Comparative studies on antioxidant properties of wild and cultured shrimps

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Received: June 2020 Accepted: October 2020

Abstract

The aim of the study was to compare the antioxidant potential on edible parts of wild *Penaeus* species (*P. semisulcatus*, *P. indicus*, *P.monodon* and *Metapenaeus monoceros*) and culture *Penaeus* species (*P. vannamei*, *P.monodon*). Antioxidant properties of the samples were assessed using 1, 1- diphenyl-2- picrylhydrazyl and hydrogen peroxide radical scavenging assays for ascorbic acid equivalents. Various concentrations of methanolic extract of the sample (4.0mL) were mixed with 1.0mL of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.1mM and IC_{50} value was calculated. From the results, the culture species *P.vannamei* was recorded maximum inhibition 97.69% at 4000 µg /mL followed by *P.monodon* (culture) 96.62% with IC_{50} value at low concentration 2500 µg /mL. On comparing wild Penaeus species, *P. indicus* shown the maximum inhibition of 92.68% at 4000 µg /mL with IC_{50} at 2000 µg /mL and *Metapenaeus monoceros* shown 92.56% at 4000 µg /mL, IC_{50} value 2500 µg /mL, respectively. Hence the study revealed that both wild and cultured shrimps are rich in antioxidant property observed by DPPH radical scavenging activity. Shrimp could be a unique source of the antioxidant and prevent the reduction of oxidative stress.

Keywords: Shrimp, DPPH, Antioxidant

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Introduction

Nutrition is defined as the science of food and its relationship with health. It is a basic human need and a prerequisite for a healthy life. A proper diet is essential from the very early stages of life for growth, development and for a state of overall well-being. Food consumption, which largely depends on production and distribution, determines nutrition and health of the population (Kamala *et al*., 2011). Apart from supplying nutrients, food provides other components (non-nutrient phytochemicals), which have a positive impact on health. According to UNICEF data (October 2019), India is home to 46.6 million stunted children, a third of world's total as per Global Nutrition Report 2018. Nearly half of all under-5 child mortality in India is an attributable to under nutrition.

India has a long history of aquaculture and fisheries practices to produce food for human consumption, the earliest descriptions of which can be traced to the Kautilya's Arthashastra (321–300 B.C.), one of the ancient books on economics from the era of King Someswara's Manasoltara (1127 A.D.) (Pallapothu, 2012; Babu *et al*., 2013; Pankaj Birnale and Rathod, 2017).

Shrimp is one of the most delicious sea foods and has a vital role in almost every nation's cultural cuisines. Looking from nutritional perspective it is predicted that shrimp has high protein content, low saturated fatty acid content, high source of vitamin B12, potential source of omega 3 highly unsaturated fatty acids (HUFA) and enriched with wide range of antioxidants like astaxanthine (Dayal,J.S *et al.*, 2013).

Antioxidants are compounds that play a major role in tolerating oxidative stress exerted over any cells (Gill and Tuteja, 2010). Oxidative stress is implied on cells by group of oxidation moieties like Reactive oxygen species (ROS). ROS and other free radicals which contribute for higher oxidation rate could lead to disastrous oxidative damage to cellular and sub-cellular components like proteins, lipids, lipo-proteins, deoxy ribonucleic acids etc (Singh *et al.*, 2015).

Inadequate nutritious food consumption, unintentionally cause various health issues such as cancer, cardiac, cataract, premature aging, Alzheimer's disease, arthritis and other degenerative diseases (Kikuzaki *et al*., 2002). Nutraceutical contents of seafood and their potential benefits in human nutrition and healthcare are current commercial status. However various studies and reviews revealed that suitable amount of antioxidant in any balance diet could overcome this great impact rendered by free radicals and ROS (Diplock,A.T *et al.*, 1998; Bose and Agrawal, 2007; Cynthia,A *et al*., 2007; Bohn, 2019). Still comparative studies related to the antioxidant activity of the various shrimp species belonging to different habitat and cultivation sites is lagging. Hence the present study focused on to compare the antioxidant potential of marine (wild catch) and cultured shrimp

species belonging to different habitats and cultivation sites using DPPH assay.

