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LIPID AND PROTEIN OXIDATION IN THE MUSCLE TISSUE OF RAINBOW TROUT AFTER *IN VITRO* TREATMENT BY EXTRACTS DERIVED FROM GREATER CELANDINE (*CHELIDONIUM MAJUS* L.) COLLECTED FROM RURAL AND URBAN AGGLOMERATIONS OF POMERANIAN REGION

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Abstract

Consistent with our previous studies, we continue to evaluate the antioxidant potential of representatives belonging to the Papaveraceae family collected from the coastal region of northern Poland using different cell models, namely the muscle tissue of rainbow trout. Therefore, in the current study, oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of oxidative modification of proteins (OMP), total antioxidant capacity (TAC)] were used for evaluating the antioxidant activity of extracts derived from stalks and roots of *Chelidonium majus* L. (CM) at final dose 2.5 mg/mL. The muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) was used in the current study. The supernatant of the muscle tissue was used to incubate with extracts derived from stalks and roots of CM (in a ratio of 39:1, final concentration 2.5 mg/mL) at room temperature. The control samples (muscle tissue) were incubated with 100 mM Tris-HCl

buffer (pH 7.2) (in a ratio of 39:1). The results of the current study revealed that extracts derived from stalks and roots of CM exhibited cytotoxic effects on cellular structures of muscle tissue by increasing the level of lipid peroxidation and decreased of the total antioxidant capacity. These results suggest the possibility of using CM extracts at a final dose of 2.5 mg/mL for pro-oxidant effects and warrant further studies to evaluate their therapeutic potential. The levels of aldehydic and ketonic derivatives of OMP were significantly decreased after *in vitro* incubation with the extracts derived from stalks and roots of CM collected only from rural agglomerations. Screening of plants for other biological activities, including antioxidant activity, is essential and may be effective in the search for preventive measures in the pathogenesis of some diseases, as well as in the prevention and treatment of some disorders in human and veterinary medicine.

Key words: coastal regions, rainbow trout, muscle tissue, lipid peroxidation, protein damage, total antioxidant capacity

INTRODUCTION

Coastal regions are tremendously important for the worldwide economy. Sixty percent of the world's major cities are located in coastal zones, and 40% of all people on the planet live within 100 km of a coastal zone (Nicholls et al. 2007). Approximately 40% of the EU's population lives within 50 km of the sea. Almost 40% of the EU's GDP is generated in these maritime regions, and a staggering 75% of the volume of the EU's foreign trade is conducted by sea. Coastal zones are the most productive and valued ecosystems on the planet (Crossland et al. 2005). In coastal areas, the closely intertwined relationship between people and coastal resources reinforces the most pressing questions of boundaries and sustainability, sustainability, and development in our world today.
Kashubia is a region in the western part of Pomerania, which stretches from the Baltic Sea to the Tuchola Forest. It is one of the most floristically diverse areas in Poland. The northern Kashubian region has a picturesque cliff coastline, while the central and southern parts are full of lakes, hills, and forests. Kartuzy is a Kashubian town in Pomeranian Province, the seat of Kartuzy County and Kartuzy urban-rural municipality. The vegetation cover is an integral part of the environment of the Kashubia region. The plants of the Baltic coastal zone also include medicinal plants with a wide range of uses. Papaveraceae is a family of vascular plants also found in the Baltic coastal area. Recent studies have reported that plants from this group have antioxidant properties, among others (Gilca et al. 2010).
Chelidonium majus L. (Papaveraceae), or greater celandine, is an important plant in western phytotherapy and in traditional Chinese medicine. Crude extracts of *C. majus* (CM), as well as purified compounds derived from it, exhibit a wide spectrum of biological activities (anti-inflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective, etc.) that support some of the traditional uses of CM. However, herbal medicine also claims that this plant has several important properties which have not yet been scientifically studied, i.e. CM is supposed to have diuretic, antitussive, and eye-regenerative effects (Zielińska et al. 2018). On the other hand, CM also has scientifically proven effects, e.g. anti-osteoporotic activity, and radioprotection, which are not mentioned in traditional sources (Nawrot 2017).

