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Research Article

Nutritional composition, protein characterisation, and in vitro antioxidant activity of the marine polychaete *Perinereis nuntia* (Lamarck, 1818) from the Bay of Bengal

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The present study explores *Perinereis nuntia* from the Bay of Bengal Coast, India, for its nutritional quality, ecological significance, and bioactivity. Proximate analysis demonstrated high moisture (88.76 %), protein (54.33 %), and fat/lipid (27.1 %) levels, suggesting its potential as a nutritive aquafeed for broodstock rearing. Elemental analysis highlighted abundant micronutrients, including zinc (463.25 ppm) and iron (447.18 ppm). Heavy metals were detected, with Pb (34.05±7.53 ppm) being the highest, followed by Ni (13.12±0.69 ppm). Cr and Cd were detected at very low levels. Elevated Pb, Ni, Zn, and Fe levels indicate notable bioaccumulation potential, supporting its use as a bioindicator. Protein extraction with RIPA buffer resulted in a low yield (1.47 mg/g tissue), with evidence of aggregation indicated by UV-Vis spectroscopy, Dynamic Light Scattering (DLS), and FTIR analysis. Despite this, the protein extract exhibited notable DPPH radical-scavenging activity (87.65 %) and moderate Fe^{3+} reducing capacity (10.78 %) in the FRAP assay. This indicates that *P. nuntia* has preliminary antioxidant activity. The results highlight *P. nuntia* as a promising multidimensional bioresource, serving as a nutrient source and bioindicator with broader bioactive potential. Additional optimisation of protein extraction methods and extensive biosafety analysis are essential for safe and sustainable use.

[**Keywords**: Antioxidant activity, Aquafeed potential, Bioindicator, *Perinereis nuntia*, Protein extraction, Sustainable marine resource]

Introduction

Marine polychaetes, albeit often overlooked amongst marine invertebrates, are increasingly seeking tremendous attention not only for their ecological significance but also for their remarkable biochemical efficacy and high nutritional value. They inhabit a wide range of marine environments, from intertidal mudflats and seagrass meadows to abyssal plains and hydrothermal vents[1]. Their potential to thrive under diverse environmental conditions reflects exceptional adaptability. Owing to their varied ecological roles, trophic strategies, and life-history patterns, polychaetes constitute an integral component of benthic ecosystems worldwide[2].

Marine bioresources are chiefly explored for sustainable utilisation across multiple applications. Amongst these, polychaetes are noted for their nutritional richness and potential to derive functional biomolecules. According to the Food and Agriculture Organization (FAO), worldwide aquaculture production reached 223.2 million tonnes in 2022[(ref. 3)]. The sudden expansion of aquaculture underscores the need to explore alternative and lesser-known marine organisms, such as polychaetes, to ensure sustainable production systems.

Polychaetes have long been employed as live feed in aquaculture. An optimal feed source should satisfy the nutritional demands of cultured organisms, be scalable for mass production, and be sustainably produced. Polychaetes demonstrate a well-established nutritional profile that supports broodstock growth, development, and reproductive maturation. Their positive impact on reproductive ability is often referred to as the "polychaete effect"[4].

In addition to their nutritional importance, polychaetes serve as critical bioindicators, forming a significant link within marine food webs. They play a pivotal role in energy transfer across trophic levels and may critically contribute to biomagnification events[5,6]. As they are largely used as feed for commercially important fish species to improve yield[7], their quality and safety become chiefly important for various applications. Due to disparities in feeding patterns and habitat choices, polychaete

species demonstrate variable capacities for heavy metal accumulation[8,9].

Despite their ecological and nutritional value, polychaetes are also known for their remarkable bioactive properties. Marine invertebrates rely extensively on intrinsic immune mechanisms, as their adaptive immunity is considerably less developed[10]. Numerous studies have reported the antioxidant activity in marine polychaetes. Zamzam *et al*.[11] showed DPPH radical scavenging activity and appreciable total phenolic content in *Diopatra claparedii* and *Marphysa moribidii*. Similarly, antioxidant properties have been identified in the mucus of *Myxicola infundibulum*[12].

Beyond these observations, studies on the antioxidant potential of polychaetes remain scanty. Several studies depend on crude extracts or mucus samples devoid of standardised extraction and quantification protocols. Detailed characterisation of extracted proteins and examination of structure-activity relationships remain scarce. More than 12,776 species of polychaetes have been documented worldwide[13], indicating a vast yet largely unexplored reservoir of potential natural antioxidants. However, the majority of these species remain insufficiently investigated.

With increasing global interest in marine-derived antioxidants for mitigating oxidative stress-related disorders such as liver damage, neurodegeneration, and ageing[14], addressing this research gap has become particularly important. By assessing antioxidant activity using DPPH and FRAP assays, the current study contributes to the body of knowledge on antioxidant-rich polychaetes and evaluates their suitability for nutraceutical and pharmaceutical applications[12].

Perinereis nuntia (Lamarck, 1818), an Indo-Pacific polychaete species, represents one such underexplored candidate[15]. The current study investigates the proximate composition and bioactive potential of *P. nuntia* collected from the Bay of Bengal region. Specimens were collected and identified taxonomically, and their distribution was assessed to determine ecological relevance. Proximate analysis was conducted to evaluate nutritional value, while heavy metal estimation along with macro- and micronutrient profiling was performed to assess biosafety and elemental composition.

Although polychaetes have been extensively studied for their fatty acid profiles due to their association with broodstock development, limited attention has been given to their protein composition. The study also evaluates the suitability of the RIPA buffer for protein extraction from *P. nuntia*, followed by its quantification to assess extraction efficiency. The extracted proteins were subsequently characterised using UV-visible spectroscopy, Dynamic Light Scattering (DLS) for average particle size and zeta potential analysis, and Fourier-Transform Infrared (FTIR) spectroscopy. Functional bioactivity was finally evaluated using DPPH and FRAP radical-scavenging assays.

Overall, this investigation provides an integrated understanding of the nutritional, ecological, and biomedical relevance of *P. nuntia*, highlighting its promise as a sustainable marine bioresource with potential applications in health-promoting and value-added sectors.

Materials and Methods

Sample collection, identification, and storage

Samples of *P. nuntia* were collected from the Bay of Bengal coast from the intertidal region of Thatiyakula Palem Beach, Kona Forest, Andhra Pradesh, India (17°12'43.4" N, 82°29'19.0" E). Approximately 500 g (wet weight) of mixed-sized adult samples were collected, packed in a thermocol box to maintain cold conditions, and transported to the laboratory. Upon arrival, the worms were thoroughly washed with distilled water and then with phosphate-buffered saline (PBS; pH 7.4) to minimise contamination. Excess water was gently wiped away with tissue paper.

