Valorization of Shrimp Shell Waste: Comparative Phytochemical Composition and Bioactivity of *Parapenaeus longirostris* and *Aristeus antennatus*

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Abstract

The valorization of marine by-products represents a promising approach for discovering novel bioactive compounds. This study presents a comparative phytochemical and bioactivity assessment of shrimp shell waste extracts from Parapenaeus longirostris and Aristeus antennatus species, which are prevalent on Algerian coasts. The objective of this study is to evaluate their potential as a source of biologically active compounds, with a particular focus on those compounds that possess anti-inflammatory and antidiabetic properties. The methodology entailed the extraction of chemical compounds. The subsequent procedure involved the utilization of shrimp residues, which were then subjected to a comprehensive compositional analysis. This analysis was then followed by an evaluation of their biological activity. The results obtained demonstrated the presence of biologically effective compounds, with the extracts exhibiting notable anti-inflammatory and antidiabetic activity. White shrimp (P. longirostris) was found to be particularly efficacious in both activities, showing particular promise in diabetes-related inhibition. This finding indicates the potential of valorization. It is evident that these marine by-products have the potential to serve as a promising natural resource for the development of value-added products. It is evident that these initiatives facilitate novel advancements in the domains of health and industry, thus underpinning the blue economy within the paradigm of sustainable development.

Keywords: *Shrimp shell waste, antidiabetic activity, anti-inflammatory property.*

Introduction

The aquatic environment, characterized by its immense biological richness, is a prolific source of high-value chemical and biological compounds. These compounds possess unique physicochemical properties and hold significant potential for diverse applications in critical sectors, particularly nutrition and human health (Kim *et al.,* 2008; Wang *et al.,* 2019). Marine organisms are rich in important nutrients, such as minerals, lipids, amino acids, proteins, carotenoids, and polysaccharides, especially in certain parts of their bodies. Often considered as processing by-products, these components, such as heads, viscera, skins, and crustacean shells, represent underutilized reservoirs of bioactive compounds (Lee, 1999; Liaset *et al.,* 2000; Aspmo *et al.,* 2005; Babu *et al.,* 2008).

The disposal of marine processing co-products poses significant environmental and economic challenges worldwide, with such waste representing approximately 50% of the total weight of global aquatic resource production (Kristinsson and Rasco, 2000a; Je *et al.,* 2007; Randriamahatody *et al.,* 2011). In Algeria, a significant amount of shrimp waste primarily from white shrimp (*Parapenaeus longirostris*) and red shrimp (*Aristeus antennatus*), are generated. Currently, these materials are largely discarded, contributing to environmental contamination and the forfeiture of significant economic prospects. Given their high consumption

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rates and substantial contribution to local fisheries, these two shrimps species hold considerable economic and fisheries importance in Algeria (Cherif and Benabdi, 2017).

Valorizing these co-products is essential, as this strategy not only supports environmental protection and pollution reduction but also enhances business profitability and minimizes resource waste (Valdimarsson and James, 2001). Converting such residues into raw or intermediate materials facilitates enables the development of high value-added products (Singh *et al.,* 2014; Zhang *et al.,* 2014). These fishery by-products are now considered as valuable primary sources for manufacturing compounds applicable to animal nutrition (Rebeca *et al.,* 1991), cosmetics and human health (Liceaga and Li-Chan, 1999; Kristinsson and Rasco, 2000a, 2000b).

The inherent value of these residues lies in their richness in bioactive compounds. They are notable sources of high-quality proteins (Ibrahim *et al.,* 1999), lipids (Dumay *et al.,* 2006), chitosan and its derivatives (Chung *et al.,* 2020), astaxanthin (Ambati *et al.,* 2014; Liu *et al.,* 2020), and bioactive peptides (Wang *et al.,* 2019; Hu *et al.,* 2021). Furthermore, these by-products contain diverse phytochemicals, including flavonoids (Jain and Garg, 2020; Kumar *et al.,* 2021), saponins (Zang *et al.,* 2021), tannins (Ma *et al.,* 2018; Chen *et al.,* 2022), terpenoids (Sharma *et al.,* 2021), coumarins (Zeng *et al.,* 2022), and glycosides (Li *et al.,* 2019). Collectively, these compounds exhibit significant therapeutic and preventive properties, particularly in the areas of anti-inflammation and antidiabetic activities.

The primary objective of this study is to valorize shrimp by-products (white and red) from the Algerian region by identifying and extracting bioactive compounds and subsequently evaluating their potential applications, particularly for their anti-inflammatory property and antidiabetic activity using the α-amylase inhibition method.

Materials and Methods

Biological materials

The collection and analysis of biological materials and samples is an essential component of scientific research in this field. The two species of shrimp, white shrimp (*Parapenaeus longirostris*) and red shrimp (*Aristeus antennatus*), utilized in this study were obtained in July 2024 from commercial fishing activities in the fishing port of Bou Haroun, located in the province of Tipaza. The samples were subsequently transported in a cooler to the biochemistry laboratory at the University of Khemis Miliana. There, the shrimp were washed and peeled, and the shell wastes were dried at room temperature away from sunlight and then powdered using an electric mortar.