In the current study, the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of oxidative modification of proteins (OMP), total antioxidant capacity (TAC)] in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) were used in the study *in vitro* for assessing the antioxidant activity of root and stalk extracts (final concentration 2,5 mg/mL) derived from CM collected in urban and rural agglomerations of Kartuzy district in the Pomeranian province (northern part of Poland).

2-Thiobarbituric acid reactive substance (TBARS) assay is a method to detect lipid oxidation. This assay measures malondialdehyde (MDA), which is a split product of an endoperoxide of unsaturated fatty acids resulting from the oxidation of lipid substrates. The MDA reacts with 2-thiobarbituric acid (TBA) forming a pink chromogen (TBARS), which is measured at 532-535 nm (Kumar et al. 2018).

Radicals (e.g. HO•, CO_3^{-}, NO_2•, ROO•, RO•, R•, and many others), two-electron oxidants (e.g. peroxides, O_2, O_3, ONOOH, HOCl, and related species), and metal–oxo complexes can all modify proteins, although the reactivity and selectivity of these oxidants are highly variable. Reactions of secondary products (e.g. aldehydes, quinones, and dehydroalanine) are a further source of modifications. Together, these generate a wide variety of post-translational modifications that alter amino acid and protein composition and structure, charge, hydrophobicity/hydrophilicity, folding, and function (Grimsrud et al. 2008, Shu et al. 2019).

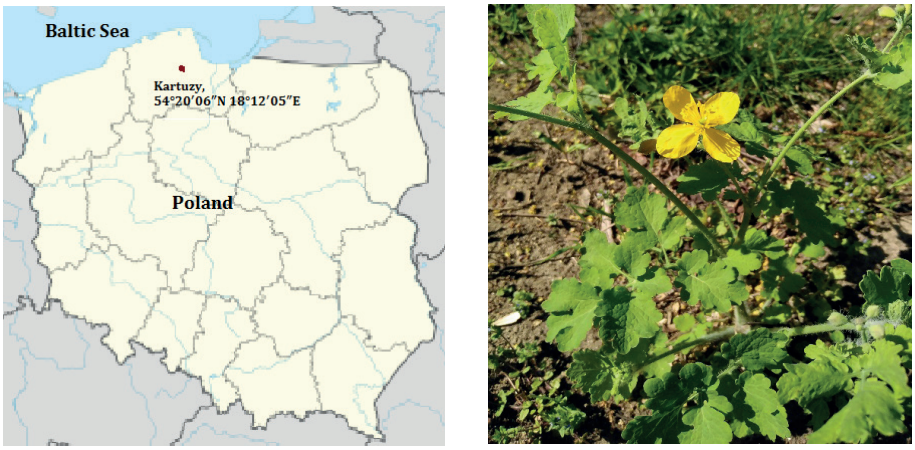
One of the strategies most commonly used to assess a free radical-antioxidant balance in chemical and biological systems is the determination of the total antioxidant capacity (TAC). TAC determinations are simple, inexpensive, and able to evaluate the capacity of known and unknown antioxidants and their additive, synergistic, and/or antagonistic actions, in chemical and biological systems (Fraga et al. 2014, Ialongo 2017). The measure of total antioxidant capacity considers the cumulative action of all the antioxidants present in plasma and body fluids. This results in an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance *in vivo* between oxidants and antioxidants (Ghiselli et al. 2000). In the current study, to determine total antioxidant capacity (TAC), the previous measurement of TBARS after oxidation of Tween 80 to malondialdehyde (MDA) is used. The presence of the sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80 resulting in a decrease in TBARS levels. As a result of the above method, a trimetin complex is formed between TBA and MDA, which by characteristic pink coloration.

MATERIALS AND METHODS

Collection of plant material

Plant materials (Fig. 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) (Fig. 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 River, comprises the highest parts of the Baltic Sea Plate (www.kartuzy.pl). Plants were collected from urban (n = 5) and rural agglomerations (n = 15) on the territory of the Kartuzy district.



A B

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

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 were stored at –20°C until use.

