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Biomarkers of Oxidative Stress and Antioxidant Defence in Muscle Tissue of *Acipenser oxyrinchus* Oxyrinchus Mitchill after *in vitro* Treatment with Extracts from the Roots and Stems of *Chelidonium majus* L.

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Greater celandine (*Chelidonium majus* L., GC) exhibits a range of biological activities that support its traditional use in folk medicine and highlight its potential therapeutic applications in modern healthcare. This study was designed to investigate the effects of GC root and stem extracts on lipid peroxidation, oxidative protein modification, total antioxidant capacity (TAC) and antioxidant enzyme activities (superoxide dismutase, SOD, catalase, glutathione peroxidase, GPx) in Atlantic sturgeon muscle tissue after *in vitro* treatment. The plant material (stems and roots) was collected from natural habitats on the territory of the South Park in Słupsk, Pomeranian Province (northern part of Poland). The supernatant obtained from Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill) muscle tissue was used for incubation with GC root and stem extracts *in vitro* (at a final concentration of 5 mg·mL^{-1}) at room temperature. The untreated control samples (muscle tissue) were incubated with 100 mM Tris-HCl buffer (pH 7.2) in the same way as the GC-treated samples. The incubation time was 2 hours. Biomarkers of lipid peroxidation and protein oxidation, TAC, and antioxidant enzyme activities were analysed in the incubated homogenates (control untreated samples and samples treated with GC root and stem extracts). We observed an increase in the levels of aldehyde and ketone derivatives of oxidatively modified proteins with a simultaneous increase in TAC levels after *in vitro* incubation of sturgeon muscle tissue with GC stem and root extracts. The intensity of lipid peroxidation was not altered. SOD and catalase activities were statistically significantly decreased, while GPx activity was not altered, suggesting the maintenance of antioxidant activity of sturgeon muscle tissue by this antioxidant enzyme. The results of this study highlight the pro-oxidative potential of GC extracts at the doses studied as potent natural oxidants to enhance antioxidant defences, but these processes are accompanied by the induction of oxidative protein modification in sturgeon muscle tissue. Future research should focus on elucidating the specific bioactive compounds responsible for the observed effects, optimising extraction methods to enhance the bioavailability of antioxidants, and evaluating the long-term effects of dietary supplementation with GC extracts on fish performance and product quality.

Keywords: Greater celandine, stem, root, Atlantic sturgeon, lipid peroxidation, protein oxidation, total antioxidant capacity

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Introduction

In recent years, there has been a growing interest in exploring natural compounds with potential health benefits, particularly those derived from medicinal plants. Among these, greater celandine (*Chelidonium majus* L., GC) has attracted attention due to its rich phytochemical composition and traditional medicinal uses. Of particular interest are its roots and stems, which have been studied for their potential antioxidant properties (Zielińska et al., 2018; Krizhanovska et al., 2021; Segneanu et al., 2024). GC is a perennial herbaceous plant of the Papaveraceae family. It is native to Europe and parts of Asia, but has been introduced into North America and other regions. The plant typically grows in wasteland, roadsides and disturbed habitats (Gilca et al., 2010; Zielińska et al., 2018). GC is characterised by its distinctive lobed leaves, bright yellow flowers with four petals and long, slender seed pods. The plant exudes a bright yellow-orange latex when cut, which has been used in traditional medicine for its reputed medicinal properties (Zielińska et al., 2018).

Antioxidants play a crucial role in cellular defence mechanisms against oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defences (Birben et al., 2012; Afzal et al., 2023). Oxidative stress has been implicated in several pathological conditions, including cardiovascular disease, cancer and ageing (Liguori et al., 2018). The search for potent antioxidants from natural sources has therefore gained considerable momentum in recent years (Parcheta et al., 2021). GC contains several phytochemicals, including alkaloids, flavonoids and phenolic compounds, which have antioxidant activity. These antioxidants help to neutralise ROS and protect cells from oxidative damage, potentially reducing the risk of chronic diseases such as cancer and cardiovascular disease (Colombo and Bosisio, 1996; Arora and Sharm, 2013). Compounds found in GC have been shown to have anti-inflammatory properties by inhibiting inflammatory mediators and enzymes (Lee et al., 2007; Mikołajczak et al., 2015). This activity may contribute to the traditional use of the plant in the treatment of inflammatory conditions such as arthritis and dermatitis (Lee et al., 2007; Yang et al., 2011).

