Evaluation of antioxidant activity of extracts from the roots and shoots of *Scutellaria alpina* L. and *S. altissima* L. in selected blood cells

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D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899-5276 (print), ISSN 2451-2680 (online) *Adv Clin Exp Med*. 2019;28(4):453–460

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Funding sources
None declared

Conflict of interest
None declared

Acknowledgment
Special thanks to Dr. Joanna Ruszkowska (Medical University of Lodz), Mrs. Malwina Borys and the staff of the diagnostic laboratory in Konstantynów Łódzki for technical assistance.

Received on April 18, 2017
Reviewed on July 30, 2017
Accepted on October 13, 2017

Published online on August 7, 2018

Cite as
Grzegorczyk-Karolak I, Kontek B, Kontek R, Wysokińska H, Olas B. Evaluation of antioxidant activity of extracts from the roots and shoots of *Scutellaria alpina* L. and *S. altissima* L. in selected blood cells. *Adv Clin Exp Med*. 2019;28(4):453–460. doi:10.17219/acem/78589

DOI
10.17219/acem/78589

Abstract

Background. It is widely known that reactive oxygen species (ROS) can cause oxidative damage in cells and have been linked to the pathogenesis of oxidative diseases, such as atherosclerosis, ischemia, neurodegenerative disease, diabetes, or cancer. Recently, much attention has been focused on preventive strategies for oxidative stress and related diseases. Plants represent a source of bioactive compounds whose antioxidant activity may be useful in protecting against pro-oxidative reactions.

Objectives. The study determines the in vitro biological activity of the ethanolic extracts from the shoots and roots of *Scutellaria* species (*S. altissima* and *S. alpina*) in selected blood cells (blood platelets and lymphocytes).

Material and methods. Platelet activity, both resting and after thrombin stimulation, was used to indicate the ability of the plant extracts to inhibit the production of superoxide anion radicals ($O_2^{\bullet -}$) and platelet lipid peroxidation. The generation of superoxide anion radicals was measured with cytochrome c reduction. Lipid peroxidation in blood platelets was measured by the level of thiobarbituric acid reactive substances (TBARS). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was used to determine the protective effect of *Scutellaria* extracts on lymphocyte cells against oxidative damage induced by hydroxyl radicals.

Results. Extracts (5–50 µg/mL) containing phenolic compounds from both *Scutellaria* species distinctly reduced nonenzymatic lipid peroxidation and arachidonic acid metabolism by blood platelets in vitro. When given at the tested concentration, the extracts reduced the generation of $O_2^{\bullet -}$ in resting blood platelets and platelets activated by thrombin in vitro. All *Scutellaria* extracts (10 µg/mL) containing phenolic compounds also protected human lymphocytes against oxidative stress induced by hydrogen peroxide (H_2O_2).

Conclusions. The present study suggests that the natural extracts from *S. altissima* and *S. alpina* have antioxidant properties and, therefore, may be beneficial in the prevention of diseases in which blood platelets and lymphocytes are involved, i.e., cancer or inflammatory and infective diseases.

Key words: oxidative stress, lymphocytes, blood platelets, polyphenols, *Scutellaria*

Introduction

Reactive oxygen radicals are released under conditions of stress and cause a number of pathological changes in all cells of the human organism. In recent years, increased attention has been given to the antioxidant activity of plants. Many studies suggest that several medicinal plants containing polyphenolic compounds can protect cells against destructive oxidative damage and limit the risk of various diseases associated with oxidative stress. Natural antioxidants are known to be radical scavengers or radical-chain breakers, which can inhibit or delay the oxidation process.

Extracts from *Scutellaria* plants have been used in traditional Chinese medicine for their hepatoprotective, anti-inflammatory, antihistaminic, hyperlipidemic, antibacterial, antiviral, antitumor, and other pharmacological properties for centuries.[1] Today it is known that the dominant role in these therapeutic effects may be attributed to their antiradical properties. Flavones present in *Scutellaria* extracts, which can bind and eliminate heavy metals and scavenge free radicals, are strong antioxidants. Experiments have shown that the flavonoids present in skullcap plants protect hepatocytes against necrosis and mutagenesis induced by toxins, high doses of paracetamol or hydrogen peroxide (H_2O_2).[2–4] *Scutellaria* metabolites have demonstrated protective activity towards nervous cells and have effectively limited the development of neurodegenerative diseases. For example, baicalin is known to have a neuroprotective effect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a factor causing changes in dopaminergic neurons, which can translate into the occurrence of the Parkinson's disease.[5] *Scutellaria* extracts have also exhibited a protective effect on erythrocytes and prevented their membranes from free radical damage.[6] However, no studies have examined the effects of skullcap extracts on the viability and function of the other cellular components of blood under conditions of oxidative stress.