Experimental fish

Clinically healthy rainbow trout with a mean body mass of 80-120 g were used in the experiments. Rainbow trout is a species of fish in the salmonid family (Salmonidae). It is a prized farmed fish. Its breeding in Poland has developed especially in the area of Gdańsk Pomerania, where it is an alternative to the fishing industry of the Baltic Sea (www.fishbase.org). The experiments were performed in water at 14.5 ± 0.5°C and pH 7.2-7.4. The dissolved oxygen level was about 9 ppm with an additional oxygen supply, with a water flow of 25 L/min, and a photoperiod of 12 h per day. The same experimental conditions were used during the whole research. The water parameters were maintained under constant surveillance. The fish were held in square tanks (150 fish per tank) and fed a commercial pelleted diet.

Muscle tissue samples

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.

Experimental design

The supernatant of the muscle tissue was used to incubate with extracts derived from stalks and roots of *C. majus* (in a ratio of 39:1, final concentration 2.5 mg/mL) at room temperature. The control samples (muscle tissue) were incubated with 100 mM Tris-HCl buffer (pH 7.2) (in a ratio of 39:1). The incubation time was 2 hours. Biomarkers of oxidative stress were studied in the incubated homogenates (control samples and in samples with extracts derived from stalks and roots of CM).

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 the 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 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, 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 (Zar 1999). The data were analyzed using Statistica software, version 13.3 (TIBCO Software Inc., Kraków, Poland) (Zar 1999).

RESULTS AND DISCUSSION

In our study, we have investigated the effects of extracts derived from roots and stalks of CM collected from rural and urban regions of Kartuzy province (central Pomeranian region) on the levels of biomarkers of lipid peroxidation measured as TBARS, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the muscle tissue of rainbow trout as a model *in vitro* for assessment of antioxidant properties of CM. The results are shown in Figures 2-5.

By analyzing the level of biomarkers of lipid peroxidation, we obtained very precise results. We observed a statistically significant elevation in TBARS levels after *in vitro* incubation with rainbow trout muscle tissue compared to the control samples (196.72 ± 1.34 nmol/mg protein) for extracts of CM collected from the urban area for root extracts (221.33 ± 0.89 nmol/mg protein) and for stalks extracts (228.2 ± 3.91 nmol/mg protein). A similar increase in TBARS levels was observed for extracts of CM collected from the rural area for root extracts (223.4 ± 1.21 nmol/mg protein) and for stalk extracts (225.25 ± 1.32 nmol/mg protein). Levels of lipid peroxidation biomarkers were increased by approximately 15% ($p < 0.05$) compared to the control untreated samples (Fig. 2).

Analyzing levels of protein oxidation after *in vitro* incubation of rainbow trout muscle tissue with greater celandine extracts, we noted a statistically significant decrease in aldehydic derivatives of oxidatively modified proteins for stalk extracts (11.93 ± 0.23 nmol/mg protein) and root extracts (12.25 ± 0.14 nmol/mg protein) of CM collected from rural agglomerations only. There was a decrease of approximately 16.17% ($p < 0.05$) compared to untreated controls. In contrast, we observed a statistically non-significant elevation of levels of aldehydic derivatives of OMPs after incubation with muscle tissue for stalk extracts (13.14 ± 0.16 nmol/mg protein) compared with the control samples (12.68 ± 0.16 nmol/mg protein). After incubation with root extracts, we observed no change compared to the control samples (12.68 ± 0.16 nmol/mg protein) (Fig. 3). We obtained similar results when we examined the levels of ketonic derivatives of oxidatively modified proteins, where we also noted a statistically significant reduction in levels of ketonic derivatives of oxidatively modified proteins for

stalk extracts (10.06 ± 0.5 nmol/mg protein) and root extracts (10.73 ± 0.42 nmol/mg protein) of CM collected from rural agglomerations compared to the control samples (12.4 ± 0.62 nmol/mg protein) (Fig. 4). There was a 16.17% ($p < 0.05$) decrease from the untreated controls.

