



Research Article



Effects of extracts derived from roots and stems of *Chelidonium majus* L. on oxidative stress biomarkers in the model of equine plasma

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Greater celandine (*Chelidonium majus* L., Papaveraceae) is a perennial herbaceous plant, with an upright and spreading stem, large leaves, and yellow flowers collected on the tops of the stems in rare umbel inflorescence. The main aim of the study was an assessment of the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification (OMP), total antioxidant capacity (TAC)] and also activity of antioxidant enzymes (catalase, ceruloplasmin) in the equine plasma after treatment by extracts derived from roots and stems of *C. majus* collected from rural and urban agglomerations. Plant materials were collected from natural habitats on the territory of the Kartuzy district in the Pomeranian province (northern part of Poland). Our results demonstrated that statistically significant reductions in lipid peroxidation byproducts were noted after incubation with extracts derived from roots of *C. majus* collected from both urban (by 35 %, $p < 0.05$) and rural (by 34 %, $p < 0.05$) agglomerations compared to the control samples. Stem extracts derived from *C. majus* also reduced TBARS levels, but only extracts derived from *C. majus* were collected from the rural areas; a statistically significant decrease (by 21 %, $p < 0.05$) was observed compared to the control samples. The lowest values in the content of the aldehydic derivatives of OMP were observed after incubation with extracts derived from roots of *C. majus* collected from both rural and urban areas. On the other hand, levels of ketonic derivatives of OMP were significantly increased after incubation with extracts derived from stems of *C. majus* collected from both rural and urban areas compared to the control samples, in contrast to extracts derived from roots of *C. majus* collected from urban areas, where there was a statistically significant reduction in ketonic derivatives of OMP (by 15 %, $p < 0.05$) compared to the control samples. A significant increase in the TAC levels was observed after incubation with root and stem extracts of *C. majus* collected from both urban and rural areas, but the highest values were observed after incubation with extracts derived from roots of *C. majus* collected from rural areas (by 66.7 %, $p < 0.05$) compared to the control samples. Stem extracts of *C. majus* collected from urban agglomerations were found to be most effective in increasing catalase activity (by 115 %, $p < 0.05$). Both root and stem extracts of *C. majus* collected from rural areas caused a statistically significant reduction in ceruloplasmin levels. These *in vitro* studies indicate that extracts from this plant are a significant source of natural antioxidants that could prevent the progression of various disorders caused by oxidative stress. However, the proportions of secondary metabolites responsible for the antioxidant activity of *C. majus* extracts are currently unclear. Therefore, further studies are needed to isolate and identify the antioxidant compounds present in the plant extracts. Screening of *C. majus* plant for other biological activities including antioxidant activities is essential and may be effective for searching the preventive agents in the pathogenesis of some metabolic diseases.

Keywords: *Chelidonium majus*, root and stem extracts, equine plasma, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity, catalase, ceruloplasmin

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Introduction

Organismal life encounters reactive oxidants from internal metabolism and environmental toxicant exposure. Reactive oxygen and nitrogen species cause oxidative stress and are traditionally viewed as being harmful (Forrester et al., 2019). Reactive oxygen species (ROS) regulate cellular homeostasis and act as prime modulators of cellular dysfunction contributing to disease pathophysiology. ROS are byproducts of numerous enzymatic reactions in various cell compartments, including the cytoplasm, cell membrane, endoplasmic reticulum (ER), mitochondria, and peroxisome, as part of basal metabolic function (Allen and Bayraktutan, 2009; Goncharov et al., 2015; Forrester et al., 2019). Oxidative stress is now recognized to play a central role in the pathophysiology of many different disorders (Bedard and Krause, 2007).

Living cells are under constant oxidative attack from reactive oxygen species, which can cause, among other things, lipid peroxidation or increase the level of byproducts of excessive protein oxidation. The lipid peroxidation chain reactions products display high biological activity (Brieger et al., 2012; Paik et al., 2017). It destroys DNA, proteins, and enzyme activity as well as acts as a molecular to activate signaling pathways initiating cell death (Su et al., 2019). As proteins are highly abundant and react rapidly with many oxidants, they are highly susceptible to, and major targets of, oxidative damage. This can result in changes to protein structure, function, and turnover and loss or (occasional) gain of activity (del Río et al., 1992).

Accumulation of oxidatively-modified proteins, due to either increased generation or decreased removal, has been associated with both ageing and multiple diseases (Singh, 1996). Different oxidants generate a spectrum of broad, sometimes characteristic, post-translational modifications (Hawkings and Davies, 2019).

Equine erythrocytes are uniquely susceptible to oxidant-induced damage due to the use of inefficient mechanisms to correct and protect against oxidative damage (Harvey et al., 2003). Oxidants typically damage erythrocytes by oxidizing the heme iron in hemoglobin, reactive sulfhydryls, or unsaturated lipids in the membranes. The oxidation of the heme iron in hemoglobin to the ferric (Fe^{3+}) state generates methemoglobin, which is incapable of transporting oxygen (Baskurt and Meiselman, 1999; Boyer et al., 2002). Oxidation of sulfhydryl groups in the globin portion of hemoglobin can induce protein denaturation and the formation of Heinz body aggregates. The

oxidation of sulfhydryl groups and unsaturated lipids can also compromise the erythrocyte membrane integrity (Wright et al., 1999). Reduced glutathione (GSH) can protect erythrocytes against oxidant injury, being oxidized itself to a disulfide; however, horses have a reduced ability to regenerate reduced GSH, compared with other mammals, likely due to the decreased activity of glutathione reductase in equine erythrocytes. Under normal conditions, equine erythrocytes have sufficient capacity to prevent oxidative damage (Robin and Harley, 1967; Medeiros et al., 1984).

