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Dose-dependent changes in the levels of oxidative stress biomarkers in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after *in vitro* treatment by extracts derived from stalks and roots of great celandine (*Chelidonium majus* L.)

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Consistent with our previous studies, we continue to evaluate the antioxidant potential of representatives belonging to the Papaveraceae family collected from the northern part of Poland using a muscle tissue model of rainbow trout (*Oncorhynchus mykiss* Walbaum). Therefore, in the present study, oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), protein oxidative modification carbonyl derivative content, total antioxidant capacity (TAC)] were used to evaluate the antioxidant activity of stalk and root extracts of *Chelidonium majus* L. (CM) at doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, and 0.63 mg.mL^{-1}. Muscle tissue homogenates of rainbow trout were used in this study. Phosphate buffer was used as a positive control. After incubation of the mixture at 25 °C for 2 h with continuous mixing, samples were used for biochemical studies. Results of our study revealed that a dose of CM extracts of 0.63 mg.mL^{-1} showed the highest antioxidant activity in the muscle tissue of rainbow trout. The extracts derived mainly from the roots of CM collected from rural areas were effective in reducing the levels of oxidative stress biomarkers by reducing lipid peroxidation markers, which may suggest that the active substances such as alkaloids (chelidonine, sanguinarine, berberine), flavonoids, phenols in these plants can effectively protect the membrane structures in muscle cells of salmonids. We also observed statistically significant reductions in levels of both aldehydic and ketonic derivatives of oxidatively modified proteins in muscle tissue of rainbow trout after incubation with CM extracts at this dose compared to the controls. The comparison of these results shows that CM extracts can effectively inhibit protein damage by scavenging free radicals and/or activation of antioxidant defenses. The secondary metabolites of CM, i.e. polyphenols and alkaloids, are most likely responsible for this effect. Using doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} of both root and stalk extracts *in vitro* study, statistically significant increases in levels of TBARS and OMP were observed. Screening of species belonging to the family Papaveraceae for other biological activities, including antioxidant activity, is essential and may be effective in the search for preventive measures in the pathogenesis of diseases caused by oxidative stress in human and veterinary medicine.

Keywords: *Chelidonium majus*, rainbow trout, total antioxidant capacity, lipid peroxidation, oxidatively modified proteins, muscle tissue, dose-dependent changes

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Introduction

Nature gifts medicinal plants with the untapped and boundless treasure of active chemical constituents with significant therapeutic potential that makes these plants a beneficial source in the phytomedicines (Yurdakok-Dikmen et al., 2018). Phytomedicines are believed to have benefits over conventional drugs and are regaining interest in current research (Li et al., 2021). The development of health products of phytomedicine has often stemmed from traditional or historical use, or from long-term evidence that consumption of phytomedicine is associated with better health outcomes. Phytomedicine is a collection of therapeutic knowledge that is deeply rooted in culture and forms the basis for an early version of pharmacopeias based largely on natural products of predominantly plant origin as sources of antioxidants used in veterinary and human medicine (Cheng et al., 2016).

Oxidative stress plays a key role in the onset of many diseases in humans, but also in animals. Reactive oxygen species and reactive nitrogen species are continuously produced in the body through oxidative metabolism, mitochondrial bioenergetics, and immune function (Tan et al., 2018). They can be bound to nucleic acids, enzymes, membrane lipids, proteins, and other small molecules lowering their biological potential (Poljsak et al., 2013; Tan et al., 2018). Antioxidants can act as chain breakers, scavenging chain initiating radicals like hydroxyl, alkoxyl, or peroxyl, quenching singlet oxygen, decomposing hydroperoxides, and chelating prooxidative metal ions (Pisoschi and Pop, 2015). Epidemiological studies confirm that the incidence of oxidative stress-related conditions is lowered by using herbs rich in compounds possessing high antioxidant activity (Pisoschi et al., 2016; Carocho and Ferreira, 2013).

Recent studies have reported on the antioxidant properties of plants belonging to the Papaveraceae family (Krošlák et al., 2017; Zhang et al., 2020; Nile et al., 2021). *Chelidonium majus* L. (CM, Papaveraceae), or greater celandine, is an important plant in western phytotherapy and in traditional Chinese medicine (Zielińska et al., 2018). Crude extracts of CM, as well as purified compounds derived from it, exhibit a broad spectrum of biological activities (antiinflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective and antioxidant) that support some of the traditional uses of CM plants (Colombo and Bosisio, 1996; Nawrot, 2017; Zielińska et al., 2018; Huang et al., 2019; Popovic et al., 2021; Krzyżek et al., 2021). However, herbal medicine also claims that this plant has several important properties which have not yet been scientifically studied (Colombo and Bosisio, 1996; Gilca et al., 2010; Zielińska et al., 2018). The results of CM studies offered new insights into the preliminary steps regarding the development of a high-value product for phytomedicine applications through promising metabolic variations with antioxidant and anticancer potentials (Nile et al., 2021).

In our previous study, we assessed if the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification (OMP), total antioxidant capacity (TAC)] and also activities of antioxidant enzymes (catalase, ceruloplasmin) in the equine plasma after treatment by extracts derived from roots and stalks of CM collected from rural and urban agglomerations. Our results demonstrated that statistically significant reductions in lipid peroxidation biomarkers were noted after incubation with extracts derived from roots of CM collected from both urban (by 35%, $p < 0.05$) and rural (by 34%, $p < 0.05$) agglomerations compared to the untreated samples. Stalk extracts derived from CM also reduced TBARS levels, but only extracts derived from CM collected from the rural areas; a statistically significant decrease (by 21%, $p < 0.05$) was observed compared to the control untreated samples. The lowest values in the content of the aldehydic derivatives of OMP were observed after incubation with extracts derived from roots of CM 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 stalks of CM collected from both rural and urban areas compared to the control samples, in contrast to extracts derived from roots of CM 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 sample. A significant increase in the TAC levels was observed after incubation with root and stalk extracts of CM collected from both urban and rural areas, but the highest values were observed after incubation with extracts derived from roots of CM collected from rural areas (by 66.7%, $p < 0.05$) compared to the control samples. Stalk extracts of CM collected from urban agglomerations were found to be most effective in increasing catalase activity (by 115%, $p < 0.05$). Both root and stalk extracts of CM 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 (Stefanowski et al., 2021).