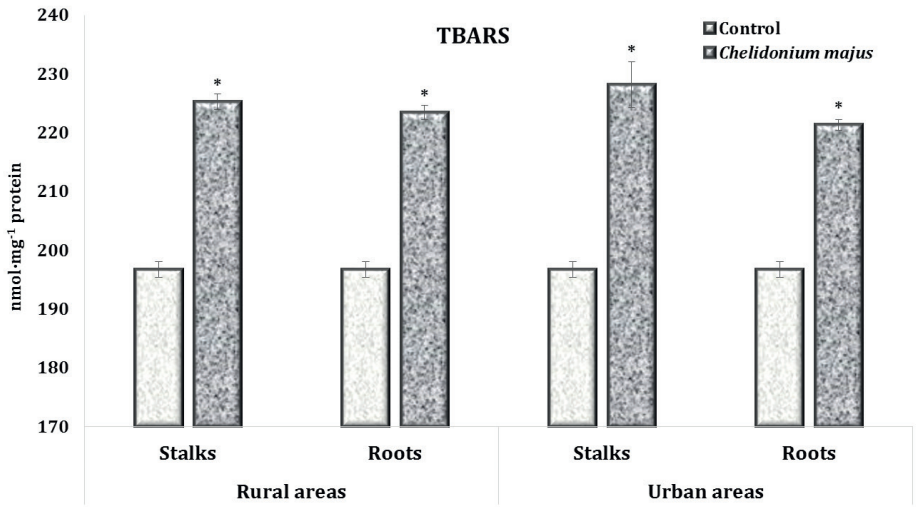


Fig. 2. The content of 2-thiobarbituric acid reactive substances (TBARS) as a biomarker of lipid peroxidation in the muscle tissue of rainbow trout after *in vitro* incubation with extracts derived from stalks and roots of CM collected from urban and rural agglomerations of Pomeranian region (M ± m, n = 8)

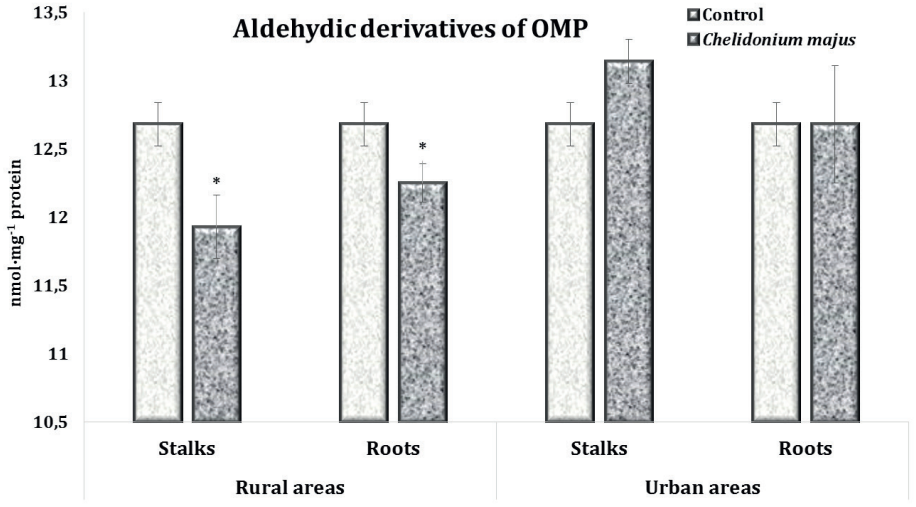


Fig. 3. The content of aldehydic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts derived from stalks and roots of *C. majus* collected from urban and rural agglomerations of Pomeranian region (M ± m, n = 8)

We noted different results after incubation the muscle tissue homogenate with extracts prepared from CM collected from urban areas – increase in the level of ketonic derivatives of OMP for stalk extracts (13.74 ± 0.37 nmol/mg protein) and for root extracts (13.77± 0.65 nmol/mg protein) compared to the control samples (12.4 ± 0.62 nmol/mg protein) (Fig. 4).