A complex system of antioxidant defenses has evolved that generally holds this attack in balance. In recent years, an important increase in the attempts to find natural sources of molecules with biological potential has been noticed (Storz, 2005; Liou and Storz, 2010). Polyphenols from plants are such molecules, which have proven important antioxidant activity (Unuofin and Lebelo, 2020). Antioxidants are important agents involved in the protection against oxidative stress that has proven to be one of the most important causes of many diseases nowadays (Diniz do Nascimento et al., 2020). Therefore, in recent years, many researchers have focused on this direction, with important results in the curative or adjuvant treatment of some diseases (Ratnam et al., 2006; Pisoschi et al., 2016). Several medicinal plants have been proven to contain significant amounts of polyphenols, which added an important value to their use in the therapy of numerous diseases (Carocho and Ferreira, 2013).

Plants naturally produce many new metabolic compounds that have been an invaluable source of pharmacological discovery for centuries (Rahman, 2007). Plants, particularly those of the Papaveraceae family, produce many antioxidant factors including a wide range of natural defensive compounds such as phenols, terpenoids, alkaloids, polyacetylenes, lectins, and carotenoids (Păltinean et al., 2017). *Chelidonium majus* (CM) is a perennial herbaceous plant, with an upright and spreading stem, large leaves, and yellow flowers collected on the tops of the stems in rare umbel inflorescence. The plant is widely present in Europe and Asia, North America, and a part of Northwest Africa (Krahulcová, 1982). The plant contains, as major secondary metabolites, isoquinoline alkaloids, such as sanguinarine, chelidonine, chelerythrine, berberine, and coptisine. Other compounds structurally unrelated to the alkaloids have been isolated from the aerial parts: several flavonoids and phenolic acids (Colombo and Bosisio, 1996). Crude extracts of CM, as well as purified compounds derived from it, exhibit a broad spectrum of bioactive properties with a potential

for the protection of human health, such as anti-inflammatory, antimicrobial, cytotoxic, analgesic, antioxidant, antiulcer, acetylcholinesterase- and butyrylcholinesterase-inhibitory, and hepatoprotective activities (Lee et al., 2007; Kuenzel et al., 2013). The high spectrum of antioxidant properties for CM that are increasingly being used suggests the necessity of further investigations regarding their influence on organs and tissue function, including the evaluation of molecular mechanisms involved to exploit them for potential therapeutic benefits (Lenfeld et al., 1981; Kokoska et al., 2002; Havelek et al., 2016).

The originality of this work is that it is a study on the antioxidant activity of CM using equine blood as an adequate model. Furthermore, to increase the rationale for the possible introduction of greater celandine extracts into phytotherapy, the present study also evaluated the level of ceruloplasmin and catalase activity after dosing of the greater celandine extracts. However, with the advent of modern and synthetic drugs and supplements, many of these natural plant-derived antioxidant compounds have remained unexplored. This is the main reason why characterization and testing the biological activity of extracts obtained from this plant is essential for their introduction in therapy as phytopharmaceuticals.

Therefore, in the present study, the oxidative stress biomarkers [2-thiobarbituric acid reactive substances, carbonyl derivatives of oxidative modification of proteins, total antioxidant capacity], as well as activity of antioxidant enzymes (catalase, ceruloplasmin) in the equine plasma, were used for assessing the antioxidant activity of root and stem extracts derived

from *Chelidonium majus* collected in urban and rural agglomerations of Kartuzy district in the Pomeranian province (northern part of Poland).

Material and methodology

Collection of plant material

The plants of *Chelidonium majus* were harvested from natural habitats on the territory of the Kartuzy district (54° 20' N 18° 12' E) in the Pomeranian province (northern part of Poland) (Figure 1A). Kartuzy is located about 32 kilometers (20 miles) west of Gdańsk and 35 km (22 miles) south-east of the town of Łębork on a plateau at an altitude of approximately 200 meters (656 feet) above sea level on average. The plateau, which is divided by the Radaune lake, comprises the highest parts of the Baltic Sea Plate (<http://www.kartuzy.pl/>). Plants were collected from urban (n = 5) and rural agglomerations (n = 15) on the territory of the Kartuzy district.

Preparation of plant extracts

Freshly collected roots and stems were washed, weighed, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. The extracts were stored at -20 °C until use.

