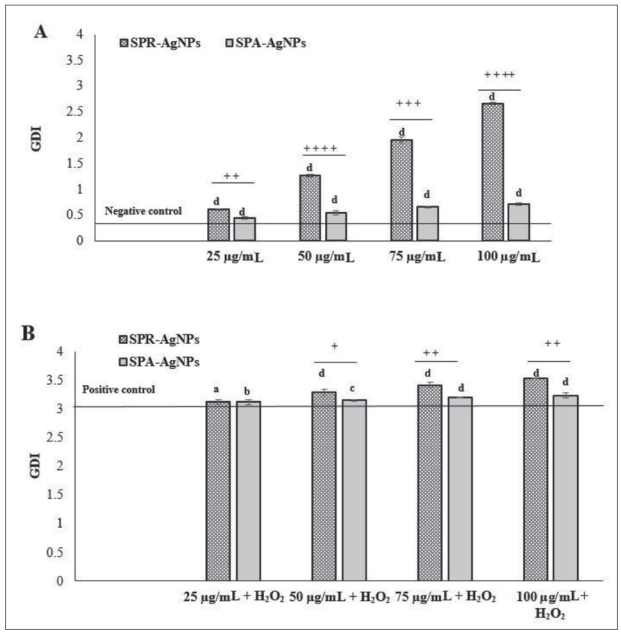

Fig. 4. The level of DNA damage in human lymphocytes induced with AgNPs biosynthesized from *S. pratensis* root and aerial part extracts (**A**) and H_2O_2 and AgNPs biosynthesized from *S. pratensis* root and aerial part extracts in combination (**B**). Significant differences relative to the negative control (for a) or positive control (for b) were determined by ANOVA (^{a}P<0.05; ^{b}P<0.001; ^{c}P<0.0001; ^{d}P<0.0005); significant differences between AgNPs biosynthesized from root and aerial part extracts were determined by Student's *t*-test ($^{+}$P<0.05; $^{++}$P<0.01; $^{+++}$P< 0.001; $^{++++}$P<0.0005).

All tested concentrations of SPR extract increased H_2O_2-induced GDI in lymphocytes from 3.05±0.06 to 3.42±0.04, compared to the positive control (3.01±0.01) (ANOVA, P<0.05). As the extract concentration increased, the percentage of total damaged cells also increased and was highest at 100 µg/mL (65.33±2.31) (Figs. 2b and 3b). Pearson's correlation coefficient showed a significant negative correlation between extract concentrations and GDI (r=-0.955, P=0.000).

H_2O_2-induced GDI was reduced in a dose-dependent manner (r=0.970; P=0.000) when cells were treated with SPA extract (ANOVA, P<0.05). The percentage of class 4 was reduced with increasing extract concentrations. Thus, the highest tested extract concentration reduced the percentage of total damaged cells by almost 2.7-fold compared to the positive control (48.30 vs. 18.00) (Fig. 3b). *Salvia* extracts showed different effects on H_2O_2-induced DNA damage. The SPR extract increased the H_2O_2-induced GDI at all tested concentrations, while the SPA extract decreased GDI (Student's *t*-test, P<0.005).

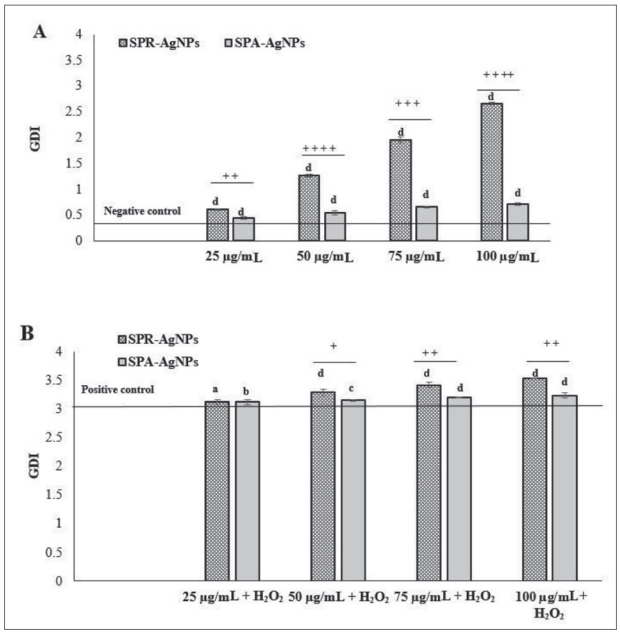

Fig.5. Distribution of comet classes in human lymphocytes induced with AgNPs biosynthesized from *S. pratensis* root and aerial part extracts (**A**) and H_2O_2 and AgNPs biosynthesized from *S. pratensis* root and aerial part extracts in combination (**B**).