The current study is a continuation of our study according to the assessment of antioxidant effects of extracts derived from roots and stalks of CM collected from rural and urban agglomerations in the northern part of Poland (Kartuzy district, Pomeranian voivodeship). Thus, the aim of our study was evaluation the oxidative stress biomarkers, i.e. 2-thiobarbituric acid reactive substances, carbonyl derivatives of protein oxidative modification, total antioxidant capacity in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after treatment by extracts in different doses derived from roots and stalks of CM collected from rural and urban agglomerations for estimation the optimal doses of extracts exhibiting the antioxidant activity.

Material and methodology

Collection of plant material

Plant materials (Figure 1B) 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 Lębork 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

The collected roots and stalks were brought into the laboratory for biochemical studies. Freshly washed samples of plants were 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 extract was stored at -20 °C until use.

Experimental fish and muscle tissue samples

Clinically healthy rainbow trout with a mean body mass of 80–120 g were used in the experiments. The muscle tissue samples were homogenized in ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogenizer immersed in an ice water bath. Homogenates were centrifuged at 3,000 rpm for 15 min at 4 °C. After centrifugation, the supernatant was collected and frozen at −20 °C until analyzed. All enzymatic assays were carried out at 22 ±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate. The reactions were started by adding the tissue supernatant.

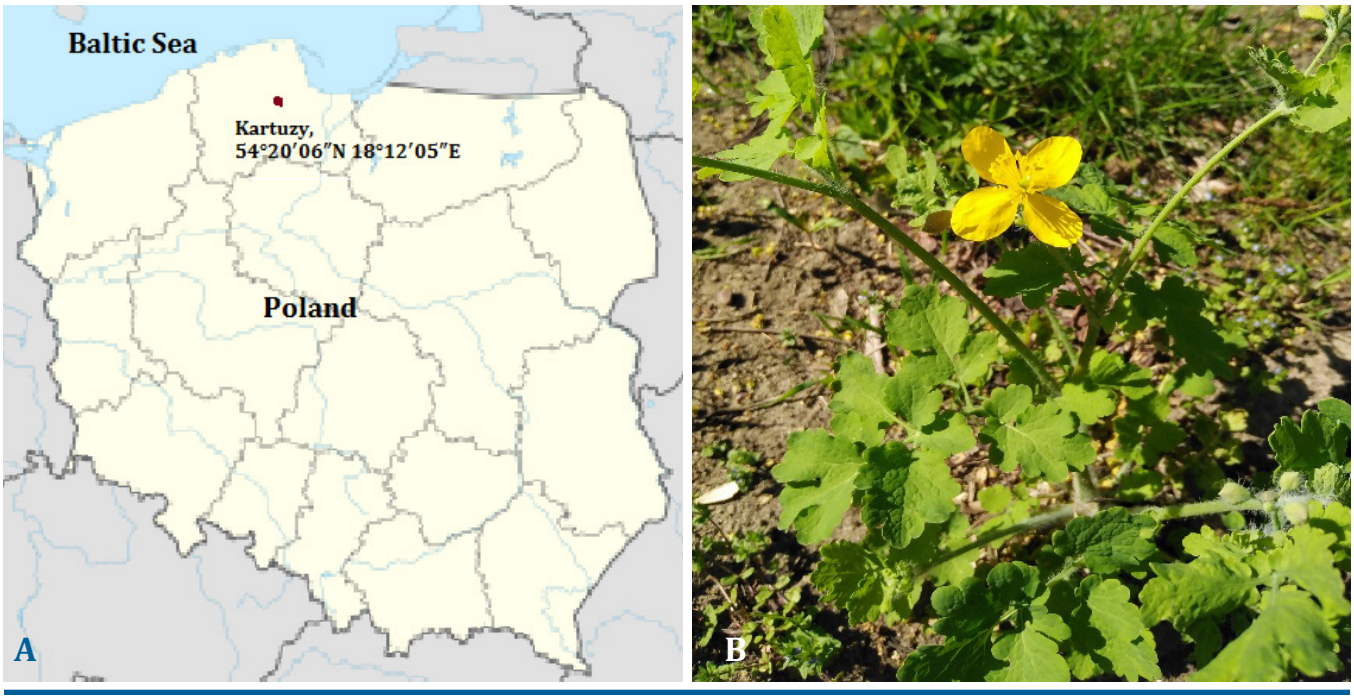

Figure 1 Location of Kartuzy in the map of Poland (A), where the greater celandine (B) was collected

Experimental design

The supernatant of the muscle tissue was used to incubate with extracts derived from stalks and roots of CM at four final concentrations, i.e. 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1} and 0.63 mg.mL^{-1}, respectively, at room temperature. The control samples (muscle tissue) were incubated with 100 mM Tris-HCl buffer (pH 7.2). The incubation time was 2 h. Biomarkers of oxidative stress were studied in the incubated homogenates (control samples and in samples with extracts derived from stalks and roots of CM). Tissue homogenates were used for the determination of the levels of 2-Thiobarbituric acid reactive substances (TBARS), oxidative modification of proteins (OMP), antioxidant defense enzymes, and total antioxidant capacity (TAC). The method described by Bradford (1976) with bovine serum albumin as a standard was used for the quantification of proteins. Absorbance was recorded at 595 nm.