Extract preparation

In this study, powders of shell waste from two species of shrimp underwent ethanol extraction using the following protocol: A precise quantity of 10 g of the powder was weighed, and 100 ml of absolute ethanol was added as the extraction solvent. To achieve optimal homogeneity within the mixture and enhance the efficiency of the extraction process, both samples were subjected to a magnetic stirrer at a constant speed for a duration of two hours (Maia *et al.,* 2023).

Phytochemical screening

This qualitative analysis aims to identify the various chemical compounds (secondary metabolites) present in a plant extract. The methodology under consideration is contingent upon the implementation of colorimetric or precipitation reactions. The objective of this study is to reveal the presence of various classes of phytochemical compounds, including but not limited to alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, glycosides, and terpenes (Shaikh and Patil, 2020).

Anti-inflammatory activity in vitro using protein denaturation method

The *in vitro* anti-inflammatory activity was studied using the bovine serum protein denaturation method, as previously described by Rahman and Eswaraiah (2015). The reaction mixture (0.5 ml) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of shrimp shell waste extracts at different concentrations. Subsequently, the samples were exposed to an incubation temperature of 37°C for a duration of 30 min, and the temperature was increased to keep the samples at 57°C for 3 min. Subsequent to cooling, 2.5 ml of sodium phosphate buffered solution (pH 6.3) was added to each tube.

Turbidity was measured spectrophotometrically at 660 nm for the control test. In this test, 0.05 ml of distilled water was used as a substitute for the extracts. It has been established that the product control test did not contain bovine serum albumin. The percentage inhibition of protein denaturation is calculated by the following equation:

$$\text{Percentage of inhibition } (\%) = [(Abs_{Control} - Abs_{treaties})/(Abs_{Control})] \times 100$$

The results were also expressed as IC50 values.

Inhibition test of enzymatic activity of α-amylase

A volume of 250 µl of each shrimp shell waste extract, with varying concentrations, was combined with 250 µl of α-amylase solution that contained 3mg.ml^{-1} of the enzyme. The mixture was then subjected to incubation at a temperature of 25 °C for a duration of 10 min. Subsequently, 250 µl of starch solution (1% w/v in 0.02 M sodium phosphate buffer) was added to the reaction mixture and incubated once more at 25 °C for 10 min. The reaction was then terminated by the addition of 500 µl of 3,5-dinitrosalicylic acid (DNSA) reagent. The reaction mixture was subjected to a heating procedure of 5 min, followed by a cooling cycle to room temperature, and the measurement of the absorbance was conducted at 540 nm (Lordan *et al.,* 2013). The inhibitory activity was calculated according to the following formula:

$$\% \text{ inhibition activity} = [(Abs_{sample} - Abs_{control} / Abs_{sample})] \times 100$$

Note: Abs control: the absorbance of the reaction control (containing all the reagents except sample tested). Abs sample: the absorbance of the sample tested.

The results were also expressed as IC50 values.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical significance was assessed using one-way ANOVA followed by Tukey post-hoc test. Differences were considered statistically significant at $P<0.05$.

Results and Discussion

Phytochemical screening

A comparative phytochemical screening was conducted, and the results are presented in Table 1. This analysis revealed notable similarities and differences in the chemical composition of shell waste extracts from *P. longirostris* and *A. antennatus*.

The results of our study indicate that the chemical profiles of the two extracts are similar, with the presence of flavonoids, tannins, alkaloids, glycosides, saponins, and terpenoids. These secondary metabolites are widely recognized for their various biological and pharmacological activities. It is noteworthy that no phenolic compounds were detected in any of the extracts within the limits of the applied detection methods. However, the fundamental differences lie in the presence of reducing sugars, which were identified exclusively in the *P. longirostris* extract, whereas they were not detected in the *A. antennatus* extract.

Anti-inflammatory activity in vitro

The extract of *P. longirostris* exhibited superior efficacy and statistically significant differences ($P<0.05$) compared to *A. antennatus* extract, whose IC50 values are 1.113±0.04 and 2.0315±0.44 mg.ml^{-1}, respectively, while the extracts demonstrated noteworthy activity, their efficacy was considerably lower than that of synthetic reference drugs, such as sodium diclofenac. It is important to note that a statistically insignificant difference ($P>0.05$) was observed in efficacy between sodium diclofenac and prednisolone, according to the analysis of their IC50 values (0.380±0.14 and 0. 333 ± 0.06 mg.ml^{-1} respectively) (Figure 1).