Horses

Eighteen healthy adult horses from the central Pomeranian region in Poland (village Strzelinko, N 54° 30' 48.0" E 16° 57' 44.9"), aged 8.9 ±1.3 years

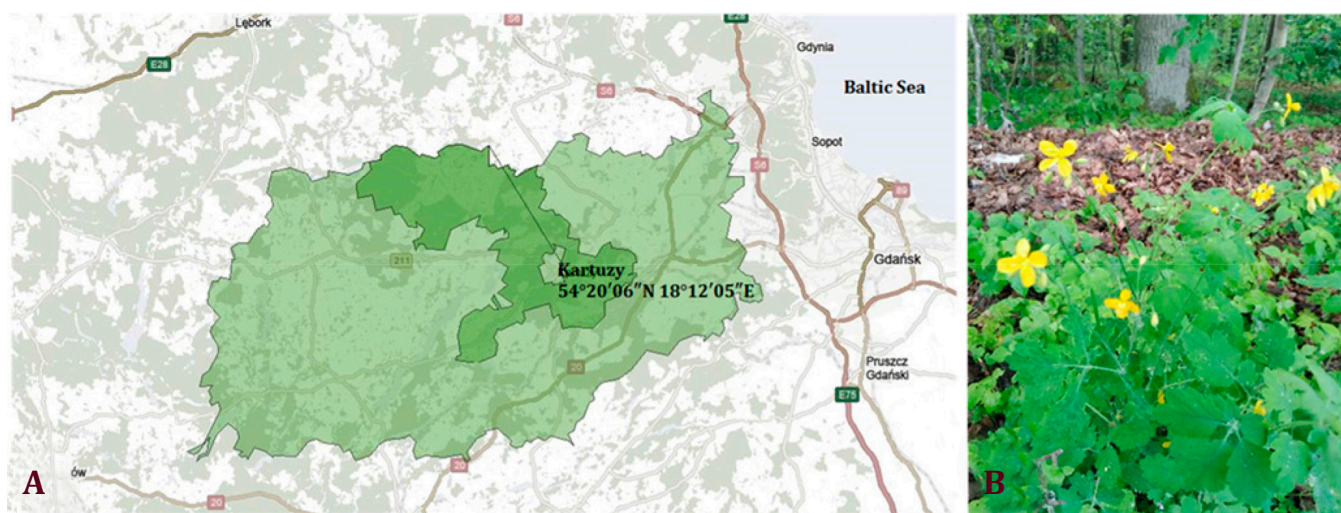


Figure 1 Location of Kartuzy in the map of northern Poland (A), where the greater celandine (B) was collected (Photo by Nataniel Stefanowski)

old, including 6 Hucul pony, 5 Thoroughbred horses, 2 Anglo-Arabian horses, and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical, and vital parameters, which were within reference ranges. The females were non-pregnant.

Collection of blood samples

Blood was drawn from the jugular vein of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM). Blood was stored in tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3,000 rpm for 5 min to remove plasma. The pellet of blood was re-suspended in 4 mM phosphate buffer (pH 7.4). A volume of 0.1 mL of the plant extract was added to 1.9 mL of equine plasma. For positive control, 4 mM phosphate buffer (pH 7.4) was used. After incubating the mixture at 37 °C for 60 min with continuous stirring, it was centrifuged at 3,000 rpm for 5 min. Plasma aliquots were used in the study.

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with TBA under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56 \cdot 10^5$ mM/cm as the extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay

To evaluate the protective effects of extracts derived from root and stem extracts derived from CM collected from urban and rural agglomerations against free radical-induced protein damage in equine erythrocytes and plasma, a content of carbonyl derivatives of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the plasma was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives

of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) and as modified by Dubinina and co-workers (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP₃₇₀) and 430 nm (ketonic derivatives, OMP₄₃₀).

Measurement of total antioxidant capacity (TAC)

The TAC level in the sample was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). Sample inhibits the Fe²⁺/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated relatively the absorbance of the blank sample.

Assay of catalase activity

Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of H₂O₂ in the reaction mixture using a spectrophotometer at the wavelength of 410 nm by the method of Koroliuk et al. (1988). One unit of catalase activity is defined as the amount of enzyme required for decomposition of 1 μmol H₂O₂ per min per mL of blood.

Assay of Ceruloplasmin level

The ceruloplasmin (CP, EC 1.16.3.1) level in the plasma was measured spectrophotometrically at 540 nm, as described by Ravin (1961). The assay mixture contained 0.1 mL of plasma, 0.4 M sodium acetate buffer (pH 5.5), and 0.5 % *p*-phenylenediamine. The mixture was incubated at 37 °C for 60 min. Before cooling at 4 °C for 30 min, the mixture was added to 3 % sodium fluoride for inhibition. Ceruloplasmin was expressed in mg per dL of plasma.

Statistical analysis

The arithmetic means ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). All statistical calculation was performed on separate data from

each individual with Statistica 13.3 software (StatSoft, Krakow, Poland).

Results and discussion

The cellular components, that make up the cell membrane, the most exposed to the harmful action of free radicals are lipid structures, lipoproteins, and proteins. Damage of the abundant and thus most susceptible polyunsaturated fatty acid (PUFA) is termed lipid peroxidation (Srivastava and Shrivastava, 2016). The most widely used assay for lipid peroxidation is malonic dialdehyde (MDA) formation as a secondary lipid peroxidation product, with the 2-thiobarbituric acid reactive substances test (Xiong et al., 2020).

