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BIOMARKERS OF LIPID AND PROTEIN OXIDATION IN THE MUSCLE TISSUE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) TREATED *IN VITRO* WITH ROOT AND STEM EXTRACTS OF GREATER CELANDINE (*CHELIDONIUM MAJUS* L.)

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The main aim of the present study was to evaluate the oxidative stress biomarkers [TBARS, carbonyl derivatives of oxidative modification of proteins (OMP), total antioxidant capacity (TAC)] in the muscle tissue of rainbow trout (Oncorhynchus mykiss Walbaum) after in vitro incubation with the root and stem extracts derived from greater celandine (Chelidonium majus L., CM) (at final concentrations of 5 and 2.5 mg/mL) collected in South Park in Słupsk in the Pomeranian Province (northern part of Poland). The current study demonstrated the increase in TBARS levels after in vitro incubation of rainbow trout muscle tissue with stem and root extracts of CM at a final concentration of 5 mg·mL^{-1} compared to untreated control samples. There was a statistically significant increase in TBARS levels compared to controls. We obtained similar results after in vitro incubation with root and stem extracts of CM at a final concentration of 2.5 mg·mL^{-1} with rainbow trout muscle tissue, where we also observed a statistically non-significant increase in TBARS levels. There was a decrease in the levels of aldehydic derivatives and ketonic derivatives of OMP in muscle tissue after incubation with extracts from roots and stems of CM at final concentrations of 5 mg·mL^{-1} compared to untreated controls. There was a decrease in the levels of aldehydic and ketonic derivatives of OMP in muscle tissue after incubation with extracts from roots and stems of CM at final concentrations of 2.5 mg·mL^{-1} compared to untreated controls. TAC levels in rainbow trout muscle tissue after in vitro incubation with extracts of CM roots and stems at final concentrations of 5 and 2.5 mg·mL^{-1} were not statistically significantly increased. The present study investigated the antioxidant potential of CM. Extracts from CM roots and stems exert their activity by inhibiting protein damage.

Key words: *Greater celandine (Chelidonium majus L.), rainbow trout (Oncorhynchus mykiss Walbaum), lipid peroxidation, oxidatively modified proteins, total antioxidant capacity*

БІОМАРКЕРИ ОКИСНЕННЯ ЛІПІДІВ І БІЛКІВ У М'ЯЗОВІЙ ТКАНИНІ РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS* WALBAUM) ПІСЛЯ ІНКУБАЦІЇ *IN VITRO* З ЕКСТРАКТАМИ КОРЕНІВ І СТЕБЕЛ ЧИСТОТІЛУ ВЕЛИКОГО (*CHELIDONIUM MAJUS* L.)

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Метою цього дослідження була оцінка in vitro біомаркерів окиснювального стресу (продукти, які реагують з 2-тіобарбітуровою кислотою (TBARS), карбонільні похідні окиснювально змодифікованих білків (ОМБ), загальна антиоксидантна активність (ТАС)) у м'язовій тканині райдужної форелі (Oncorhynchus mykiss Walbaum) після інкубації з екстрактами коренів і стебел, отриманими з чистотілу великого (Chelidonium majus L., СМ) (у кінцевих концентраціях 5 і 2,5 мг/мл), зібраних у Південному парку в Слупську (Поморське воєводство, північна частина Польщі). У цьому дослідженні ми продемонстрували підвищення рівня TBARS після in vitro інкубації м'язової тканини райдужної форелі з екстрактами стебел та коренів СМ у кінцевій концентрації 5 мг·мл$^{-1}$ порівняно з необробленими контрольними зразками. Отримано статистично істотне збільшення рівня TBARS порівняно з контролем. Ми отримали подібні результати після in vitro інкубації м'язової тканини райдужної форелі з екстрактами коренів та стебел СМ у кінцевій концентрації 2,5 мг·мл$^{-1}$, де також виявлено статистично неістотне підвищення рівня TBARS. Спостерігали зниження рівнів альдегідних і кетонових похідних ОМБ у м'язовій тканині після інкубації з екстрактами коренів і стебел СМ в кінцевих концентраціях 5 мг·мл$^{-1}$ та 2,5 мг·мл$^{-1}$ порівняно з необробленим контролем. Продемонстровано також статистично неістотне підвищення рівнів ТАС у м'язовій тканині райдужної форелі після in vitro інкубації з екстрактами коренів і стебел СМ у кінцевих концентраціях 5 і 2,5 мг·мл$^{-1}$. Таким чином, екстракти з коренів і стебел СМ проявляють свою активність, пригнічуючи пошкодження білків у м'язовій тканині райдужної форелі.