Specimens were observed under a stereo zoom microscope (Labomed CZM6, Labomed Inc., USA) for morphological assessment. Key diagnostic features, including prostomium configuration, arrangement of paragnaths in Areas I-VIII, parapodial cirri, and chaetal morphology, were examined following the descriptions provided by WoRMS[16] and Glasby & Hsieh[15]. Due to their relatively small body size, specimens were processed as pooled biomass for all biochemical analyses rather than individually. Cleaned samples were stored at -20 °C until further analysis.

Proximate analysis

The proximate content was determined using the standard protocol set by the Association of Official Analytical Chemists (AOAC), 2023[(ref. 17)].

Moisture

Moisture content was determined using Shimadzu Moisture Analyser (Moc63u). 1 g of tissue was placed on the pan and dried at 120 °C to a constant weight. Moisture content was analysed automatically.

Ash

1 g dried powder sample was placed in a weighed porcelain crucible (W1) in a muffle furnace and was burnt for 12 h until white ash remained. The weight was taken after cooling the crucibles (W2). Ash content was determined by:

$$Ash\% = \frac{Weight\ of\ Ash\ (W2 - W1)}{Weight\ of\ Sample} \times 100$$

Protein

Total protein content was estimated by the Kjeldahl method[17]. 2 g of dried sample was digested by employing the acid digestion method. The digest was diluted, and distillation was performed using a Kjeldahl apparatus. The released ammonia was trapped in boric acid and then titrated with 0.1 N sulfuric acid to obtain the nitrogen content. Protein was estimated indirectly using the nitrogen percentage.

$$Protein\ \% = 6.25 \times nitrogen\%$$

Fat/Lipid

Total fat content was determined by the Soxhlet method[17]. 2 g of dried sample was taken in a thimble and weighed (W1). Extraction was done using petroleum ether for 5 h. After extraction, the thimble was placed in hot-air oven for 48 h to evaporate the petroleum ether completely and then weighed (W2).

$$Fat\ \% = \frac{Weight\ of fat(W1 - W2)}{Weight\ of\ the\ sample} \times 100$$

Carbohydrate (Nitrogen-free extract)

Carbohydrate content was calculated as Nitrogen-Free Extract (NFE) by subtracting the sum of percentages of protein, lipid, and ash from 100:

$$NFE\% = 100 - (protein\% + lipid\% + ash\%)$$

Heavy metal and nutrient analysis

Heavy metal and nutrient content in *P. nuntia* were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The analysis was conducted at the Biotechnology Centre, Bannerghatta, Bengaluru. Samples were acid-digested[7,9], and the digested solutions were analysed for micro-nutrients and macronutrients, including N, P, K, Ca, Mg, S, Cu, Zn, Mn, Fe, Pb, Cr, Cd, and Ni.

Protein extraction and quantification

Approximately 1 g of *P. nuntia* tissue was stored at -20 °C, thawed on ice, and transferred to a chilled homogeniser tube. 10 mL of RIPA buffer and 100 µL of 1X protease inhibitor cocktail were added to the reaction mixture. RIPA buffer was chosen as it is commonly used in mammalian and tissue-based proteomics for efficiently extracting cytosolic and membrane proteins[18]. The sample was homogenised properly on ice using a mechanical homogeniser. Further, the homogenate was incubated on ice for 30 min, vortexed every 5 min, and centrifuged at 11,000 rpm for 30 min at 4 °C.

The supernatant (protein extract) was collected, and 4 volumes of acetone LR were added. The mixture was left overnight for improved precipitation. The next day, centrifugation was carried out at 11,000 rpm for 25 min at 4 °C. The pellet obtained was resuspended in double-distilled water. This suspension was aliquoted into chilled microcentrifuge tubes and stored at -20 °C until further use[18].

Protein concentration was determined using the Lowry method, with Bovine Serum Albumin (BSA) as a standard[19]. Absorbance was measured at 660 nm using a UV-visible spectrophotometer (Shimadzu-1900).

Protein characterisation

The extracted protein was subjected to various characterisation methods to confirm its presence and assess the quality of the extraction.

UV-visible spectroscopy

The extract was scanned between 200–700 nm to obtain peaks in a UV-1900 spectrophotometer (Shimadzu).

Dynamic Light Scattering (DLS)

Average particle size and Zeta potential were analysed using DLS (ZEN 3600, Malvern Instruments). The detection sensitivity was 0.1 mg/mL. Average particle size was assessed based on the Dynamic Light Scattering (DLS) principle, whereas the Zeta potential was measured based on the Electrophoretic Light Scattering (ELS) principle.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR was employed to examine the functional group of the extracted molecule and its polyelectrolytic interactions. Completely air-dried

pellets obtained by acetone precipitation were used for analysis. FTIR analysis was performed using QATR-S (Shimadzu IRSpirit) in the 4000–400 cm^{-1} range.

In vitro antioxidant assays

Antioxidant activity was assessed using DPPH radical-scavenging and Ferric Reducing Antioxidant Power (FRAP) assays.

DDPH

0.5 mL of the test sample was taken, and the volume was made up to 1.5 mL using methanol. To this, 2 mL of methanolic DPPH was added, and the mixture was incubated in the dark for 30 min. After incubation, absorbance was taken at 517 nm, using a UV-Vis 1100 spectrophotometer[11].

$$\% \text{ DPPH Scavenged} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

FRAP

0.5 mL of the test sample was first diluted to 1.5 mL using distilled water. Then, 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide solution were added to the mixture. The solution was well vortexed and then left in a water bath at 50 °C for 20 min. The reaction was arrested by adding 2.5 mL of 10 % trichloroacetic acid. The mixture was then centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant was pipetted into a fresh test tube, and 0.5 mL of 0.1 % ferric chloride solution was added. The absorbance of the final reaction mixture was measured at 700 nm using a UV-Vis-1100 spectrophotometer[20].

$$\%Fe^{3+} \text{ Scavenged} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Statistical analysis

Proximate analysis and protein extraction yield (Lowry method) were done in triplicate (n = 3), whereas crude protein content (Kjeldahl method) and elemental analysis were done in duplicates (n = 2). Data are presented as mean ± standard deviation. Statistical analysis was done using IBM SPSS Statistics (Version 21.0).

Results

Morphological identification

The sample exhibited morphological characteristics typical of the *P. nuntia* species group, such as bar-shaped paragnaths in Area VI and cones and bars in Area IV. Parapodial and chaetal features were in accordance with nereidid morphology[15]. Recent taxonomic revisions[21] indicate that the *P. nuntia* group comprises several closely related species. This identification is referred to as *P. nuntia* sensu lato[21].