Protein oxidation reactions involve, among others, ROS free radicals and result in oxidative modification of amino acid side chains, peptide cleavage under the influence of reactive oxygen species, reactions of peptides with lipids and products of carbohydrate oxidation, and formation of carbonyl derivatives of proteins (Himmelfarb et al., 2000).

Figure 2 shows the TBARS levels obtained by incubating equine plasma in the presence of aqueous extracts derived from the root and stem of CM collected from rural and urban agglomerations. Statistically significant reductions in levels of lipid peroxidation byproducts were noted after incubation with root extracts of CM collected from both urban (by 35 %, $p < 0.05$) and rural (by 34 %, $p > 0.05$) agglomerations compared to the

control samples. Stem extracts derived from CM also reduced TBARS levels, but only those collected in rural areas; there was a statistically significant decrease by 21 % ($p < 0.05$).

The aldehydic and ketonic derivatives of oxidatively modified proteins in the equine plasma after *in vitro* incubation with root and stem extracts derived from *C. majus* collected from rural and urban areas of Pomeranian region were present in Figure 3.

A similar result (Figure 3) was observed in the levels of aldehydic derivatives of oxidatively modified proteins, where the lowest value was observed after incubation with extracts derived from roots of CM collected from both rural and urban areas (by 7 and 8 %, respectively, $p > 0.05$) compared to the control samples. On the other hand, levels of ketonic derivatives of OMP showed that stem extracts of CM collected from both urban and rural areas, significantly increased levels of protein oxidation compared to the control samples (by 16 and 17 %, respectively, $p > 0.05$) in contrast to root extracts of CM collected from urban areas, where there was a statistically significant reduction in ketonic derivatives of oxidatively modified proteins by 15 % ($p < 0.05$) compared to the control samples.

Measurement of total antioxidant capacity (Figure 4) after incubation with CM extracts showed surprising results. Statistically significant increases in TAC levels were observed after incubation with root and stem extracts of CM collected from both urban and rural

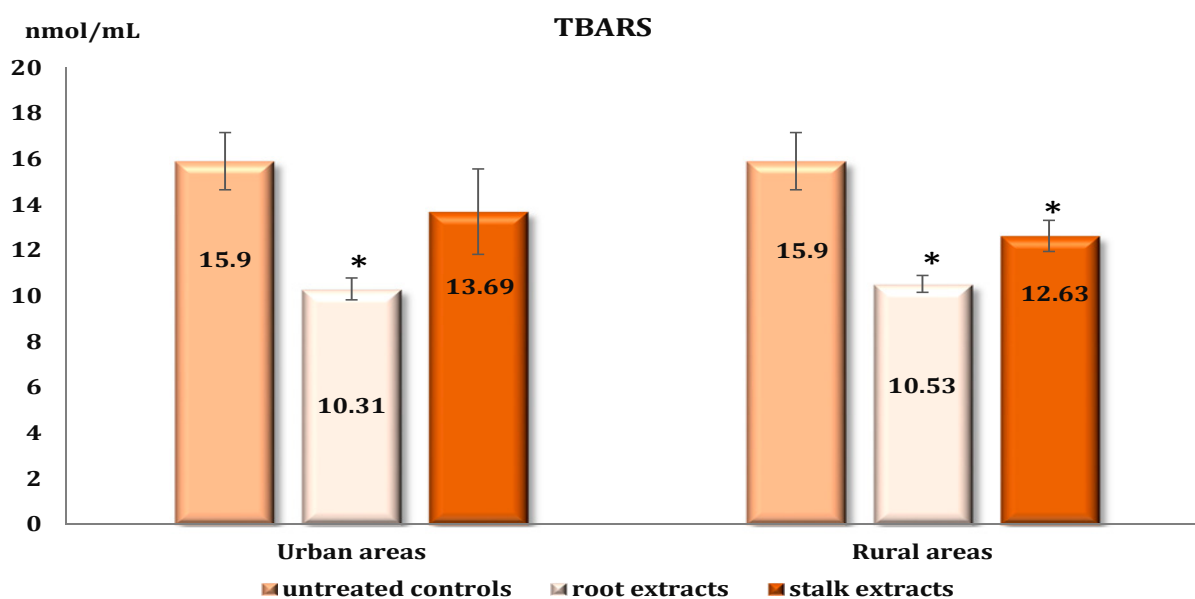


Figure 2 The TBARS content as a biomarker of lipid peroxidation in the equine plasma after *in vitro* incubation with root and stem extracts obtained from *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region ($M \pm m$, $n = 8$)
* – statistically significant differences ($p < 0.05$) compared to the control samples