Ключові слова: *Чистотіл великий (Chelidonium majus L.), райдужна форель (Oncorhynchus mykiss Walbaum), перекисне окиснення ліпідів, окиснювально модифіковані білки, загальна антиоксидантна активність*

Herbal medicines, including crude herbs, herbal compounds, crude herbal extracts and herbal active ingredients, are widely known for their unique fragrance, taste or therapeutic properties. Herbal medicines are widely used to improve health and resist disease in humans, livestock and poultry (Mosihuzzaman M., 2012; Valladão G. M. et al., 2015). In recent decades, they have also been widely used in fish aquaculture and have become a preferred therapy to replace antibiotics and chemicals because of their excellent therapeutic efficacy, low toxicity, few adverse effects, diverse drug targets and less possibility of developing drug resistance (Reverter M. et al., 2014). Herbs can be used in many ways, such as garden fresh, dried, powdered, juices or extracted (in vari-

ous solvents such as water, alcohol, acetone, ether, etc.) or essential oils. Herbal medicines are used either as decoctions (single) or concoctions (mixed) or in combination with other medicines for effective fish health management (Harikrishnan R. 2003).

Several studies have shown the importance of a strong immune system in commercial fish species to minimise mortality during fish disease outbreaks. These agents have the potential to enhance the host's immune system to prevent disease infection or for treatment purposes. Today, phytotherapeutic agents are synthesised in combination with natural ingredients, especially naturally occurring compounds found in plants. Immunostimulants are mainly given to fish to boost their immune system and make them more resistant to disease (Galina J. et al., 2019; Liao W. et al., 2022; Zhang W. et al., 2022). Compared to chemical drugs and antibiotics, medicinal plants have fewer side effects, cause little drug resistance and have low toxicity to the aquatic environment. Most medicinal plants can effectively improve the growth performance of aquatic animals and are therefore becoming increasingly valued and widely used in aquaculture (Liao W. et al., 2022).

Chelidonium majus L. (Papaveraceae), or greater celandine, is an important plant in Western phytotherapy and traditional Chinese medicine. Crude extracts of *C. majus* (CM), as well as purified compounds derived from it, exhibit a wide range of biological activities (anti-inflammatory, antimicrobial, antitumour, analgesic, hepatoprotective, etc.) that support some of the traditional uses of CM (Gilca M. et al., 2010; Capistrano I. R. et al., 2015; Mikołajczak P. Ł. et al., 2015). However, herbal medicine also claims that this plant has some important properties that have not yet been scientifically investigated, i.e. CM possesses diuretic, antitussive and ocular regenerative effects (Zielińska S. et al., 2018, 2019). On the other hand, CM also has scientifically proven effects, such as anti-osteoporotic activity and radioprotection, which are not mentioned in traditional sources (Boyko V. N. & Zholus R. B. 1998; Gilca M. et al., 2010).

In the present study, 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 to assess the antioxidant activity of root and stem extracts (at final concentrations of 5 and 2.5 mg/ml) derived from CM collected in Słupsk (Pomeranian Province, northern part of Poland). The current research is funded by the Ministry of Science and Higher Education (Poland). This study was carried out as part of the project "*Greater Celandine (Chelidonium majus L.) as a source of bioactive substances for pharmaceutical use*" (Student Science Associations Create Innovations programme, 2023-2024).

Materials and methods.

Plant materials. The plant materials were collected from natural habitats on the territory of the South Park in Słupsk (54°28′08,5″N 17°02′56,0″E) in the Pomeranian Province (northern part of Poland). This area has been adapted for recreational purposes by creating a guarded swimming area, a permanent fireplace, benches and baskets, a place for camping and physical games, an access road and a car park. The collected roots and stems were taken to the laboratory for biochemical analysis. Freshly washed plant samples were weighed, crushed and homogenised in 0.1M phosphate buffer (pH 7.4) (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 an average body weight of 110-150 g were used in the experiments. Fish sampling for the current study was carried out at the Department of Salmonid Research, Stanisław

Sakowicz Inland Fisheries Institute in Olsztyn (Rutki, Poland). The experiments were conducted in water at 14.5 ± 0.5 °C and pH 7.2-7.4. The dissolved oxygen level was approximately 9 ppm with supplemental oxygen supply, with a water flow of 25 L/min and a photoperiod of 12 h per day. The same experimental conditions were used throughout the study. Water parameters were continuously monitored. Fish were housed in square tanks (150 fish per tank) and fed a commercial pelleted diet.

Muscle tissues were collected after decapitation of the fish. Minced muscle tissue was rinsed from blood with cold isolation buffer (100 mM Tris-HCl, pH 7.2) and homogenised on ice in an H500 homogeniser using a motorised pestle. The homogenates were centrifuged at 3,000 rpm for 15 minutes at 4°C. After centrifugation, the supernatant was collected and frozen at -25°C until analysis. Protein content was determined by the method described by Bradford (1976) using bovine serum albumin as standard (Bradford M. M. 1976). The absorbance was recorded at 595 nm. All enzymatic assays were performed at 22 ± 0.5 °C (n = 8). Reactions were initiated by the addition of tissue supernatant.