Measurement of DNA damage in human lymphocytes induced with biosynthesized AgNPs

Both AgNPs significantly increased the GDI at all tested concentrations compared to the negative control (ANOVA, P<0.005) (Fig. 4a). With increasing concentration, the percentage of undamaged cells (81.67±2.08) decreased to 5.67±1.08 for SPR-AgNPs and 65.67±2.52 for SPA-AgNPs. Class 4 was most prevalent at 100 µg/mL of both AgNPs (28.33±2.08 for SPR-AgNPs and 5.33±1.53 for SPA-AgNPs) (Fig. 5a). Pearson's correlation coefficient showed a strong positive correlation between AgNPs concentrations and GDI (r=0.993, P=0.000 for SPR-AgNPs; r=0.973, P=0.000 for SPA-AgNPs).

Although both AgNPs exhibited genotoxic effects, SPR-AgNPs produced consistently higher GDI values across all concentrations compared to SPA-AgNPs. At the highest tested concentration, SPR-AgNPs induced a 3.7-fold greater GDI than SPA-AgNPs (2.66±0.03 vs. 0.71±0.02; Student's t-test: t=73.000, P<0.05) (Table 2).

All tested concentrations of both AgNPs increased the H_2O_2-induced GDI in comparison to the positive control cells (ANOVA, P<0.05) (Fig. 4b). The highest GDI was at 100 µg/mL, ranging from 3.53 to 3.56 for SPR-AgNPs and 3.21-3.28 for SPA-AgNPs. The analysis of comet class distribution showed that with increasing AgNPs concentration, the percentage of class 4 increased from 48.30 to 62.67% for SPR-AgNPs and from 49.67 to 58.67% for SPA-AgNPs (Figure 5b). A significantly positive correlation was found between AgNP concentration and GDI for both SPR-AgNPs (r=0.985, P<0.001) and SPA-AgNPs (r=0.927, P<0.001).

SPR-AgNPs induced higher GDI, especially at higher tested concentrations, compared to SPA-AgNPs (Student's *t*-test: t=6.725, P<0.05 for 50 µg/mL; t=10.568, P<0.005 for 75 µg/mL; t=20.877, P<0.05 for 100 µg/mL). At the lowest tested concentration (25 µg/mL), the same GDI was observed for AgNPs from both plant parts (P>0.05).

Comparative analysis of the genotoxic potential of *S. pratensis* extracts and biosynthesized AgNPs

Table 2 shows the results of the comparative analysis of the effect of *S. pratensis* extracts and the AgNPs biosynthesized from the corresponding extract. Both AgNPs showed a higher GDI than the extract from which they were obtained. The highest GDI (2.66±0.03) was recorded at 100 µg/mL of SPR-AgNPs, which was four times greater than that observed at the same

Table 2. Comparative analysis of the level of genome damage of human lymphocytes induced by aqueous extracts from different parts of *S. pratensis* and biosynthesized AgNPs

Concentration (µg/ml)	GDI			
	Root		Aerial part	
	S. pratensis	AgNPs	*S. pratensis*	AgNPs
Negative control (0)	0.24±0.01	0.24±0.01	0.24±0.01	0.24±0.01
Positive control (H_2O_2)	3.01±0.01	3.01±0.01	3.01±0.01	3.01±0.01
25	0.42±0.01	0.60±0.02^{b}	0.27±0.01	0.44±0.02
50	0.47±0.02	1.26±0.0^{d}	0.38±0.01	0.54±0.04
75	0.59±0.01	1.95±0.06^{d}	0.40±0.01	0.65±0.02^{a}
100	0.66±0.03	2.66±0.03^{d}	0.50±0.01	0.71±0.02^{b}
25 + H_2O_2	3.05±0.06	3.12±0.04	2.38±0.07	3.12±0.04^{a}
50 + H_2O_2	3.14±0.03	3.29±0.06	2.23±0.02	3.15±0.02^{d}
75 + H_2O_2	3.24±0.06	3.42±0.04^{a}	2.00±0.01	3.20±0.0^{d}
100 + H_2O_2	3.42±0.04	3.54±0.0^{a}	1.59±0.11	3.24±0.04^{d}

^{a,b,d}significant difference of GDI *Salvia pratensis* extracts and biosynthesized AgNPs (Student's *t*-test, ^{a}P < 0.05; ^{b}P < 0.01; ^{d}P < 0.0005)

concentration of SPR extract (0.66±0.03) (Student's t-test: t=57.374, P<0.0005). Furthermore, H_2O_2-induced GDI was significantly higher following treatment with SPR-AgNPs at all tested concentrations, and with SPA-AgNPs at the two highest concentrations (75 and 100 µg/mL), compared to their respective parent extracts (Student's t-test, P<0.05).

DISCUSSION

S. pratensis is a medicinal plant with a long history of use, and its application has been recognized over the years. It contains different classes of bioactive constituents, with phenolic acids and flavonoids being the primary active compounds. Both extracts contained relatively high total phenolic levels, though the SPR extract had a lower flavonoid content. These results contrast with previously published results for methanolic extracts from the aerial parts and roots of *S. pratensis*, where the root extract had a higher total phenolic content than the aerial part extract [2]. This discrepancy may be attributed to the different solvent polarities, with methanol being less polar than water, potentially allowing for a more efficient extraction of certain phenolic compounds from the SPR sample. Compared to [17], who reported a total phenolic content of 162 mg GAE/g dry extract and a total flavonoid content of 39.47 mg QUE/g dry extract in aqueous extracts of *S. officinalis*, the results of this study show a significantly higher content of phenols and flavonoids in *S. pratensis*.

