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Biomarkers of oxidative stress in the muscle tissue of atlantic salmon (*Salmo salar* L.) treated *in vitro* by extracts of *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 Atlantic salmon (*Salmo salar* L.). Therefore, in the present study, oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), total antioxidant capacity (TAC)) and also activity of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) were used for evaluating the *in vitro* antioxidant activity of root and stalk extracts derived from great celandine *Chelidonium majus* L. (CM) collected in urban and rural agglomerations of Kartuzy district (Pomeranian province, northern part of Poland). Freshly collected roots and stalks were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in the proportion of 1:19, w/w) at room temperature. Incubation of salmon muscle tissue with extracts derived from both the stalks and roots of CM harvested from rural areas resulted in a decrease in lipid peroxidation. The incubation of salmon muscle tissue with extracts derived from both the stems and roots of CM harvested from rural areas resulted in a decrease in lipid peroxidation. Similarly, the use of extracts derived from the roots of CM collected from urban areas resulted in a decrease in TBARS levels. These results suggest that it can be argued that the presence of secondary plant metabolites in CM extracts protects structures of cell membranes against the damaging effects of free radicals. On the other hand, analysis of levels of protein oxidation after incubation of muscle tissue with CM extracts showed that extracts derived from both roots and stalks of CM harvested from urban areas reduced levels of ketonic derivatives of oxidatively modified proteins. Analyzing the total antioxidant capacity after the incubation with CM extracts under *in vitro* conditions, we concluded that extracts mainly derived from the stalks of CM harvested from both urban and rural areas effectively increase TAC levels. These results are reflected after analysis of antioxidant enzyme activity, where we observed statistically significant increases in superoxide dismutase and catalase activity. On the other hand, the incubation of CM extracts with muscle tissue resulted in a statistically significant decrease in glutathione peroxidase activity compared to the control samples.

Keywords: root and stalk extracts, Atlantic salmon, muscle tissue, biomarkers of oxidative stress, antioxidant enzymes

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Introduction

Many proteins contain redox-sensitive thiols, and reactions of thiol systems occur largely by non-radical two-electron transfers. Accumulating data show that central thiol-disulfide couples are maintained under non-equilibrium conditions in biological systems. This presents a condition wherein changes in abundance and distribution of redox catalysts and changes in rates of generation of relevant oxidants (e.g., peroxides) and precursors for NADPH supply can account for pathological effects of oxidative stress through altered functions of enzymes, receptors, transporters, transcription factors, and structural elements, without free radicals (Go and Jones, 2013). Free radicals or reactive oxygen species (ROS) are generated by oxygen metabolism which is balanced by the rate of oxidant formation and the rate of oxidant elimination (Sinha and Dabla, 2015). Oxidative stress is a result of an imbalance between the generation of reactive oxygen species and the antioxidant defence systems. Oxidative stress is involved in the development and progression of clinical and experimental tissue failure (Sies, 2015; Herrmann and Dick, 2012). Oxidative stress is defined as a dysregulation between the production of reactive oxygen species and the endogenous antioxidant defence mechanisms, the so-called 'redox state'. When present in low concentrations, ROS plays a critical function in cell homeostasis. However, excess ROS causes cellular dysfunction, protein and lipid peroxidation, and DNA damage, and eventually leads to irreversible cell damage and death (Khatri et al., 2018). Oxidative stress is predominantly caused by a host of lifestyle-related factors, the majority of which are modifiable. Antioxidant regimens and lifestyle modifications could both be plausible therapeutic approaches that enable the burden of oxidative stress-induced tissue damage to be overcome (Jones, 2006; van der Pol et al., 2018).

Better knowledge of oxidative balance in fish tissues and its application to fisheries and aquaculture science (i.e., breeding fit fish) is needed in the face of global environmental change, high fishing pressure, increased aquaculture production, as well as increased concern for fish welfare (Johnston, 1999; Palstra and Planas, 2011). Oxidative stress-related diseases can contribute to increased fish mortality (Bisht et al., 2017). Therefore, there is a need to search for measurements to prevent oxidative imbalance in fish. It is suggested that medicinal plants containing secondary metabolites such as alkaloids, polyphenols, and vitamins, among others, can contribute to eliminating the harmfulness of oxidative stress (Koleva et al., 2018). Recent scientific reports have demonstrated that plants belonging to the Papaveraceae family contain several compounds possessing antioxidant properties. Great celandine *Chelidonium majus* L. (CM) (Papaveraceae) has a long history of being useful for the treatment of many diseases. This plant is of great interest for its use also in Chinese herbal medicine (Zielińska et al., 2018). 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. CM extracts and their purified compounds exhibit antiviral, antitumor, antimicrobial, and antioxidative properties in both *in vitro* and *in vivo* studies (Arora and Sharma, 2013).

Therefore, the current study aimed to assess the *in vitro* antioxidant activity of root and stalk extracts derived from CM collected in urban and rural agglomerations of Kartuzy district (Pomeranian province, northern part of Poland). For this purpose, we used 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 (superoxide dismutase, catalase, glutathione peroxidase) in the muscle tissue of Atlantic salmon (*Salmo salar* L.) incubated *in vitro* with CM extracts.

Materials and methodology

Collection of plant material

Plant materials 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). The plant collection covered the period from June to July 2020. For our studies, we collected CM plants in phases beginning with flowering (flower buds visible) and full flowering (yellow flowers blooming, young fruits small and developing). Kartuzy is located about 32 kilometres (20 miles) west of Gdańsk and 35 km (22 miles) south-east of Lębork town 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 stalks were washed, weighed, crushed, and homogenized in 0.1M phosphate

buffer (pH 7.4) (in the proportion of 1:19, w/w) at room temperature. The extracts were then filtered and used for analysis. The extracts were stored at -25 °C until use.