Studies have shown that GC extracts may have hepatoprotective effects by promoting liver regeneration, improving liver function, and reducing liver damage caused by toxins or disease (Biswas et al., 2004, 2008; Gilca et al., 2010). These effects are attributed to the plant's ability to modulate liver enzymes and reduce oxidative stress in liver cells (Paul et al., 2013a,b). GC extracts have been shown to have antimicrobial activity against various bacteria, fungi, and parasites (Zuo et al., 2008; Meng et al., 2009; Krzyżek et al., 2021; Stefanowski et al., 2021a-c). This antimicrobial activity may be beneficial in the treatment of microbial infections, although further research is needed to elucidate the mechanisms of action and potential therapeutic applications (Zielińska et al., 2019; Sadecki et al., 2024). Some studies suggest that greater celandine extracts may have anti-cancer properties, including cytotoxic effects on cancer cells and inhibition of tumour growth (Warowicka et al., 2019; Musidlak et al., 2022). These effects are attributed to the plant's bioactive compounds, which have apoptotic, anti-proliferative, and anti-angiogenic effects on cancer cells (Gilca et al., 2010; Park et al., 2015; Deljanin et al., 2016; Zielińska et al., 2018). However, more research is needed to assess the safety and efficacy of greater celandine in cancer therapy.

The Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill) is a species of great ecological and economic importance, but its muscle tissue is susceptible to oxidative damage, which can affect its quality and nutritional value (Taylor et al., 2016; Logan-Chesney et al., 2018). Understanding the effects of natural antioxidants in mitigating oxidative stress in sturgeon muscle tissue could have significant implications for both human health and the aquaculture industry. The use of natural antioxidants from plant sources may have implications for fisheries management and seafood industry practices. By preserving the quality and shelf-life of fish products, these antioxidants may contribute to the economic viability of fisheries and promote consumer satisfaction with seafood products.

This study aimed to investigate the effects of GC root and stem extracts on lipid peroxidation, oxidative protein modification, and total antioxidant capacity, as well as the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase in Atlantic sturgeon muscle tissue after *in vitro* treatment. By elucidating the mechanisms underlying the antioxidant activity of GC extracts, we hope to contribute to the growing body of knowledge on natural antioxidants and their potential applications in both human health and food preservation.

Material and methodology

Collection of plant material and preparation of plant extracts

The plant material (stems and roots) was 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) (Figure 1).

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 0.1 M phosphate buffer (pH 7.4) (1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. Extracts were stored at -25 °C.

Tissue samples and experimental design

Clinically healthy Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill) with an average body mass of 450–500 g were used in the experiments and were obtained from the Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute (Rutki, Poland). Fish muscle tissue samples were homogenised in an ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogeniser immersed in an ice-water bath. The homogenates were centrifuged at 3,000 rpm for 15 min at 4 °C. After centrifugation, the supernatants were collected and frozen at -25 °C until analysis. All enzymatic assays were performed at 21 ±0.5 °C in duplicate. The protein content of the samples was determined by the Bradford method (1976).

The muscle tissue supernatant was used for incubation with root and stem extracts *in vitro* (in a ratio of 9 : 1) at room temperature. The untreated control samples (muscle tissue) were incubated with 100 mM Tris-HCl buffer (pH 7.2) (in a 9 : 1 ratio). The incubation time was 2 hours. Biomarkers of lipid peroxidation and protein oxidation, total antioxidant capacity, as well as the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) were analysed in the incubated homogenates (control untreated samples and samples with root and stem extracts). The biochemical assays used in this study have been described in our previous publications (Tkachenko et al., 2019; Buyun et al., 2023).

Determination of 2-thiobarbituric acid reactive substances (TBARS)

The level of lipid peroxidation was determined by quantifying the concentration of TBARS for the determination of malonic dialdehyde (MDA) concentration. Briefly, 2.1 mL of sample homogenate was added to 1 mL of 20% trichloroacetic acid (TCA) and 1 mL of 0.8% 2-thiobarbituric acid (TBA). The mixture was heated for 10 minutes in a boiling water bath. After cooling, the mixture was centrifuged at 3,000 rpm for 10 min. The absorbance of the supernatant was measured at 540 nm. The concentration of MDA (nmol·mg^{-1} protein) was calculated using an extinction coefficient of 1.56 10^5 mM^{-1}·cm^{-1}.