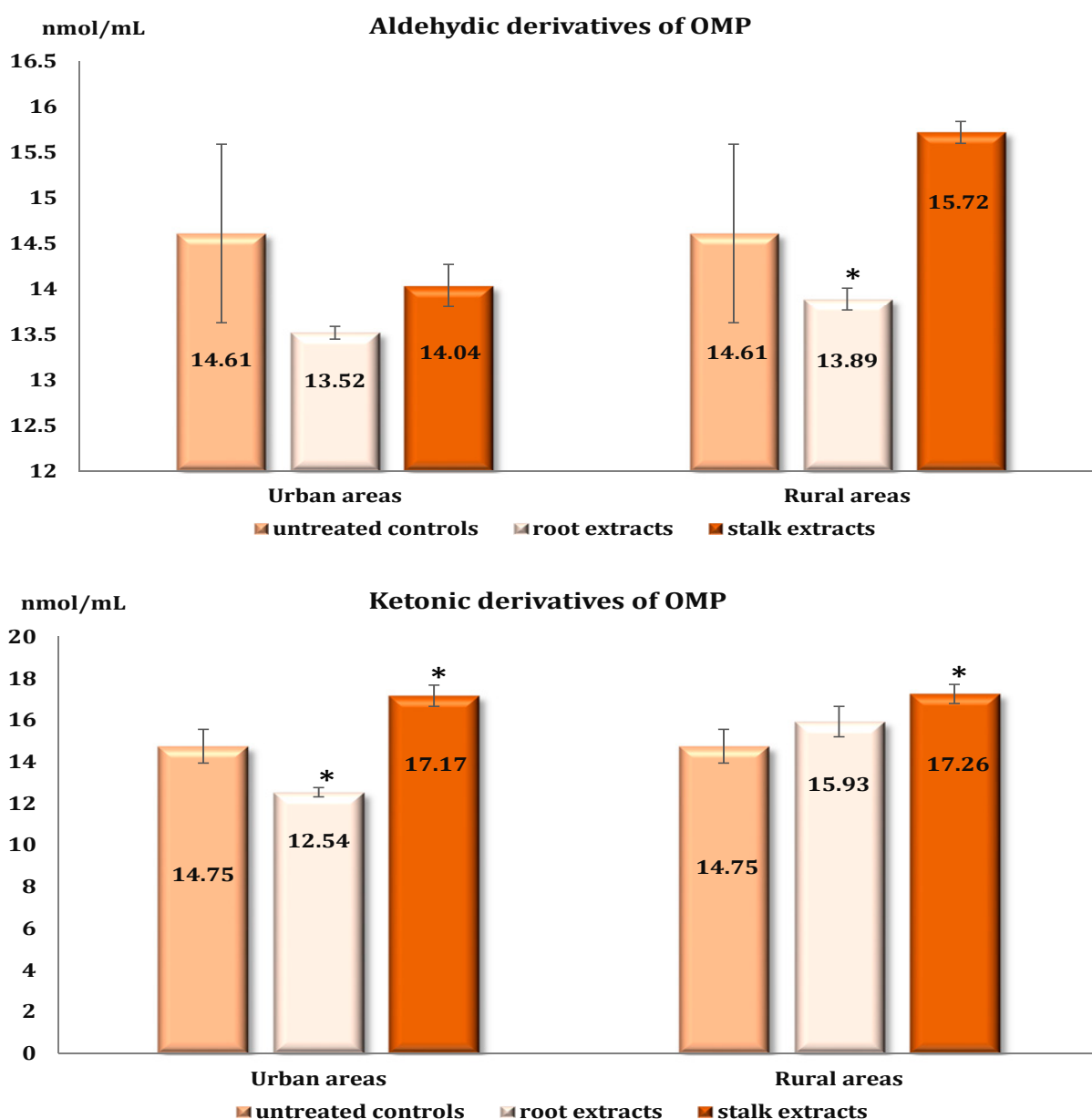


Figure 3 The aldehydic and ketonic derivatives of oxidatively modified proteins in the equine plasma after *in vitro* incubation with root and stem extracts obtained from *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region ($M \pm m$, $n = 8$)
 *- statistically significant differences ($p < 0.05$) compared to the control samples

areas. Moreover, the highest value was observed after incubation with root extracts of *C. majus* collected from rural areas (increase by 67 %, $p < 0.05$ compared to the control samples).

Antioxidants are substances that prevent the oxidation of other compounds. Enzymatic antioxidants consist of superoxide dismutase and catalase. The enzymatic antioxidants have more effective protective effects against active and massive oxidative attacks due to the ability to decompose ROS (He et al., 2017). Therefore, this set of antioxidants play important role in diseases

prevention. Catalase (CAT), is among the most important antioxidant enzyme against hydrogen peroxides (Muhlisin et al., 2016). In our study, stem extracts of CM collected from urban agglomerations were found to be most effective in increasing catalase activity (by 115 %, $p < 0.05$) (Figure 5). Root extracts of CM collected from rural agglomerations also significantly increased catalase levels by 65 % ($p < 0.05$). Probably, the increase in catalase activity has resulted in a 65 % increase in TAC level ($p < 0.05$) (Figure 4).