Assay of 2-thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was evaluated by TBARS according to the method described by Kamyshnikov (2004) with some modifications. TBARS were calculated as nmoles of malonic dialdehyde (MDA) per mg of protein.

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

To evaluate the protective effects of the extracts derived from stalks and roots of CM against free radical-induced protein damage in muscle samples, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in samples 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 et al. (1990) and as modified by Dubinina et al. (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, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in the samples 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). The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis

Statistical analysis of the data obtained was performed by employing the mean ± S.E.M. 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 Kruskal-Wallis one-way analysis of variance. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica software, version 8.0 (StatSoft, Poland) (Zar, 1999).

Results and discussion

The content of 2-thiobarbituric acid reactive substances as a biomarker of lipid peroxidation in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses (5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, 0.63 mg.mL^{-1}) derived from stalks and roots of CM collected from urban and rural agglomerations was presented in Figure 2. The highest significant increase in TBARS levels after incubation with CM extracts at dose 5 mg.mL^{-1} was observed for root extracts of CM collected from urban areas (232.56 ±2.64 nmol.mg^{-1} protein) compared to the untreated samples (196.72 ±1.34 nmol.mg^{-1} protein). An increase of TBARS level was 18.2% ($p < 0.05$). Similar results were obtained for TBARS level in the muscle tissue of rainbow trout after incubation with root extracts of CM (5 mg.mL^{-1}) collected from rural areas (223.97 ±1.45 nmol.mg^{-1} protein) compared to the untreated samples, where it was statistically significantly increased by 13.9% ($p < 0.05$). Similar results were observed for TBARS level in the muscle tissue of rainbow trout after incubation with stalk extracts of CM collected from both urban (223.79 ±2.3 nmol.mg^{-1} protein) and rural areas (223.71 ±0.84 nmol.mg^{-1} protein) compared to the untreated samples. There was a statistically significant increase in lipid peroxidation biomarkers by 13.8 and 13.7% ($p < 0.05$) for stalk extracts of CM collected from both urban and rural agglomerations, respectively (Figure 2).

According to CM extracts at a dose of 2.50 mg.mL^{-1}, statistically significantly increase in TBARS levels compared to the untreated samples were observed for all extracts. After *in vitro* incubation of muscle tissue with root extracts of CM collected from urban (221.33 ±0.89 nmol.mg^{-1} protein) and rural areas

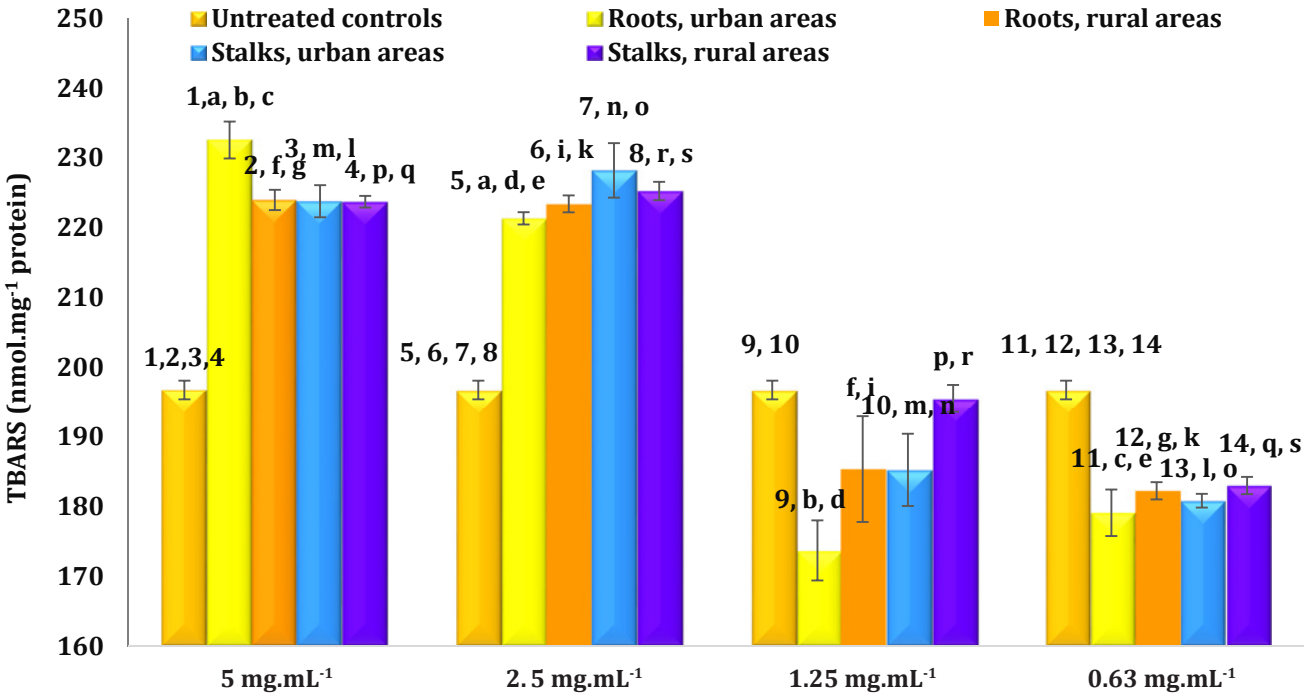

Figure 2 The content of 2-thiobarbituric acid reactive substances as a biomarker of lipid peroxidation in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of *Chelidonium majus* collected from urban and rural agglomerations (n = 8)
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
Changes are statistically significant ($p < 0.05$) in relations: 1 – untreated controls *vs.* extracts derived from roots collected in urban areas (5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from roots collected in rural areas (5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from stalks collected in urban areas (5 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from stalks collected in rural areas (5 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from roots collected in urban areas 2.5 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}); 8 – untreated controls *vs.* extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}); 9 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 10 – untreated controls *vs.* extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}); 11 – untreated controls *vs.* extracts derived from roots collected in urban areas (0.63 mg.mL^{-1}); 12 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 13 – untreated controls *vs.* extracts derived from stalks collected in urban areas (0.63 mg.mL^{-1}); 14 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); b – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); c – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from roots collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); e – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); f – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); g – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); i – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); k – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); m – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); l – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); n – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); o – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); p – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); q – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); r – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); s – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