Figure 1 A flowering Greater celandine (A) and yellow milky sap on cut stems (B)
Photo: Oleksandr Lukash

Carbonyl derivative content of oxidative protein modification (OMP) assay

To evaluate the protective effects of GC root and stem extracts against free radical-induced protein damage in Atlantic sturgeon muscle tissue, the content of aldehydic and ketonic oxidative modification protein (OMP) derivatives was determined by spectrophotometric measurement. The rate of oxidative protein destruction was estimated from the reaction of the resulting carbonyl derivatives of the amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) with some modifications. DNFH was used to determine carbonyls in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm and 430 nm (aldehydic and ketonic derivatives).

Total Antioxidant Capacity (TAC) measurement

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. The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The absorbance of the resulting solution was measured at 532 nm. The absorbance of the blank was defined as 100%. The content of TAC in the sample (%) was calculated with concerning the absorbance of the blank samples.

Measurement of Superoxide dismutase activity

The activity of superoxide dismutase (SOD, E.C. 1.15.1.1) was determined by its ability to dismutate superoxide produced during the auto-oxidation of quercetin in an alkaline medium (pH 10.0). The 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 reduction of H_2O_2 in the reaction mixture using a spectrophotometer at a wavelength of 410 nm. One unit of catalase activity is defined as the amount of enzyme required to reduce 1 µmol of H_2O_2 per minute 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 utilisation of GSH (the reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB). The rate of GSH reduction was monitored spectrophotometrically at 412 nm. GPx activity is expressed as nmol GSH per minute per mg protein.

Statistical analysis

Statistical analysis of the data obtained was performed using the mean ± standard error of the mean (S.E.M.). All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$). To find significant differences (significance level, $p < 0.05$) between groups, the Kruskal-Wallis rank test was applied to the data (Zar, 1999). All statistical analyses were performed using STATISTICA 13.3 software (TIBCO Software Inc., USA).

Results and discussion

Figure 1 shows the TBARS and TAC levels in muscle tissue of Atlantic sturgeon after *in vitro* incubation with extracts from roots and stems of GC collected from the Pomeranian region. When analysing the TBARS levels after *in vitro* treatment with GC extracts, the following results were obtained. There was a reduction in TBARS levels after *in vitro* incubation of sturgeon muscle tissue with GC stem extracts (65.06 ±5.95 nmol·mg^{-1} protein) compared to untreated control samples (66.15 ±5.33 nmol·mg^{-1} protein). There was a statistically non-significant decrease in TBARS levels of 1.7% ($p > 0.05$) compared to controls (Figure 2). Other results were obtained after *in vitro* incubation of sturgeon muscle tissue with GC root extracts, where we observed a statistically non-significant increase in TBARS of 4% ($p > 0.05$) compared to control samples (68.77 ±6.50 nmol·mg^{-1} protein vs. 66.15 ±5.33 nmol·mg^{-1} protein) (Figure 2).

The opposite trends in TAC levels were observed after *in vitro* incubation of sturgeon muscle tissue with stem and root extracts of GC collected from the Pomeranian region. The use of stem extracts of GC resulted in a statistically significant highest increase in TAC levels (26.56 ±1.31%) (by 63.2%, $p < 0.05$) compared to control samples (16.28 ±0.99%). After *in vitro* incubation of sturgeon muscle tissue with GC root extracts, we observed a statistically significant increase in TAC levels (by 45.8%, $p < 0.05$) compared to control samples (23.73 ±1.22% vs. 16.28 ±0.99%) (Figure 2).

The aldehydic and ketonic derivatives of oxidatively modified proteins in Atlantic sturgeon muscle tissue after *in vitro* incubation with extracts from roots and stems of GC collected from the Pomeranian region are shown in Figure 3.