Proximate content

The moisture content was found to be (88.76±0.19 %), ash (9.65±0.15 %), protein (54.33±0.185 %), fat (27.1±0.0 %), and carbohydrate (by the difference) was found to be (8.915±0.335 %), depicted in Figure 1.

Nutrient and heavy metal composition

The profile of heavy metals in *P. nuntia* revealed that lead content was 34.05±7.53 ppm, cadmium (0.055±0.055 ppm), chromium (9.45±1.19 ppm), and nickel (13.12±0.69 ppm) (Fig. 2).

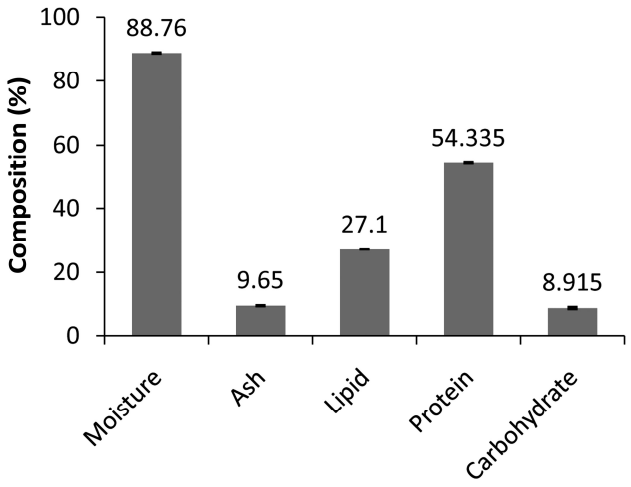


Fig. 1 — Proximate composition (%) of *P. nuntia*

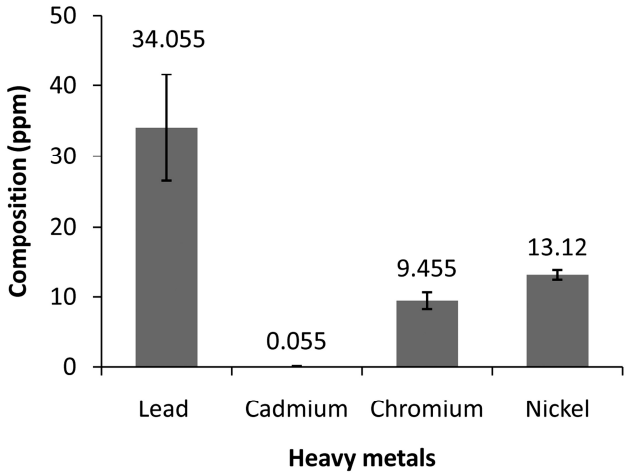


Fig. 2 — Heavy metal composition (ppm) of *P. nuntia*

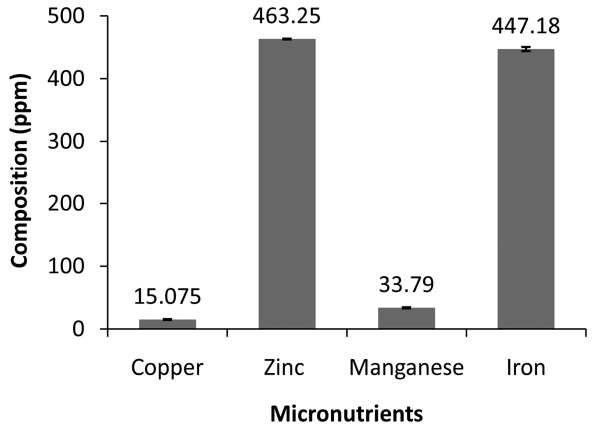


Fig. 3 — Micronutrient content (ppm) in *P. nuntia*

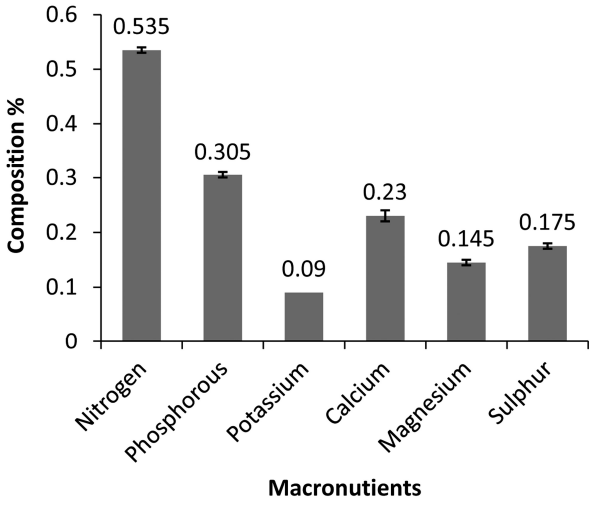


Fig. 4 — Macronutrient content (%) in *P. nuntia*

Micronutrients detected were copper (15.07± 0.65 ppm), zinc (463.25±0.73 ppm), manganese (33.79± 0.91 ppm), and iron (447.1800±3.32 ppm) (Fig. 3).

Macronutrients detected were nitrogen (0.5350± 0.005 %), phosphorus (0.3050±0.005 %), potassium (0.09±0.0 %), calcium (0.23±0.1 %), magnesium (0.145±0.005 %), and sulphur (0.175±0.005 %) (Fig. 4).

Protein extraction and quantification:

On quantification by Lowry's method, the amount of protein in 1 g of tissue was found to be 1.47±0.54 mg (wet weight basis) by substituting absorbance in the equation $Y = 0.0022x + 0.0184$ obtained from the standard curve.

Characterisation of the protein extract

UV-Vis spectrum

UV-Vis spectrum of *P. nuntia* showed well-defined absorbance peaks at 219, 250, 270, and 340 nm (Fig. 5).

DLS analysis

The extracted protein exhibited a z-average particle size of 270.2 nm with a Polydispersity Index (PDI) of 0.45. The average zeta potential of the protein sample was -26.9 mV.

FTIR spectrum

The FTIR spectrum showed typical protein vibrational bands. The amide A band appeared at 3384 cm^{-1}. The amide II band was obtained at 1583 cm^{-1}. The amide III bands were visible at 1342 cm^{-1}, 1288 cm^{-1}, and 1190 cm^{-1} (Fig. 6).

Bioactivity

Antioxidant properties

The protein extract of *P. nuntia* demonstrated radical-scavenging activity, with 87.648 % DPPH scavenging and 10.78 % Fe^{3+} scavenging.