(223.4 ±1.21 nmol.mg^{-1} protein), a statistically significant increase in TBARS levels compared to untreated samples (196.72 ±1.34 nmol.mg^{-1} protein) was noted. Based on the results, there was an increase in TBARS levels by 12.5% ($p < 0.05$) and 13.6% ($p < 0.05$) for root extracts collected both in urban and rural areas, respectively. Similar results were obtained after using stalk extracts of CM collected from both urban (228.2 ±3.91 nmol.mg^{-1} protein) and rural areas (225.25 ±1.32 nmol.mg^{-1} protein), where we observed a statistically significant elevation in TBARS levels compared to the untreated samples (196.72 ±1.34 nmol.mg^{-1} protein); the increase in TBARS levels was by 16% ($p < 0.05$) and 14.5% ($p < 0.05$) for stalk extracts of CM collected from both urban areas and rural areas.

By reducing the dose of CM extracts sequentially to 1.25 mg.mL^{-1}, we obtained remarkably different results compared to the previous cases. After incubating

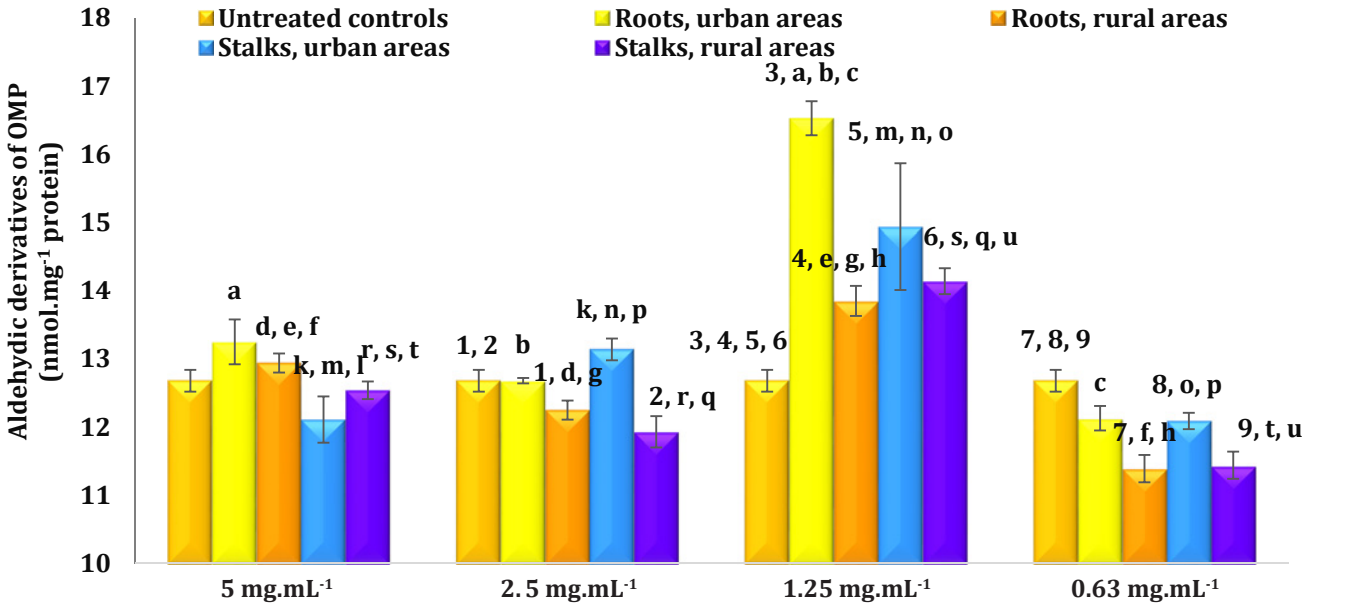

Figure 3 The content of aldehydic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of *Chelidonium majus* collected from urban and rural agglomerations (n = 8)

Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)

Changes are statistically significant ($p < 0.05$) in relations: 1 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from roots collected in rural areas (1.25 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 8 – untreated controls *vs.* extracts derived from stalks collected in urban areas (0.63 mg.mL^{-1}); 9 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); b – extracts derived from roots collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); c – extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); e – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); f – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); g – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); h – extracts derived from roots of CM collected in rural areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); k – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); m – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); l – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); n – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); o – extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); p – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); q – extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); r – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); s – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); t – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); u – extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

extracts from both roots and stalk extracts of CM collected only in the urban area, we obtained statistically significant changes [(173.74 ±4.31 nmol.mg^{-1} protein) and (185.28 ±5.18 nmol.mg^{-1} protein)] compared to the untreated samples (196.72 ±1.34 nmol.mg^{-1} protein). We recorded statistically significant reductions in TBARS level by 11.7% and 5.8% ($p < 0.05$) for both root and stalk extracts of CM collected in urban areas.