Blood platelet activation results in the production of reactive oxygen species (ROS), which play a crucial role in hemostasis and thrombosis. Reactive oxygen species can behave as 2nd messengers during platelet activation and participate in signaling pathways.[7] Several sources of ROS in blood platelets are postulated, one of which being the metabolites of arachidonic acid.[8] Under pathological conditions, oxidants generated by activated blood platelets or inflammatory cells may promote oxidative stress and influence platelet functions, which can damage platelet structure.[9] In fact, one of the therapeutic strategies used in the treatment and prophylaxis of cardiovascular disease is the prevention of platelet activation during exposure to oxidative stress. Another important component of the blood are lymphocytes, which act as the basis of the specific resistance of the organism by initiating the immune response. Lowering their amounts and activity leads to the inhibition of the immune system.[10]

Previously, we have demonstrated the antioxidant properties of *Scutellaria* extract in human blood plasma.[11] The aim of the paper is to determine the in vitro protective effect of ethanolic extracts, derived from the shoots and roots of *Scutellaria altissima* and *S. alpina*, on blood platelets and lymphocytes. The aim of the study was to investigate the antioxidant activity of skullcap extracts against the effect of a strong biological oxidant, H_2O_2, a known hydroxyl radical donor, on human lymphocytes. The study also examines the effect of *Scutellaria* extract on lipid peroxidation, and on superoxide anion ($O_2^{\cdot -}$) production in resting blood platelets and platelets activated by thrombin, a strong physiological agonist. Experimental models used in this study are similar to reactions which take place in human cells under conditions of oxidative stress or during blood platelet activation.

Material and methods

Plant material

The roots and aerial parts of *S. altissima* L. and *S. alpina* L. were used for the study. The plants had previously been growing for 2 years under field conditions in the Medical Plant Garden of the Department of Pharmacognosy, Medical University of Lodz, Poland. The plants were identified on the basis of the Flora Europaea by I. Grzegorczyk-Karolak and voucher specimens were deposited in Department of Biology and Pharmaceutical Botany, Medical University of Lodz, Poland.[12]

Preparation of extracts

The lyophilized plant material (1 g) was pre-extracted with chloroform overnight. After filtration, the plant material was extracted 3 times in a 30 mL ethanol–water (7:3) mixture for 15 min in an ultrasonic bath. The extracts were combined and evaporated under reduced pressure.

Total phenolic determination

Total phenolic content was measured using the Folin-Ciocalteu method as described by Singleton and Rossi.[13] Briefly, 400 µL of each extract was mixed with 2 mL of Folin-Ciocalteu reagent (diluted 10-fold) and 1.6 mL of 7.5% sodium carbonate (Na_2CO_3). The absorbance was determined by spectrophotometry at 765 nm (Beijing Rayleigh Corp., Beijing, China) after 30 min of incubation at room temperature. The results were expressed as mg gallic acid equivalents (GAE) per gram of dry extract. The calibration curve was obtained by preparing a gallic acid solution in the concentration range 1–400 mg/mL. The results are mean values of 9 independent experiments.

Blood platelet isolation

Peripheral blood was collected from 5 non-smoking volunteers into ACD solution (citric acid/citrate/dextrose; 5:1; v/v; blood/ACD). Donors were healthy men and women aged 35–45 years, with normal body mass (body mass index [BMI] between 22 and 25). They did not take any medications or addictive substances, including tobacco, alcohol, antioxidant supplementation, aspirin, or any other anti-platelet drugs. They did not take treatment for any kind of systemic disease in their histories. The protocol was approved by the Committee for Research on Human Subjects, University of Lodz, Poland (reference: 2/KBBN-UŁ/III/2014). Platelet-rich plasma (PRP) was prepared by centrifugation of fresh human blood at 250 × g for 10 min at room temperature. Platelets were then sedimented by centrifugation at 500 × g for 10 min at room temperature. The platelet pellet was washed twice with Tyrode's buffer containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 140 mM sodium chloride (NaCl), 3 mM potassium chloride (KCl), 0.5 mM magnesium chloride ($MgCl_2$), 5 mM sodium bicarbonate ($NaHCO_3$), and 10 mM glucose (pH 7.4), and the platelets were suspended in Tyrode's buffer. Spectrophotometric evaluation found the concentration of platelets in platelet suspensions to be about 5×10^8/mL.[14] The suspensions of blood platelets were incubated with plant extracts at final concentrations of 0.5–50 µg/mL (15 min, at 37°C), with or without the addition of thrombin (5 U/mL, 5 min, 37°C).