Experimental design. The supernatant of the muscle tissue was used to incubate with extracts obtained from roots and stems of CM (with a final plant concentration in extracts of 5 and 2.5 mg per mL) at room temperature. The untreated control 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 were studied in the incubated homogenate (control untreated group and in samples with extracts obtained from roots and stems 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 reactive substances (TBARS) to determine the concentration of malonic dialdehyde (MDA) (Kamyshnikov V. S., 2004). This method is based on the reaction of the degradation product of lipid peroxidation, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to form a coloured adduct which is measured spectrophotometrically. The nmol of MDA per mg of protein was calculated using an extinction coefficient of $1.56 \cdot 10^5$ mM^{-1} cm^{-1}.

Carbonyl derivatives of protein oxidative modification (OMP) content. In order to evaluate the protective effects of extracts derived from stems and roots of CM against free radical-induced protein damage in muscle samples, an assay for the content of carbonyl derivatives of protein oxidative modification (OMP) was performed based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the samples. The rate of oxidative modification of proteins was estimated from the reaction of the resulting carbonyl derivatives of the amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine R. L. and co-workers (1990) and modified by Dubinina E. E. and co-workers (1995). DNFH was used to determine the carbonyl content of 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 level of TAC in the samples was estimated by measuring the level of 2-thiobarbituric acid reactive substances (TBARS) after oxidation of Tween 80. This level was determined spectrophotometrically at 532 nm (Galaktionova L. P. et al., 1998). The content of TAC in the sample (%) was calculated with reference to the absorbance of the blank.

Statistical analysis. The mean ± S.E.M. values were calculated for each group to determine the significance of the difference between the groups. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors tests (p

>0.05). The significance of differences between levels of oxidative stress biomarkers (significance level, p <0.05) was tested using the Mann-Whitney *U* test (Zar J. H. 1999). All statistical calculations were performed on separate data from each individual using STATISTICA 13.3 software (TIBCO Software Inc., USA).

Results. Figure 1 shows TBARS levels in rainbow trout muscle tissue after *in vitro* incubation with extracts from CM roots and stems at final concentrations of 5 and 2.5 mg·mL^{-1}. When TBARS levels were analysed after *in vitro* treatment with CM extracts, the following results were obtained. There was an increase in TBARS levels after *in vitro* incubation of rainbow trout muscle tissue with stem and root extracts of CM at a final concentration of 5 mg·mL^{-1} (69.23 ± 2.89 and 68.61 ± 3.15 nmol·mg^{-1} protein) compared with untreated control samples (58.71 ± 2.12 nmol·mg^{-1} protein). There was a statistically significant increase in TBARS levels of 16.9% and 17.9% (p < 0.05) respectively compared to controls (Fig. 1).