When analysing the levels of protein oxidation after incubation of Atlantic sturgeon muscle tissue with

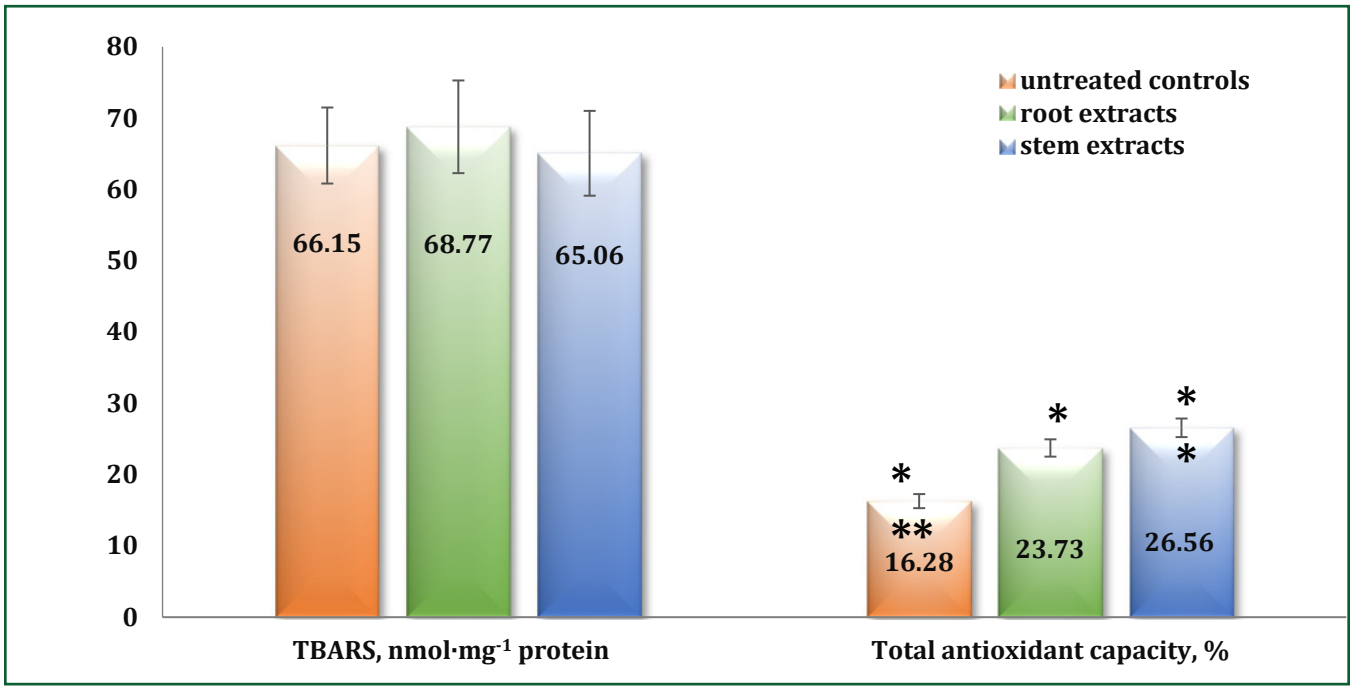

Figure 2 TBARS level as a biomarker of lipid peroxidation and total antioxidant capacity in Atlantic sturgeon muscle tissue after *in vitro* incubation with extracts from roots and stems of GC collected from the Pomeranian region (M ±m, n = 8)
* and ** – changes are statistically significant compared to the untreated controls

GC extracts, an increasing trend was observed. We observed an increase in the levels of aldehydic derivatives of oxidatively modified proteins after *in vitro* incubation of sturgeon muscle tissue with GC stem extracts (1.55 ±0.24 nmol·mg^{-1} protein) compared with the control samples (0.68 ±0.13 nmol·mg^{-1} protein), where there was a statistically significant increase of 128% ($p < 0.05$). Similarly, *in vitro* incubation of muscle tissue with GC root extracts resulted in a similar increase in the levels of aldehydic derivatives of OMP compared to controls (1.44 ±0.20 nmol·mg^{-1} protein vs. 0.68 ±0.13 nmol·mg^{-1} protein).

