Klara PIOTROWSKA ORCID 0000-0001-9287-7616

DOI: 10.15199/180.2026.1.5

Faculty of Animal Science, Department of Animal Breeding and Nutrition, Institute of Animal Sciences, Warsaw University of Life Sciences – SGGW, Ciszewskiego 8 Street, 02-786 Warsaw, Poland
Author e-mail: klara_piotrowska@sggw.edu.pl

OPEN ACCESS

Submission received: 14 March 2026 / Revised: 18 March 2026 / Accepted: 27 March 2026 / Published: 31 March 2026

ANTIOXIDANT ACTIVITY OF CRAYFISH EXOSKELETON EXTRACTS AND THEIR POTENTIAL PROTECTIVE EFFECT AGAINST OXIDATIVE STRESS IN HT-22 NEURONAL CELL – PRELIMINARY STUDY

DZIAŁANIE PRZECIWUTLENIAJĄCE EKSTRAKTÓW Z PANCERZY RAKÓW I ICH POTENCJALNE DZIAŁANIE OCHRONNE PRZED STRESEM OKSYDACYJNYM W KOMÓRKACH NEURONALNYCH HT-22 – BADANIE WSTĘPNE

***Summary**: Crayfish exoskeletons represent a by-product of freshwater crustacean processing and may constitute a valuable source of bioactive compounds, particularly carotenoids such as astaxanthin. The aim of this study was to evaluate the antioxidant properties of extracts obtained from the exoskeletons of three crayfish species (Faxonius limosus, Astacus leptodactylus and Procambarus clarkii) and to preliminarily assess their biological activity in a cellular model of oxidative stress. Extracts were obtained using acetone or a mixture of hexane and 2-propanol (3:2). Antioxidant activity was determined using the ABTS assay and expressed as Trolox equivalents. Among the tested samples, the highest antioxidant activity was observed for extracts obtained from Procambarus clarkii. In addition, preliminary XTT assay results suggested that crayfish extracts may partially protect cells against hydrogen-peroxide-induced oxidative stress.*

***Keywords**: oxidative stress, neurons, dementia, bioactive substances*

Streszczenie: Pancerze raków stanowią produkt uboczny przetwórstwa słodkowodnych skorupiaków i mogą być cennym źródłem związków bioaktywnych, zwłaszcza karotenoidów, takich jak astaksantyna. Celem niniejszego badania była ocena właściwości przeciwutleniających ekstraktów uzyskanych z pancerzy trzech gatunków raków (*Faxonius limosus*, *Astacus leptodactylus* i *Procambarus clarkii*) oraz wstępna ocena ich aktywności biologicznej w komórkowym modelu stresu oksydacyjnego. Ekstrakty uzyskano przy użyciu acetonu lub mieszaniny heksanu i 2-propanolu (3:2). Aktywność przeciwutleniającą określono za pomocą testu ABTS i wyrażono jako ekwiwalenty Troloxu. Spośród badanych próbek najwyższą aktywność przeciwutleniającą zaobserwowano w przypadku ekstraktów uzyskanych z *Procambarus clarkii*. Ponadto wstępne wyniki testu XTT sugerowały, że ekstrakty z raków mogą częściowo chronić komórki przed stresem oksydacyjnym wywołanym przez nadtlenek wodoru.

Słowa kluczowe: stres oksydacyjny, neurony, demencja, substancje bioaktywne

Introduction

Crayfish are a common food source for humans in some regions. There was also a tradition of crayfish consumption in Poland. Currently, *Astacus leptodactylus* crayfish are primarily farmed for human consumption in Poland, although imported *Procambari clarkii* are also available for sale. In turn, an invasive species of crayfish, originally from the USA, the spiny crayfish, lives in our waters. This crayfish is small and contains significantly less meat than the previously mentioned species, making it less attractive for direct consumption. However, Śmietana et al. [10] demonstrated that crayfish meat has beneficial nutritional values. Advantages include its leanness, while its fat content is characterized by a very favorable ratio of total polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA). Also carotenoids play a significant role in the biological value of crayfish, and are present primarily in the exoskeleton and, to a lesser extent, in the meat. Astaxanthin, which has strong antioxidant, anti-inflammatory, and potentially neuroprotective effects, is responsible for crayfish's nutritional value [3, 4, 6]. Astaxanthin exerts its protective effects through multiple mechanisms, including direct scavenging of reactive oxygen species and modulation of redox-related signaling pathways such as Nrf2 and NF-κB, as well as regulation of apoptosis and inflammation [11]. Astaxanthin is known to exert strong antioxidant activity by enhancing the activity of endogenous antioxidant enzymes,

such as superoxide dismutase (SOD) and catalase (CAT), thereby improving cellular defense mechanisms. These combined effects contribute to the protection of cells against oxidative damage [2].