Superoxide anion radical measurement

The generation of $O_2^{\cdot-}$ in the control platelets and in platelets incubated with tested extracts was measured by an inhibitable reduction of cytochrome c using superoxide dismutase (1 µg/mL), as described earlier.[15] Briefly, an equal volume of modified Tyrode's buffer containing cytochrome c (160 µM) (Sigma-Aldrich, St. Louis, USA) was added to a platelet suspension. After incubation, the platelets were sedimented by centrifugation at 2,000 × g for 5 min and the supernatants were transferred to cuvettes. Any reduction in cytochrome c was measured spectrophotometrically at 550 nm. To calculate the molar concentration of $O_2^{\cdot-}$, the molar extinction coefficient for cytochrome c was taken as 18,700/M × cm.

Lipid peroxidation measurement

Lipid peroxidation was quantified by measuring the concentration of thiobarbituric acid reactive substances (TBARS) (Sigma-Aldrich). Incubation of platelets was stopped by cooling the samples in an ice bath. Samples of platelets were transferred to an equal volume of 20% (v/v) cold trichloroacetic acid in 0.6 M HCl and centrifuged at 1,200 × g for 15 min. The clear supernatant was mixed with 0.12 M thiobarbituric acid in 0.26 M Tris at pH 7.0 in a ratio of 5:1 by volume and immersed in a boiling water bath for 15 min. Absorbance was measured at 532 nm (Spectrophotometer UV/Vis Helios alpha; Unicam, Cambridge, UK).[16] The TBARS concentration was calculated using the molar extinction coefficient (ε = 15,600/M × cm).

Lymphocyte cultures

Lymphocytes were isolated from peripheral human blood obtained from medication-free, regular non-smoking donors at the blood bank (Łódź, Poland) by centrifugation in a density gradient with Histopaque 1077 (Sigma-Aldrich) at 300 × g for 15 min. The protocol was approved by the Committee for Research on Human Subjects, University of Lodz, Poland (reference No.: 2/KBBN-UŁ/III/2014). The cells were suspended in Roswell Park Memorial Institute (RPMI) 1640 culture medium with Glutamax, 15% inactivated fetal bovine serum (FBS), 1% penicillin and streptomycin, and 1% mitogen PHA (added 24 h before application of the tested compound). The lymphocytes were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

3-(4,5-dimethylthiazol-2-yl)-2,5--diphenyltetrazolium bromide assay

Lymphocyte viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[17] The MTT test is based on the reduction of tetrazolium to colored formazan by dehydrogenase inside living cells. The assay only detects living cells and the signal generated is dependent on their degree of activation. A 24-hour culture of lymphocytes seeded on to 96-well microplates at 10^3 cells/well was treated with extracts of shoot and root *S. altissima* and *S. alpina* at a concentration of 10 µg/mL. Fifteen min before the end of the incubation period, H_2O_2 solutions at concentrations of 0.1, 0.5, 1 or 1.5 mM were added to the wells. After this time, 20 µL of fresh MTT solution, i.e., 5 mg/mL MTT in sterile phosphate-buffered saline (PBS), was added to each well and the plates were incubated for the next 3 h. Following this, a 100 µL mixture of 20% sodium dodecyl sulfate (SDS) and 50% dimethylformamide (DMF) was added to each well and left for 24 h. The absorbance of purple formazan solution was measured spectrophotometrically using a microplate reader (BioTek Instruments Inc., Winooski, USA) at 595 nm. A lymphocyte culture treated with H_2O_2, but without any plant extracts, was used as a positive control, while a culture of cells not exposed to any of the tested compounds was used as a negative control. Any observed reduction of MTT after treatment with the tested compounds was compared to the negative control, which represented a 100% MTT reduction.[18] All results were presented as the means of the replicates from 6 independent experiments.