After undergoing dose reduction of CM extracts to 0.63 mg.mL^{-1}, we have obtained even more satisfactory results. After incubation of muscle tissue with root extracts of CM collected from urban areas (179.13 ±3.33 nmol.mg^{-1} protein), we obtained statistically significantly the lowest TBARS levels compared to untreated controls (196.72 ±1.34 nmol.mg^{-1} protein), i.e. by 8.9% ($p < 0.05$). Similar results were noted also for stalk extracts of CM collected from urban areas (180.87 ±0.99 nmol.mg^{-1} protein), where we also obtained a statistically significant reduction in TBARS levels (8.1%, $p < 0.05$) compared to the untreated controls (196.72 ±1.34 nmol.mg^{-1} protein). A statistically significantly reduced in the TBARS level was demonstrated after incubation of muscle tissues with root (182.29 ±1.23 nmol.mg^{-1} protein) and stalk extracts (183.04 ±1.24 nmol.mg^{-1} protein) of CM collected from rural agglomerations. Statistically

significant reduced levels of lipid peroxidation biomarkers were 7.3% ($p < 0.05$) and 7% ($p < 0.05$) for root and stalk extracts compared to controls (Figure 2).

The content of aldehydic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM collected from urban and rural agglomerations was presented in Figure 3.

After *in vitro* incubation of muscle tissue with root and stalk extracts at a final dose of 5 mg.mL^{-1}, we did not note statistically significant changes in the level of aldehydic derivatives of OMP compared to the untreated controls. Another situation was observed after reducing the extract dose to 2.5 mg.mL^{-1}. After incubation with root extracts of CM collected from rural areas, we noted a statistically significant decrease in the level of aldehydic derivatives of OMP (12.25 ±0.14 nmol.mg^{-1} protein) compared to untreated controls (12.68 ±0.16 nmol.mg^{-1} protein); a decrease was by 3.4% ($p < 0.05$). A similar trend was observed after incubation of muscle tissue homogenate with stalk extracts of CM collected from rural areas, where we obtained a statistically significant decrease in the level of aldehydic derivatives of OMP (11.93 ±0.23 nmol.mg^{-1} protein) compared to untreated control (by 5.9%, $p < 0.05$).

By reducing the extract dose to 1.25 mg.mL^{-1}, incubation of muscle tissue homogenates with root extracts of CM collected from both urban and rural agglomerations, we obtained a statistically significant increase in the level of aldehydic derivatives of OMP [(16.53 ±0.25 nmol.mg^{-1} protein) and (13.85 ±0.22 nmol.mg^{-1} protein), respectively] compared to the untreated samples (12.68 ±0.16 nmol.mg^{-1} protein); increase was by 30.4% ($p < 0.05$) and 9.2% ($p < 0.05$) for root extracts of CM collected from both urban areas and rural areas, respectively. Statistically significant changes were also obtained after incubation of muscle tissue with stalk extracts of CM collected from both urban (14.94 ±0.93 nmol.mg^{-1} protein) and rural areas (14.14 ±0.19 nmol.mg^{-1} protein), where there was an increase in the level of aldehydic derivatives of OMP in relation to the untreated samples by 17.8% ($p < 0.05$) and by 11.5% ($p < 0.05$) for stalk extracts of CM collected from both urban and rural agglomerations, respectively.

Using extracts at a dose of 0.63 mg.mL^{-1}, there was a statistically significant reduction in levels of aldehydic derivatives of OMP after incubation of muscle tissue homogenate with root extracts of CM collected from rural areas (11.39 ±0.2 nmol.mg^{-1} protein) and stalk extracts of CM collected from both urban (12.09 ±0.12 nmol.mg^{-1} protein) and rural agglomerations (11.4 ±0.2 nmol.mg^{-1} protein) compared to controls (12.68 ±0.16 nmol.mg^{-1} protein). There was a statistically significant decrease in levels of aldehydic derivatives of OMP by 10.2% ($p < 0.05$) for root extracts of CM collected from rural areas and by 4.7% ($p < 0.05$) and 9.8% ($p < 0.05$) for stalk extracts of CM collected from urban and rural agglomerations, respectively (Figure 3).

Comparing values obtained after using root extracts of CM collected from urban areas, we noted a statistically significant reduction in levels of aldehydic derivatives of OMP by 19.8% ($p < 0.05$) compared to the values obtained after using a dose of 1.25 mg.mL^{-1}. A statistically significant increase in level of aldehydic derivatives of OMP by 30.4% ($p < 0.05$) was observed after using the above-mentioned extract at the dose of 1.25 mg.mL^{-1} compared to those obtained at the dose of 2.5 mg.mL^{-1}. A comparison of the values obtained after using root extracts of CM collected from urban areas at a dose of 1.25 mg.mL^{-1} *vs.* those obtained at the dose of 0.63 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 36.3% ($p < 0.05$) (Figure 3).

The use of root extracts of CM in a dose of 2.5 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 5.3% ($p < 0.05$) compared to those values using extracts at the dose of 5 mg.mL^{-1}, which also showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 6.6% ($p < 0.05$) compared to values obtained after incubating with extracts at a dose of 1.25 mg.mL^{-1}. A comparison of values obtained from the above-mentioned extract using *in vitro* at the dose of 5 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 13.6% ($p < 0.05$) compared to results obtained using a dose of 0.63 mg.mL^{-1}. A comparison of the values obtained after using root extracts of CM at a dose of 1.25 mg.mL^{-1} *vs.* those obtained at the dose of 2.5 mg.mL^{-1} and 0.63 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 13.1% ($p < 0.05$) and 21.6% ($p < 0.05$). The use of stalk extracts of CM collected from urban areas at a dose of 5 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 7.8% ($p < 0.05$) compared to those values using extracts in the dose of 2.5 mg.mL^{-1}, which also showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 18.9% ($p < 0.05$) compared to values obtained after incubating