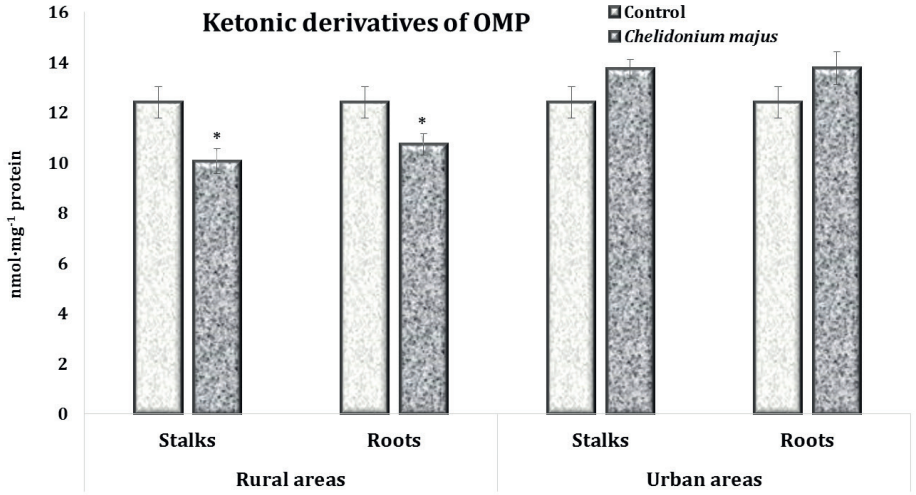


Fig. 4. The content of ketonic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts derived from stalks and roots of *C. majus* collected from urban and rural agglomerations of Pomeranian region (M ± m, n = 8)

Analyzing the results of total antioxidant capacity (TAC) in the muscle tissue of rainbow trout after *in vitro* incubation with CM extracts, we noted a decrease in TAC level for stalk extracts (47.26 ± 3.54%) and root extracts (38.86 ± 3.52%) derived from CM collected from rural agglomerations, while statistical significant reduction occurred only for root extracts compared to the control samples (56.1 ± 1.54%). Stalk and root extracts derived from CM collected from urban areas increased TAC levels, i.e. (58.22 ± 5.24%) and (51.65 ± 5.62%) respectively, compared to untreated controls (Fig. 5).
In our previous study (Stefanowski et al. 2021a), we have determined the antioxidant properties of these extracts using the 2-thiobarbituric acid reactive substances (TBARS) as biomarkers of lipid peroxidation in the *in vitro* study using the blood samples of the rainbow trout. When blood was incubated with root extracts derived from CM collected from urban and rural agglomerations, the lipid peroxidation levels were increased compared to the untreated controls. Stalk extracts derived from CM collected from both rural and urban areas showed the highest inhibitory effect (decrease in lipid peroxidation was 11%, $p < 0,05$ compared to the untreated controls).
Results obtained in our previous study also revealed that there is a possibility of using extracts derived from stalks and roots of CM in aquaculture due to inhibition of protein damage by scavenging free radicals. The lipid peroxidation (TBARS as biomarkers) in the blood of rainbow trout after incubation with extracts derived from stalks and roots

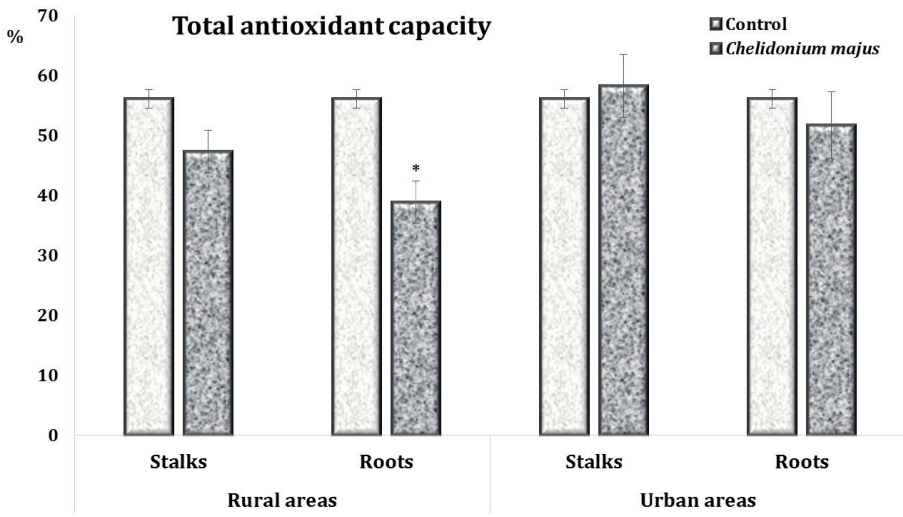


Fig. 5. The total antioxidant capacity in the muscle tissue of rainbow trout after *in vitro* incubation with extracts derived from stalks and roots of *C. majus* collected from urban and rural agglomerations of Pomeranian region (M ± m, n = 8)