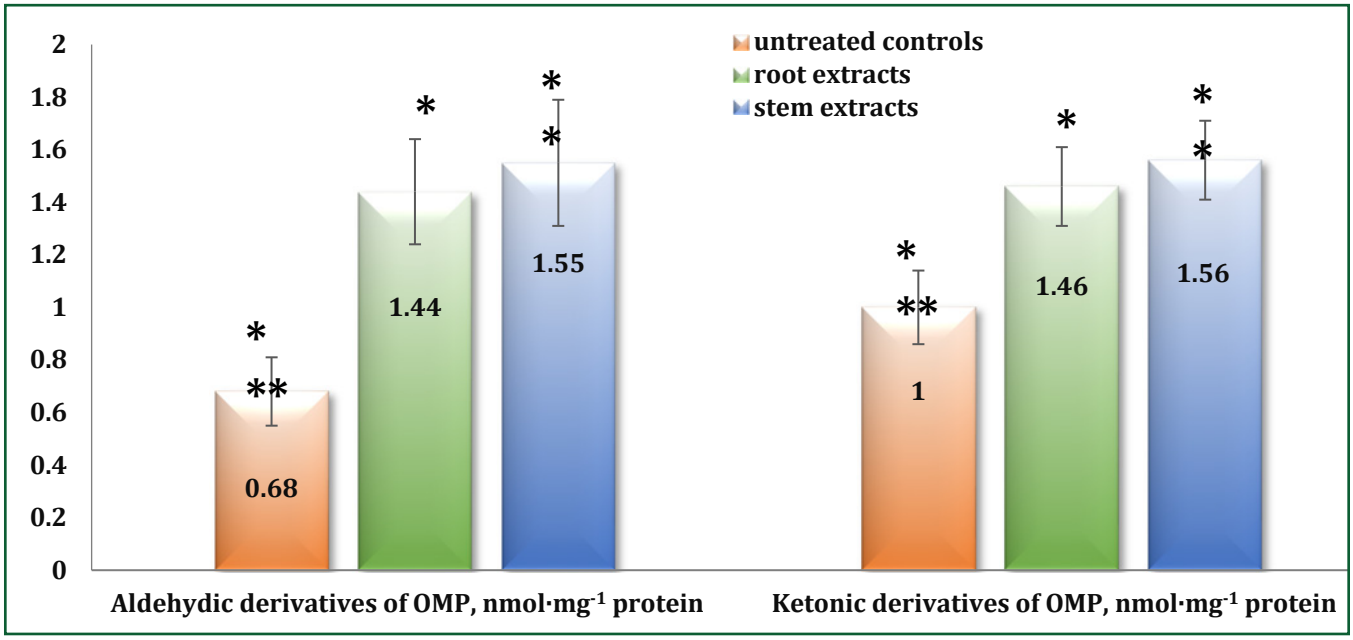

Figure 3 Aldehydic and ketonic derivatives of oxidatively modified proteins (OMP) in Atlantic sturgeon muscle tissue after *in vitro* incubation with extracts from roots and stems of GC collected from the Pomeranian region (M ±m, n = 8)
* and ** – changes are statistically significant compared to the untreated controls

The percentage increase was 111.8% ($p < 0.05$) (Figure 3). Similarly, *in vitro* incubation of muscle tissue with GC root extracts resulted in an increase in the levels of ketonic derivatives of OMP compared to controls (1.46 ±0.15 nmol·mg^{-1} protein vs. 1.0 ±0.14 nmol·mg^{-1} protein). After incubation of sturgeon muscle tissue with GC stem extracts, we also observed a similar increase in the levels of ketonic derivatives of OMP compared to control samples (1.56 ±0.15 nmol·mg^{-1} protein vs. 1.0 ±0.14 nmol·mg^{-1} protein). The percentage increase was 55.8% ($p < 0.05$) (Figure 3).

Activities of antioxidant enzymes in Atlantic sturgeon muscle tissue after *in vitro* incubation with extracts from roots and stems of GC collected from the Pomeranian region are presented in Table 1.