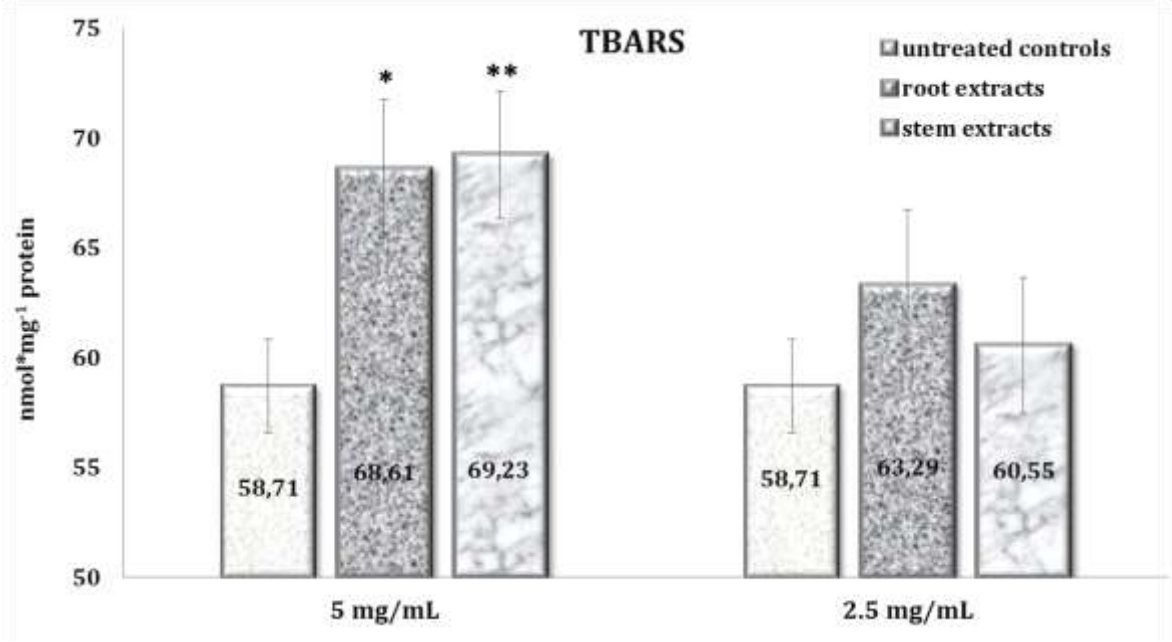

Fig. 1. **TBARS levels as a biomarker of lipid peroxidation in rainbow trout muscle tissue after *in vitro* incubation with root and stem extracts of greater celandine (*Chelidonium majus* L.) at final concentrations of 5 and 2.5 mg·mL^{-1} (M ±m, n = 8).**

** and ** – changes are statistically significant compared to the untreated controls.*

Similar results were obtained after *in vitro* incubation of rainbow trout muscle tissue with root extracts of CM at a final concentration of 2.5 mg·mL^{-1}, where we also observed a statistically non-significant increase in TBARS of 7.81% (p > 0.05) compared with control samples (63.29 ± 3.44 nmol·mg^{-1} protein *vs.* 58.71 ± 2.12 nmol·mg^{-1} protein). Similar trends were observed after *in vitro* incubation of rainbow trout muscle tissue with stem extracts of CM at a final concentration of 2.5 mg·mL^{-1}. The use of stem extracts of CM (60.55 ± 3.11 nmol·mg^{-1} protein) resulted in a statistically non-significant increase in TBARS levels (by 3.14%, p > 0.05) compared to control samples (58.71 ± 2.12 nmol·mg^{-1} protein) (Fig. 1).

The levels of aldehydic and ketonic derivatives of oxidatively modified proteins in rainbow trout muscle tissue after in vitro incubation with root and stem extracts of greater celandine at final concentrations of 5 and 2.5 mg·mL^{-1} are shown in Figure 2.