of CM showed, that extracts derived only from stalks reduced the TBARS level in the extracts-treated blood, only these results were statistically not significant (Stefanowski et al. 2021b). Furthermore, the use of such plant products as antioxidants and immunostimulants in aquaculture systems may also have environmental value because of their biodegradability. The results of the study suggested the high antioxidant capacity of CM screened give reason to believe that application of these plants signifies a rational curative strategy to prevent and cure various fish diseases involving oxidative stress by increasing the ability of a fish organism to adapt (Stefanowski et al. 2021c, d).

In our previous study (Stefanowski et al. 2021a), we have assessed the oxidative stress biomarkers (TBARS, carbonyl derivatives of OMP, total antioxidant capacity) and activity 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 levels of lipid peroxidation biomarkers (TBARS) 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. Stalk extracts from CM also reduced TBARS levels, but only extracts of CM collected only from the rural areas. The lowest values in the content of the aldehydic derivatives of OMP have been observed after incubation with root extracts 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 stalk extracts of CM collected from both rural and urban areas compared to the control samples, 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 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 root extracts 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 may be helpful in preventing the progression of various levels of oxidative stress (Stefanowski et al. 2021a).

The results of CM offered new insights in the preliminary steps regarding the development of a high-value product for phytomedicine applications through promising metabolic variations with antioxidant and anticancer potentials. Comparative analysis of metabolic variations, antioxidant potential, and cytotoxic effects in different parts of CM was demonstrated by other researchers. Nile and co-workers (2021) have investigated metabolic variations, antioxidant potential, and cytotoxic effects in the different plant parts like the leaf, stem, flower, pod, and root of CM using spectroscopic and chromatographic methods. Total phenolics and flavonoids were studied in the different parts of CM, leaf showed higher flavonoid content (137.43 mg/g), while the pod showed the highest phenolic (23.67 mg/g) content when compared with the stem, flower, and root. In the ABTS 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 assay. Similar to the phospho-molybdenum assay, the flower revealed higher antioxidant activity (46.82%) than the other plant parts. The in vitro SRB assay facilitated the evaluation of the cytotoxic effect against the HeLa and CaSki human cervical cancerous cells. The extract displayed a dose-dependent inhibitory effect on both the cell lines. The highest cytotoxic effect was observed in the pod and flower extracts post 48 h of exposure at 1000 µg/mL. The results of CM offered new insights in 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).

Aerial parts of CM are traditionally used in the treatment of gallstones and dyspepsia, however, several cases of hepatotoxicity are reported. Mazzanti and co-workers (2009) evaluated the effects on liver function of a CMs extract, obtained from the herbal material responsible for one case of hepatotoxicity. Experiments were performed in Wistar rats, after oral administration of doses corresponding to 1.5 and 3g/(kg day) of herbal drug, for 2 or 4 weeks. Blood samples were collected to perform biochemical analysis, whereas liver samples were used for histomorphological and immunohistochemical examination along with the determination of oxidative stress parameters. No significant modification in animal body weight, food consumption, enzyme activities, hepatic histomorphology, and malonic dialdehyde (MDA) formation, at either time or dosage level. Conversely, CM induced a slight but significant decrease in GSH levels and superoxide dismutase (SOD) activity, especially at the high dose. Thus, CM at doses about 50 and 100 times higher than those generally used in humans, does not alter hepatic function. However, the reduction in GSH levels and SOD activity suggests

particular attention in the use of CM or its preparations in situations (pharmacological treatments, physio-pathological conditions, etc.) that can compromise liver function (Mazzanti et al. 2009).