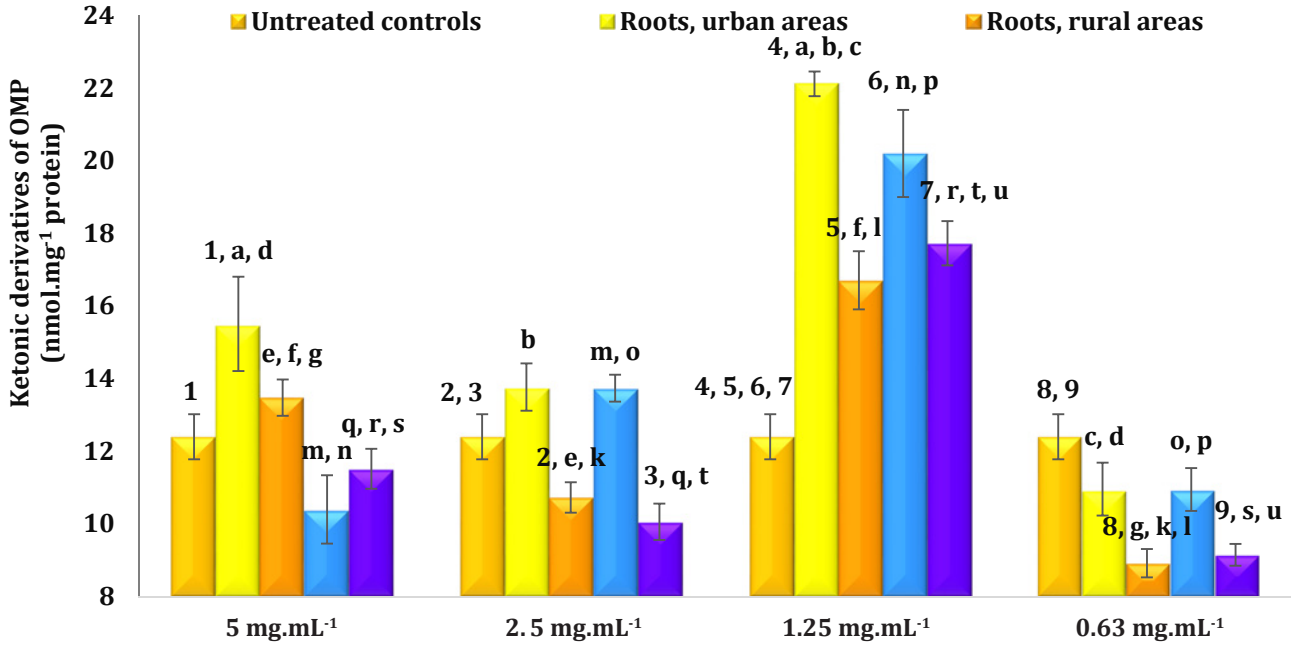

Figure 4 The content of ketonic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM collected from urban and rural agglomerations (n = 8)

Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)

Changes are statistically significant ($p < 0.05$) in relations: 1– untreated controls *vs.* extracts derived from roots collected in urban areas (5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from roots collected in rural areas (1.25 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}); 8 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 9 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); b – extracts derived from roots collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); c – extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); e – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); f – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); g – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); k – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); m – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); n – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); o – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); p – extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); q – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); r – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); s – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); t – extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); u – extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

with extracts at a dose of 1.25 mg.mL^{-1}. The use of stalk extracts of CM collected from urban areas at a dose of 1.25 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 23.6% ($p < 0.05$) compared to those values using extracts in the dose of 0.63 mg.mL^{-1}, which also showed a statistically significant increase in levels of aldehydic derivatives of OMP by 13.7% ($p < 0.05$) compared to values obtained after incubating with extracts at a dose of 2.5 mg.mL^{-1}. A comparison of the values obtained after using stalk extracts of CM collected from rural areas at a dose of 2.5 mg.mL^{-1} *vs.* those obtained at the dose of 5 mg.mL^{-1} and 1.25 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 4.9% ($p < 0.05$) and 15.6% ($p < 0.05$), respectively. A stalk extract of CM collected from rural areas at the dose of 0.63 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 19.1% ($p < 0.05$) compared to those obtained at a dose of 1.25 mg.mL^{-1}, while compared to the dose of 2.5 mg.mL^{-1} showed an decrease in levels of aldehydic derivatives of OMP by 4.1% ($p < 0.05$) (Figure 3).

The content of ketonic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM

collected from urban and rural agglomerations was presented in Figure 4.

Focusing on a dose of 5 mg.mL^{-1} of CM extracts after *in vitro* incubation with the muscle tissue of rainbow trout, we observed a statistically significant increase in ketonic derivatives after incubation with root extracts of CM collected from urban areas (15.51 ±1.3 nmol. mg^{-1} protein) compared to untreated samples (12.4 ±0.62 nmol.mg^{-1} protein); the increase was 25.1% ($p < 0.05$). Another situation was observed after the analysis of the dose of 2.5 mg.mL^{-1}, where there was a statistically significant reduction in ketonic derivatives of OMP by 13.5% ($p < 0.05$) for root extracts of CM collected from a rural agglomeration was observed compared to the controls [(10.73 ±0.42 nmol.mg^{-1} protein) *vs.* (12.4 ±0.62 nmol.mg^{-1} protein)]. For the stalk extract of CM collected from rural areas, we recorded the lowest statistically significant value of ketonic derivatives by 18.9% ($p < 0.05$) compared to the controls [(10.06 ±0.5 nmol.mg^{-1} protein) *vs.* (12.4 ±0.62 nmol.mg^{-1} protein)] (Figure 4).

In subsequent steps, decreasing the dose to 1.25 mg. mL^{-1}, we obtained a statistically significant increase in ketonic derivatives of CM after incubation of muscle tissue homogenates with root extracts of CM collected from both urban (22.12 ±0.34 nmol.mg^{-1} protein) and rural areas (16.71 ±0.8 nmol.mg^{-1} protein) compared to the untreated samples (12.4 ±0.62 nmol.mg^{-1} protein); increase was 78.4 and 34.8% ($p < 0.05$), respectively. Similarly, after incubation of muscle tissue with stalk extracts of CM collected from both urban (20.2 ±1.2 nmol.mg^{-1} protein) and rural agglomerations (17.73 ±0.61 nmol.mg^{-1} protein), we observed a statistically significant increase in levels of ketonic derivatives of OMP by 62.9 and 43% ($p < 0.05$), respectively, compared to the control samples (12.4 ±0.62 nmol.mg^{-1} protein). Analyzing the dose of 0.63 mg.mL^{-1}, we recorded a statistically significant decrease in levels of ketonic derivatives of OMP (by 28.1%, $p < 0.05$) in the muscle tissue after treatment with root extracts of CM collected from rural areas (8.92 ±0.39 nmol.mg^{-1} protein) compared to untreated samples (12.4 ±0.64 nmol.mg^{-1} protein) (Figure 4).