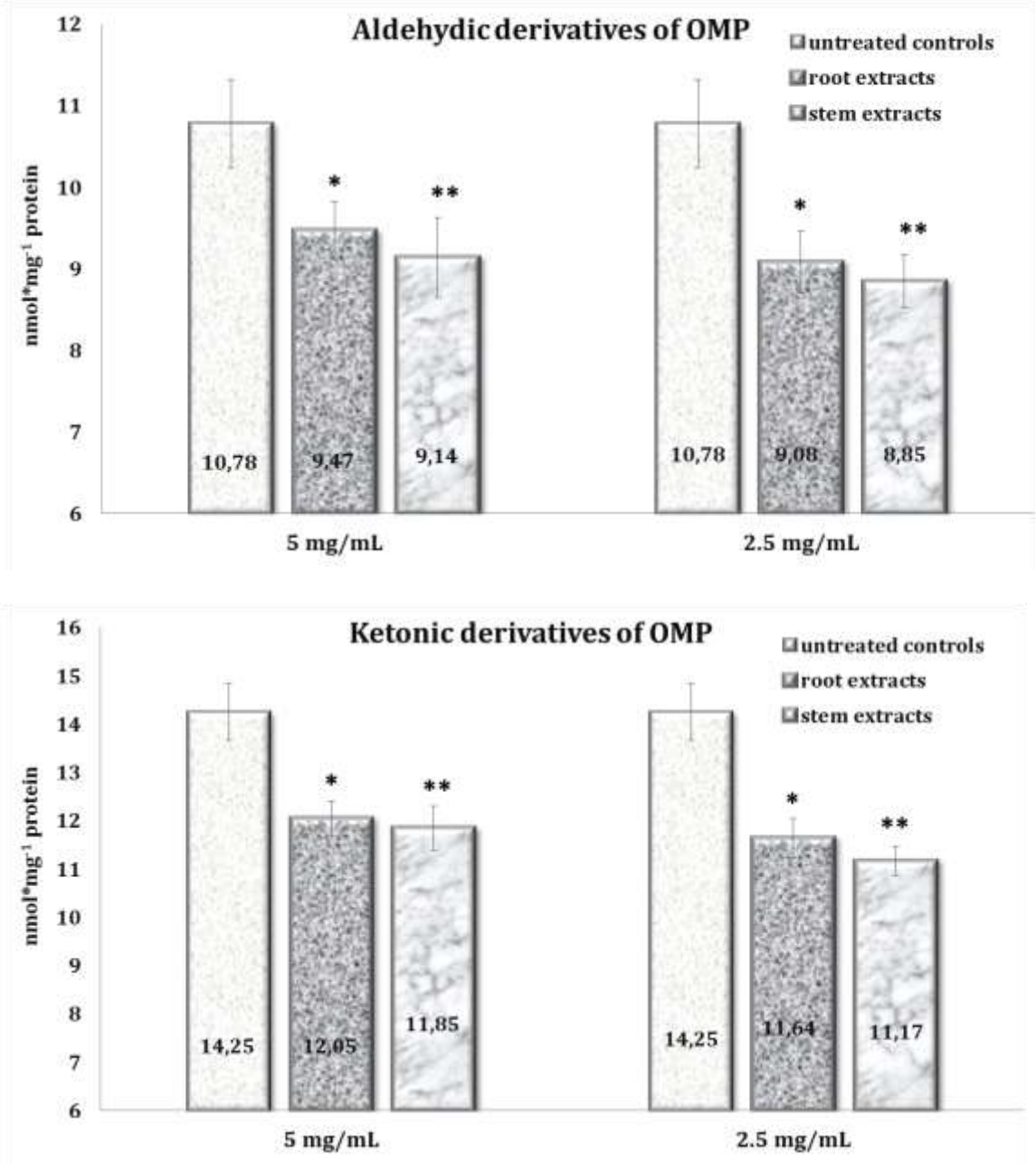

Fig. 2**. Levels of aldehydic and ketonic derivatives of oxidatively modified proteins in rainbow trout muscle tissue after *in vitro* incubation with root and stem extracts of greater celandine (*Chelidonium majus* L.) at final concentrations of 5 and 2.5 mg·mL^{-1} (M ±m, n = 8).**

** and ** – changes are statistically significant compared to the untreated controls.*

When analysing the level of protein oxidation after *in vitro* incubation of rainbow trout muscle tissue with celandine extracts at final concentrations of 5 mg·mL^{-1}, we found a statistically significant decrease in the levels of aldehydic and ketonic derivatives of oxidatively modified proteins for stem extracts (9. 14 ± 0.49 and 11.86 ± 0.46 nmol·mg^{-1} protein) and root extracts (9.47 ± 0.35 and 12.05 ± 0.36 nmol·mg^{-1} protein) compared to untreated control samples (10.78 ± 0.54 and 14.25 ± 0.59 nmol·mg^{-1} protein) (Fig. 2). There was a decrease of 12.16% and 15.22% ($p < 0.05$) in the levels of aldehydic derivatives of OMP in muscle tissue after incubation with extracts from roots and stems of CM at final concentrations of 5 mg·mL^{-1} compared to untreated controls.

Similarly, for extracts from roots and stems of CM at final concentrations of 5 mg·mL^{-1}, a decrease in the levels of ketonic derivatives of OMP in muscle tissue of 15.44% and 16.78%, respectively, was observed ($p < 0.05$) compared to untreated controls (Fig. 2).

Also, for extracts at final concentrations of 2.5 mg·mL^{-1}, we found a statistically significant decrease in the levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle tissue for stem extracts (8.85 ± 0.33 and 11.17 ± 0.31 nmol·mg^{-1} protein) and root extracts (9.08 ± 0.38 and 11.64 ± 0.41 nmol·mg^{-1} protein) compared to the untreated control samples (10.78 ± 0.54 and 14.25 ± 0.59 nmol·mg^{-1} protein). There was a decrease of 15.77% and 17.91% ($p < 0.05$) in the levels of aldehydic derivatives of OMP in the muscle tissue after incubation with extracts from roots and stems of CM at final concentrations of 2.5 mg·mL^{-1} compared to untreated controls. Similarly, for extracts from roots and stems of CM at final concentrations of 2.5 mg·mL^{-1}, a decrease in the levels of ketonic derivatives of OMP in muscle tissue of 18.31% and 21.62% ($p < 0.05$), respectively, was observed compared to untreated controls (Fig. 2).

The total antioxidant capacity in the muscle tissue of rainbow trout after *in vitro* incubation with extracts from the roots and stems of greater celandine at final concentrations of 5 and 2.5 mg·mL^{-1} is shown in Figure 3.