Data analysis

Several tests were used in the statistical analysis. In order to eliminate uncertain data, Dixon's Q test was performed. Significant differences were assessed with the Kruskal-Wallis test. Differences were considered significant at $p < 0.05$. All the values in this study were expressed as mean ± standard error (SE). The statistical analysis was performed with STATISTICA v. 10.0 software (StatSoft Inc., Tulsa, USA).

Results

We have observed that *Scutellaria* extracts may decrease the oxidative alteration of lipids and the level of ROS in both resting blood platelets and those activated by thrombin. However, the exposure of blood platelets to thrombin (5 U/mL) resulted in stronger oxidative changes than observed in the resting blood platelets (controls). Thrombin, which is a known effective inducer of platelet activation, increased both $O_2^{\cdot-}$ production (2.26 nM of $O_2^{\cdot-}/10^8$ of platelets vs 1.6 nM of $O_2^{\cdot-}/10^8$ of platelet) and lipid peroxidation (0.7 nM/mL of platelets vs 0.49 nM/mL of platelets).

Cytochrome c reduction was used to test the ability of analyzed extracts to influence ROS generation in platelets. Extracts from the shoots and roots of *S. altissima* and *S. alpina* at concentrations of 0.5–50 µg/mL decreased the production of $O_2^{\cdot-}$ in resting blood platelets and those activated by thrombin in vitro in a dose-dependent manner (Fig. 1). Root and shoot extracts from both species had similar inhibitory effects at the same concentrations. The highest dose of skullcap extracts reduced $O_2^{\cdot-}$ production in platelets by about 30% (Table 1). The *Scutellaria* extracts were also observed to bestow a protective effect at lower concentrations (5 µg/mL); however, only slight inhibition of $O_2^{\cdot-}$ generation was observed at the lowest concentration (0.5 µg/mL).

The TBARS level was measured as nonenzymatic lipid peroxidation in resting blood platelets and as enzymatic lipid peroxidation of arachidonic acid in blood platelets stimulated by thrombin. The tested extracts decreased

Table 1. Inhibitory effects of extracts from shoots and roots of *Scutellaria altissima* and *S. alpina* (50 µg/mL; 15 min) on the production of $O_2^{\cdot-}$ in blood platelets

Plant material	Inhibition of the production of $O_2^{\cdot-}$ in resting blood platelets [%]	Inhibition of the production of $O_2^{\cdot-}$ in blood platelets activated by thrombin [%]
S. altissima shoots	35.4 ±6.07[a]	32.9 ±2.93[a]
S. altissima roots	35.3 ±3.96[a]	34.6 ±2.29[a]
S. alpina shoots	33.3 ±4.01[a]	33.3 ±2.34[a]
S. alpina roots	33.0 ±4.26[a]	32.7 ±2.74[a]

The results are mean values ± standard error (SE) of 3 replicates for each of the 5 donors. Means with the same letters for the same column are not significant according to the Kruskal-Wallis test at $p < 0.05$.