Discussion

Proximate content and its utility for broodstock development

Polychaetes have been used as live feed since ancient times. The proximate composition of *P. nuntia* in this study indicates high levels of protein and fats. The values are consistent with values reported in several studies, *e.g*., protein in wild *P. nuntia* (52.8 %) and in *Perinereis cultifera* (54.24 %). However, Li *et al*.[22] reported higher protein in *P. nuntia* (59.46 %). High protein content is important for meeting the high metabolic requirements of broodstock for reproductive development. The 27.1 % fat content reported in this study is relatively high. It is on par with 27.4 % reported for wild *P. nuntia*[23] and much higher than *P. nuntia* (21.38 %) reported by Li *et al*.[22] and *P. cultifera* (6.15 %) as reported by Sivalakshmi *et al*.[24]. These differences may be due to differences in the region. The 88.76 % moisture content indicates that the sample must be very fresh, although it is well above some published values for *P. nuntia* (76.3–81.3 %)[22,23] and *P. cultifera* showing moisture in the range of 85.33–87.76 %. The ash (9.65 %) and carbohydrate (8.91 %) levels obtained during the current approach are comparable to values reported by Techaprempreecha *et al*.[23] and Li *et al*.[22]. These values fall within typical ranges for high-quality animal-based feeds[23]. This denotes that *P. nuntia* is a rich source of energy and key nutrients essential for reproductive success in shrimp.

Studies have demonstrated that *Penaeus monodon* fed with polychaetes produces much higher sperm counts than with commercial diets[25]. They have also