Comparing the dose of 5 mg.mL^{-1}, we observed a statistically significant decrease in ketonic derivatives of OMP after incubation of muscle tissue with root extracts of CM collected from urban areas by 29.9% ($p < 0.05$), increase by 12.6% ($p < 0.05$) and increase by 41.5% ($p < 0.05$) compared to those using a dose of 1.25 mg.mL^{-1}, 2.5 mg.mL^{-1} and 0.63 mg.mL^{-1}, respectively. Root extracts at a dose of 0.63 mg.mL^{-1} after incubation of muscle tissue homogenates showed a statistically significant decrease in ketonic derivatives of OMP (by 29.3%, $p < 0.05$) compared to the values obtained using a dose of 5 mg.mL^{-1}. When comparing values obtained to root extracts of CM collected from rural areas at a dose of 5 mg.mL^{-1}, we recorded a statistically significant increase in ketonic derivatives of OMP by 25.6% ($p < 0.05$) compared to those obtained using a dose of 2.5 mg.mL^{-1}, while the dose of 1.25 mg. mL^{-1} showed a statistically significant decrease in ketonic derivatives of OMP by 19.3% ($p < 0.05$). On the other hand, using the dose of 5 mg.mL^{-1} of the root extracts of CM collected from the rural areas showed a statistically significant increase in ketonic derivatives of OMP by 51.1% ($p < 0.05$) compared to the dose of 0.63 mg.mL^{-1}, while a statistically significant increase in ketonic derivatives of OMP by 25.6% ($p < 0.05$) compared to the dose of 2.5 mg.mL^{-1} was observed. The stalk extracts of CM collected from urban areas at a dose of 5 mg.mL^{-1} showed a statistically significant decrease in ketonic derivatives of OMP by 24.3% ($p < 0.05$) compared to those obtained at a dose of 2.5 mg.mL^{-1}, while a statistically significant decrease in ketonic derivatives of OMP by 48.5% ($p < 0.05$) was noted compared to those obtained at a dose of 1.25 mg.mL^{-1} (Figure 4).

Using the dose of 2.5 mg.mL^{-1} of the stalk extracts of CM collected from urban areas showed a statistically significant increase in ketonic derivatives of OMP by 25.5% ($p < 0.05$) compared to the dose of 0.63 mg.mL^{-1}. On the other hand, a dose of 0.63 mg.mL^{-1} of the above CM extracts showed a statistically significant decrease in levels of ketonic derivatives of OMP by 45.8% ($p < 0.05$) compared to those values obtained using a dose of 1.25 mg.mL^{-1}. We also observed that a dose of 0.63 mg.mL^{-1} of the stalks extracts of CM collected from rural agglomerations showed a statistically significant decrease in the levels of ketonic derivatives of OMP by 48.4% ($p < 0.05$) compared to those values obtained using a dose of 1.25 mg.mL^{-1}, while statistically significant decrease in the levels of ketonic derivatives of OMP (by 20.6%, $p < 0.05$) was observed compared to a dose of 5 mg.mL^{-1}. A dose of 1.25 mg.mL^{-1} of stalk extracts from CM collected from rural agglomerations statistically significantly increased the levels of ketonic derivatives of OMP (by 76.2%, $p < 0.05$) compared to values obtained at a dose of 2.5 mg.mL^{-1}, while a statistically significant increase in the levels of ketonic derivatives of OMP (by 53.9%, $p < 0.05$) was observed compared to values obtained at a dose of 5 mg.mL^{-1} (Figure 4).