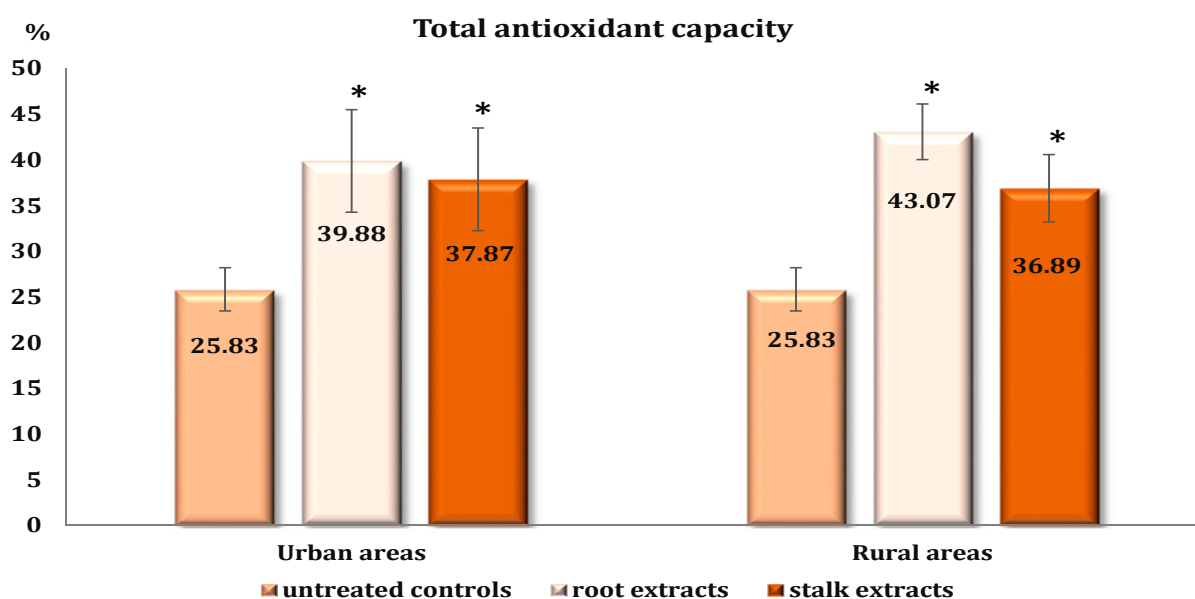


Figure 4 The total antioxidant capacity in the equine plasma after *in vitro* incubation with root and stem extracts obtained from *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

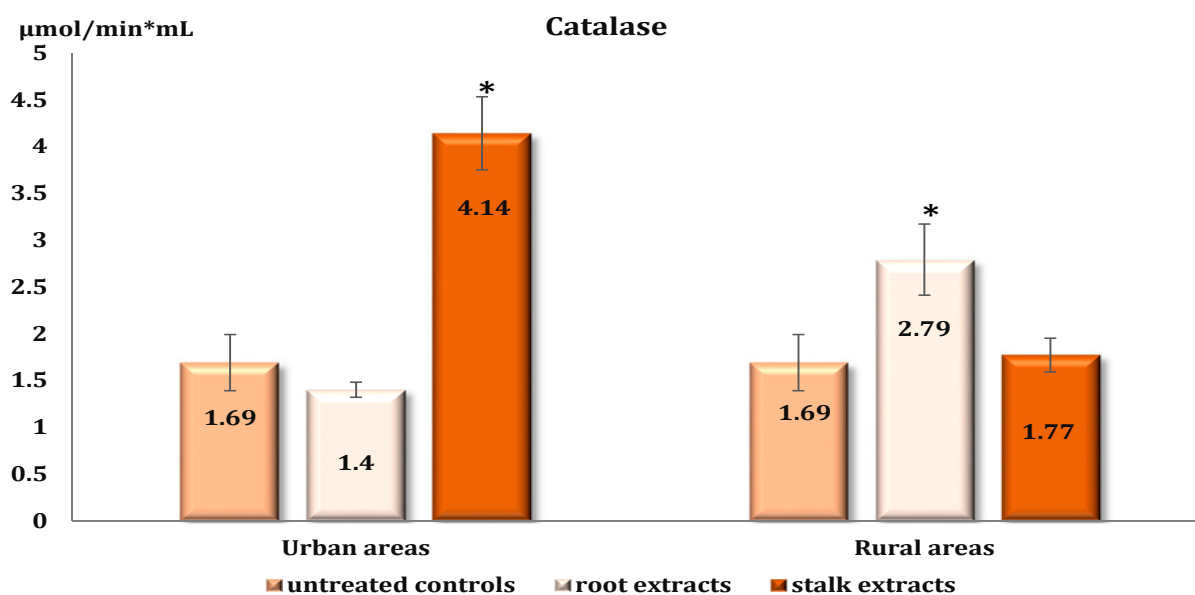


Figure 5 The catalase activity in the equine plasma after *in vitro* incubation with root and stem extracts obtained from *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

Ceruloplasmin (CP) is a copper-binding glycoprotein that is the major ferroxidase in liver-derived plasma (Arenas de Larriva et al., 2020). It is characterized as a copper (Cu)-containing protein that binds 40–70 % of the Cu in plasma and is mainly produced by the liver. This protein is a member of the multicopper oxidase family, an evolutionarily conserved group of proteins

that use copper to couple substrate oxidation with the four-electron reduction of oxygen to water (Jeremy and Shukla, 2014). Apart from playing a role in copper and iron metabolism, CP is an acute-phase reactant that may work as an antioxidant but can also generate free radicals that may lead to several illnesses (Dadu et al., 2013). In our study, root and stem extracts of