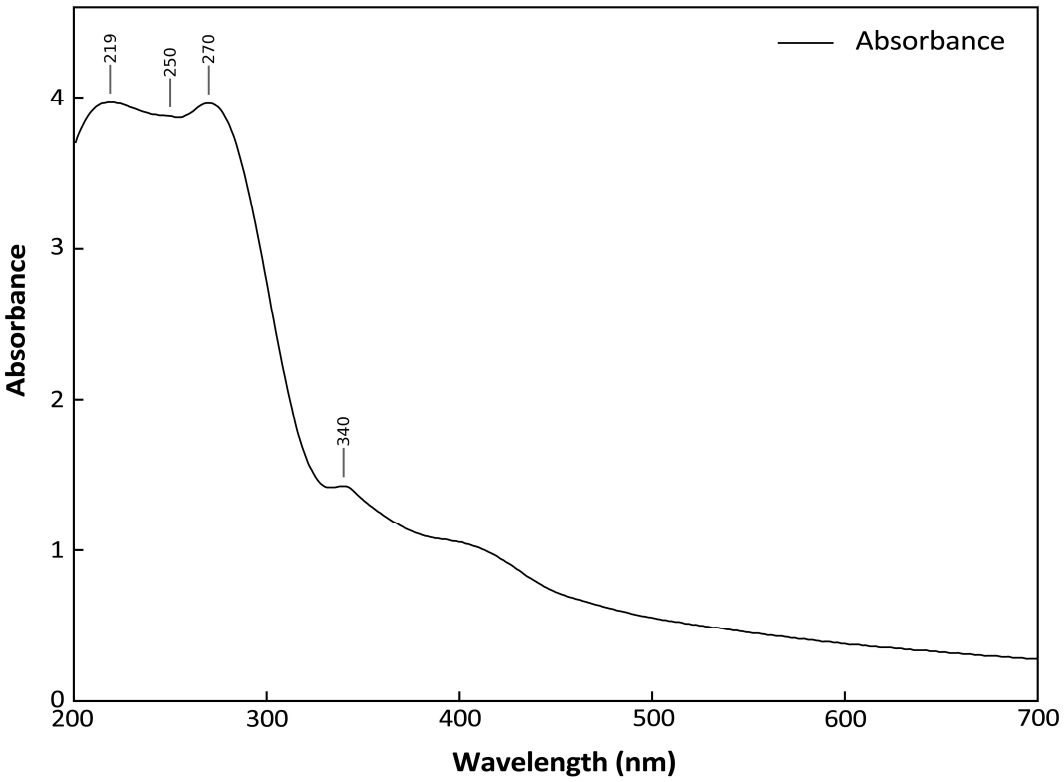


Fig. 5 — UV-Visible spectrum for protein extract of *P. nuntia*

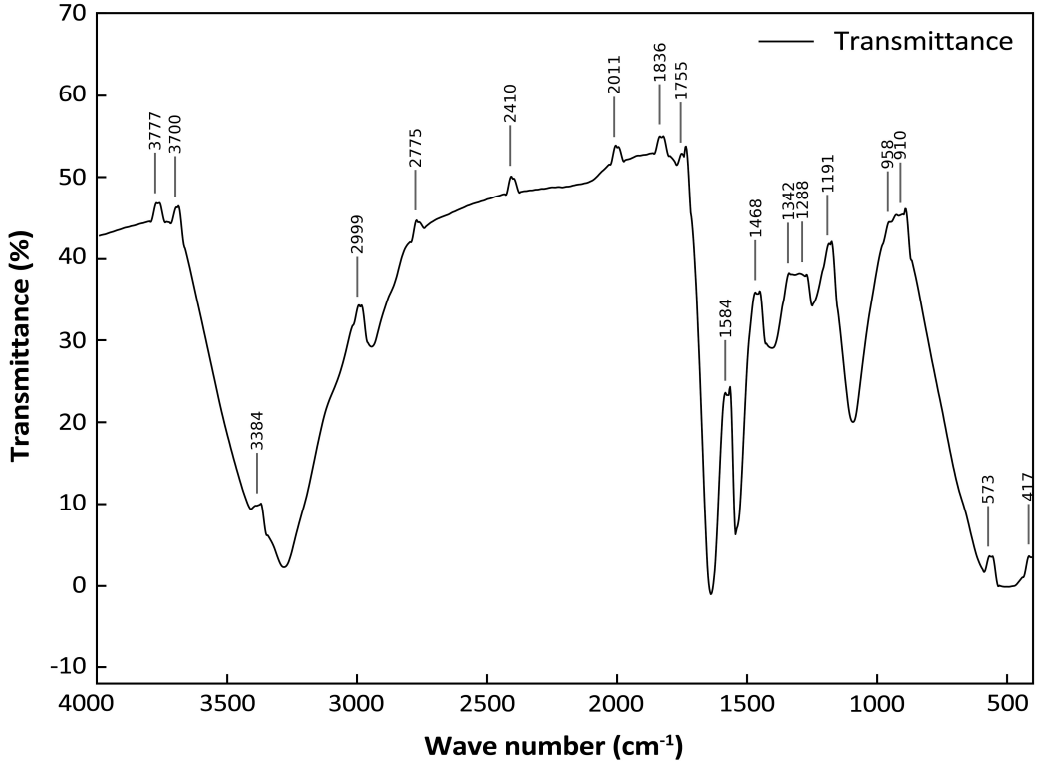


Fig. 6 — FTIR plot for protein extract of *P. nuntia*

Table 1 — Heavy metals accumulation in polychaetes from the Indo-Pacific region

Species	Location	Heavy metal concentration (ppm)								Interpretation	References
		Pb	Cr	Cd	Ni	Hg	Cu	Zn	Mn		
Nereis succinea	Hurghada city- Red Sea coast Marine Biological Station (MBS-1)	0.59	-	0.26	-	-	0.31	-	0.18	Worms collected from a relatively clean site had less accumulation	Said *et al*., 2017[ref. 6]
	Hurghada city – Red Sea coast Fishing Port at Sakala (FPS-2)	0.78	-	0.85	-	-	0.95	-	0.87	Worms collected from comparatively more polluted areas showed higher accumulation	
Wawo worms	Alang-Ambon coastal waters, Indonesia	1.82	-	1.36	-	7.30	-	-	-	Hg wasn't detected in the water column. Yet the highest amount of Hg was detected in worms. More Pb and Cd were detected in worms than in the water column	Liline & Rumahlatu, 2018[ref. 29]
	Latuhalat – Ambon coastal waters, Indonesia	1.62	-	1.21	-	6.81	-	-	-	More heavy metals were detected in worms than in the water column. Indicates bioaccumulation	
	Rutong – Ambon coastal waters, Indonesia	1.54	-	2.26	-	6.48	-	-	-	More heavy metals were detected in worms than in the water column. Indicates bioaccumulation	
Laor worms	Tobelo coastal water	-	-	10.15	-	-	0.85	29.54		These metals account for metabolism and physiological processes in Laor worms	Wairata *et al*., 2020[ref. 28]
Perinereis nuntia	Bay of Bengal coast. Thatiyakula Palem beach, Andhra Pradesh, India	34.05	9.45	0.26	13.12	-	15.07	463.25	33.79	Elevated levels are found for all heavy metals, indicating high pollution and significant bioaccumulation by the worms. Zinc levels are specifically alarming	Current study

been shown to reduce the overall percentage of abnormal sperm. They benefit the acrosome reaction percentage, critical sperm viability, and the fertilising capacity of sperm in males[26]. Whereas *P. monodon* females benefit from improved ovarian development[27].

Heavy metal and nutrient composition

Sediments are an essential part of marine ecosystems, and heavy metals, both essential and non-essential, accumulate in sediments[6]. Many ecotoxicology studies have shown that polychaetes can accumulate heavy metals and serve as good bioindicators of sediment contamination[6,7]. They can efficiently accumulate metals such as Pb, Cr, Ni, Zn, Fe, etc., reflecting environmental pollution[6,28]. A study shows that *N. succinea* accumulates more in polluted areas[6]. Another study from Indonesia found that Wawo worms can accumulate high levels of Hg, Pb, and Cd even when these metals are not detected in the water column. The study highlights their value for marine pollution assessment[29].

Polychaetes form an important part of the marine food chain[5]. Humans can also be affected if they consume marine organisms living in a contaminated environment. Heavy metals in seawater, sediment, and polychaetes are largely correlated[8]. This makes them important for understanding pollution impacts and trophic transfer[7].

In this study, the heavy metal profile of *P. nuntia* showed notably elevated levels, including lead (34.05±7.53 ppm), chromium (9.45±1.19 ppm), and nickel (13.12±0.69 ppm) while cadmium was present at a lower level (0.055±0.055 ppm). These values were above the FAO/WHO safe limit of 1.0–2.0 ppm (for Cr), 2.2 ppm (for Cd), and 2.0 ppm (for Pb) in commercial fish feed. The values were above the EU safe limit of 0.1–8.0 ppm for Ni[30]. Table 1 further supports these findings by showing that *P. nuntia*

exhibits higher concentrations of several heavy metals compared to other polychaetes reported from less polluted regions.

Relative to such feed safety levels, the heavy metal contents of Pb, Cr, and Ni in *P. nuntia* were exceedingly high and are of concern if they are to be used in aquaculture. It is therefore crucial to monitor and potentially pre-treat or purify *P. nuntia* if it can be used as a sustainable feed source. Such levels of heavy metals could also pose ecological and health risks through bioaccumulation and trophic transfer unless properly controlled[30].

In terms of nutritional value, the availability of essential micronutrients such as zinc, iron, manganese, and copper, supports the nutritional value of *P. nuntia* as a feed ingredient. These micronutrients are essential for maintaining multiple physiological processes in shrimp and crabs, such as antioxidant defence, immune response, enzyme activation, and nutrient metabolism[4]. Zinc is a component of carbonic anhydrase, an enzyme required for shell formation. Iron contributes to oxygen transport, metabolic pathways, and cellular defence mechanisms[31]. All these trace elements aid in synchronising moulting cycles and hardening exoskeletons[4]. The high micronutrient content of *P. nuntia* aligns with observations in the *Marphysa* and *Halla* species[4], suggesting its potential as a sustainable feed. Nevertheless, as they have high bioaccumulation potential, their safe use is recommended to prevent long-term heavy metal accumulation in consumers such as humans[6,32].

The macronutrient content of *P. nuntia* (N: 0.53± 0.005 %; P: 0.30±0.005 %; K: 0.09±0 %; Ca: 0.23±0.01 %; Mg: 33.79±0.005 %; S: 0.15±0.005 %) correlates with *Marphysa* spp. and *Halla* spp., which have been used in nutritional aquaculture applications[4]. In general, the data validate both the potential of *P. nuntia* as an aquafeed ingredient with potential benefits and the status of sensitive bio-monitor species. Further purification, selective blending in feed formulations and habitat monitoring are suggested to achieve a balance between nutritional value and food safety. Thus, while *P. nuntia* shows promising nutritional properties; its direct use as wild-harvested aquafeed should be approached with caution. The species can be cultured in controlled or captive systems to avoid exposure to heavy metals.

Protein extraction and quantification

It was observed that the protein content of the extract was low (1.47±0.54 mg/g tissue). Even after using RIPA, a commonly used lysis technique, total protein recovery from *P. nuntia* was very low. The results obtained were reproducible across replicates.

The RIPA buffer has been known to solubilise cytoplasmic, nuclear, and membrane-bound proteins[18]. Nevertheless, a critical finding of this study is that it does not efficiently extract the Extracellular Matrix (ECM) and structural proteins (insoluble in nature). It also pointed out that RIPA-extracted fractions from breast tumours contained virtually all ECM proteins, which were recovered only by subsequent extraction with a urea-based buffer[18].