Based on LC-MS data, hydroxycinnamic acids constitute the largest proportion of phenolic compounds identified, with rosmarinic acid being the dominant compound in both extracts. Greater diversity and higher quantities of hydroxycinnamic acid derivatives were identified in the SPA extract, in which rosmarinic acid was also the most abundant individual compound. This finding contrasts with our previous study, in which methanolic root extracts contained significantly higher levels of rosmarinic acid [2]. In addition to hydroxycinnamic acid derivatives, numerous flavonoid glycosides and their aglycone forms were identified, predominantly in SPA. These results are consistent with earlier reports on the methanolic extracts of *S. pratensis* [2], although the present study revealed an even greater diversity of flavonoid glycosides. Furthermore, [18] demonstrated that salvianolic acid B, salvianolic acid A, lithospermic acid, and caffeic acid represented the dominant phenolic constituents in the aqueous aerial part extract of *S. miltiorrhiza*, accounting for approximately 19% of the total phenolic content.

In our previous study [4], FTIR spectroscopy was used to identify the functional groups in *Salvia pratensis* extracts and their corresponding AgNPs, which were subsequently applied in genotoxicity assessment. FTIR spectra of both extracts and their derived AgNPs displayed characteristic bands corresponding to phenolic -OH groups, aromatic rings, C=O, ester and amide functionalities, and C-O-C vibrations typical of flavonoids. The presence of these bands in the AgNP spectra at positions comparable to those observed in the extract spectra indicates that these bioactive constituents play a crucial role not only in the reduction of Ag^+ ions but also in the stabilization and capping of the formed nanoparticles. According to the FTIR analysis, the spectra of the plant extracts and the corresponding AgNPs were comparable, showing absorption bands at similar wavenumbers. However, the bands observed in the AgNP spectra exhibited lower intensity compared to those of the crude extracts. This reduction in intensity likely reflects the partial consumption of phytochemicals during the reduction process and their interaction with and binding to the nanoparticle surface, which may alter the vibrational environment of the associated functional groups, while confirming that FTIR analysis provides qualitative insight into their involvement [4].

Evaluating the genotoxic potential of medicinal plants is essential for determining their safety. The comet assay, a highly sensitive method for detecting DNA strand breaks at the level of individual cells, is widely applied in plant extract studies [19]. In the present study, both the root and aerial part aqueous extracts of *S. pratensis* exhibited genotoxic effects at all tested concentrations, except for the lowest concentration of the SPA extract (25 µg/mL), which did not induce significant DNA damage. Since the experiments were performed on human lymphocytes, the findings primarily reflect the genotoxic response of circulating blood cells, and further studies on other relevant cell types are warranted. According to available literature, this is the first report on the genotoxic potential of *S. pratensis* aqueous extracts. These findings are consistent

with those of Sevindik and Rencuzogullari [20], who demonstrated that *S. fruticosa* leaf extract induced genotoxic effects in human lymphocytes, as evidenced by increased sister chromatid exchange, chromosome aberrations, and micronucleus formation. Similarly, the aqueous extracts of *S. officinalis* caused a 3-fold increase in micronucleus frequency in cultured human peripheral blood lymphocytes compared to untreated controls [21].

While genoprotective effects of *Salvia* extracts have been relatively well documented [21,22], data specific to *S. pratensis* remain scarce. Srećković et al. [2] assessed the DNA-protective effects of methanolic root and aerial extracts of *S. pratensis* at concentrations of 25-400 μg/mL against hydroxyl radical-induced damage in herring sperm DNA. Both *S. pratensis* extracts showed a high level of protection against DNA damage caused by free radicals. The SPR extract showed a synergistic effect with H_2O_2, as H_2O_2-induced GDI was significantly and dose-dependently increased across all tested concentrations. In contrast, the SPA extract exhibited a strong protective effect, reducing H_2O_2-induced GDI in a dose-dependent manner at all tested concentrations.

When comparing the genotoxic effect of the extracts from both plant parts (SPR and SPA), the SPR extract showed a stronger genotoxic effect than the SPA extract at all tested concentrations. In the treatment with H_2O_2, the SPR extract exhibited a synergistic effect with H_2O_2, whereas the SPA extract demonstrated a pronounced protective effect. This may be attributed to the higher total phenolic and flavonoid content of the SPA extract, along with greater concentrations of several individual polyphenolic constituents, relative to the SPR extract. LC-MS analysis showed that the SPA extract had a higher content of hydroxybenzoic acid than the SPR.