Experimental fish and muscle tissue samples

Clinically healthy Atlantic salmon (*Salmo salar* L.) with a mean body mass of 85–190 g were used in the experiments. The fish samples for the current study were carried out in the Department of Salmonid Research, Inland Fisheries Institute (Rutki, Poland). The muscle tissues were sampled after the decapitation of the fish. The minced muscle tissue was rinsed clear of blood with cold isolation buffer (100 mM Tris-HCl, pH 7.2) and homogenized in a homogenizer H500 with a motor-driven pestle on ice. Homogenates were centrifuged at 3,000 rpm for 15 min at 4 °C. After centrifugation, the supernatant was collected and frozen at -25 °C until analyzed. Protein contents were determined with the method described by Bradford (1976) with bovine serum albumin as a standard. Absorbance was recorded at 595 nm. All enzymatic assays were carried out at 22 ±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) (n = 8). The enzymatic reactions were started by adding the tissue supernatant.

Experimental design

The supernatant of the muscle tissue was used to incubate with extracts obtained from roots and stalks of CM (in a final concentration of extracts of 2.5 mg per mL) at room temperature. The control untreated samples (muscle tissue) were incubated only with 100 mM Tris-HCl buffer (pH 7.2) (in the same ratio). The incubation time was 2 hours. Biomarkers of oxidative stress and antioxidant defences were studied in the incubated homogenate (control untreated group and in samples with extracts obtained from roots and stalks of CM).

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 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. The nmol of MDA per mg of protein was calculated using $1.56 \cdot 10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

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

To evaluate the protective effects of extracts derived from roots and stalks of CM collected in urban and rural agglomerations against free radical-induced protein damage in the muscle tissue of Atlantic salmon, a content of aldehydic and ketonic derivatives of oxidative modification of proteins (OMP) based on the spectrophotometric measurement was done. 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 carbonyls 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 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 sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated concerning the absorbance of the blank samples.

Measurement of superoxide dismutase activity

Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was assessed by its ability to dismutate superoxide produced during quercetin auto-oxidation in an alkaline medium (pH 10.0) by Kostiuk et al. (1990) method. Activity is expressed in units of SOD per mg of protein.

Measurement of catalase activity

Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 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 the decomposition of 1 μmol H_2O_2 per min per mg of protein.

Measurement of glutathione peroxidase activity

Glutathione peroxidase (GPx, EC 1.11.1.9) activity was determined by detecting the non-enzymatic utilization of GSH (the reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) according to by the method of Moin (1986). The rate of GSH reduction was followed spectrophotometrically at 412 nm. GPx activity is expressed as nmol GSH per min per mg of protein.

Statistical analysis

The mean ± 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 (TIBCO Software Inc., Krakow, Poland).

Results and discussion

Figure 1 demonstrates the TBARS levels in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas of the Pomeranian region. Analyzing the TBARS levels after the *in vitro* treatment of CM extracts, we obtained the following results. There was a reduction in TBARS levels after *in vitro* incubation of salmon muscle tissue with root extracts of CM collected from rural areas (145.69 ±9.66 nmol.mg^{-1} protein) compared to the untreated control samples (154.56 ±7.1 nmol.mg^{-1} protein). There was a statistically no-significant decrease in TBARS levels by 5.82% ($p > 0.05$) compared to the controls (Figure 1).

We obtained similar results after *in vitro* incubation with root extracts of CM collected from urban agglomerations with salmon muscle tissue, where we also observed a statistically no-significant decrease in TBARS by 5.85% ($p > 0.05$) compared to the control samples (145.52 ±3.84 nmol.mg^{-1} protein vs. 154.56 ±7.1 nmol.mg^{-1} protein). The opposite trends were observed after *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from urban areas. The use of stalk extracts of CM collected from urban areas (159.69 ±5.05 nmol.mg^{-1} protein) resulted in a statistically no-significant highest increase in TBARS levels (by 3.32%, $p > 0.05$) compared to the control samples (154.56 ±7.1 nmol.mg^{-1} protein). After *in vitro* incubation of salmon muscle tissue with stalk extracts of CM harvested from a rural agglomeration, we observed a statistically no-significant decrease in TBARS levels (by 3.03%, $p > 0.05$) compared to the control samples (149.88 ±3.58 nmol.mg^{-1} protein vs. 154.56 ±7.1 nmol.mg^{-1} protein) (Figure 1).