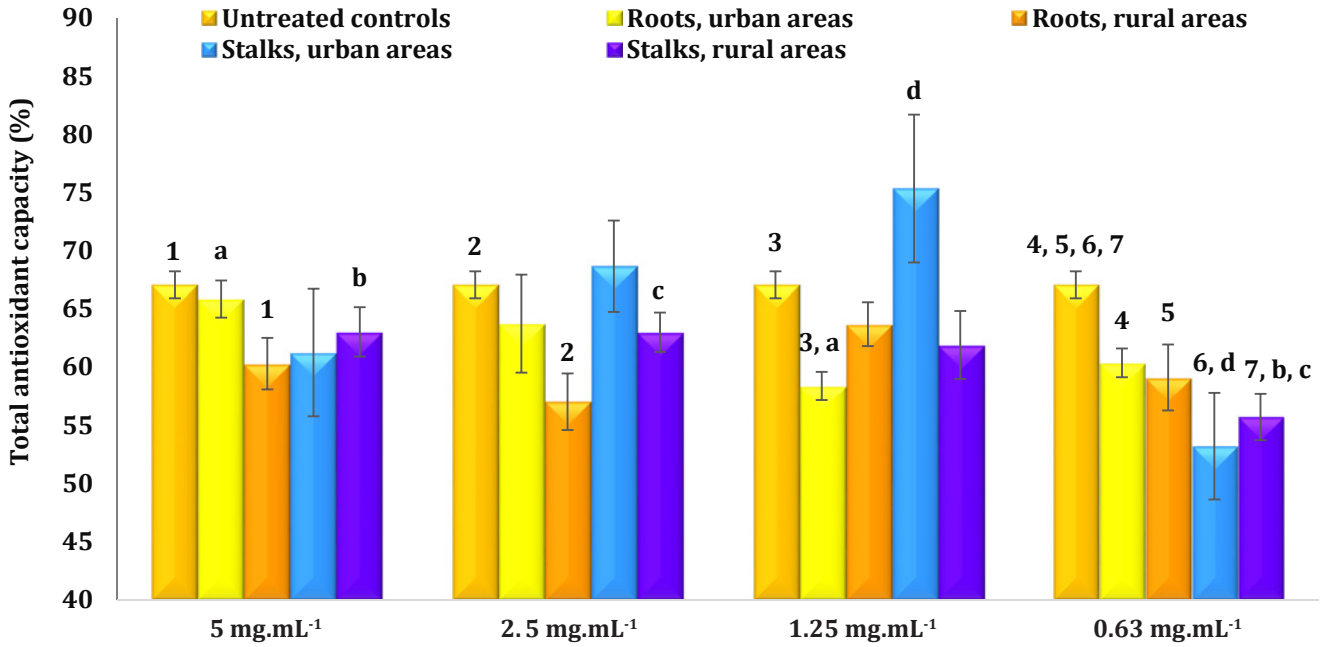

Figure 5 The total antioxidant capacity in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses (5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, 0.63 mg.mL^{-1}) derived stalks and roots of *Chelidonium majus* collected from urban and rural agglomerations (n = 8)
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
Changes are statistically significant (p <0.05) in relations: 1 – untreated controls *vs.* extracts derived from roots collected in rural areas (5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from roots collected in urban areas (0.63 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from stalks collected in urban areas (0.63 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); b – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); c – extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

The total antioxidant capacity in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM collected from urban and rural agglomerations was presented in Figure 5.

After incubation of muscle tissue with root extracts of CM collected from rural areas at a dose of 5 mg.mL^{-1} we observed a statistically significant decrease by 10.1% (p <0.05) in total antioxidant capacity (60.3 ±1.6%) compared to the untreated controls (67.07 ±1.16%). We obtained similar results after incubation with the same extracts at a dose of 2.5 mg.mL^{-1}, i.e. (57.03 ±2.43%) compared to the untreated controls (67.07 ±1.16%), where a statistically significant decrease in TAC was 14.97% (p <0.05). A statistically significant change occurred only for root extracts of CM collected from urban agglomerations at a dose of 1.25 mg.mL^{-1} after incubation with muscle tissue homogenates, where we also observed a statistically significant decrease in TAC levels (58.39 ±1.21%) compared to the untreated controls (67.07 ±1.16%) by 5.04% (p <0.05).

By reducing the dose to 0.63 mg.mL^{-1}, we observed a statistically significant decrease in TAC levels after incubation with both root (60.37 ±1.24%) and stalk (53.21 ±4.58%) extracts from CM collected from urban agglomerations compared to the control samples (67.07 ±1.16%) by 9.99% (p <0.05) for root and by 20.66% (p <0.05) for stalk extracts. Similarly, after incubation of muscle tissue at the above dose with root and stalk extracts from CM collected from rural areas, we recorded a statistically significant decrease in total antioxidant capacity [(59.11 ±2.83) and (55.72 ±1.99%), respectively compared to the untreated controls (67.07 ±1.16%)] by 11.87 and 16.92% (p <0.05), respectively (Figure 5).

Comparing a dose of 5 mg.mL^{-1} of root extracts of CM collected from urban areas to a dose of these extracts of 1.25 mg.mL^{-1}, we observed a statistically significant increase in TAC level by 12.8% (p <0.05). A dose of 5 mg.mL^{-1} of stalk extracts of CM collected from rural areas statistically significantly increased the TAC level by 13.1% (p <0.05) compared to a dose of 0.63 mg.

mL^{-1}, while a statistically significant increase in the total antioxidant capacity by 0.05% ($p < 0.05$) was observed compared to a dose of 2.5 $mg.mL^{-1}$. On the other hand, stalk extracts of CM collected from urban agglomerations at a dose of 1.25 $mg.mL^{-1}$ resulted in statistically significantly increased TAC levels (by 41.6%, $p < 0.05$) compared to values obtained using a dose of 0.63 $mg.mL^{-1}$ (Figure 5).

Conclusions

The purpose of our study was evaluation the oxidative stress biomarkers, i.e. 2-thiobarbituric acid reactive substances, carbonyl derivatives content of protein oxidative modification, total antioxidant capacity in the muscle tissue of rainbow trout after treatment by extracts in different doses derived from roots and stalks of CM collected from rural and urban agglomerations for estimation the optimal doses of extracts exhibiting the antioxidant activity. From our study, a dose of CM extracts of 0.63 $mg.mL^{-1}$ showed the highest antioxidant activity in the muscle tissue of rainbow trout. The extracts derived mainly from the roots of CM collected from rural areas were effective in reducing the levels of oxidative stress biomarkers by reducing lipid peroxidation markers, which may suggest that the active substances such as alkaloids (chelidonine, sanguinarine, berberine), flavonoids, phenols in these plants can effectively protect the membrane structures in muscle cells of salmonids. We also observed statistically significant reductions in levels of both aldehydic and ketonic derivatives of oxidatively modified proteins in muscle tissue of rainbow trout after incubation with CM extracts at this dose compared to the controls. The comparison of these results shows that CM extracts can effectively inhibit protein damage by scavenging free radicals and/or activation of antioxidant defenses. The secondary metabolites of CM, i.e. polyphenols and alkaloids, are most likely responsible for this effect. Using doses of 5 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ of both root and stalk extracts *in vitro* study, statistically significant increases in levels of TBARS and OMP were observed. This phenomenon may explain the use of destructive effects of CM on the membrane structures of cancer cells, due to the presence of a wide range of active compounds and other secondary metabolites.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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