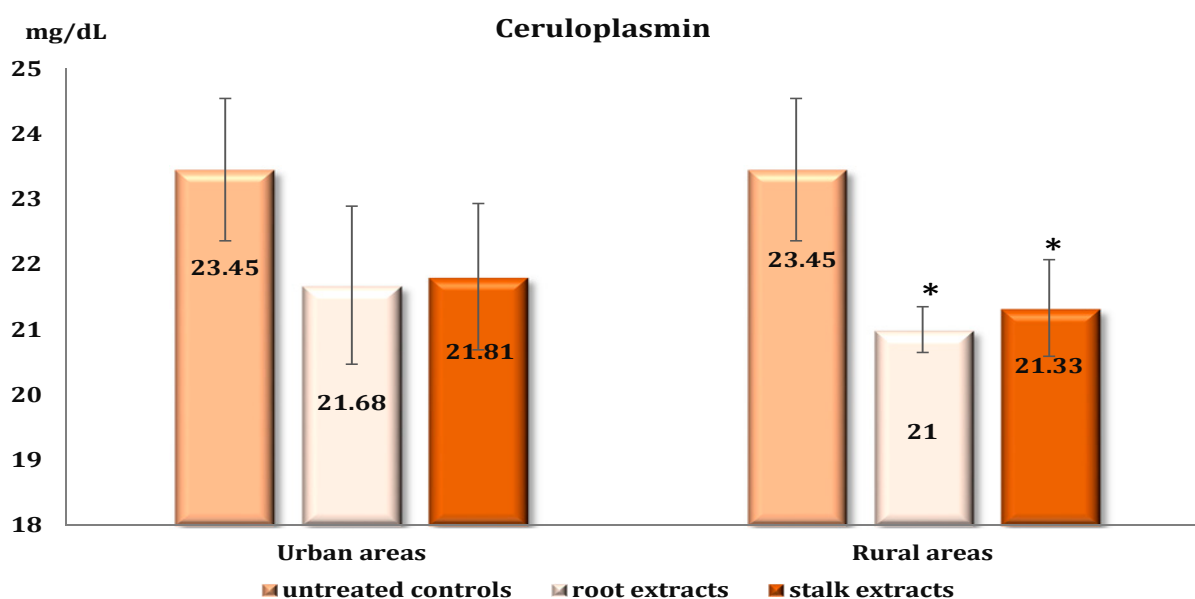


Figure 6 The ceruloplasmin level in the equine plasma after *in vitro* incubation with root and stem extracts obtained from *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ±m, n = 8)
*– statistically significant differences (p < 0.05) compared to the control samples

CM collected from rural areas caused a statistically significant reduction in ceruloplasmin levels by 10 and 9 %, respectively (p < 0.05) (Figure 6).

In the current study, we investigated the effects of CM extracts on lipid peroxidation and biomarkers of oxidatively modified proteins, as well as on antioxidant defense in equine plasma. Our study suggests that the crude extracts obtained from CM roots exhibit effective antioxidant activity when incubated with equine plasma. The protective effect of CM extracts is evident from the improvement of antioxidant enzymes activity exemplified by catalase and increase in total antioxidant capacity. The antioxidant defense system was improved with the suppression of aldehydic and ketonic derivatives of oxidatively modified proteins after incubation with CM extracts. CM extracts have also shown anti-inflammatory activity expressed by decreasing plasma ceruloplasmin levels.

In our previous study (Stefanowski et al., 2021a, b) on muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum), we also demonstrated the antioxidant activity of CM extracts. Our results showed that extracts of CM collected from both urban and rural areas statistically significantly reduced the level of aldehydic derivatives of OMB by 18.8 % (p < 0.05). The analysis of the levels of ketonic derivatives of OMP showed that extracts of CM collected from both urban and rural areas statistically significantly decreased the level of ketonic derivatives of OMP by 20.6 and 21.5 %, respectively (for urban

areas), as well as 26.7 and 12.5 % (for rural areas). Lower levels of lipid peroxidation were observed after incubation with stem extracts, while those collected from rural areas showed the lowest result (by 11 %). Root extracts of CM collected from urban and rural areas increased TBARS levels. Analysis of oxidatively modified protein in the blood of rainbow trout after *in vitro* incubation with root and stem extracts showed that extracts can inhibit the production of oxidative carbonyls by scavenging free radicals.

Phytochemical constituents in the Papaveraceae family are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer (Zielińska et al., 2018). CM contains, as major constituents, isoquinoline alkaloids (such as sanguinarine, chelidonine, chelerythrine, berberine, protopine, and coptisine), flavonoids, and phenolic acids (Gilca et al., 2010). Both crude extracts of CM and purified compounds derived from it exhibit a wide variety of biological activities (anti-inflammatory, antimicrobial, immunomodulatory, antitumoral, choleric, hepatoprotective, analgesic, etc.) which are in concordance with the traditional uses of CM (Gilca et al., 2010; Zielińska et al., 2018).

Phenols are a large class of secondary metabolites. Phenolics (including flavonoids) are among the most active antioxidants, as well as the most important stabilization factors of the oxidative processes.