Owing to the intricate tissue structure of *P. nuntia*, a large amount of protein may have remained insoluble in RIPA buffer, thereby limiting yield. This observation indicates buffer choice as a crucial factor in proteomics. A two-stage extraction process, as suggested by Ngoka[18], using RIPA initially followed by a urea-based buffer, could enhance extraction efficiency in future investigations involving polychaetes.

Characterisation

UV Spectrum

The strong absorbance at 219 nm corresponds to the π-π* transitions of peptide bonds, indicative of protein backbone structures. Peaks at 250 nm and 270 nm are associated with aromatic amino acids such as tyrosine and tryptophan. They are commonly found in protein-rich extracts. The 340 nm absorbance, however, can indicate residual non-protein chromophores, like remaining lipids or interfering substances. The breadth and overlap of peaks indicate a lack of purification[33]. The noise can also be due to contamination by detergent residues such as SDS and Triton X-100[(ref. 18)]. UV-Visible spectroscopy, though not very specific, is a convenient primary tool for protein detection and assessing sample integrity. Therefore, combining it with other methods is recommended to verify protein purity and concentration[34].

Average particle size (nm) and zeta potential (mV)

The average particle size obtained (270.2 nm) through DLS is higher than that of usual globular proteins (1–5 nm)[33]. The polydispersity index obtained was also quite high. This reflects high aggregation and heterogeneity in the sample. Multimodal distribution reaffirms heterogeneous aggregate populations.

The average zeta potential (-26.9 mV) indicates moderate colloidal stability, as reading below ±30 mV

indicates very little repulsion to keep particles separate (Malvern Instruments). The two populations (-31.3 mV and -10.3 mV) also account for the observed heterogeneity and aggregation. Even though the RIPA extraction technique is commonly utilised, it can cause aggregation. It may even result in partial protein profiles, particularly for high molecular weight proteins.

FTIR

The FTIR spectrum shows characteristic protein vibrational bands, providing information about the nature of the sample. The amide A band at 3384 cm^{-1} is mainly due to the N-H stretching vibration of the peptide backbone. The position of this band can indicate the degree of hydrogen bonding[35].

A key observation was the absence of a distinct amide I band in the 1600–1700 cm^{-1} range. The amide I band is largely caused by the C=O stretching vibration of the amide group. It is highly sensitive to the secondary structure of protein (Helical or sheet conformation)[35]. Its absence strongly suggests poor structural integrity. This might be due to the extraction method employed, which might have led to mild protein denaturation, aggregation, or loss of specific conformational elements, thereby diminishing the characteristic amide I peaks[36,37].

In addition, the amide II band was seen at 1583 cm^{-1} due to coupled N-H bending and C-N stretching vibrations[35]. Several amide III bands were also observed at 1342 cm^{-1}, 1288 cm^{-1}, and 1190 cm^{-1}. This region includes C-N stretching, N-H bending, and C-H bending modes[35]. Characterisation of proteins employing FTIR analysis on *P. nuntia* is limited. Moreover, this study concludes that RIPA extraction resulted in protein extraction; however, the quality and quantity of the extracts are not up to the mark. The lack of well-defined structural features (missing amide I bond) may compromise the protein's functional and bioactive properties to a certain extent. Therefore, the current approach reveals the efficacy of RIPA buffer-mediated polychaete-based protein extraction and spurs insights to open up the scope to find and optimise better protein extraction methods from polychaetes for future studies.

Antioxidant properties

Perinereis nuntia protein extract exhibited notable antioxidant activity against DPPH (87.65 %). In contrast, ferric ion (Fe^{3+}) reduction was relatively low at 10.78 %, signifying minimal electron-donating activity under the tested conditions. Antioxidant capacity is usually correlated with peptide structural characteristics, such as hydrophobicity, aromatic amino acids, and molecular size. These are known to improve radical-scavenging efficiency[38]. This may be due to adaptive physiological responses to environmental stress. Interestingly, gene expression analysis has indicated superinduction of antioxidant genes, including superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), in *P. nuntia* when exposed to hydrocarbon pollutants, implying an evolved oxidative defence system[39].

Moreover, marine invertebrates are known as reservoirs of natural antioxidants, with potential health and therapeutic benefits[40]. The DPPH scavenging activity observed in the present study suggests that *P. nuntia* protein extracts may contain bioactive constituents; however, further purification and structural characterisation are required before drawing any conclusion on functional applications.

For contextual comparison, methanol extracts of two other polychaetes, *Diopatra claparedii* and *Marphysa moribidii*, were found to exhibit lower DPPH inhibition percentages of 38.80 % and 25.54 %, respectively (at 5000 µg/mL)[11]. However, direct comparisons across extraction methods should be interpreted with caution. Within these limitations, the DPPH response observed in this study implies appreciable free radical scavenging activity. The relatively low ferric Fe^{3+} ion reduction capacity aligns with reports on other marine invertebrates, such as *S. spallanzani* and *M. squamiger*, which also demonstrate metal chelation and antioxidant activities, pointing toward a heterogeneous mechanism involved[41].

Conclusion

The study identifies *Perinereis nuntia* as a promising marine bioresource with multifaceted applications. Its protein and fat content support its potential as a nutritive aquafeed for broodstock development. It is also a good bioindicator owing to its ability to bioaccumulate heavy metals such as Pb, Ni, Cr, Cu, Mn, Zn, etc. Although the RIPA buffer extraction resulted low protein yield, the extracted proteins showed notable antioxidant activity. This suggests the possible presence of bioactive compounds. Since the specimens were wild-collected, heavy metal accumulation remains a concern; therefore, captive culture may improve their suitability for aquafeed applications. Future studies

should focus on optimising extraction techniques and conducting detailed biosafety evaluations for sustainable application and safe utilisation.

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Conflict of Interest

The authors declare no competing or conflict of interest.

Ethical Statement

This study did not involve human participants, human-derived materials, or experimental animals. Therefore, ethical approval was not required. All experimental procedures were conducted in accordance with applicable institutional and laboratory guidelines.

Author Contributions

KS: Conceptualisation, methodology, investigation, data curation, formal analysis, writing-original draft, and visualisation. PA: Conceptual guidance, project administration, resources, supervision, and writing-review & editing. KBA: Conceptual guidance, protocol design for protein extraction, and conceptual guidance.