When we examined the activity of superoxide dismutase in the muscle tissue of sturgeons incubated *in vitro* with stem extracts of GC, we recorded a decrease in the activity of this antioxidant enzyme with a value of 294.16 ±13.53 U·mg^{-1} protein compared to the control samples (418.76 ±18.30 U·mg^{-1} protein). There was a statistically significant increase in SOD activity of 29.8% ($p < 0.05$) compared to the control samples. Similar results were obtained after incubation of sturgeon muscle tissue with GC root extracts, where there was also a statistically significant decrease in SOD activity (by 25.7%, $p < 0.05$) compared to the control samples (310.97 ±13.74 U·mg^{-1} protein vs. 418.76 ±18.30 U·mg^{-1} protein) (Table 1). Similar results were obtained for the catalase activity. After incubation of sturgeon muscle tissue with GC root extracts, there was also a statistically significant decrease in catalase activity (by 17.3%, $p < 0.05$) compared to the control samples (6.80 ±0.47 µmol·min^{-1}·mg^{-1} protein vs. 8.22 ±0.48 µmol·min^{-1}·mg^{-1} protein). We also observed a statistically significant reduction in catalase activity in muscle tissue incubated *in vitro* with GC stem extracts (by 24.5%, $p < 0.05$) compared to control samples (6.21 ±0.49 µmol·min^{-1}·mg^{-1} protein vs. 8.22 ±0.48 µmol·min^{-1}·mg^{-1} protein) (Table 1). After incubation of sturgeon muscle tissue with GC stem extracts, we observed a 4.2% increase in GPx activity ($p > 0.05$) compared to control samples (501.93 ±33.87 nmol·min^{-1}·mg^{-1} protein vs. 481.68 ±31.95 nmol·min^{-1}·mg^{-1} protein). We also observed a statistically non-significant increase in GPx activity in muscle tissue incubated *in vitro* with GC root extracts (by 4.4%, $p > 0.05$) compared to control samples (502.85 ±53.47 nmol·min^{-1}·mg^{-1} protein vs. 481.68 ±31.95 nmol·min^{-1}·mg^{-1} protein) (Table 1).

The results of this study indicate the potential effects of GC root and stem extracts on biomarkers of oxidative stress and antioxidant defences in Atlantic sturgeon muscle after *in vitro* treatment. The observed changes in biomarkers of oxidative stress, such as lipid peroxidation and protein modification, provide insight into the pro-oxidative properties of GC extracts at the dose studied (5 mg per mL) (Figure 2 and 3). A potential role for these extracts in activating oxidative damage in sturgeon muscle tissue is suggested by the significant increase in protein modification (Figure 3). This is particularly relevant in the context of aquaculture, where oxidative stress can be detrimental to fish health and product quality. The increase in TAC in sturgeon muscle tissue following treatment with GC extracts suggests a possible up-regulation of endogenous antioxidant defence mechanisms (Figure 2). This may involve activation of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, as well as scavenging of free radicals by non-enzymatic antioxidants present in the extracts. Understanding the mechanisms underlying this modulation may provide valuable insights into the physiological responses of aquatic organisms to natural antioxidants.

In our previous study, we evaluated the biomarkers of oxidative stress in blood samples collected from healthy volunteers after *in vitro* incubation with extracts derived from stems and roots of GC (Tkaczenko et al., 2023). The results of this study showed a statistically significant decrease in TBARS levels in blood samples for root extracts at the final dose of GC extracts at both 5 and 2.5 mg·mL^{-1}. We observed similar trends after *in vitro* incubation of blood samples with stem extracts of CM (at a final dose of 2.5 mg·mL^{-1}), where there was a statistically significant reduction in TBARS concentration compared to the untreated control

Table 1 Activities of antioxidant enzymes in Atlantic sturgeon muscle tissue after *in vitro* incubation with extracts from roots and stems of GC collected from the Pomeranian region (M ±m, n = 8)

Antioxidant enzymes	Untreated control samples	Root extracts	Stem extracts
SOD, U·mg^{-1} protein	418.76 ±18.30	310.97 ±13.74*	294.16 ±13.53**
Catalase, µmol H_2O_2·min^{-1}·mg^{-1} protein	8.22 ±0.48	6.80 ±0.47*	6.21 ±0.49**
GPx, nmol GSH·min^{-1}·mg^{-1} protein	481.68 ±31.95	502.85 ±53.47	501.93 ±33.87

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

samples. We also observed a statistically non-significant increase in TAC levels after *in vitro* incubation of blood samples with stem extracts of CM (at a final dose of 5 and 2.5 mg·mL^{-1}) compared to control samples, while there was a statistically non-significant reduction in TAC levels after *in vitro* incubation of blood samples with root extracts of GC (at a final dose of 2.5 mg·mL^{-1}) compared to untreated control samples (Tkaczenko et al., 2023).