Plants may contain mutagenic, teratogenic, or carcinogenic constituents capable of interacting with DNA and increasing susceptibility to disease [23]. Given that plant extracts comprise hundreds of individual compounds, identifying those responsible for a specific biological activity is essential. The observed results may therefore reflect complex interactions among extract constituents, including potential synergistic or antagonistic effects. Several studies have demonstrated that the genotoxic or comutagenic activity of individual extract constituents can be attenuated by other co-occurring compounds [24]. Phenolic compounds are characterized by their dual role, as they can transform from antioxidants to prooxidants [25]. Several factors contribute to this, such as the structure of the compound itself, the low molecular mass, and the higher number of hydroxyl groups in their aromatic rings. In addition, studies have shown that the prooxidant potential of phenolics increases with a high pH and in the presence of transition metal ions such as Cu^{2+} or Fe^{3+} [26,27]. Phenolic compounds, as prooxidants, can weaken the antioxidant defenses of the nucleus, leading to oxidative DNA damage, which could explain their mutagenicity [26]. It should be noted that flavonoids in higher concentrations can have genotoxic effects by intercalating into DNA or inhibiting DNA topoisomerase, thereby interfering with the DNA replication process [28].

The use of plants in nanoparticle synthesis has attracted considerable research interest, particularly given the widespread application of AgNPs across various fields and the consequent increase in human exposure. This underscores the importance of investigating the genotoxic effects of green-synthesized AgNPs and characterizing their toxicological profiles to ensure safe use, which was among the key objectives of the present study. This is the first study to investigate these effects of AgNPs synthesized from *S. pratensis* extracts, so our results represent a first in this field of research. Recent studies have demonstrated the antioxidant [6], cytotoxic [29,30], antibacterial [6,29,30], and antifungal [5] activities of biosynthesized AgNPs from *Salvia* extracts. However, our results show that both AgNPs (SPR and SPA) have a genotoxic effect on human lymphocytes in all tested concentrations in human lymphocytes *in vitro*. The effect of AgNPs on H_2O_2-induced genotoxicity was also investigated, and the results showed that all tested concentrations of both AgNPs significantly increased DNA damage compared to the positive control (H_2O_2 alone). The results suggest that the AgNPs synthesized with *Salvia* extracts exhibit a synergistic effect with H_2O_2. The effects of AgNPs are primarily a consequence of oxidative stress and interaction with cellular structures, which subsequently lead to DNA damage. Numerous studies have reported that the main mechanisms of their genotoxic effect are based on the induction of

reactive oxygen species, disruption of mitochondrial function, interaction with DNA, and interference with DNA repair systems [31].

Both SPR- and SPA-AgNPs exhibited greater genotoxicity than their corresponding extracts, whether applied alone or in combination with H_2O_2. The higher genotoxic effect of AgNPs is probably the result of the release of Ag^+ ions inside cells from nanoparticles. Although this study relies on previously characterized AgNPs, it should be noted that some of the observed effects may result from released Ag^+ ions. Future mechanistic studies should distinguish between particle-specific and ionic silver effects. In a previously published study on the synthesis and characterization of SPR-AgNPs and SPA-AgNPs [4], it was shown that they consist predominantly of silver, with a smaller portion consisting of carbon- and oxygen-containing compounds. Considering the investigated nanoparticles are primarily made of silver and that their surface is mostly coated with phytocompounds, it is most likely that the majority of their genotoxic effect originates from the release of silver ions. AgNPs have been shown to bind to and penetrate cell membranes by altering their permeability, releasing Ag^+ ions that damage cellular structures and molecules and promote reactive oxygen species (ROS) formation [32,33]. Although both types of investigated nanoparticles are predominantly composed of silver, their genotoxic potential differed. While the Ag^+ ions released from AgNPs can induce genotoxic effects, the obtained results indicate that the plant extracts used in the synthesis of these nanoparticles may also modulate their genotoxic potential. Accordingly, SPR-AgNPs exhibited a higher degree of genotoxicity compared to SPA-AgNPs, which correlates with the genotoxic effects of the extracts used for their synthesis. A limitation of this study is the small donor sample size (n=3), which may not fully capture inter-individual variability, and larger studies are needed to confirm these findings. Future investigations incorporating targeted mechanistic assays will be necessary to clarify these pathways and deepen understanding of the biological activity of the synthesized AgNPs, and despite the noted limitations, the present study provides a valuable foundation for such work.

CONCLUSIONS

Both aqueous *S. pratensis* extracts and their biogenic AgNPs induced DNA damage in human lymphocytes. When combined with H_2O_2, both AgNPs and the root extract exhibited a synergistic effect, further enhancing oxidative DNA damage, whereas only the aerial part extract showed a clear genoprotective effect. These outcomes likely reflect the complex phytochemical nature of the extracts, in which individual phenolic and flavonoid constituents, as well as their interactions, contribute to the overall biological response. This study demonstrated that *S. pratensis* aqueous extracts are a notable source of phenolic acids, but their genotoxic potential, particularly at higher concentrations, warrants careful consideration. The presence of phytochemicals on the nanoparticle surface highlights the need to evaluate each plant-mediated AgNP preparation individually, rather than assuming it to be inherently safer than conventionally synthesized AgNPs. As the experiments were conducted on human lymphocytes as a model of systemic exposure, further studies in additional human cell types and *in vivo* models are essential to fully assess the safety of these materials for potential biomedical applications.