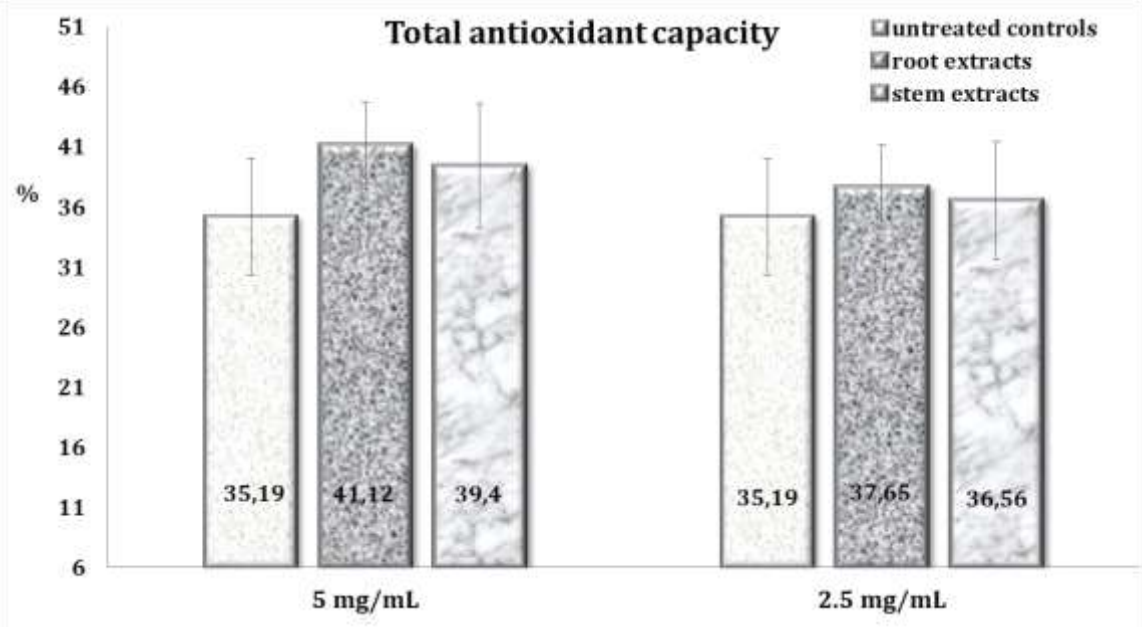

Fig. 3. **Total antioxidant capacity in the muscle tissue of rainbow trout after *in vitro* incubation with extracts derived from roots and stems of greater celandine (*Chelidonium majus* L.) at final concentrations of 5 and 2.5 mg·mL^{-1} (M ±m, n = 8).**

Figure 3 shows TAC levels in rainbow trout muscle tissue after *in vitro* incubation with extracts from CM roots and stems at final concentrations of 5 and 2.5 mg·mL^{-1}. When TAC levels were analysed after *in vitro* treatment with CM extracts, the following results were obtained. There was an increase in TAC levels after *in vitro* incubation of rainbow trout muscle tissue with stem and root extracts of CM at a final concentration of 5 mg·mL^{-1} (39.40 ± 5.17 and 41.12 ± 3.62 %) compared to the untreated control samples (35.19 ± 4.88 %). There was a statistically non-significant increase in TAC levels of 12% and 16.9% respectively ($p > 0.05$) compared to controls (Fig. 3).

Similar results were obtained after *in vitro* incubation of rainbow trout muscle tissue with root extracts of CM at a final concentration of 2.5 mg·mL^{-1}, where we also

observed a statistically non-significant increase in TAC of 7% ($p > 0.05$) compared with control samples (37.65 ± 3.50% *vs*. 35.19 ± 4.88%). Similar trends were observed after *in vitro* incubation of rainbow trout muscle tissue with stem extracts of CM at a final concentration of 2.5 mg·mL^{-1}. The use of stem extracts of CM (36.56 ± 4.91 %) resulted in a statistically non-significant increase in TAC levels (by 3.9%, $p > 0.05$) compared to the control samples (35.19 ± 4.88 %) (Fig. 3).

Discussion. The main aim of the present study was to evaluate the oxidative stress biomarkers [TBARS, carbonyl derivatives of oxidative modification of proteins (OMP), total antioxidant capacity] in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after *in vitro* incubation with the root and stem extracts derived from CM (at final concentrations of 5 and 2.5 mg/ml) collected in Słupsk.

The imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant capacity is referred to as oxidative stress. Free radicals can also be generated in living organisms as a result of external factors (e.g. UV radiation, ionising radiation) and during the defence reactions of the body's immune system (Jones D. P., 2008). Free radicals are also produced during normal physiological processes in various compartments of the cell. Under physiological conditions, these processes are tightly controlled by the body through the action of enzymatic and non-enzymatic defence mechanisms (Forman H. J. & Zhang H., 2021). Free radicals are also known to mediate important cellular functions such as cell growth, proliferation, differentiation and apoptosis (Pizzino G. et al., 2017). Disturbance of these mechanisms under the influence of various pathogenic factors or external influences leads to a significant increase in the concentration of radicals in the body and, consequently, to the occurrence of pathological reactions leading to cell and tissue damage (Apel K. & Hirt H., 2004). The destructive effects of radicals can affect virtually all biomolecules present in the body, causing damage at the molecular level and to cellular organelles. *In vitro*, free radicals cause chemical modifications and damage proteins (aggregation and denaturation), lipids (peroxidation), carbohydrates and nucleotides, inducing changes in the structure of DNA leading to mutations or cytotoxic effects, etc. (Sheu S. S. et al., 2006). The 2-thiobarbituric acid reactive substances (TBARS) assay is widely used as a general measure of lipid peroxidation in biological fluids. It is often considered to be a good indicator of the level of oxidative stress within a biological sample, provided that the sample has been properly handled and stored (Aguilar Diaz De Leon J. & Borges C. R., 2020).