The concentrations of secondary metabolites in CM depend on the phenological stage of the plant. Jakovljevic and co-workers (2013) have investigated the total phenolic content, the concentration of flavonoids, and antioxidant activity in extracts of the CM during different phenological stages (stage of rosette, the initial flowering stage, the stage of fully formed flowers, and stage of fruits formation). Five different extracts of the whole plant, for each phase, were obtained by extraction with water, methanol, acetone, ethyl acetate, and petroleum ether. The concentration of total phenolic content was determined using the Folin-Ciocalteu's reagent and obtained values were the highest in the rosette stage (60.96 mg GA/g). The concentration of flavonoids was the highest in the initial stage of flowering (291.58 mg RU/g). The antioxidant activity was determined *in vitro* using a DPPH reagent. The highest antioxidant activity was expressed in the rosette stage (50.72 mg/ml) (Jakovljevic et al. 2013).

The crude extract of CM was observed to inhibit tumor cells from migration and induce cell cycle arrest and apoptosis. Alkaloids are the active components of CM. Chelidonine, a benzophenanthridine alkaloid, is the major alkaloid in CM with the capability of performing various biological activities, including antitumor, anti-inflammatory, antibacterial, analgesic, insecticide, and spasmolytic effects. Chelidonine inhibits various cancers, including leukemia, liver cancer, breast cancer, lung cancer, and so on (Huang et al. 2020). Also, Paul and co-workers (2013) have evaluated the possible protective potentials of chelidonine and its poly lactide-co-glycolide (PLGA) encapsulated nano-form against cadmium chloride ($CdCl_2$) induced oxidative stress and hepatotoxicity in mice, *ex vivo* and *in vivo*. Acute exposure to $CdCl_2$ (1.0 mg/kg b.w; i.p., twice a week for 30 days) generated oxidative stress in mice through the accumulation of reactive oxygen species and increased lipid peroxidation, and levels of certain liver marker enzymes (alanine aminotransferase ALT, aspartate aminotransferase AST, alkaline phosphatase ALP) with a decrease in levels of GSH and certain other antioxidant enzymes (superoxide dismutase SOD, catalase CAT, glutathione reductase GR) in the liver. Treatment with nano-chelidonine for 30 days after $CdCl_2$ intoxication significantly reduced oxidative stress and lipid peroxidation and restored levels of GSH, cholesterol, triglyceride, and antioxidant enzymes, showing ameliorative changes in histopathology of the liver. The expression pattern of certain inflammatory and apoptotic signal proteins also indicated better hepatoprotective abilities of nano-chelidonine, making it a more suitable protective drug than chelidonine against cadmium toxicity in mice (Paul et al. 2013).

The benzophenanthridine alkaloids sanguinarine and chelerythrine of CM, are potent inhibitors of 5-lipoxygenase in polymorphonuclear leukocytes and 12-lipoxygenase in mouse epidermis, while the activity of soybean lipoxygenase is not influenced. The extract of the herb of CM also inhibits the 5-lipoxygenase. Chelidonine, which cannot form pseudobases, is inactive against lipoxygenase enzymes. Pro- and antioxidant actions of benzophenanthridine alkaloids can be excluded from the lack of deoxyribose degradation, reactivity against free radicals, and inhibition of lipid peroxidation,

suggesting that the inhibitory effects against lipoxygenase enzymes appear to be due to specific enzyme interaction rather than a nonspecific redox mechanism (Vavrecková et al. 1996).

The study of Liao and co-workers (2018) have demonstrated that chelidonine may suppress the LPS-induced inflammatory response both *in vitro* and *in vivo*, which was related to TLR4/NF-κB signaling pathway disturbed by chelidonine. Chelidonine significantly suppressed lipopolysaccharide (LPS)-induced the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2), as well as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) mRNA and protein expression. In addition, pro-inflammatory cytokines induced by LPS, such as tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) were also attenuated by chelidonine. What's more, LPS-induced activation and degradation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) followed by translocation of the p65 from the cytoplasm to the nucleus were attenuated by chelidonine. Furthermore, chelidonine even significantly inhibited Toll-like receptor 4 (TLR4) expression induced by LPS. Finally, chelidonine strikingly decreased serum TNFα, IL-6 and PGE_2 levels in LPS stimulated mice (Liao et al. 2018). Also, the neuroprotective effect of ethanolic extract of CM may be mediated through its antioxidant and free radical scavenging activity as proved by the data on ferric-reducing power ability and DPPH radical scavenging activity (Ra Kasem et al. 2022).