Funding: This work was supported by the Serbian Ministry of Science, Technological and Innovation (Agreement Nos. 451-03-34/2026-03/200122; 451-03-33/2026-03/200122 and 451-03-136/2025-03/200007).

Author contributions: MRJ: Writing – original draft, Investigation. JTV: investigation, methodology, visualization, formal analysis, writing – review & editing. NS: writing – original draft, investigation. UG: writing – review and editing, investigation. DM: writing – review and editing, investigation. VM: writing – review and editing, investigation. OMDj: writing – review and editing, validation, conceptualization, funding acquisition.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The data supporting this article is available in the online datasets: https://hdl.handle.net/21.15107/rcub_ibiss_8091 and https://www.serbiosoc.org.rs/NewUploads/Uploads/Radovic%20Jakovljevic%20et%20al_Anonimised%20Dataset.pdf

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SUPPLEMENTARY MATERIAL

Table S1. LC-MS data on phenolic compounds identified in *S. pratensis* aerial part (SPA) and root (SPR) aqueous extracts

No	Compound name	t_R, min	Molecular formula, [M - H]$^-$	Exact mass, m/z	Calculated Mass, m/z	Δ mDa	MS^2 Fragments, (% Base Peak)	Peak areas	SPR
								SPA	
Hydroxybenzoic acids									
1	**Gallic acid hexoside**	0.65	$C_{13}H_{15}O_{10}$-	331.06720	331.06315	4.05	125.02292(8), 151.00180(20), **169.01224**(100), 211.02229(28), 271.04282(25), 331.06348(21)	1696763	216062
2	**Gallic acid**	0.69	$C_7H_5O_5$-	169.01425	169.01217	2.08	**125.02296**(100), 169.01219(48)	3625178	NI
3	**Vanillic acid hexoside**	0.75	$C_{14}H_{17}O_9$-	329.08783	329.08386	3.97	108.02035(14), 123.04375(36), 152.00977(16), **167.03304**(100)	14124435	6943387

4	**Vanillic acid**	0.90	$C_8H_7O_4$-	167.03498	167.03298	2.00	123.04369(97), **167.03290**(100)	10836244	59270248
5	**Dihydroxybenzoic acid**	1.13	$C_7H_5O_4$-	153.01933	153.01754	1.79	**109.02827**(100), 123.04378(18), 153.01761(35)	64742743	46078342
6	**Hydroxybenzoic acid 1**	1.34	$C_7H_5O_3$-	137.02442	137.02303	1.39	93.03350(5), **137.02289**(100)	138721149	152001118
7	**Hydroxybenzoic acid 2**	2.31	$C_7H_5O_3$-	137.02442	137.02289	1.52	93.03350(3), **137.02280**(100)	55090388	110956107
8	**Syringic acid**	5.61	$C_9H_9O_5$-	197.04555	197.04299	2.56	135.04366(14), 138.03069(16), 153.05389(10), 166.99664(26), 179.03294(9), **182.01988**(100)	20912519	26620431
9	**Hydroxybenzoic acid 3**	6.48	$C_7H_5O_3$-	137.02442	137.02280	1.62	**93.03350**(100), 137.02287(72)	18829544	NI
Hydroxycinnamic acids									
10	**Caffeic acid hexoside**	4.81	$C_{15}H_{17}O_9$-	341.08783	341.08312	4.72	135.04356(16), **179.03297**(100)	24219914	43549547
11	***p*-Coumaric acid hexoside**	5.10	$C_{15}H_{17}O_8$-	325.09292	325.08895	3.97	119.04883(50), 145.02768(5), **163.03812**(100)	38659601	NI
12	**Caffeoyl-glyceric acid**	5.32	$C_{12}H_{11}O_7$-	267.05103	267.04775	3.27	135.04356(14), **179.03282**(100)	10643383	8960990
13	**Caffeoyl-glycerol**	5.43	$C_{12}H_{13}O_6$-	253.07176	253.06867	3.09	135.04356(57), **161.02263**(100), 179.03275(34), 253.06921(71)	7658315	NI
14	**Caffeoyl-threonic acid**	5.44	$C_{13}H_{13}O_8$-	297.06168	297.05791	3.77	**135.02835**(100), 179.03290(7)	731777931	905026
15	**Ferulic acid**	5.48	$C_{10}H_9O_4$-	193.05063	193.04820	2.43	117.03326(8), **134.03577**(100), 149.05911(7), 178.0251(12), 193.04843(8)	116778122	NI
16	**Feruloyl threonic acid 1**	5.54	$C_{14}H_{15}O_8$-	311.07727	311.07352	3.74	117.01801(21), 134.0358(40), 149.05878(13), 178.02516(5), **193.04836**(100)	294477257	2641033
17	**Ferulic acid hexoside**	5.57	$C_{16}H_{19}O_9$-	355.10358	355.09846	5.12	134.03583(23), 149.05914(24), 178.02467(25), **193.04840**(100)	3104440	2395542
18	**Dan Shen Suan C**	5.60	$C_{18}H_{17}O_9$-	377.08783	377.08300	4.84	135.04359(14), 137.02272(7), **161.02248**(100), 179.03288(16), 197.04323(42)	338606197	21467251
19	**Caffeoyl-glycolic acid**	5.69	$C_{11}H_9O_6$-	237.04046	237.03747	2.99	135.04356(20), **179.03281**(100)	NI	30158959
20	**Salvianolic acid F**	5.70	$C_{17}H_{13}O_6$-	313.07129	313.06794	3.35	135.04356(95), **179.03281**(100)	4076550	267955524