References

1 Villalobos-Guerrero T F, Huč S, Tilic E, Hiley A S & Rouse G W, A remarkable new deep-sea nereidid (Annelida: Nereididae) with gills, *PLoS ONE*, 19 (3) (2024) p. e0297961. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0297961

2 Pamungkas J, Species richness and macronutrient content of Wawo worms (Polychaeta, Annelida) from Ambonese waters, Maluku, Indonesia, *Biodivers Data J*, 3 (2015) p. e4251. https://bdj.pensoft.net/article/4251/

3 FAO, *FAO Report: Global fisheries and aquaculture production reaches a new record high*, World Wide Web electronic publication. Accessed online at: https://www.fao.org/newsroom/detail/fao-report-global-fisheries-and-aquaculture-production-reaches-a-new-record-high/en; (Accessed on July 2025).

4 Wang X, Sui Z, Xu X, Chai Y & Wang H, Nutritional value and biochemical composition of two new tropical polychaete species: Potential use as feed ingredients, *J World Aquac Soc*, 56 (1) (2025) p. e13122. https://doi.org/10.1111/jwas.13122

5 Balasubramanian S, Soniya S, Ahamed S J, Babu M M, Rajan R A, *et al*., Taxonomic List of Macro Benthic Polychaetes of Kanyakumari Coast, *Uttar Pradesh J Zool*, 44 (23) (2023) 416–429. https://mbimph.com/index.php/UPJOZ/article/view/3802

6 Said R E M, AbdAllah T A, Mohsen A M & Wl-Shimy N A, Efficiency of Polychaete *Nereis (Neanthes) succinea* as Biomonitor for Heavy Metals Pollution in the Red Sea, Egypt, *Adv Biosci Bioeng*, 5 (5) (2017) 86–91. https://www.sciencepublishinggroup.com/article/10.11648/j.abb.20170505.13

7 Bat L & Kurt G, Use of polychaeta species as biomonitor for heavy metal pollution in the marine environments, In: *Environmental Radioactivity In Turkish Environment*, edited by Bayram T, Zayachuk Y & Gupta D K, (Sivas Cumhuriyet Üniversitesi Matbaası, Sivas), 2020, pp. 259–281. https://www.researchgate.net/publication/347838331_USE_OF_POLYCHAETA_SPECIES_AS_BIOMONITOR_FOR_HEAVY_METAL_POLLUTION_IN_THE_MARINE_ENVIRONMENTS_In_Tuncay_Bayram_Yevhen_Zayachuk_Dharmendra_K_Gupta_Eds_Radioactivity_in_Turkish_Environment

8 Rozirwan R, Az-Zahra S A F, Khotimah N N, Nugroho R Y, Putri W A E, *et al*., Ecological Risk Assessment of Heavy Metal (Pb, Cu) Contamination in Water, Sediment, and Polychaeta (*Neoleanira tetragona*) from Coastal Areas Affected by Aquaculture, Urban Rivers, and Ports in South Sumatra, *J Ecol Eng*, 25 (1) (2024) 303–319. http://www.jeeng.net/Ecological-Risk-Assessment-of-Heavy-Metal-Pb-Cu-Contamination-in-Water-Sediment-and,175365,0,2.html

9 Bat L, Şahin F & Öztekin A, Assessment of Heavy Metals Pollution in Water and Sediments and Polychaetes in Sinop Shores of The Black Sea, *J Agric Nat*, 22 (5) (2019) 806–816. http://dergipark.org.tr/en/doi/10.18016/ksutarimdoga.v22i45606.535882

10 Safronova V N, Panteleev P V, Kruglikov R N, Bolosov I A, Finkina E I, *et al.*, Novel BRICHOS-related Defensin-like Antimicrobial Peptide from the Marine Polychaeta *Arenicola marina*, *Russ J Bioorg Chem*, 50 (3) (2024) 629–643. https://link.springer.com/10.1134/ S10681620240 30087

11 Zamzam N I, Kamarudin N I, Idris I, Harun N A, Ismail W I W, *et al*., Investigation of antioxidant activity and chemical fingerprint of marine polychaete based on atrftir metabolomics, *UMT J Undergrad Res*, 3 (4) (2021) 81–88. https://journal.umt.edu.my/index.php/umtjur/article/view/241

12 Stabili L, Licciano M, Giangrande A, Gerardi C, De Pascali S A, *et al*., First Insight on the Mucus of the Annelid *Myxicola infundibulum* (Polychaeta, Sabellidae) as a Potential Prospect for Drug Discovery, *Mar Drugs*, 17 (7) (2019) p. 396. https://www.mdpi.com/1660-3397/17/7/396

13 Read G F K (ed), *World Polychaeta Database*, World Wide Web electronic publication. Accessed online at: https://www.marinespecies.org/polychaeta/aphia.php?p=stats (Accessed on July 2025)

14 Yang X R, Qiu Y T, Zhao Y Q, Chi C F & Wang B, Purification and Characterization of Antioxidant Peptides Derived from Protein Hydrolysate of the Marine Bivalve Mollusk *Tergillarca granosa*, *Mar Drugs*, 17 (5) (2019) p. 251. https://www.mdpi.com/1660-3397/17/5/251

15 Glasby C J & Hsieh H L, New Species and New Records of the Perinereis nuntia Species Group (Nereididae: Polychaeta) from Taiwan and Other Indo-West Pacific Shores, *Zool Stud*, 45 (4) (2006) 553–577.

16 WoRMS - World Register of Marine Species, *Perinereis nuntia (Lamarck, 1818)*, World Wide Web electronic publication. Accessed online at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=130411; (Accessed on July 2025).

17 AOAC International, *Official Methods of Analysis*, 22nd Edn, World Wide Web electronic publication. Accessed online at: https://www.aoac.org/official-methods-of-analysis/; (Accessed on July 2025)

18 Ngoka L C M, Sample prep for proteomics of breast cancer: proteomics and gene ontology reveal dramatic differences in protein solubilization preferences of radioimmunoprecipitation assay and urea lysis buffers, *Proteome Sci*, 6 (1) (2008) p. 30. http://proteomesci.biomedcentral.com/articles/10.1186/1477-5956-6-30

19 Lowry Oliver H, Rosebrough Nira J, Farr A L & Randall Rose J, Protein measurement with the Folin phenol reagent, *J Biol Chem*, 193 (1) (1951) 265–275. https://www.jbc.org/article/S0021-9258(19)52451-6/pdf

20 Spiegel M, Kołodziejczyk W, Kapusta K & Sroka Z, Antioxidant Activity of Selected Phenolic Acids–Ferric Reducing Antioxidant Power Assay and QSAR Analysis of the Structural Features, *Molecules*, 25 (13) (2020) p. 3088. https://www.mdpi.com/1420-3049/25/13/3088

21 Elgetany A H, Struck T H & Glasby C J, Three new species of the genus *Perinereis* (Annelida, Nereididae) from Egyptian coasts, *ZooKeys*, 1132 (2022) 163–188. https://doi.org/10.3897/zookeys.1132.87629

22 Li S, Liu H, Huang W, Yang S, Xie M, *et al*., Effect of three polychaetes on growth and reproductive performance, biochemical indices and histology of different tissues in the female Pacific white shrimp, *Litopenaeus vannamei* broodstock, *Anim Nutr*, 21 (2025) 49–62. https://www.sciencedirect.com/science/article/pii/S2405654525000046

23 Techaprempreecha S, Khongchareonporn N, Chaicharoenpong C, Aranyakananda P, Chunhabundit S, *et al*., Nutritional composition of farmed and wild sandworms, *Perinereis nuntia*, *Fuel Energy Abstr*, 169 (3) (2011) 265–269.