Materials and methods

Sample collection and processing

The Wild Shrimps species for the present study were *Penaeus monodon, Penaeus semisulcatus, Penaeus indicus , Metapenaeus monoceros* each species batch weight of 1 kg were freshly (wild catch from fisherman) collected from the landing centres of different sites, southeast coast of India The cultured shrimp species of *Penaeus monodon, Penaeus vannamei* were collected from the near aqua farms

The wild shrimp sample *Penaeus monodon* was collected from the Mandapam region (9°16'32.56"N, 79°7'25.03"E), *Penaeus semisulcatus and Penaeus indicus* from the Tuticorin region (8° 45' 50.9976" N, 78° 8' 5.4024" E) and *Metapenaeus monoceros* from Chennai region (13° 4' 2.7804" N, 80° 14' 15.4212" E). The cultured shrimp samples were collected from the aqua farm at Nagapattinam (10°45'56.19"N, 79°50'32.6"E) (*P.monodon*) and Pattukkottai (10°25'24.89"N, 79°19'10.16"E) (*Penaeus vannamei*). The collected samples were taken to the laboratory as per the sampling guidelines (Li, Y *et al*., 2013). The shrimp samples were washed thoroughly in running water and then dissected to separate the fillet parts. The studies of antioxidant activity of the cultured and wild shrimp species were determined from the fillet part of the shrimps. The separated fillet parts were dried in hot air oven at 30^{o}C for 2 hours. The dried samples were pulverized using standard pulverizer, the powdered fillet samples were stored in air tight container for further studies and analysis.

Shrimp fillet antioxidant Extraction

The antioxidant pools of all the total six shrimp samples of both wild (*Penaeus monodon, Penaeus semisulcatus, Penaeus indicus, Metapenaeus monoceros*) and cultured (*Penaeus monodon, Penaeus vannamei*) were extracted from the fillets samples by performing homogenization with 80% methanol for 5 min. The crude extract was centrifuged at 11200 g for 10 min at 4 °C, and the supernatant was stored in air tight brown bottles, the procedure was repeated again with the solvent until the complete removal of antioxidants pools. All the supernatants obtained were pooled together and the final mixture was filtered through a membrane filter of pore size 0.25µm. To avoid oxidation of the moieties in the bottle, pure N_2 gas was sparged into the bottle and the extract was stored in -21°C (±1°C) until the antioxidant activity was determined.

Determination of antioxidant activity (DPPH Assay)

The free radical scavenging activity of the shrimp fillets of both wild (*Penaeus monodon, Penaeus semisulcatus, Penaeus indicus, Metapenaeus monoceros*) and cultured (*Penaeus monodon, Penaeus vannamei*)

methanolic extracts were quantitatively assessed using the DPPH radical method adopted by spectrophotometry. Extracts that exhibited strong antioxidant capacity using the DPPH method proposed by (Anwar *et al.*, 2010). Briefly 0.1mM solution of DPPH in methanol was prepared and 0.1mL of this solution was added to 0.5mL of samples in various concentrations. The mixtures were incubated in dark for 30 minutes and the absorbance was measured at 517nm. The DPPH radical-scavenging activity was calculated. The commercial known antioxidant, ascorbic acid was used for comparison or as a positive control (Blois MS, 1958). The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage plotted against extract concentration (2, 4, 6, 8, 10µg/mL). IC_{50} of Ascorbic acid is 5.8 µg (Fig.1)

The percentage of DPPH decolorization of the sample was calculated according to the equation:

$$\text{Percentage decolorization} = [1-(ABS_{sample}/ ABS_{control}] \times 100$$

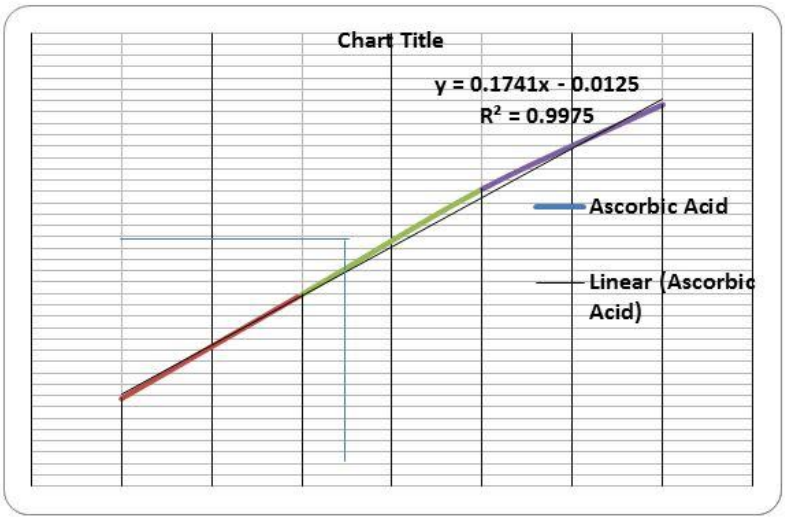

Figure 1: Standard curve.