The use of natural extracts as dietary supplements or additives in aquafeeds holds great promise for improving the health and performance of farmed fish. By reducing oxidative stress and enhancing antioxidant defences, these extracts may contribute to improved growth, immunity, and stress tolerance in aquatic species (Dügenci et al., 2003; Xavier et al., 2020; Dadras et al., 2023; Quagliardi et al., 2024). In addition, the potential role of GC extracts in preserving the quality and shelf-life of fish products warrants further investigation, as this may have implications for fisheries management and seafood industry practices (Hasan et al., 2021). For example, Yao et al. (2011) investigated the *in vivo* anthelmintic activity of chelidonine from GC against *Dactylogyrus intermedius* in *Carassius auratus*. *D. intermedius* is an important monogenean parasite of the gills of cyprinids and can cause severe economic losses in aquaculture and ornamental fish farming. The ethanolic extract of the whole plant of GC showed significant anthelmintic activity against *D. intermedius* [EC_{50} (median effective concentration) value = 71.5 mg·L^{-1}]. A quaternary benzo[c]phenanthridine alkaloid with significant activity against *D. intermedius* was obtained and identified as chelidonine. *In vivo* anthelmintic efficacy tests showed that chelidonine was 100% effective against *D. intermedius* at a concentration of 0.9 mg·L^{-1}, with an EC_{50} value of 0.48 mg·L^{-1} after 48 h exposure, which is more effective than the positive control, mebendazole (EC_{50} value = 1.3 mg·L^{-1}). In addition, the median 48-h lethal concentration (LC_{50} for chelidonine against the host *Carassius auratus* was 4.54 mg·L^{-1}). The resulting therapeutic index for chelidonine was 9.46. The researchers' findings provide evidence that chelidonine may be a potential source of new anti-parasitic drugs to control *Dactylogyrus* (Yao et al., 2011).

Chelerythrine may be selected as a lead compound for the development of new drugs against *D. intermedius*. In the study by Li et al. (2011), bioactivity guide fractionation was used to identify active compounds from GC against *D. intermedius*. Petroleum ether, ethyl acetate, chloroform and *n*-butanol extracts of GC were tested for *in vivo* anthelmintic activity. Among them, only the *n*-butanol extract showed promising anthelmintic activity and was therefore subjected to further isolation and purification using various chromatographic techniques. A compound with potent activity was obtained and identified as chelerythrine by hydrogen, carbon-13 nuclear magnetic resonance spectrum, and electron ionisation mass spectrometry. *In vivo* anthelmintic efficacy tests showed that chelerythrine was 100% effective against *D. intermedius* at a concentration of 1.60 mg·L^{-1}, with LC_{50} values of 0.68 mg·L^{-1} after 48 h exposure. The 48 h LC_{50} (acute toxicity tests) of chelerythrine was 3.59 mg·L^{-1} for grass carp.

Isoquinoline alkaloids (IQs) from another species of the Papaveraceae family, *Macleaya cordata* (Willd.) R.Br., are promising natural products for improving the growth performance and overall health of farm animals. Bussabong et al. (2021) demonstrated the benefits of using *M. cordata* IQs as feed additives to improve growth performance, survival, immune response and resistance to vibriosis in Pacific white shrimp. These researchers investigated the effects of two formulations of IQ extract, provided in either powder form (IQ-E) or water-soluble granulated form (IQ-WS), containing the main active ingredient sanguinarine at concentrations of 0.5 and 1%, respectively, on the growth, survival, immune response and resistance to *Vibrio parahaemolyticus* infection of Pacific white shrimp (*Litopenaeus vannamei*). The results showed that all IQ-fed shrimp in experiment 1 had significantly improved survival rates and immune parameters (total haemocyte count and phagocytic, phenoloxidase and superoxide dismutase activities) compared to the control group, although growth performance was similar in all groups. In experiment 2, all IQ-fed groups showed better growth performance and survival rates compared to the positive control. In contrast to the positive control group, no histopathological lesions were found in the hepatopancreas and intestine (Bussabong et al., 2021).