24 Sivalakshmi R, Madhavi M, Jothika A, Anupama V & Devi P L, Biochemical Analysis of the Polychaete, *Perineries cultifera* as a Potential Live Feed in Aquaculture, *Uttar Pradesh J Zool*, 45 (19) (2024) 1–9. https://mbimph.com/index.php/UPJOZ/article/view/4492

25 Yotbuntueng P, Jiemsup S, Deenarn P, Tobwor P, Yongkiettrakul S, *et al*., Differential distribution of eicosanoids and polyunsaturated fatty acids in the Penaeus monodon male reproductive tract and their effects on total sperm counts, *PLoS One*, 17 (9) (2022) p. e0275134. https://doi.org/10.1371/journal.pone.0275134

26 Leelatanawit R, Uawisetwathana U, Khudet J, Klanchui A, Phomklad S, *et al*., Effects of polychaetes (*Perinereis nuntia*) on sperm performance of the domesticated black tiger shrimp (*Penaeus monodon*), *Aquaculture*, 433 (2014) 266–275. https://www.sciencedirect.com/science/article/abs/pii/S0044848614003299

27 Deenarn P, Tobwor P, Vichai V, Phomklad S, Chaitongsakul P, *et al*., Polychaete consumption increased prostaglandin biosynthesis in female *Penaeus monodon*, *Reproduction*, 160 (6) (2020) 873–885. https://pubmed.ncbi.nlm.nih.gov/33112802/

28 Wairata J, Tutup F W O & Mangimbulu J C, Evaluation of Fatty Acid and Amino Acid in Laor Worms (Polychaetes) from Tobelo, Indonesia Coastal Water, *Asian J Biol Sci*, 13 (2) (2020) 127–133. https://www.scialert.net/abstract/?doi=ajbs.2020.127.133

29 Liline S & Rumahlatu D, Bioaccumulation of heavy metals (Cd, Pb, and Hg) in wawo worms (Polychaeta, Annelida) from Ambon Coastal Waters, Indonesia, *Indian J Geo-Mar Sci*, 48 (12) (2019) 1881–1887. http://nopr.niscpr.res.in/handle/123456789/52804

30 Saha B, Mottalib M A & Razee A A, Assessment of selected heavy metals concentration in different brands of fish feed available in Bangladesh, *J Bangladesh Acad Sci*, 42 (2) (2018) 207-210. https://doi.org/10.3329/jbas.v42i2.40054

31 Luo J, Zhu T, Jin M, Cheng X, Yuan Y, *et al*., Influence of dietary zinc on growth, zinc bioaccumulation and expression of genes involved in antioxidant and innate immune responses in juvenile mud crabs (*Scylla paramamosain*), *Br J Nutr*, 124 (7) (2020) 681–692. https://doi.org/10.1017/S0007114520001531

32 Raynal B, Hoos S, Baron B, Lenormand P & England P, Quality assessment and optimization of purified protein samples: why and how? *Microb Cell Fact*, 13 (1) (2014) p. 180. https://doi.org/10.1186/s12934-014-0180-6

33 Bhattacharjee S, DLS and zeta potential – What they are and what they are not? *J Contr Release*, 235 (2016) 337–351. https://www.sciencedirect.com/science/article/abs/pii/S0168365916303832

34 Rodger A, UV Absorbance Spectroscopy of Biological Macromolecules, In: *Encyclopedia of Biophysics*, (Springer, Berlin, Heidelberg), 2018, pp. 1–5. https://link.springer.com/rwe/10.1007/978-3-642-35943-9_780-1

35 Hayashi T & Mukamel S, Two-Dimensional Vibrational Lineshapes of Amide III, II, I and A Bands in a Helical Peptide, *J Mol Liq*, 141 (3) (2008) 149–154. https://pmc.ncbi.nlm.nih.gov/articles/PMC2897153/

36 Ji Y, Yang X, Ji Z, Zhu L, Ma N, *et al*., DFT-Calculated IR Spectrum Amide I, II, and III Band Contributions of N-Methylacetamide Fine Components, *ACS Omega*, 5 (15) (2020) 8572–8578. https://doi.org/10.1021/acsomega.9b04421

37 DeFlores L P, Ganim Z, Nicodemus R A & Tokmakoff A, Amide I′−II′ 2D IR Spectroscopy Provides Enhanced Protein Secondary Structural Sensitivity, *J Am Chem Soc*, 131 (9) (2009) 3385–3391. https://doi.org/10.1021/ja8094922

38 Ramu Ganesan A, Mohan Ram M S G, Balasubramanian B, Kim I H, Seedevi P, *et al*., Marine invertebrates' proteins: A recent update on functional property, *J King Saud Univ Sci*, 32 (2) (2019) 1496–1502. https://doi.org/10.1016/j.jksus.2019.12.003

39 Won E J, Rhee J S, Shin K H, Jung J H, Shim W, *et al*., Expression of three novel cytochrome P450 (CYP) and antioxidative genes from the polychaete, *Perinereis nuntia* exposed to water accommodated fraction (WAF) of Iranian

crude oil and Benzo[α]pyrene, *Mar Environ Res*, 90 (2013) 75–84. https://doi.org/10.1016/j.marenvres.2013.05.014

40 Chai T T, Law Y C, Wong F C & Kim S K, Enzyme-Assisted Discovery of Antioxidant Peptides from Edible Marine Invertebrates: A Review, *Mar Drugs*, 15 (2) (2017) p. 42. https://doi.org/10.3390/md15020042

41 Pan Y L, Rodrigues M J, Pereira C G, Engrola S, Colen R, *et al*., Exploring the Biotechnological Value of Marine Invertebrates: A Closer Look at the Biochemical and Antioxidant Properties of Sabella spallanzanii and Microcosmus squamiger, *Animals*, 11 (12) (2021) p. 3557. https://doi.org/10.3390/ani11123557