21	**Clinopodic acid K**	5.71	$C_{18}H_{15}O_9$-	375.07168	375.06694	4.75	133.02792(48), 134.03568(34), 135.04352(49), 177.01712(21), 178.02496(65), **179.03276**(100)	13346908	771295861
22	***p*-Coumaric acid**	5.79	$C_9H_7O_3$-	163.04007	163.03809	1.98	**119.04887**(100), 163.03821(14)	48668813	2602154
23	***p*-Coumaroyl-threonic acid**	5.82	$C_{13}H_{13}O_7$-	281.06668	281.06336	3.32	119.04886(40), 135.02835(17), **163.03815**(100)	71153279	NI
24	**Salvianolic acid K hexoside**	5.85	$C_{33}H_{33}O_{18}$-	717.16727	717.16073	6.54	**135.04355**(100), 161.02248(66), 179.03305(28), 197.04320(67), 313.06784(34), 359.09399(69)	NI	36604929
25	**Sagerinic acid**	5.92	$C_{36}H_{31}O_{16}$-	719.16188	719.15278	9.10	135.04356(12), **161.02251**(100), 179.0329(13), 197.04326(35)	173408514	NI
26	**Yunnaneic acid E**	5.95	$C_{27}H_{23}O_{14}$-	571.10936	571.10217	7.19	**135.04356**(100), 179.03293(42), 197.04321(79), 215.10524(20), 241.08415(72), 285.07318(20)	451564728	41492056
27	**Erigeroster B**	5.99	$C_{26}H_{23}O_{13}$-	543.11444	543.10746	6.98	**135.04359**(100), 179.03284(24), 197.0432(47), 213.08966(18), 231.10001(21), 257.07892(27)	125193504	NI
28	**Yunnaneic acid D**	6.03	$C_{27}H_{23}O_{12}$-	539.11953	539.11287	6.66	**135.04358**(100), 161.02248(79), 179.03278(42), 197.04326(59), 271.09418(17), 297.07327(53)	454992797	21482229
29	**Feruloyl threonic acid 2**	6.04	$C_{14}H_{15}O_8$-	311.07727	311.07348	3.78	117.03323(4), 134.03578(53), 135.02832(8), 149.05904(14), **193.04832**(100)	314672083	NI
30	**Grandifolia I**	6.04	$C_{32}H_{31}O_{16}$-	671.16189	671.15356	8.33	135.04353(6), **161.02246**(100), 179.03293(11), 193.04826(36), 197.04317(42)	136085091	NI
31	**Yunnaneic acid F**	6.12	$C_{29}H_{25}O_{14}$-	597.12501	597.11764	7.37	**135.04355**(100), 179.03287(22), 197.04324(27), 267.09940(36), 293.07880(8), 311.08884(18)	560488978	71114647