Flavonoids include many pharmacological and biological properties. In phytopharmacy, flavonoids are the active ingredients, of plant origin, with strong biological effects, while in dietetics and food industries they present important phytonutrients, preservatives, spices, and aromatics substances (Stanković et al., 2010, 2011; Jakovljevic et al., 2013). The highest concentration of total phenolic compounds was observed by Jakovljevic et al. (2013) in the spring when the CM was in the rosette stage with well-formed, thick leaves, but when the whole plant is not yet sufficiently developed. When the plant enters the flowering stage, there is a lack of that concentration, which again increases when the plant begins with the formation of fruit. The concentration of flavonoids is the greatest just before flowering and before fruiting. During flowering, the concentrations of these secondary metabolites are the lowest. The antioxidant activity and total phenol concentrations are the highest in the spring months during the rosette stage (Jakovljevic et al., 2013).

Neither strong nor direct relations were found between the antioxidant activity of the plant and the concentrations of flavonoid content and phenolic compounds. This could be due to the complexity of the substances which cause the antioxidant activity of this plant. The composition evolution in two opposite ways simultaneously for two compounds characterized by high individual activities could lead to the compensatory effect of the final activity (Gourine et al., 2010; Jakovljevic et al., 2013).

The analysis conducted by Nawrot et al. (2016) confirmed the presence of the protein components of the antioxidant defense system in the CM latex. These proteins form the first line of defense against different stress conditions and help to prevent the attack of different pathogens, which are highly abundant in the milky sap. Peroxidase 12-like and isoflavone reductase homolog were present only in the milky sap. The presence of class III plant peroxidase, glyoxalase, quinone reductase, and ubiquitin in CM latex was previously reported by Nawrot et al. (2007a, b).

Lee et al. (2007) in animal studies showed, that CM methanol extract significantly suppressed the progression of collagen-induced arthritis in mice. This action was characterized by a decreased production of TNF- α , IL-6, Interferon(IFN)- γ , B cells, $\gamma\delta$ -T cells (in spleen), and an increased proportion of CD4+CD25+ regulatory T cells. The serum levels of IgG and IgM RA factors were decreased. Song et al. (2002) in *in vitro* studies showed an interesting immunomodulatory potential exhibited by a protein-bound polysaccharide

extracted from *C. majus* (CM-Ala), which showed mitogenic activity on spleen cells, bone marrow cells, and increased the number of granulocyte macrophage-colony forming cells (GM-CFC). When CM extract was used in combination with recombinant IFN- γ , there was a marked combined induction of NO and TNF- α production in mouse peritoneal macrophages.

Plants are an important origin of natural substances that are the raw material for various pharmaceutical and therapeutic applications due to the presence of phytochemicals, such as alkaloids. Alkaloids, which are found in different plant species, possess numerous biological activities. Some alkaloids have strong cytotoxic effects on various cancer cells (Deljanin et al., 2016). The CM root has higher all determined alkaloid contents as compared to extract that is obtained from herb (Petruczynik et al., 2019). The highest contents of chelerythrine, sanguinarine, and berberine possessing very high cytotoxic activity were found in the root extract. These isoquinoline alkaloids might have synergistic high cytotoxic activity on cancer cell lines. Extracts that were obtained from CM exhibit very strong cytotoxicity (Zielińska et al., 2020). The studies of Petruczynik and co-workers (2019) indicated the strong cytotoxicity of these extracts against Pancreatic Cell Lines (PANC-1, IC₅₀, 20.7 $\mu\text{g}/\text{mL}$) and Cell Line human Caucasian colon adenocarcinoma grade II (HT-29, IC₅₀, 20.6 $\mu\text{g}/\text{mL}$), and moderate cytotoxic activity against Cell Line human breast adenocarcinoma (MDA-MB-231, IC₅₀, 73.9 $\mu\text{g}/\text{mL}$), Cell Line human from human lung (carcinoma, A-549), HeLa, and Celosaurus cell line SGC-7901 cell lines. Their studies have shown that the extracts from CM are also cytotoxic against other cell lines (FaDu, SCC-25, MCF-7, MDA-MB-231, and CRL1634). FaDu and SCC-25 cell lines belong to the so-called head and neck squamous cell carcinomas are often resistant to chemotherapy, even including targeted drug therapy.

Conclusions

The results showed that the extracts obtained from the roots and stems of *C. majus* exhibited effective antioxidant activity when incubated with equine plasma. The protective effect of CM root extracts is evident from the improvement of antioxidant defenses and increase in total antioxidant capacity. The antioxidant defense system was improved with the suppression of aldehydic and ketonic derivatives of oxidatively modified proteins after incubation with root and stem extracts. CM extracts have shown anti-inflammatory activity expressed as a decrease in plasma ceruloplasmin levels. The pronounced effect

of CM root extract can be attributed to the secondary metabolites it contains, such as polyphenols and flavonoids. Finally, further studies are needed to reveal the exact cellular mechanisms of the effects of CM extract on erythrocyte and plasma function. These *in vitro* studies indicate that extracts from this plant are a significant source of natural antioxidants that could prevent the progression of oxidative stress. However, the proportions of secondary metabolites responsible for the antioxidant activity of CM extracts are currently unclear. Therefore, further studies are needed to isolate and identify the antioxidant compounds present in the plant extracts.

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