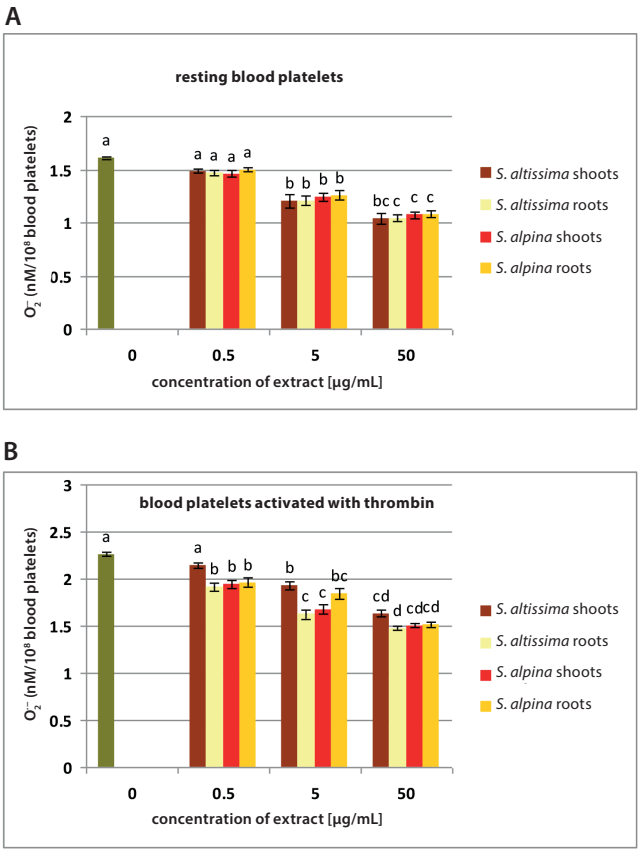

Fig. 1. The effects of shoot and root extracts of *Scutellaria altissima* and *S. alpina* (0.5–50 µg/mL; 15 min, 37°C) on $O_2^{\cdot-}$ generation in resting blood platelets (A) and blood platelets activated by thrombin (5 U/mL, 5 min, 37°C) (B). The data represents means of 3 replicates for each of the 5 donors ± standard error (SE). Means with the same letters for treatment (resting blood platelets or blood platelets activated by thrombin) are not significant according to the Kruskal-Wallis test at $p < 0.05$

TBARS level. After a 15-minute pre-incubation of blood platelets with both shoot and root extracts of *S. altissima* and *S. alpina* at tested concentrations (0.5–50 µg/mL), the amount of TBARS in resting platelets and thrombin-activated blood platelets was seen to diminish. The activity of the 4 tested extracts was concentration-dependent (Fig. 2). Differences were found between resting platelets and induced platelets regarding lipid peroxidation. In the presence of the highest extract concentrations (50 µg/mL), TBARS production in activated platelets was reduced by about 43% in the shoot extract of *S. altissima* and about 40% in the root extract of *S. altissima* (Table 2). The tested extracts demonstrated less effective antioxidant action regarding the protection of the resting blood platelet lipids; extracts at a concentration of 50 µg/mL inhibited the peroxidation of 22–27%. Even the lowest concentration of the tested extracts (5 µg/mL) was able to reduce TBARS production by about 20% in activated platelets. In control experiments, dimethyl sulfoxide (DMSO) (the solvent) added to platelet suspensions at a final concentration below 0.05% did not influence platelet activation in the studied assays.

Table 2. Inhibitory effects of extracts from shoots and roots of *Scutellaria altissima* and *S. alpina* (50 µg/mL; 15 min) on blood platelet lipid peroxidation

Plant material	Inhibition of lipid peroxidation in resting blood platelets [%]	Inhibition of lipid peroxidation in blood platelets activated by thrombin [%]
S. altissima shoots	21.5 ±2.03[a]	43.2 ±4.39[a]
S. altissima roots	21.3 ±2.96[a]	40.6 ±3.44[a]
S. alpina shoots	23.6 ±2.87[a]	40.3 ±3.44[a]
S. alpina roots	27.4 ±3.25[a]	40.5 ±3.38[a]

The results are mean values ± standard error (SE) of 3 replicates for each of the 5 donors. Means with the same letters are not significant according to the Kruskal-Wallis test at $p < 0.05$.