32	**Salvianolic acid K**	6.15	$C_{27}H_{23}O_{13}$-	555.11444	555.10744	7.00	135.04353(80), **161.02245**(100), 179.03285(20), 197.04320(35), 295.05786(7)	3222228855	10100793509
33	**Rosmarinic acid hexoside**	6.23	$C_{24}H_{25}O_{13}$-	521.12959	521.12339	6.21	135.04355(10), **161.02245**(100), 179.03282(29), 197.04318(32), 323.07339(48)	57009362	1221428017
34	**Salvianolic acid F**	6.37	$C_{17}H_{13}O_{6}$-	313.07176	313.06804	3.73	133.02795(88), 134.03577(77), 135.04356(97), 178.02505(63), **179.03282**(100)	10125778	23297286
35	**Cimicifugic acid M**	6.52	$C_{20}H_{17}O_{9}$-	401.08783	401.08208	5.75	**135.04352**(100), 178.02510(4), 179.03278(6)	NI	46220846
36	**Lithospermic acid**	6.53	$C_{27}H_{21}O_{12}$-	537.10388	537.09641	7.47	109.02824(22), **135.04355**(100), 161.02252(39), 179.03279(30), 197.04324(25), 295.05768(32)	978295793	1863922395
37	**Rosmarinic acid**	6.57	$C_{18}H_{15}O_{8}$-	359.07727	359.07213	5.14	135.04361(6), **161.02251**(100), 179.03285(20), 197.04326(33)	12179359570	6808398772
38	**Caffeic acid**	6.59	$C_{9}H_{7}O_{4}$-	179.03498	179.03237	2.61	**135.04358**(100), 179.03293(26)	27474477	118452927
39	**Orthosiphoic acid A hexoside**	6.62	$C_{33}H_{31}O_{16}$-	683.16188	683.15289	8.99	**135.04356**(100), 161.02246(29), 323.07355(25)	9317284	36949249
40	**Benzyl-caffeate**	6.63	$C_{16}H_{13}O_{4}$-	269.08193	269.07879	3.14	134.03575(14), **135.04353**(100), 269.00635(31)	5614448	7819046
41	**Bis-hydroxybenzoic acid hexoside**	6.64	$C_{20}H_{19}O_{10}$-	419.09850	419.09282	5.68	93.03345(12), **137.02278**(100), 281.06339(5)	56065389	NI
42	**3,4-Dihydroxyphenacyl caffeate**	6.66	$C_{17}H_{13}O_{7}$-	329.06610	329.06265	3.46	134.03578(11), 135.04356(17), 175.03784(18), 178.02499(17), **179.03281**(100), 219.02737(11)	NI	50737707
43	**Lithospermic acid hexoside**	6.66	$C_{33}H_{31}O_{17}$-	699.15679	699.14789	8.90	135.04358(98), **161.02249**(100), 179.03284(37), 197.04317(87), 295.05707(13), 359.09467(50)	NI	38420642
44	**Salvianolic acid C1**	6.67	$C_{26}H_{21}O_{11}$-	509.10896	509.10243	6.53	**135.04352**(100), 179.03290(18), 197.04311(21), 239.06859(16), 285.07339(33), 311.05234(15)	605429012	256602830

45	**Clinopodic acid A**	6.80	$C_{18}H_{15}O_7$-	343.08235	343.07756	4.79	135.04359(59), **145.02783**(100), 161.02251(72), 179.03290(32), 181.04851(42), 197.04321(45)	89709883	19607911
46	**Salvianolic acid Z methyl ester**	6.81	$C_{28}H_{25}O_{13}$-	569.12959	569.12334	6.25	135.04358(65), **137.02280**(100), 161.02248(99), 179.03293(22), 193.04842(27), 197.04315(27)	10494527	51489345
47	**Methyl orthosiphoic acid A hexoside**	6.93	$C_{34}H_{33}O_{16}$-	697.17743	697.16886	8.56	135.04358(16), **161.02246**(100), 179.03287(22), 197.04337(18), 323.07379(9), 337.08911(15)	5315465	35586646
48	**Methyl rosmarinate**	6.98	$C_{19}H_{17}O_8$-	373.09232	373.08780	4.52	**135.04358**(100), 160.01468(12), 161.02238(15), 175.03796(75), 179.03294(34), 197.04327(56)	145999931	229404422
49	**Salvianolic acid A**	6.99	$C_{26}H_{21}O_{10}$-	493.11355	493.10771	5.84	135.04356(44), **161.02246**(100), 179.03278(18), 197.04321(36)	NI	4602540229
50	**Sagecoumarin**	7.05	$C_{27}H_{19}O_{12}$-	535.08823	535.08155	6.67	135.04369(12), 161.02260(53), **177.01723**(100), 179.03314(12), 197.04333(15)	64945190	5625189
51	**Salvianolic acid**	7.15	$C_{26}H_{21}O_{10}$-	493.11355	493.10771	5.84	135.04349(46), **161.02251**(100), 179.03302(20), 197.04317(35)	NI	830513910
Flavonoid glycosides									
52	**Luteolin 8-*C*-hexoside (Orientin)**	5.81	$C_{21}H_{19}O_{11}$-	447.09331	447.08738	5.94	297.03683(10), **327.04715**(100), 357.05685(65)	143959194	NI
53	**Apigenin 8-*C*-hexoside (Vitexin)**	5.98	$C_{21}H_{19}O_{10}$-	431.09790	431.09264	5.25	283.05771(14), **311.05228**(100), 341.06192(26)	NI	1793415
54	**Quercetin 3-*O*-(6"-rhamnosyl)-hexoside (Rutin)**	6.04	$C_{27}H_{29}O_{16}$-	609.14614	609.13826	7.87	**300.02408**(100), 301.03171(43), 609.13928(14)	13753062	NI
55	**Isorhamnetin 3-*O*-(6"-rhamnosyl)-hexoside**	6.05	$C_{28}H_{31}O_{16}$-	623.16189	623.15385	8.03	300.02414(15), **315.04718**(100)	18018152	NI
56	**Luteolin 7-*O*-hexoside**	6.13	$C_{21}H_{19}O_{11}$-	447.09331	447.08759	5.72	284.02930(19), **285.03693**(100), 447.08752(24)	407196783	NI
57	**Quercetin 3-*O*-hexoside**	6.14	$C_{21}H_{19}O_{12}$-	463.08823	463.08205	6.17	151.00201(3), **300.02411**(100), 301.03174(39)	45341951	NI
58	**Luteolin 7-*O*-hexuronide**	6.23	$C_{21}H_{17}O_{12}$-	461.07258	461.06659	5.98	**285.03702**(100)	126388953	NI