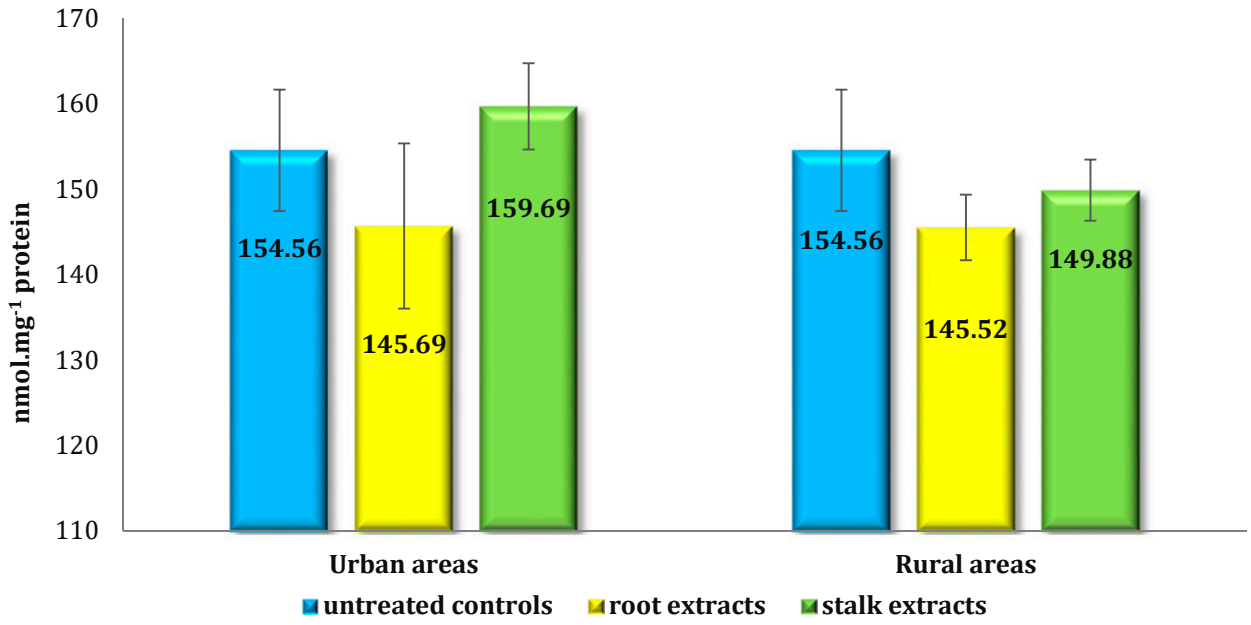

Figure 1 The TBARS level as a biomarker of lipid peroxidation in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region (M ±m, n = 8)

The aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas of the Pomeranian region were present in Figure 2.

Analyzing levels of protein oxidation after incubation of muscle tissue with CM extracts, we observed interesting results. We noted similar levels of aldehydic derivatives of oxidatively modified proteins after *in vitro* incubation of salmon muscle tissue with CM stalk extracts of CM collected from urban areas (14.24 ±0.12 nmol.mg^{-1} protein) compared with the control samples (14.7 ±0.27 nmol.mg^{-1} protein), where there was a statistically no-significant decrease by 3.13% (p >0.05). Similarly, *in vitro* incubation of muscle tissue with stalk extracts of CM harvested from rural

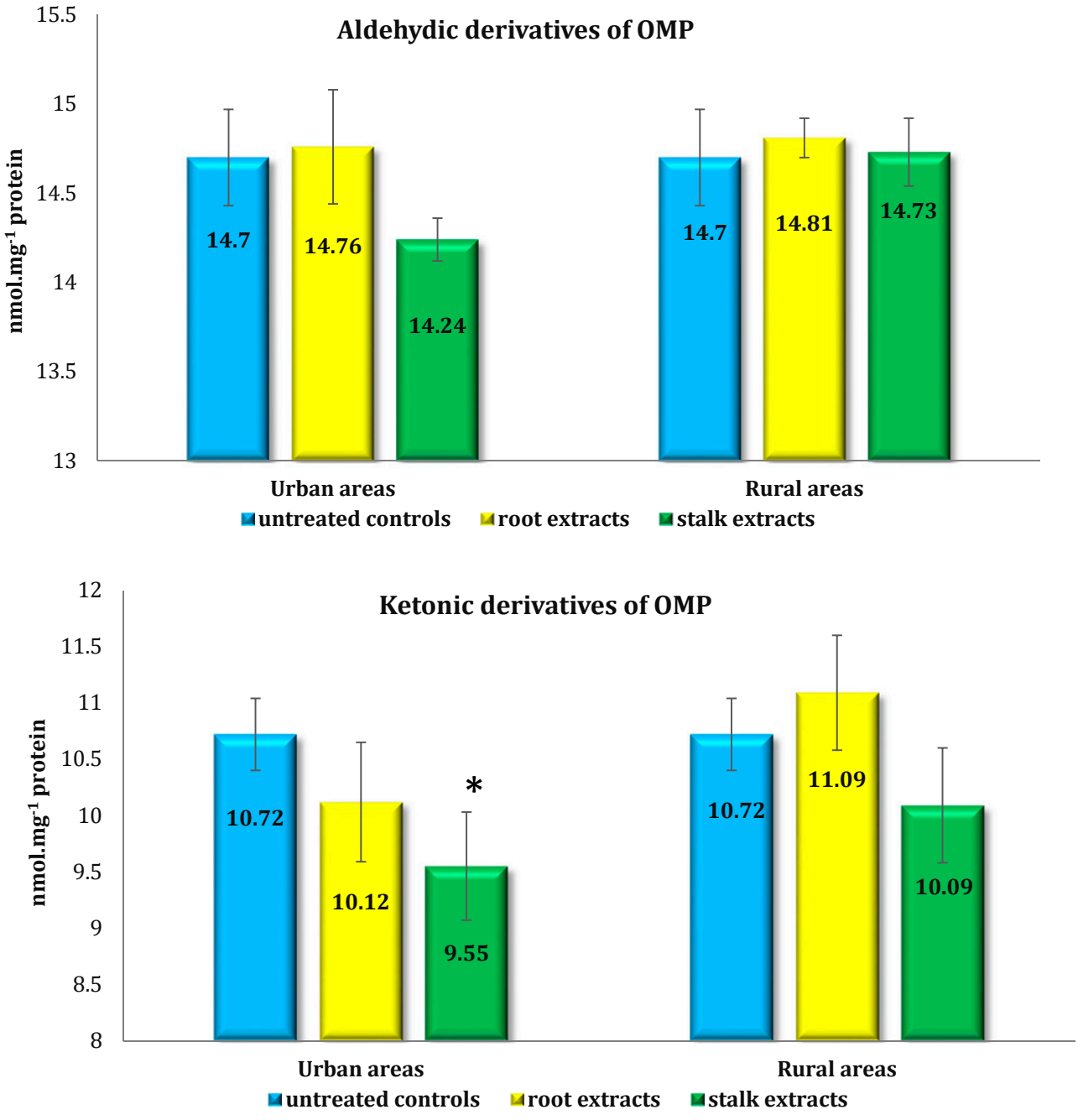

Figure 2 The aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ± m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