Previous studies by our team have shown that aqueous extracts of whole crayfish protect fibroblasts from the effects of oxidative stress [12]. However, it is known that carotenoids are an extremely valuable raw material, located mainly in the crayfish's exoskeletons [1].

It is hypothesized that crayfish exoskeleton extracts, as a source of carotenoids such as astaxanthin, exhibit antioxidant activity and may provide cytoprotective effects against oxidative stress in a cellular model. Furthermore, it is assumed that these effects are associated with both direct scavenging of reactive oxygen species and modulation of cellular antioxidant defense mechanisms.

The aim of this preliminary study was to evaluate the antioxidant activity of extracts obtained from the exoskeletons of three crayfish species (*Faxonius limosus*, *Astacus leptodactylus* and *Procambarus clarkii*), to assess their potential cytoprotective effects against hydrogen peroxide-induced oxidative stress in HT-22 neuronal cells, and to identify the most promising extracts for further studies.

Materials and Methods

Exoskeletons from three crayfish species (*Faxonius limosus*, *Astacus leptodactylus* and *Procambarus clarkii*) were freeze-dried and ground. Two extraction systems were used: acetone and a mixture of hexane:2-propanol (3:2). Extracts were tested at a concentration of 20 ppm in 99% ethanol.

The carotenoid content in crayfish shell extracts was determined using a colorimetric spectrophotometric method. The measurement was based on the characteristic absorption of astaxanthin (470 nm) in the visible region, which allows the estimation of carotenoid concentration. Based on the recorded absorbance values, the total carotenoid content was calculated and expressed as astaxanthin equivalents.

Antioxidant activity was determined using the ABTS (radical scavenging assay and expressed as μM Trolox equivalents.

Cytoprotective effects were evaluated using the XTT cell viability assay (Biotum, cat. nr. 30007). Cells were pretreated with extracts for 24 h and subsequently exposed to hydrogen peroxide (300 μM) for another 24 h.

Statistical analysis was performed for cell studies using non-parametric methods due to the small sample size (n=5). Differences between groups were assessed using the Kruskal–Wallis test, followed by Dunn's *post hoc* test with Bonferroni correction for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

All extracts exhibited measurable antioxidant activity and carotene content. The highest value was observed for the hexane:2-propanol extract from *Procambarus clarkii* (HL). Acetone extracts from *Procambarus clarkii* (AL) also showed higher activity compared with the other species (Table 1).

In the XTT assay, exposure to hydrogen peroxide reduced cell viability to approximately 52% of the control (Figure 2.).

Table 1. Antioxidant activity and astaxanthin content of crayfish exoskeleton extracts (20 ppm)

Extract	Species	Solvent	ABTS (μM Trolox eq.)	TEAC (mM Trolox/g)	Carotenoid content expressed as a astaxanthin (μg/mL)
AP	*F. limosus*	Acetone	132.00	6.60	21.07
AB	*A. leptodactylus*	Acetone	134.44	6.72	21.07
AL	*P. clarkii*	Acetone	157.89	7.89	27.67
HP	*F. limosus*	Hexane:2-propanol	146.22	7.31	23.37
HB	*A. leptodactylus*	Hexane:2-propanol	150.33	7.52	30.76
HL	*P. clarkii*	Hexane:2-propanol	164.22	8.21	42.85