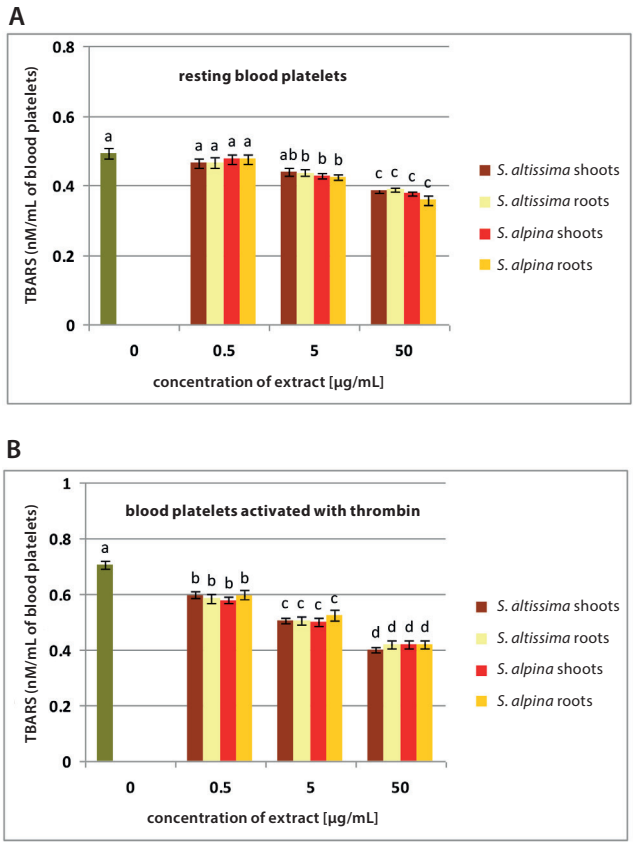

Fig. 2. The effects of shoot and root extracts of *Scutellaria altissima* and *S. alpina* (0.5–50 µg/mL; 15 min, 37°C) on the level of thiobarbituric acid reactive substances (TBARS) in resting blood platelets (A) and blood platelets activated by thrombin (5 U/mL, 5 min, 37°C) (B). The data represents the means of 3 replicates for each of the 5 donors ± standard error (SE). Means with the same letters for treatment (resting blood platelets or blood platelets activated by thrombin) are not significant according to the Kruskal-Wallis test at $p < 0.05$

The results of lymphocyte survival assessment after the treatment with tested plant extracts in the absence and in the presence of H_2O_2 are presented in Table 3. Only the lowest concentration of H_2O_2 (0.1 mM) had no significant effect on lymphocyte survival rate. Higher concentrations (0.5–1.5 mM) induced oxidative damage in the lymphocytes resulting in a 40% decrease in cell viability (Table 3). The results of the MTT assay found that all *Scutellaria* extracts at concentration of 10 µg/mL increased the viability of the human lymphocyte cells and protected them against damage induced by H_2O_2. No significant difference was found in this regard between the plant species or organ used for preparing the extract. In the presence of plant extracts, lymphocyte survival ranged from 90.2 to 98.7%, which was close to that demonstrated by cells grown without stress conditions. Dimethyl sulfoxide added to lymphocyte culture at the concentration used in the study (0.05%) did not result in any decrease in the survival rate of lymphocytes.

The main group of bioactive compounds found in *Scutellaria* extracts includes polyphenols such as flavonoids and phenylethanoids.[1] Both analyzed species have been found to contain flavonoids such as baicalin, wogonoside, luteolin, and cynaroside, as well as the phenylethanoid verbascoside.[11] Total polyphenol levels in *S. altissima* roots and shoots (30.51 ±0.32 and 30.52 ±0.09 mg GAE/g dry weight of extract, respectively) were found to be 30% lower than those identified in the *S. alpina* extracts (49.76 ±0.52 and 44.55 ±0.16 mg GAE/g dry weight of extract, respectively).

Discussion

Recently, much attention has been focused on plants containing various bioactive compounds which may have pharmacological properties, such as antioxidant activity. The present study uses lymphocytes, cells important for the immune system, and blood platelets, which are not only important components of hemostasis or the pathomechanisms of several arterial diseases, but also participate in tumor progression and allergic inflammation.[9]

Blood platelet activation can be strongly induced by thrombin, a proteolytic enzyme and platelet physiological agonist. Thrombin activates platelets by several independent and interactive signal transduction pathways.[9] Our findings confirm that thrombin stimulates $O_2^{\cdot-}$ formation and TBARS production in blood platelets (Fig. 1,2). A signal is transmitted into the cell that invokes various biochemical events, including ROS production. This signal also exerts an influence on arachidonic acid metabolism. The ensuing multistage reactions in the cyclooxygenase pathway involve the activation of phospholipase A_2, which catalyzes the cleavage of arachidonic acid from membrane phospholipids. Free arachidonic acid in platelets is converted mainly to thromboxane A_2 (TXA_2) and malonyldialdehyde (MDA).[19] Thromboxane A_2 acts as a signaling molecule (2^{nd} messengers) in the regulation of blood platelet activation and thrombus formation.