The results of the current study showed an increase in TBARS levels after in vitro incubation of rainbow trout muscle tissue with stem and root extracts of CM at a final concentration of 5 mg·mL^{-1} compared to untreated control samples. There was a statistically significant increase in TBARS levels of 16.9% and 17.9% ($p < 0.05$) respectively compared to controls. We obtained similar results after *in vitro* incubation with root and stem extracts of CM at a final concentration of 2.5 mg·mL^{-1} with rainbow trout muscle tissue, where we also observed a statistically non-significant increase in TBARS levels.

Oxidative changes in proteins are an inherent effect of aerobic cellular metabolism and cannot be eliminated despite numerous protective systems. The accumulation of oxidised protein products impairs cell function and can even lead to cell death (Sies H., 2021). There was a decrease of 12.16% and 15.22% ($p < 0.05$) in the levels of aldehydic derivatives of OMP in muscle tissue after incubation with extracts from roots and stems of CM at final concentrations of 5 mg·mL^{-1} compared to untreated controls. Similarly, for extracts from roots and stems of CM at final concentrations of 5 mg·mL^{-1}, a decrease in the levels of ketonic derivatives of OMP in muscle tissue of 15.44% and 16.78%, respectively, was observed ($p < 0.05$) compared to untreated controls. After

incubation with extracts from roots and stems of CM at final concentrations of 2.5 mg·mL^{-1}, there was a decrease in the levels of aldehydic derivatives of OMP in muscle tissue of 15.77% and 17.91% ($p < 0.05$), respectively, compared to untreated controls. Similarly, for extracts from CM roots and stems at final concentrations of 2.5 mg·mL^{-1}, a decrease in the levels of ketone derivatives of OMP in muscle tissue of 18.31% and 21.62%, respectively, was observed compared to untreated controls ($p < 0.05$). There was no statistically significant increase in TAC levels in rainbow trout muscle tissue after *in vitro* incubation with extracts of CM roots and stems at final concentrations of 5 and 2.5 mg·mL^{-1}.

The results of the CM study provided new insights in the preliminary steps towards the development of a high value product for phytomedicine applications through promising metabolic variations with antioxidant and anticancer potentials. Comparative analysis of the metabolic variations, antioxidant potential and cytotoxic effects of different parts of CM has been demonstrated by other researchers. For example, Nile S. H. and co-workers (2021) used spectroscopic and chromatographic methods to investigate metabolic variations, antioxidant potential and cytotoxic effects in different plant parts such as leaf, stem, flower, pod and root of CM. Total phenolics and flavonoids were analysed in the different parts of the CM, leaf showed higher flavonoid content (137.43 mg·g^{-1}), while the pod showed the highest phenolic content (23.67 mg·g^{-1}) compared to the stem, flower and root. In the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) antioxidant assay, the flower extract showed 57.94% activity, while the leaf, pod and root extracts showed 39.10%, 36.08% and 28.88% activity respectively. The pod and leaf extracts showed potential activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with 45.46% and 41.61% activity respectively. Similar to the phosphomolybdenum assay, the flower showed higher antioxidant activity (46.82%) than the other parts of the plant. The *in vitro* sulforhodamine B (SRB) assay facilitated the evaluation of the cytotoxic effect against HeLa and CaSki human cervical cancer cells. The extract showed a dose-dependent inhibitory effect on both cell lines. The highest cytotoxic effect was observed in the pod and flower extracts after 48 h exposure at 1000 µg/mL. The results of the CM provided new insights in the preliminary steps towards the development of a high-value product for phytomedicine applications through promising metabolic variations with antioxidant and anticancer potentials (Nile S. H. et al., 2021).

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

Ra Kasem N. and co-workers (2022) investigated the protective effect of CM ethanolic extract (CMEE) on aflatoxin B1 (AFB1)-induced neurotoxicity in rats. CMEE exhibited antioxidant activity *in vitro* and neuroameliorative efficacy *in vivo*, as its administration in combination with AFB1 was able to significantly downregulate the elevated levels of inflammatory and apoptotic markers and restore the values of neuro-

chemical markers (AChE-ase, dopamine, and serotonin) that were worsened by AFB1 intake. The neuroprotective effect of CMEE may be mediated by its antioxidant and free radical scavenging activity, as evidenced by data on iron-reducing power and DPPH radical scavenging activity (Ra Kasem N. et al., 2022).