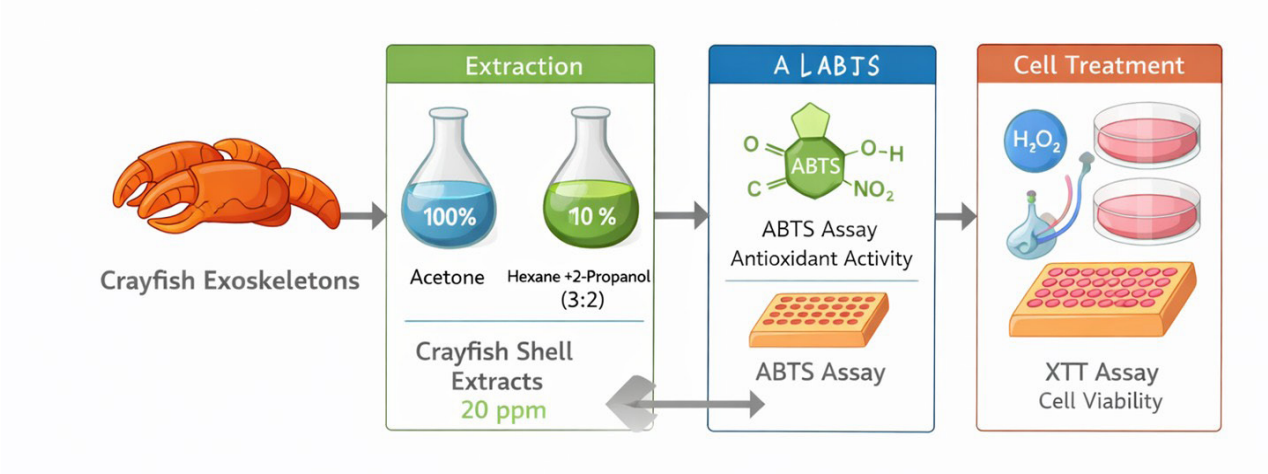

Fig. 1. Antioxidant activity of crayfish exoskeleton extracts determined using the ABTS assay

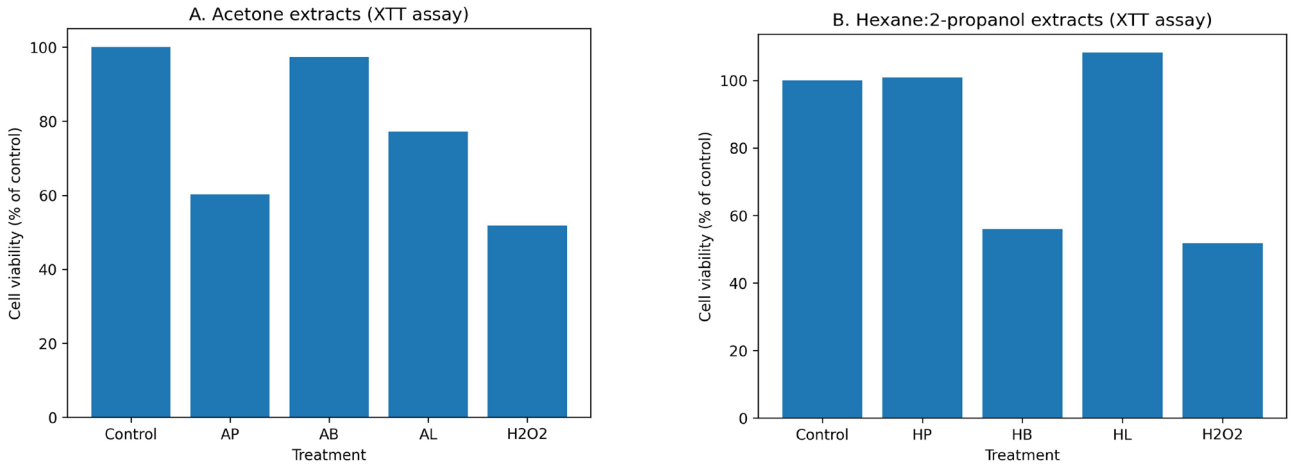

Fig. 2. Effect of crayfish extracts on cell viability determined using the XTT assay. (A) Acetone extracts: AP – Faxonius limosus, AB – Astacus leptodactylus, AL – Procambarus clarkii. (B) Hexane:2-propanol (3:2) extracts: HP – Faxonius limosus, HB – Astacus leptodactylus, HL – Procambarus clarkii. Cells were pretreated with extracts at a concentration of 20 ppm for 24 h, followed by exposure to hydrogen peroxide (H_2O_2, 300 µM) for an additional 24 h. Results are expressed as percentage of untreated control

Pretreatment with crayfish extracts partially improved cell viability, with the strongest protective effect observed for the HL extract. Interestingly, the HL extract increased cell viability above the level of the untreated control, indicating that, in addition to its protective effect against oxidative stress, it may enhance cellular proliferation or metabolic activity.