A significant finding of this study is that extracts from shoots and roots of *S. altissima* and *S. alpina* inhibit the peroxidation of arachidonic acid and ROS generation in blood platelets by enzymatic and nonenzymatic means,

Table 3. The effect of extracts from shoots and roots of *Scutellaria altissima* and *S. alpina* (10 µg/mL) in the presence of different concentration of hydrogen peroxide (H_2O_2) on lymphocyte growth after 24-hour incubation evaluated with MTT assay

Concentration of H_2O_2 [mM]	% lymphocytes survival				
	H_2O_2	*S. altissima* shoots	*S. altissima* roots	*S. alpina* shoots	*S. alpina* roots
0 (control)	100 ±5.16[a]	100 ±2.00[a]	100 ±3.02[a]	100 ±2.35[a]	100 ±1.62[a]
0.1	97.08 ±4.62[a]	97.54 ±3.18[a]	97.88 ±1.02[a]	97.57 ±2.25[a]	93.91 ±2.43[a]
0.5	63.66 ±1.71[b]	94.53 ±2.42[a]	94.08 ±2.87[a]	98.51 ±2.05[a]	91.46 ±1.97[a]
1	59.92 ±2.23[b]	94.41 ±3.45[a]	93.33 ±2.54[a]	97.79 ±2.01[a]	91.46 ±1.97[a]
1.5	59.08 ±1.96[b]	93.15 ±3.95[a]	92.33 ±3.92[a]	96.25 ±2.41[a]	90.27 ±2.23[a]

The results are mean values ± standard error (SE) of 3 replicates for each of the 6 samples. Means with the same letters are not significant according to the Kruskal-Wallis test at $p < 0.05$.

which implies that the tested extracts may inhibit cyclooxygenase activity in platelets. However, $O_2^{\cdot-}$ generation was also observed in resting platelets. Wachowicz et al. suggested that radical production can be partially associated with glutathione metabolism, or that platelet isolation and resuspension may also stimulate their activation.[20]

The antiplatelet properties of *Scutellaria* species have only been partly recognized so far. Lee et al. report that a herbal extract named Soshiho-tang containing *Scutellaria baicalensis* Georgi root demonstrated an anti-thrombotic effect via antiplatelet activity.[21] *Scutellaria baicalensis* flavonoids are also known to inhibit platelet aggregation, demonstrating an inhibition rate of 45.5% in 1 study, which was close to the value of 55% noted for the control group based on aspirin.[22] The present study found that *S. alpina* extracts were able to reduce $O_2^{\cdot-}$ production in platelets activated by thrombin by 33%, and reduce TBARS production in activated platelets by about 40%. Inhibition of lipid peroxidation, measured by the level of TBARS in blood platelets treated with $ONOO^-$, by *Aronia melanocarpa* (Michx.) Elliott, a species known for its strong antioxidant properties, was about 30%.[23] Vitamin C at a concentration of 1 mM was found to have comparable activity, inhibiting TBARS production in pig resting blood platelets by about 30%.[24]

The pharmacological properties of the *Scutellaria* plants are mainly due to the presence of polyphenolic compounds, among them flavonoids, in the plant extracts. Some studies have shown that flavonoids such as baicalin, baicalein, wogonin, or luteolin exhibited generally antioxidant activity, but little research has addressed the antiplatelet properties of the flavonoids.[1] Kubo et al. report that baicalein, baicalin, wogonin, or wogonoside inhibits the platelet aggregation induced by arachidonic acid by 48%, 31%, 46%, and 20%, respectively, at a concentration of 1 mM.[25] In comparison, the positive control, aspirin, inhibited aggregation by 30%. At a concentration of 0.5 mM, baicalein and baicalin also inhibited thrombin-induced conversion of fibrinogen to fibrin, slowing it from 193 s for controls to 413 s for baicalein and 478 s for baicalin. Incubation of fibrinogen with heparin (10 U/mL) prolonged the clotting time only to 281 s.

The 2nd blood cell type used in the present study, the lymphocytes, are an important part of the human immune defense against infection and cancer. When their functioning is disturbed, their activity can be wrongly directed against healthy human tissue, resulting in autoimmune disease.[10] The results of the MTT assay in the present study found *Scutellaria* extracts to have a protective effect against oxidative damage induced by a strong biological oxidant, H_2O_2, in lymphocyte cells. In previous studies, Zhang et al. found the aqueous extract of *S. baicalensis* to have protective effects at concentrations of 50 and 100 µg/mL against acrolein-induced oxidative stress in cultured human umbilical vein endothelial cells.[26] Shojaee et al. described the protective effects of *Scutellaria litwinowii* Bornm & Sint. ex Bornm. root extract against H_2O_2-induced DNA changes in normal fibroblasts (NIH/3T3 cell line); however, their MTT and comet assay results suggest that the methanolic *S. litwinowii* extract demonstrated a protective effect against DNA damage caused by H_2O_2 only at high extract concentrations (1,000 µg/mL).[27]