areas resulted in similar levels of aldehydic derivatives of OMP compared to the controls (14.73 ±0.19 nmol. mg^{-1} protein vs. 14.7 ±0.27 nmol.mg^{-1} protein). After incubating the salmon muscle tissue with root extracts of CM collected from both urban and rural agglomerations, we also observed similar levels of aldehydic derivatives of OMP compared to the control samples (14.76 ±0.32 nmol.mg^{-1} protein vs. 14.7 ±0.27 nmol.mg^{-1} protein for extracts of CM collected from urban areas; 14.81 ±0.11 nmol.mg^{-1} protein vs. 14.7 ±0.27 nmol.mg^{-1} protein for extracts of CM collected from rural areas).

After *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from urban areas, we recorded a statistically significant reduction in the level of ketonic derivatives of oxidatively modified proteins (by 10.91%, p <0.05) compared to the control samples (9.55 ±0.48 nmol.mg^{-1} protein vs. 10.72 ±0.32 nmol. mg^{-1} protein). We noted similar results after incubating salmon muscle tissue with root extracts of CM collected from urban areas, where there was also, but a statistically no-significant decrease (by 5.6%, p >0.05) in levels of ketonic derivatives of oxidatively modified proteins (10.12 ±0.53 nmol.mg^{-1} protein) compared to the control samples (10.72 ±0.32 nmol.mg^{-1} protein). Also, we recorded a statistically no-significant decrease in levels of ketonic derivatives of OMP after *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from rural areas (by 5.88%, p >0.05) compared to the control samples (10.09 ±0.51 nmol.mg^{-1} protein vs. 10.72 ±0.32 nmol.mg^{-1} protein). Other results were obtained after *in vitro* incubation of muscle tissue with root extracts of CM collected from rural agglomerations, where we noted a statistically no-significant increase in levels of ketonic derivatives of OMP (by 3.45%, p >0.05) compared to the control samples (11.09 ±0.51 nmol.mg^{-1} protein vs. 10.72 ±0.32 nmol.mg^{-1} protein) (Figure 2).

The total antioxidant capacity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas of the Pomeranian region is presented in Figure 3.

When measuring total antioxidant capacity, we observed a statistically significant increase in TAC levels (by 18.86%, p <0.05) after *in vitro* incubation of muscle tissue with stalk extracts of CM collected from urban areas (31.39 ±3.37%) compared to the control samples (26.41 ±1.83%). Similar results were also obtained after incubation of muscle tissue with stalk extracts of CM collected from rural agglomerations (31.59 ±1.24%), where there was a statistically significant increase in TAC levels by 19.61% (p <0.05) compared to the control samples (26.41 ±1.83%). Incubation of muscle tissue with root extracts of CM collected from both urban (30.66 ±1.72%) and rural

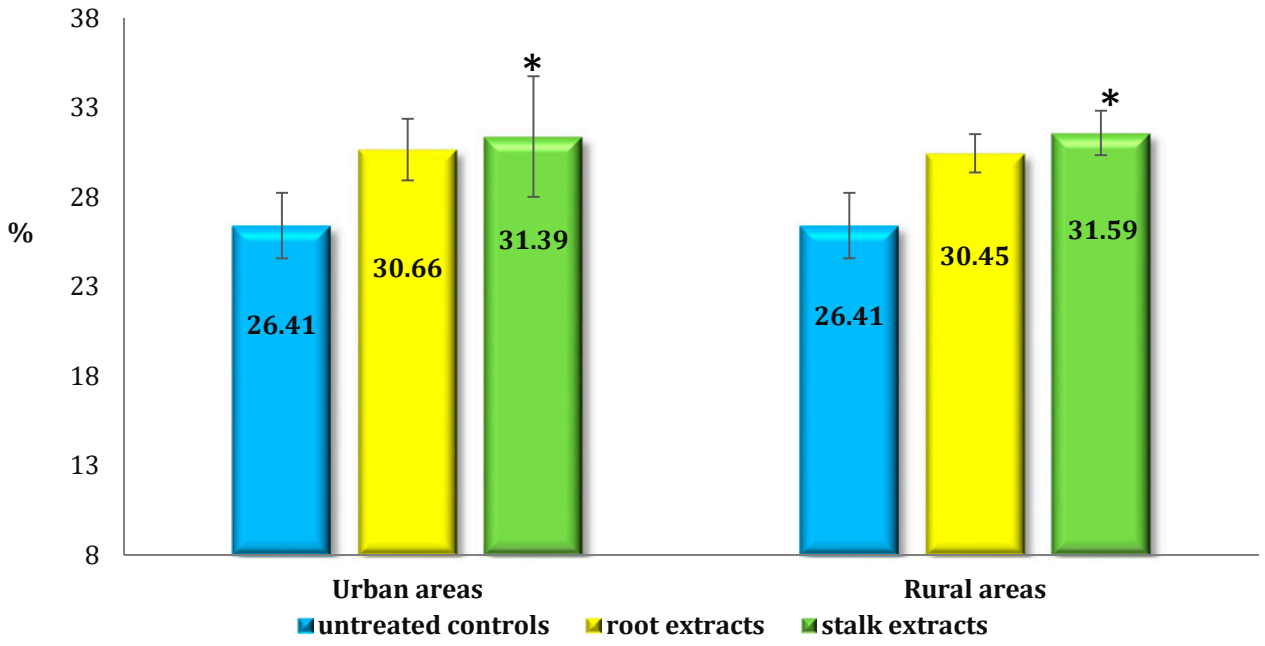

Figure 3 The total antioxidant capacity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L.CM collected from rural and urban areas of Pomeranian region (M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