The antioxidant properties of plant extracts not only have implications for aquatic animal health, but also have potential benefits for human nutrition and health. GC consists of several isoquinoline alkaloids such as chelerythrine, chelidonine, sanguinarine, etc. (Colombo and Bosisio, 1996). These alkaloids have been reported to play important roles in anti-tumour, anti-leukaemic, anti-inflammatory and some other diseases (Capistrano et al., 2015; Liao et al., 2018). Chelidonine has profound inhibitory effects on airway inflammation and this effect is caused by suppression

of IL-4, eotaxin-2 and OVA-specific IgE production through STAT6 and Foxp3 pathways (Kim et al., 2015). The anti-inflammatory activity of the GC isolates was assessed by their inhibitory effects on LPS-induced NO production in RAW264.7 macrophage cells (Park et al., 2011). Chelidonine and homochelidonine are potent inducers of cell death in cancer cell lines, highlighting their potential relevance in leukaemic cells (Havelek et al., 2016). GC-isolated chelidonine efficiently induced apoptosis in HeLa cells, possibly by altering the p38-p53 and AKT/PI3 kinase pathways (Paul et al., 2012). The results of Liao et al. (2018) showed that chelidonine could suppress the LPS-induced inflammatory response both *in vitro* and *in vivo*, which was associated with the TLR4/NF-κB signalling pathway perturbed by chelidonine. The data suggest that chelidonine may be considered as a strategy for the prevention of inflammatory diseases. Nawrot et al. (2021) describe the isolation and identification of a novel 147-amino acid GC major latex protein (CmMLP1) and present a model of its structure containing a conserved hydrophobic cavity with high affinity for berberine, 8-hydroxycheleritrine, and dihydroberberine. CmMLP1 and the three associated alkaloids decreased the *in vitro* viability of human cervical cancer cells (HPV-negative and HPV-positive). The antiviral properties of GC latex are due to both the alkaloids and the proteins contained therein, which act on different stages of the viral replication cycle. Musidlak et al. (2022) showed that the latex components, such as alkaloids and proteins, reduce human papillomavirus (HPV) infectivity and inhibit the expression of viral oncogenes (E6, E7) at the mRNA and protein levels. However, crude GC latex and its fractions do not affect the stability of structural proteins in HPV pseudovirions and do not inhibit viral attachment to the cell surface. In addition, the protein fraction induced increased TNFα secretion, which may indicate the induction of an inflammatory response (Musidlak et al., 2022).

Consumption of fish enriched with natural antioxidants from plant sources may confer health benefits, including reduced risk of chronic diseases associated with oxidative stress (Awuchi et al., 2022). In addition, further research is needed to assess the safety, bioavailability, and efficacy of GC extracts in human dietary applications. While this study provides insight into the effects of GC extracts on oxidative stress biomarkers in sturgeon muscle tissue, there are several avenues for future research. These include elucidating the specific bioactive compounds responsible for the observed effects, optimising extraction methods to enhance the bioavailability of antioxidants, and evaluating the long-term effects of dietary supplementation with GC extracts on fish growth, health, and product quality. In addition, investigating the potential synergistic effects of combining GC extracts with other natural antioxidants or functional ingredients may reveal novel strategies to enhance antioxidant defences in aquatic organisms.

Conclusions

Investigating the effects of GC root and stem extracts on biomarkers of oxidative stress and antioxidant defences in Atlantic sturgeon muscle tissue provides insight into the potential applications of natural antioxidants in aquatic animal health and aquaculture. After *in vitro* incubation of sturgeon muscle tissue with GC root and stem extracts, we observed a statistically significant increase in TAC levels with a simultaneous significant increase in the levels of aldehydic and ketonic derivatives of oxidatively modified proteins. Levels of lipid peroxidation biomarkers were the same as in untreated control samples. The results demonstrate the efficacy of GC extracts in activating oxidative protein modification while increasing TAC in sturgeon muscle tissue. These effects suggest that the bioactive compounds present in the extracts used at the dose studied have pro-oxidant properties capable of activating oxidative damage to tissues in aquatic organisms. Future research should focus on elucidating the specific bioactive compounds responsible for the observed effects, optimising extraction methods to enhance the bioavailability of antioxidants, and evaluating the long-term effects of dietary supplementation with GC extracts on fish performance and product quality. In addition, the safety, efficacy, and potential synergistic effects of combining GC extracts with other natural antioxidants should be investigated.

Conflicts of interest

The authors have no competing interests to declare.

Ethical statement

This study was conducted at the Stanislaw Sakowicz Inland Fisheries Institute (Olsztyn, Poland). The research complied with Polish animal welfare regulations and was approved by the Local Animal Experimentation Ethics Committee of the Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland.

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majus L.) as a source of bioactive substances for pharmaceutical use" (Student Science Associations Create Innovations programme, 2023-2024).

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