Although this is the first report to describe the effect of *S. altissima* and *S. alpina* extracts on lymphocyte oxidative damage, some other authors have reported that other plant species and their metabolites have a protective effect against toxic and mutagenic factors. Porrini and Riso used H_2O_2 at a concentration of 0.5 mM to stimulate oxidative stress damage in lymphocytes.[28] According the authors, the blood cells are excellent markers of the body state and could be a reliable model for studying the effect of the dietary supplementation with antioxidants on the responses of the body to factors causing oxidative stress. In the study, tomato products offered significant protection to lymphocytes against oxidative stress. Lycopene, the main carotenoid isolated from tomatoes, characterized by a great ability to quench singlet oxygen, effectively protected blood lymphocytes from NO_2 radical damage.[29] Elsewhere, Lin et al. examined the protective effects of several plant species extracts against DNA damage in lymphocytes induced by means of H_2O_2.[30] They report that the inhibition of DNA changes in the presence of tested plants ranged from 74.51% for *Bidens alba* (L.) DC. to 91.45% for *Mentha arvensis* L. extract at a concentration of 25 µg/mL.

Pool-Zobel et al., using the comet assay, found a significant decrease in endogenous levels of strand breaks in lymphocyte DNA after the intake of tomato juice containing 40 mg lycopene, carrot with 38 mg carotene, or spinach containing 11.3 mg lutein.[31] Meanwhile, American ginseng extract was effective in protecting human peripheral lymphocytes against radiation-induced oxidative stress.[32]

In the present study, a spectrophotometric method was used to determine the total phenol content in the tested plant extracts.[13] It has been previously demonstrated that the polyphenol content of *Scutellaria* extracts was significantly correlated with the antioxidant properties estimated by ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipid hydroperoxide (LPO) assays.[33] The findings suggest that the polyphenol compounds were the main contributors to the antioxidant properties of the extract, which is consistent with the findings of many other authors.[34,35] Our present findings indicate that all tested extracts exhibited similar activity in blood platelets and lymphocytes, although higher levels of polyphenolic compounds were found in *S. alpina* than *S. altissima* extracts. Also, *M. arvensis* extract was more effective against H_2O_2-induced DNA damage in lymphocyte cells than *Centella asiatica* L. extract, despite the lower content of bioactive compounds (respectively, 21.2 and 32.0 mg gallic acid/g dry weight).[30] The authors suggested that this could be connected with the synergism present among antioxidant compounds, indicating that the properties of the mixture are dependent not only on the concentrations of the antioxidants, but also on their type and the interaction between them.

The unclear relationship between the protective activity of *S. altissima* and *S. alpina* extracts on blood cells and the flavonoid content could also be connected with other factors. The scientific interest in pharmacological activity of *Scutellaria* plants has been focused mainly on the effects of their flavonoid compounds, but other groups of compounds, for example diterpenoids, could also act as antioxidant and antiplatelet compounds. Several compounds of this group have been described as having antioxidant effects, for example royleanonic acid, tanshinones or carnosol.[36–38] Diterpenoids have been identified in both *S. altissima* and *S. alpina*; however, the plants contain different diterpenoids, whose levels have not yet been evaluated in detail.[39,40] These differences could account for the results observed in the present study.

Conclusions

A significant finding of this paper is that it describes the antioxidant properties of polyphenol-rich *Scutellaria* extracts. When added at a concentration of 10 μg/mL, the extracts reduced H_2O_2-induced oxidative stress in human lymphocytes and increased cell viability to more than 90% in MTT assay. The analyzed plants may also be valuable in the protection of platelets against oxidative stress and its consequent pathological platelet activation and aggregation. Therefore, *S. altissima* and *S. alpina* may represent promising new sources of compounds offering significant benefits in diseases typified by an imbalance between oxidative reactions and the antioxidant process. However, future studies should be extended to include other groups of compounds besides flavonoids, which can play a role in the antioxidant activity of *Scutellaria* extracts.

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