The anti-inflammatory efficacy of the extracts is attributed to their chemical richness, which includes various classes of biologically active secondary compounds. Among these classes, flavonoids and Terpenoids are particularly important for their anti-inflammatory activity, with notable contributions from saponins, tannins, and coumarins. These compounds have gained scientific recognition for their anti-inflammatory properties, which act on

Table 1. The results of phytochemical screening tests

Tests	*P. longirostris* extract	*A. antennatus* extract
Alkaloids	+	+
Reducing sugar	+	-
Glycosides	+	+
Flavonoids	+	+
Phenolic compounds	-	-
Tannins	+	+
Saponins	+	+
Terpenoids	+	+
Coumarins	+	+

Note: += Present; - = Absent

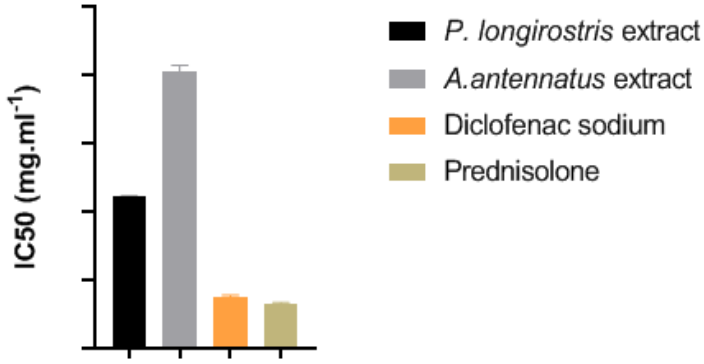

Figure 1. The results of anti-inflammatory activity (IC50 mg.ml^{-1})

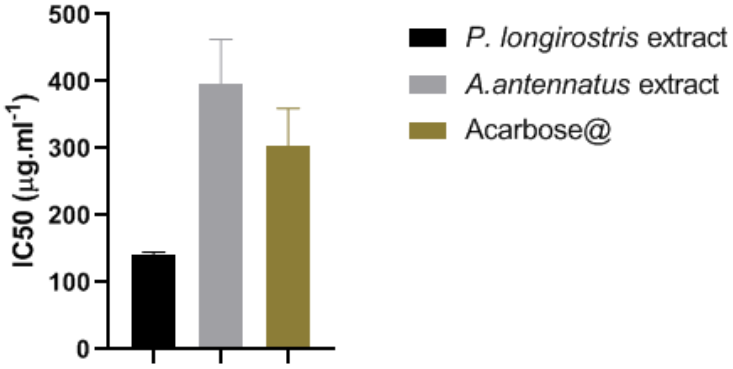

Figure 2. The results of inhibition of α-amylase activity

multiple pathways of inflammation. These pathways include the inhibition of pro-inflammatory enzymes and the reduction of oxidative stress. As demonstrated by Di Lorenzo *et al.* (2021), these categories of compounds possess various biological activities, including anti-inflammatory properties.

Inhibition of α-amylase activity

The *P. longirostris* shell waste extract (IC50= 140.16±0.10 µg.ml^{-1}) exhibited superior efficacy compared to the *A. antennatus* shrimp shell waste extract (IC50= 395.95 ±1.82 µg.ml^{-1}), as determined by statistical analysis. It is noteworthy that *A. antennatus* extract demonstrated comparable efficacy to that of Acarbose (IC50= 304.46 ± 1.18 µg.ml^{-1}) (Figure 2), with no statistically significant differences observed between the two (P>0.05). This finding underscores the promising potential of this natural extract.

A plethora of aquatic organisms, encompassing bacteria, micro- and macroalgae, sea grasses, sponges, corals, sea anemones, fish, salmon skin, a shark fusion single-domain protein, and by-products derived from fish and shellfish, have been subjected to rigorous scrutiny for their potential anti-diabetic properties. The anti-hyperglycemic and anti-diabetic characteristics of marine creatures have been the subject of research in both *in vitro* and *in vivo* studies (Lauritano and Ianora, 2016; Rayapu *et al.,* 2021).

Flavonoids have been demonstrated to exhibit a substantial hypoglycemic effect, which is achieved through a variety of mechanisms. These mechanisms include the regulation of glucose absorption (Loureiro and Martel, 2019), the inhibition of digestive enzymes (Xiao *et al.,* 2013), the regulation of intestinal microbiota (Gowd *et al.,* 2019), and the inhibition of advanced glycation end products formation (Xie and Chen, 2013).

Furthermore, tannins have been demonstrated to retard intestinal glucose absorption and the emergence of insulin-dependent diabetes mellitus by eliciting an insulin-like effect on insulin-sensitive tissues. This effect lowers glucose levels by regulating the antioxidant environment of pancreatic β-cells (Serrano *et al.,* 2009).

Conclusion

The findings of this study indicated that shrimp shell waste is a substantial reservoir of biologically active compounds of significant economic value. A comprehensive analysis was conducted to ascertain the anti-inflammatory and antidiabetic properties of the shrimp shell waste extracts. The results demonstrated that the extracts possess such properties, with the white shrimp shell waste extract (*P. longirostris*) proving to be more effective in both activities compared to that of the red shrimp shell waste (*A. antennatus*). The observed efficacy of these extracts can be attributed to their chemical richness, which is characterized by the presence of various compounds, including flavonoids, terpenoids, saponins, tannins, coumarins, and glycosides.

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