areas (30.45 ±1.07%) also resulted in a statistically no-significant increase in total antioxidant capacity by 16.09% (p >0.05) for extracts of CM collected from urban areas and by 15.3% (p >0.05) for extracts of CM collected from rural areas compared to the control samples (26.41 ±1.83%) (Figure 3).

The superoxide dismutase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas are presented in Figure 4.

When we examined the activity of superoxide dismutase in the muscle tissue of salmons incubated *in vitro* with stalk extracts of CM collected from rural agglomerations, we recorded the highest activity of this antioxidant enzyme with a value of (398.68 ±23.38 U.mg^{-1} protein) compared to the control samples (353.5 ±16.69 U.mg^{-1} protein). There was a statistically no-significant increase in SOD activity by 12.8% (p >0.05) compared to the control samples. Similar results were obtained after incubating salmon muscle tissue with root extracts of CM collected from urban areas, where there was also a statistically no-significant increase in SOD activity (by11.5%, p >0.05) compared to the control samples (394.19 ±20.75 U.mg^{-1} protein vs. 353.5 ±16.69 U.mg^{-1} protein). After incubation of salmon muscle tissue with stalk extracts of CM collected from urban areas, we noted an increase in superoxide dismutase activity by 7.2% (p >0.05) compared to the control samples (378.86 ±44.94 U.mg^{-1} protein vs. 353.5 ±16.69 U.mg^{-1} protein). Also, we recorded a statistically no-significant elevation of SOD activity in muscle tissue incubated *in vitro* with root extracts of CM harvested from rural areas (by 5.6%, p >0.05) compared to the control samples (373.43 ±22.02 U.mg^{-1} protein vs. 353.5 ±16.69 U.mg^{-1} protein) (Figure 4).

The catalase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas was presented in Figure 5.

Analyzing catalase activity after the incubation of muscle tissue with CM extracts, we obtained interesting results. We noted a statistically significant increase in catalase activity after *in vitro* incubation of salmon muscle tissue with root extracts of CM collected from urban areas (by 11.5%, p <0.05) compared to controls (394.19 ±20.75 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1} protein). Similar results were obtained after incubating muscle tissue with stalk extracts of CM collected from rural agglomerations, where there was also a statistically significant increase in CAT activity by 12.8% (p <0.05) compared to the control samples (398.68 ±23.38 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1} protein). A statistically no-significant elevation in catalase activity was observed after incubating salmon muscle tissue with stalk extracts of CM collected from urban areas (378.86 ±44.94 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1} protein) and root extracts of CM collected from rural areas (373.43 ±22.02 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1}

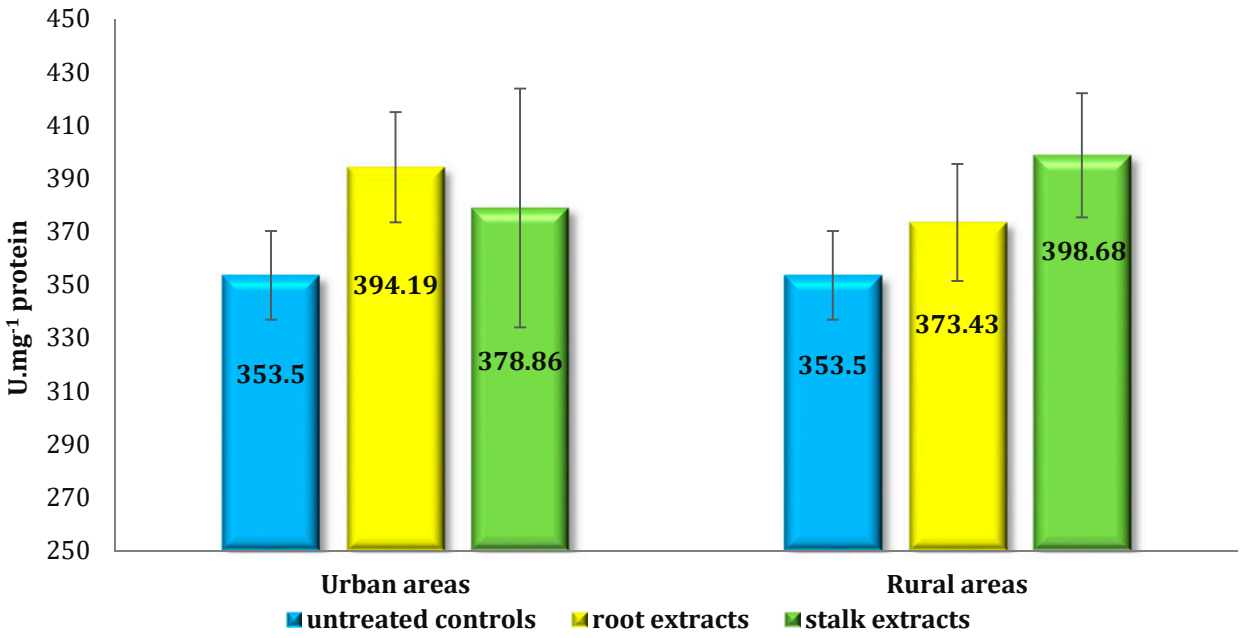

Figure 4 The superoxide dismutase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region (M ±m, n = 8)