Two new lignanamides, majusamides A and B (1 and 2), and two new alkaloids, chelidoniumine (3) and tetrahydrocoptisine N-oxide (4), together with six known hydroxycinnamic acid amides (HCCAs) were isolated from the 75% ethanol extract of CM by Huang X. Y. and co-workers (2019). The anti-inflammatory activities of all isolates on NO production in lipopolysaccharide (LPS)-induced macrophages were evaluated. Compounds 7 and 9 showed moderate inhibitory activity with IC_{50} values of 25.3 ± 0.5 and 23.5 ± 1.7 μM, respectively (Huang X. Y. et al., 2019). A new alkaloid, methyl 2'-(7,8-dihydrosanguinarine-8-yl)acetate (1), together with six known alkaloids, stylopine (2), protopine (3), norchelidonine (4), chelidonine (5), berberine (6) and 8-hydroxydihydrosanguinarine (7), were isolated from CM by Park J. E. and co-workers (2011). The anti-inflammatory activity of the isolates was evaluated by their inhibitory effects on LPS-induced NO production in RAW264.7 macrophage cells. Among them, compounds 5 and 7 showed potent inhibitory activities against LPS-induced NO production in RAW264.7 macrophage cells with IC_{50} values of 7.3 and 4.5 μM, respectively. In addition, compounds 5 and 7 inhibited the induction of COX-2 and iNOS mRNA in a dose-dependent manner, indicating that these compounds attenuated the synthesis of these transcripts at the transcriptional level (Park J. E. et al., 2011). Vavrecková C. and co-workers (1996) showed that benzophenanthridine alkaloids of CM inhibit 5- and 12-lipoxygenase by a non-redox mechanism. Pro- and antioxidant effects of benzophenanthridine alkaloids can be excluded from the lack of deoxyribose degradation, reactivity towards free radicals and inhibition of lipid peroxidation, suggesting that the inhibitory effects against LO enzymes appear to be due to specific enzyme interaction rather than a non-specific redox mechanism (Vavrecková C. et al., 1996).

The extract of CM had a strong antioxidant potential and exerted antiproliferative activity *via* apoptosis on leukaemia cells. Nadova S. and co-workers (2008) investigated whether a methanol extract isolated from the greater celandine (CME) had antioxidant activity and was able to inhibit proliferation and induce apoptosis in leukaemia cells *in vitro*. The potential antioxidant activity of CME was demonstrated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The cytotoxicity of CME was measured by the cell growth inhibition assay using the murine leukaemia cell line L1210 and human promyelocytic leukaemia cells HL-60. The apoptosis-inducing effect was determined by fluorescence microscopy (chromatin condensation and nuclear DNA fragmentation). In the DPPH assay, CME acted as a scavenger of the free radical DPPH. The results of the anti-proliferative assay clearly showed that CME had a cytotoxic effect on both leukaemia cell lines in a dose-dependent manner. In addition, the human promyelocytic HL-60 cells were more sensitive to CME treatment than the L1210 cells. Due to the presence of isoquinoline alkaloids and flavonoid components, CME may play an important role in cancer chemoprevention through its antioxidant activity as well as in modern cancer chemotherapy as a cytotoxic and apoptosis-inducing agent (Nadova S. et al., 2008).

Conclusions. The current study demonstrated the increase in TBARS levels after *in vitro* incubation of rainbow trout muscle tissue with stem and root extracts of CM at a final concentration of 5 $mg \cdot mL^{-1}$ compared to untreated control samples. There was a statistically significant increase in TBARS levels compared to controls. We obtained similar results after *in vitro* incubation with root and stem extracts of CM at a final con-

centration of 2.5 mg·mL^{-1} with rainbow trout muscle tissue, where we also observed a statistically non-significant increase in TBARS levels.

There was a decrease in the levels of aldehydic derivatives and ketonic derivatives of OMP in muscle tissue after incubation with extracts from roots and stems of CM at final concentrations of 5 mg·mL^{-1} compared to untreated controls. There was a decrease in the levels of aldehydic and ketonic derivatives of OMP in muscle tissue after incubation with extracts from roots and stems of CM at final concentrations of 2.5 mg·mL^{-1} compared to untreated controls. TAC levels in rainbow trout muscle tissue after *in vitro* incubation with extracts of CM roots and stems at final concentrations of 5 and 2.5 mg·mL^{-1} were not statistically significantly increased. The present study investigated the antioxidant potential of CM. Extracts from CM roots and stems exert their activity by inhibiting protein damage.

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