59	**Apigenin 7-O-(6"-rhamnosyl)-hexoside**	6.34	$C_{27}H_{29}O_{14}$-	577.15631	577.14982	6.48	**269.04227**(100)	3970296	NI
60	**Quercetin 3-O-[6"-(3-hydroxy-3-methylglutaryl)]-hexoside**	6.36	$C_{27}H_{27}O_{16}$-	607.13059	607.12350	7.08	135.04341(8), 179.03265(4), **300.02393**(100), 301.03159(56), 463.08249(20), 505.09213(13)	8734118	4221569
61	**Luteolin 7-O-(6"-acetyl)-hexoside**	6.39	$C_{23}H_{21}O_{12}$-	489.10338	489.09747	5.91	284.02936(34), **285.03705**(100), 429.07751(9), 489.09799(31)	223858521	NI
62	**Luteolin 7-O-(6"-malonyl)-hexoside**	6.39	$C_{24}H_{21}O_{14}$-	533.09371	533.08686	6.85	284.02921(22), **285.03711**(100), 447.08548(3), 489.09799(54)	431658868	NI
63	**Apigenin 7-O-hexoside (Apigetrin)**	6.41	$C_{21}H_{19}O_{10}$-	431.09790	431.09252	5.38	268.03445(55), **269.04230**(100), 431.09329(26)	29336027	NI
64	**Luteolin 7-O-[6"-(3-hydroxy-3-methylglutaryl)]-hexoside**	6.49	$C_{27}H_{27}O_{15}$-	591.13557	591.12815	7.42	284.02917(10), **285.03705**(100), 447.08783(30), 489.09796(62), 529.12854(22)	441421587	NI
65	**Apigenin 7-O-hexuronide**	6.53	$C_{21}H_{17}O_{11}$-	445.07766	445.07180	5.87	113.02315(14), 163.03851(4), **269.04236**(100)	397297844	4848094
66	**Hispidulin 7-O-hexoside**	6.62	$C_{22}H_{21}O_{11}$-	461.10846	461.10307	5.39	**284.02933**(100), 299.05243(88)	19397874	1773139
67	**Apigenin 7-O-[6"-(3-hydroxy-3-methylglutaryl)]-hexoside**	6.76	$C_{27}H_{27}O_{14}$-	575.14066	575.13464	6.02	268.03461(11), **269.04227**(100), 431.09286(38), 473.10306(48), 513.13385(4)	32133581	NI
68	**Apigenin 7-O-(6"-acetyl)-hexoside**	6.79	$C_{23}H_{21}O_{11}$-	473.10896	473.10255	6.41	**268.03442**(100), 269.04211(44), 311.05234(6), 473.10287(86)	70355902	2379078
69	**Luteolin 7-O-(caffeoyl)-rhamnoside**	7.05	$C_{30}H_{25}O_{13}$-	593.13019	593.12246	7.73	135.04323(3), 161.02219(4), 179.03281(5), **285.03696**(100)	18194847	1280324
Flavonoid aglycones									
70	**Luteolin**	7.09	$C_{15}H_{9}O_{6}$-	285.04046	285.03708	3.38	151.00177(2), **285.03687**(100)	94087592	2857795
71	**Apigenin**	7.48	$C_{15}H_{9}O_{5}$-	269.04555	269.04239	3.16	149.02266(4), 151.00185(4), 225.05304(3), 269.04239(100)	31217574	972872
72	**Chrysoeriol**	7.57	$C_{16}H_{11}O_{6}$-	299.05611	299.05258	3.53	**284.02948**(100), 299.05276(27)	4718135	487820
73	**Trihydroxy-dimethoxyflavone**	7.64	$C_{17}H_{13}O_{7}$-	329.06670	329.06273	3.98	299.01620(92), **314.03949**(100), 329.06299(43)	41116749	30675564

74	**Cirsimaritin**	8.07	$C_{17}H_{13}O_6$-	313.07176	313.06805	3.71	**283.02130**(100), 297.03784(18), 298.04434(92), 313.0675(31)	12840553	NI
75	**Hispidulin**	8.07	$C_{16}H_{11}O_6$-	299.05611	299.05271	3.40	284.02917(85), **299.05255**(100)	5696443	1673407
76	**Eupatorin**	8.14	$C_{18}H_{15}O_7$-	343.08235	343.07760	4.75	313.03058(45), **328.05505**(100)	2113163	811018
77	**Pinocembrin**	8.52	$C_{15}H_{11}O_4$-	255.06628	255.06327	3.02	151.00153(6), 213.05388(8), **255.06326**(100)	1727642	NI
78	**Acacetin**	8.53	$C_{16}H_{11}O_5$-	283.06120	283.05786	3.33	268.03391(24), **283.09668**(100)	8264201	1199103

NI – not identified