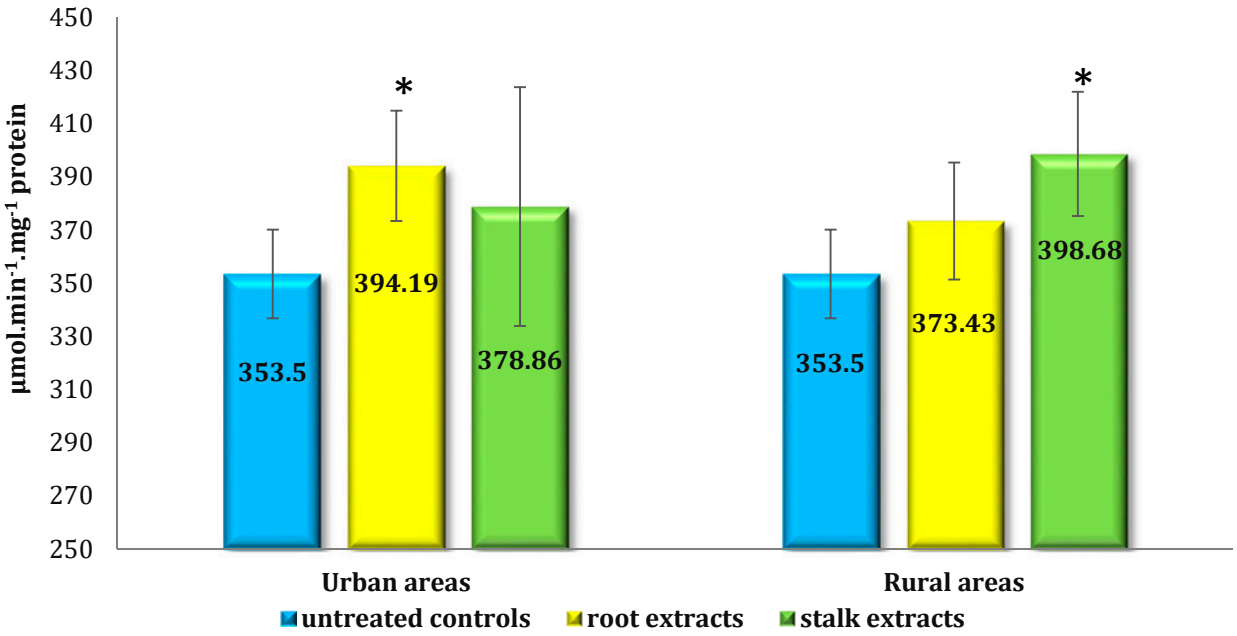

Figure 5 The catalase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region(M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control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

protein). There was an increase in CAT activity by 7.2% (p >0.05) and 5.6% (p >0.05), respectively compared to control samples (Figure 5).

The glutathione peroxidase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas was presented in Figure 6.

We observed a statistically significant reduction in the GPx activity in salmon muscle tissue after treatment with root extracts of CM harvested from both urban and rural areas compared to the control samples (190.99 ±6.47 nmol.min^{-1}.mg^{-1} protein and 188.24 ±5.26 nmol.min^{-1}.mg^{-1} protein vs. 218.89 ±7.64 nmol.min^{-1}.mg^{-1} protein, respectively). There

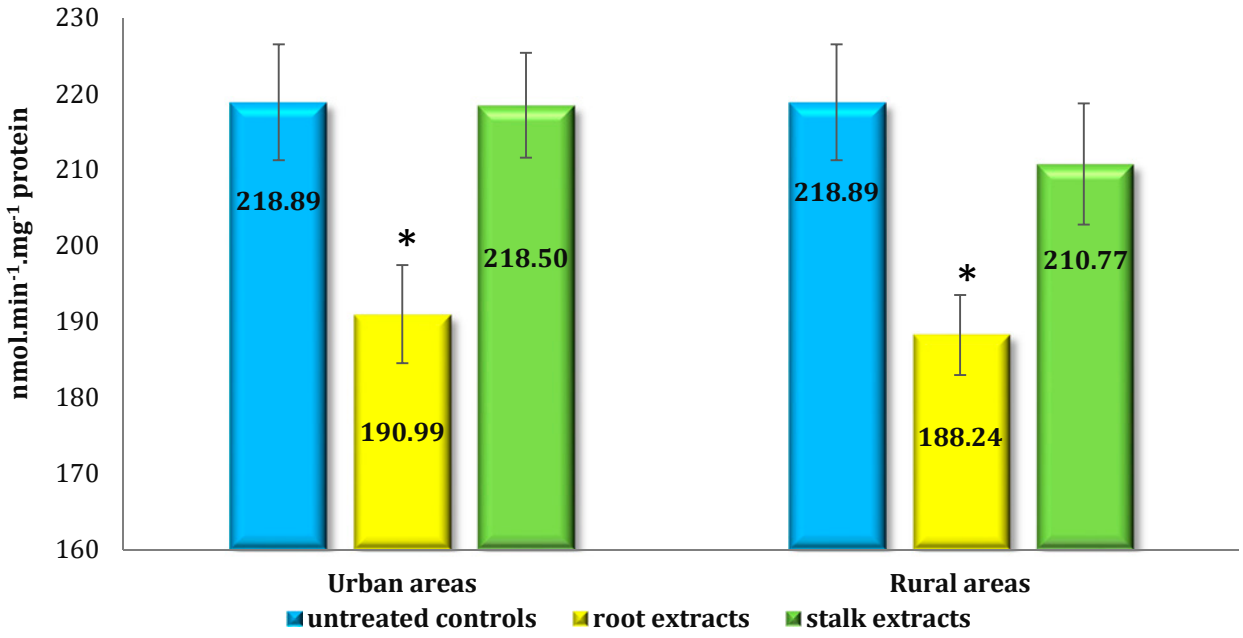

Figure 6 The glutathione peroxidase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