Post hoc analysis revealed significant differences between the control group (K) and the H_2O_2-treated group ($p = 0.002$), as well as between the HL group and the H_2O_2 group ($p = 0.004$). No statistically significant differences were observed for the remaining comparisons ($p > 0.05$), which may be attributed to the limited number of replicates and high variability in some groups.

The observed antioxidant activity is likely associated with carotenoid pigments present in crayfish exoskeletons, particularly astaxanthin. This is confirmed by data from other researchers [8]. Previous studies have demonstrated significant antioxidant activity in extracts from marine organisms such as *P. clarkii* [1], shrimp (*Litopenaeus vannamei*), other invertebrates [5] and even mikroalgal [7]. Reported ABTS values vary depending on the extraction method and solvent polarity. Preliminary XTT results also suggest that these extracts may provide protection against oxidative stress; however, further optimization of extract concentrations and hydrogen peroxide levels is required.

Similar effects have been reported in previous studies. For example, water extracts obtained from *Faxonius limosus* were shown to reduce oxidative stress and improve cell viability in fibroblast cells exposed to hydrogen peroxide. The extract decreased reactive oxygen species (ROS) production and reduced apoptosis-related markers, indicating a protective effect against oxidative damage. These findings are consistent with the results obtained in the present study, where crayfish exoskeleton extracts partially protected cells against H_2O_2-induced cytotoxicity [12].

Conclusions

Crayfish exoskeleton extracts exhibit measurable antioxidant activity and may represent a promising source of natural bioactive compounds. Extracts obtained from *Procambarus clarkii* showed the highest antioxidant potential, carotenoid content and the strongest cytoprotective effect in the preliminary cell model.

Acknowledgment

This research was funded by the National Science Centre, Poland, grant number 2024/08/X/NZ9/00525.

Bibliography

[1] Guerin M., Huntley M.E., Olaizola M., Haematococcus astaxanthin: applications for human health and nutrition, Trends in Biotechnology, 21(5), 2003, 210–216.
[2] Sachindra N.M., Bhaskar N., Mahendrakar N.S., Carotenoids in crustaceans and their biological functions, Food Chemistry, 91, 2005, 235–241.
[3] Sathasivam R., Ki J.S., Kim J.D., A review of the biological activities of microalgal astaxanthin and its potential applications, Marine Drugs, 16, 2018, 26.
[4] Zhang Y., Yang L., Zu Y., Chen X., Wang F., Liu F., Antioxidant activities of extracts from marine invertebrates, Food Chemistry, 151, 2014, 508–515.
[5] Liu Y., Zheng J., Zhang Y., Wang Z., Yang L., Antioxidant activity of extracts from marine organisms including crustaceans, Marine Drugs, 13, 2015, 2857–2871.
[6] Sathasivam R., Ki J.S., Kim J.D., Biological activities of astaxanthin and its applications, Marine Drugs, 16, 2018, 1–25.
[7] Šimat V., Bogdanović T., Poljak V., Astaxanthin from crustaceans and their byproducts: A bioactive metabolite candidate for therapeutic application, Marine Drugs, 20(3), 2022, 206.
[8] Silva D.M.L., Santos L.O., Carvalho R.M., Extraction and characterization of bioactive compounds from crustacean waste, Journal of the Brazilian Chemical Society, 32(6), 2021.
[9] Śmietana, N.; Panicz, R.; Sobczak, M.; Śmietana, P.; Nędzarek, A. Spiny-cheek crayfish, *Faxonius limosus* (Rafinesque, 1817), as an alternative food source, Animals, 11, 2021, 59.
[10] Rambaldi A., Rossi F., Bianchi M., Marine-derived astaxanthin: molecular mechanisms and biomedical applications, Marine Drugs, 2025.
[11] Zglińska, K.; Jaworski, S.; Rygało-Galewska, A.; Łozicki, A.; Roguski, M.; Matusiewicz, M.; Niemiec, T. Effect of spiny-cheek crayfish (*Faxonius limosus*) on H_2O_2-induced oxidative stress in normal fibroblast cells, Applied Sciences, 12, 2022, 8546.