Nawrot and co-workers (2016) confirmed the presence of protein components of the antioxidant defense system in CM latex. These proteins are the first line of defense against various stress conditions and help to prevent attacks by various pathogens that are present in large quantities in the milk juice.

CONCLUSIONS

Our results revealed that extracts derived from greater celandine, mainly collected from rural agglomerations of the Pomeranian region, can effectively inhibit the formation of carbonyl derivatives of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation. On the other hand, aqueous extracts derived from greater celandine collected from both rural and urban areas increased lipid peroxidation in the muscle tissue of rainbow trout after *in vitro* incubation in studied dose (statistically significant increases in TBARS levels by 15%, $p < 0.05$), which may be a potential source of useful components worthy of further investigation, including in cytotoxic studies. A decrease of the total antioxidant capacity in muscle tissue of rainbow trout after *in vitro* incubation with CM extracts derived from greater celandine collected from rural areas was observed. Therefore, future research can focus on the characterization of the active components and the effect of herb–herb combinations for future therapeutic advancements and pharmaceutical product development.

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OKSYDACJA LIPIDÓW I BIAŁEK W TKANCE MIĘŚNIOWEJ PSTRĄGA TĘCZOWEGO PO INKUBACJI *IN VITRO* Z EKSTRAKTAMI Z GLISTNIKA JASKÓŁCZE ZIELE (*CHELIDONIUM MAJUS* L.) ZEBRANEGO Z WIEJSKICH I MIEJSKICH TERENÓW POMORZA

Streszczenie

Zgodnie z naszymi wcześniejszymi badaniami kontynuujemy analizy dotyczące oceny potencjału antyoksydacyjnego przedstawicieli rodziny Papaveraceae zebranych z regionu przybrzeżnego północnej Polski, wykorzystując różne modele komórkowe, a mianowicie tkankę mięśniową pstrąga tęczowego. Dlatego też w obecnych badaniach biomarkery stresu oksydacyjnego [substancje reaktywne reagujące z kwasem 2-tiobarbiturowym (TBARS), karbonylowe pochodne oksydacyjnej modyfikacji białek (OMP), całkowita aktywność antyoksydacyjna (TAC)] zostały zastosowane do oceny aktywności antyoksydacyjnej ekstraktów uzyskanych z łodyg i korzeni glistnika jaskółcze ziele *Chelidonium majus* L. (CM) w dawce końcowej 2,5 mg/mL. W badaniach wykorzystano tkankę mięśniową pstrąga tęczowego (*Oncorhynchus mykiss* Walbaum). Supernatant z tkanki mięśniowej został użyty do inkubacji z ekstraktami uzyskanymi z łodyg i korzeni CM (w stosunku 39:1, stężenie końcowe 2,5 mg/mL) w temperaturze pokojowej. Próbki kontrolne (tkanka mięśniowa) inkubowano z 100 mM buforem Tris-HCl (pH 7,2) (w stosunku 39:1). Wyniki obecnych badań wykazały, że ekstrakty pozyskane z łodyg i korzeni CM wykazują działanie cytotoksyczne na struktury komórkowe tkanki mięśniowej poprzez zwiększenie poziomu peroksydacji lipidów i zmniejszenie całkowitej aktywności antyoksydacyjnej. Wyniki te sugerują możliwość stosowania ekstraktów z CM w końcowej dawce 2,5 mg/mL w celu osiągnięcia efektów prooksydacyjnych i uzasadniają dalsze badania, aby ocenić ich potencjał terapeutyczny. Poziom aldehydowych i ketonowych pochodnych OMP był istotnie obniżony po inkubacji *in vitro* z ekstraktami z łodyg i korzeni CM zebranych wyłącznie z aglomeracji wiejskich. Badania roślin pod kątem innych aktywności biologicznych, w tym aktywności antyoksydacyjnej, są niezbędne i mogą być skuteczne w poszukiwaniu środków zapobiegawczych w patogenezie niektórych chorób, a także w profilaktyce i leczeniu niektórych schorzeń w medycynie i weterynarii.