was a decrease in GPx activity by 12.7% ($p < 0.05$) and 14% ($p < 0.05$), respectively. After *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from both urban (218.5 ±6.9 $nmol.min^{-1}.mg^{-1}$ protein) and rural areas (210.77 ±7.98 $nmol.min^{-1}.mg^{-1}$ protein), we recorded a decrease in GPx activity, but not statistically significantly (by 0.2%, $p > 0.05$ and 3.71%, $p > 0.05$, respectively) compared to the control samples (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 defence in the muscle tissue of Atlantic salmon. Our study suggests that the extracts from both roots and stems of CM harvested from urban areas reduced the level of oxidatively modified proteins. Analyzing the total antioxidant capacity after the incubation with CM extracts under *in vitro* conditions, we concluded that extracts derived mainly from the stalks of CM harvested from both urban and rural areas effectively increase TAC levels. These results are reflected after analysis of antioxidant enzyme activity, where we observed statistically significant increases in the activity of superoxide dismutase and catalase. This may be related to the presence of antioxidant compounds contained in the plant structures of greater celandine.

In our previous study (Stefanowski et al., 2021a, c, d) on muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbum), 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 aldehyde 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 stalk 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 levels in the blood of rainbow trout after *in vitro* incubation with root and stem extracts shows that extracts can inhibit the production of oxidative carbonyls by scavenging free radicals (Stefanowski et al., 2021c, d).

In another study (Stefanowski et al., 2022) on muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbum), we also demonstrated the dose-dependent antioxidant activity of CM extracts. Results of our study revealed that a final 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 the muscle tissue of rainbow trout after incubation with CM extracts at this dose compared to the controls. The comparison of these results showed that CM extracts can effectively inhibit protein damage by scavenging free radicals and acting on antioxidant defences. The secondary metabolites of CM, i.e. polyphenols and alkaloids, are most likely responsible for this effect. Using extracts in final doses of 5 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ derived from both roots and stalks resulted in statistically significant increases in levels of TBARS and OMP (Stefanowski et al., 2022).

Pharmacologically relevant substances of CM are isoquinoline alkaloids. Generally, five groups of alkaloids were found in CM. These are the derivatives of phenanthridine (3,4-benzoisoquinoline), protoberberine, protopine, quinolizidine, and aporphine. Major phenanthridine derivatives that were found in aerial and underground parts are chelidonine and chelerythrine (Zielińska et al., 2018). Nile et al. (2021) have studied total phenolics and flavonoids in the different parts of CM. The leaves showed higher flavonoid content (137.43 $mg.g^{-1}$), while the pod showed the highest phenolic (23.67 $mg.g^{-1}$) content when compared with the stems, flowers, and roots. In the ABTS (Diammonium 2,2'-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulphonate]) antioxidant assay, the flower extract showed a 57.94% effect, while the leaf, pod, and root extract exhibited 39.10%, 36.08%, and 28.88% activity, respectively. The pod and leaf extracts demonstrated the potential effect, exhibiting 45.46 and 41.61% activity, respectively, for the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. Similar to the phosphomolybdenum assay, the flower revealed higher antioxidant activity (46.82%) than the other plant parts (Nile et al., 2021).

Some of the versatile traditional uses of CM can be explained, as in many other herbs, by anti-inflammatory potential targeting various pathways in the organism

as well as modulation of the immune response. Both have been confirmed in many studies using *in vitro* cellular models, as well as *in vivo*. The ability to inhibit inflammation or, in some cases, to stimulate immune response and mitigate excessive reactiveness can contribute to the postulated anticancer properties and improve symptoms of gastric disorders as well. Chelidonic acid was efficient in mouse models of ovalbumin-elicited allergic rhinitis (Oh et al., 2011) and ulcerative colitis (Kim et al., 2012). This compound also attenuated inflammatory responses by reducing levels and gene expression of several mediators and enzymes in colon tissues (cyclooxygenase-2, Hypoxia-inducible factor 1-α, prostaglandin E2) and in allergic mice (Interleukin (IL)-4, IL-1β, cyclooxygenase-2, caspase-1, and increase of interferon-γ). In the human mast cell line HMC-1 stimulated for inflammatory response by the phorbol ester (TPA) and calcium ionophore A23187, chelidonic acid inhibited IL-6 expression by blocking nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (Shin et al., 2011).

Most authors (Lee et al., 2007; Yang et al., 2011) using different experimental models of inflammation *in vitro* demonstrated the anti-inflammatory activity of CM extracts. It was found that mainly alkaloids contained in extracts may be responsible for these anti-inflammatory effects. The analysis of Nawrot et al. (2007a, b) confirmed the presence of the protein components of the antioxidant defence system in CM latex. These proteins form the first line of defence 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 (Nawrot et al., 2007a, b).

Zielińska et al. (2020) used the LC-MS/MS method to determine alkaloids, phenolic acids, carboxylic acids, and hydroxybenzoic acids in the CM extracts. These researchers investigated five individually tested alkaloids (coptisine, berberine, chelidonine, chelerythrine, and sanguinarine) as well as CM root extract for their effect on the secretion of interleukins (IL-1β, IL-8), and tumor necrosis factor α (TNF-α) in human polymorphonuclear leukocytes (neutrophils). Berberine, chelidonine, and chelerythrine significantly decreased the secretion of TNF-α in a concentration-dependent manner. Sanguinarine was the most potent inhibitor of IL-1β secretion. However, the overproduction of IL-8 and TNF-α and high cytotoxicity for these compounds were observed. Coptisine was highly cytotoxic and slightly decreased the secretion of the studied cytokines. According to Zielińska et al. (2020), the extract (1.25–12.5 $\mu g.mL^{-1}$) increased cytokine secretion in a concentration-dependent manner, but an increase in cytotoxicity was also noted.

Park et al. (2015) investigated the effects of CM extract on human epidermoid carcinoma A431 cells through multiple mechanisms, including induction of cell cycle arrest, activation of the caspase-dependent pathway, blocking of nuclear factor-κB (NF-κB) activation and involvement in the mitogen-activated protein kinase (MAPK) pathway. CM inhibited the proliferation of A431 cells in a dose- and time-dependent manner, increased the percentage of apoptotic cells, significantly decreased the mRNA levels of cyclin D1, Bcl-2, Mcl-1, and survivin, as well as increased p21 and Bax expression. Exposure of A431 cells to CM extract enhanced the activities of caspase-3 and caspase-9, while co-treatment with CM, the pan-caspase inhibitor Z-VAD-FMK, and the caspase-3 inhibitor, Z-DEVE-FMK, increased the proliferation of A431 cells. CM extract not only inhibited NF-κB activation, but it also activated p38 MAPK and MEK/ERK signaling. These results demonstrated that CM extract inhibited the proliferation of human epidermoid carcinoma A431 cells by inducing apoptosis through caspase activation and NF-κB inhibition *via* a MAPK-independent pathway (Park et al., 2015).

Chelidonine is known for its broad pharmacological activities that lead to anti-inflammation, anti-viral and anti-cancer effects. To be specific, chelidonine treatment induced apoptosis in T98G glioma cells, MCF-7 and SK-BR-3 breast adenocarcinoma, HepG2 hepatoma, HeLa cervical cancer, SW620 colon cancer, head and neck squamous cell carcinoma HNSCC, human gastric carcinoma SGC-7901, and leukaemia MT-4 cells, through caspase, cell cycle checkpoints, and MAP kinase pathways. In colon cancer, Caco-2, and leukaemia cell line CEM/ADR 5000, metabolic enzyme regulation by chelidonine reversed doxorubicin resistance. Chelidonine was also reported to trigger autophagy, cellular senescence, and blocking telomerase activity. The cytotoxic effect of chelidonine and its mechanisms on pancreatic cancer have not been elucidated (Paul et al., 2012; Noureini and Esmaili, 2014).

According to Orvos et al. (2015), hydroalcoholic extracts of greater celandine and its alkaloids, especially berberine, chelidonine, and sanguinarine have a significant hERG potassium channel-blocking effect. These extracts and alkaloids also prolong the cardiac action potential in dog ventricular muscle. Therefore these compounds may consequently delay cardiac repolarization, which may result in the prolongation

of the QT interval and increase the risk of potentially fatal ventricular arrhythmias.

Shen et al. (2022) suggested a potential therapeutic role of CM against ovarian cancer due to induced SKOV-3 cell death by increasing levels of activating transcription factor 3 (ATF3) and its downstream proteins Tip60 and Foxo3a. CM upregulated the expression of ATF3 and tightly regulated transcriptional regulator (Tip60) and promoted Foxo3a nuclear translocation, ultimately increasing the level of the Bcl-2-associated X protein (Bax) protein. ATF3 overexpression stimulated Tip60 expression, while ATF3 inhibition by siRNA repressed Tip60 expression. Furthermore, siRNA-mediated Tip60 inhibition significantly promoted Foxo3a phosphorylation, leading to a blockade of Foxo3a translocation into the nucleus. Thus, ATF3 mediates the regulation of Foxo3a by Tip60. Moreover, siRNA-mediated Foxo3a inhibition suppressed the expression of Bax and subsequent apoptosis (Shen et al., 2022).

Conclusions

The results revealed that the incubation of salmon muscle tissue with extracts derived from both the stems and roots of CM harvested from rural areas resulted in a decrease in lipid peroxidation. Similarly, the use of extracts derived from the roots of CM collected from urban areas resulted in a decrease in TBARS levels. These results suggest that it can be argued that the presence of secondary plant metabolites in CM extracts protects structures of cell membranes against the damaging effects of free radicals. On the other hand, analysis of levels of protein oxidation after incubation of muscle tissue with CM extracts showed that extracts derived from both roots and stalks of CM harvested from urban areas reduced levels of ketonic derivatives of oxidatively modified proteins. Analyzing the total antioxidant capacity after the incubation with CM extracts under *in vitro* conditions, we concluded that extracts mainly derived from the stalks of CM harvested from both urban and rural areas effectively increase TAC levels. These results are reflected after analysis of antioxidant enzyme activity, where we observed statistically significant increases in superoxide dismutase and catalase activity. On the other hand, the incubation of CM extracts with muscle tissue resulted in a statistically significant decrease in glutathione peroxidase activity compared to the control samples.

Conflict of interests

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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