Results

The shrimp fillets of both wild (*Penaeus monodon, Penaeus semisulcatus, Penaeus indicus, Metapenaeus monoceros*) and cultured (*Penaeus monodon, Penaeus vannamei*) methanolic extracts. The scavenging activity over free radicals was based on analyzing the scavenging activity of 1, 1-diphenyl-2- picrylhydrazyl (DPPH), a stable free radical.

The shrimp fillets of methanolic extracts showed significant DPPH scavenging activity compared with the

standard ascorbic acid (IC_{50} 5.8 µg/mL). All results were displayed in Figure 2. Concentration of the samples ranges from (1000-4000µg). The scavenging activity of the extracts increased linearly with increasing concentration. The antioxidant potential of the sample extracts ranges from 15.00% at lower concentration to maximum of 97.69% at higher concentration. The wild catch shrimp shows maximum scavenging activity of 92.68% in *P. indicus* collected at Tuticorin coastal region followed by 92.56% recorded in *M. monoceros* obtained from Chennai coastal region. When compared the results, the scavenging potential of the cultured and the wild catch shrimps, *P.vannamei* (culture) recorded the maximum percentage of inhibition 97.69% at 4000 µg /mL higher concentration followed by *P.monodon* (culture) showed 96.62% with IC_{50} value 2500 µg /mL.

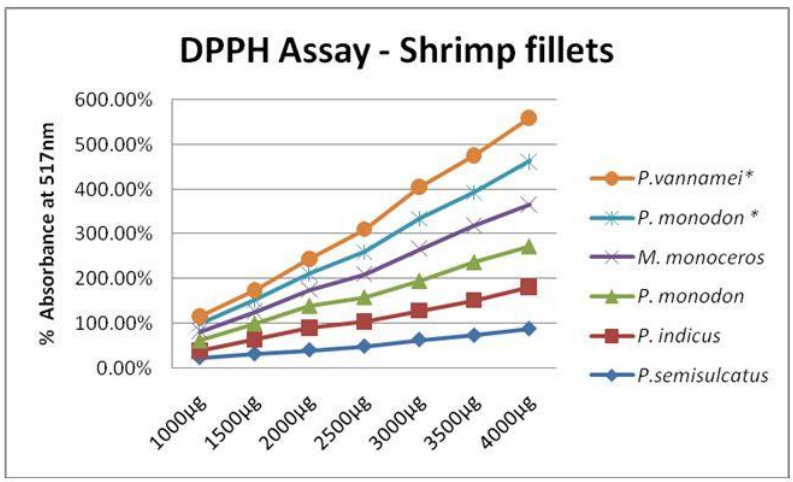

Figure 2: DPPH radical scavenging assay – Shrimp fillets. (*Cultured)

Discussion

The shrimp fillets of both wild (*Penaeus monodon, Penaeus semisulcatus, Penaeus indicus, Metapenaeus monoceros*) and cultured (*Penaeus monodon, Penaeus vannamei*) of the above mentioned shrimp sample species were collected from different locations (Li *et al*., 2013).

Anwar *et al.* (2010) recommended to using methanolic extraction procedure to extract the antioxidant molecules from tissue samples, since it would give the better yield. DPPH is a notable stable free radical when it is in methanolic solutions (Benmehdi *et al*., 2017).

Methanolic extracts of all Shrimp fillets both cultured and wild catch are allowed to react with the stable radical, DPPH, in methanol solution. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm, induced by an antioxidant (AH) after 30 min, as follows:

$$DPPH^* + AH \longrightarrow DPPH - H + A^*$$

The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC50) is a parameter widely used to measure the antioxidant activity (Sanchez-Moreno *et al*., 1998). A lower IC_{50} value corresponds with a higher antioxidant power. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997).

Comparative studies related to the antioxidant activity of the various shrimp species belonging to different habitat and cultivation sites is lagging. 2, 2 – Diphenyl – 1- picryl hydrazyl (DPPH) is used in radical scavenging measuring method as described (Yen and Duh, 1994).

Protein hydrolysates prepared by hydrolysis of shrimp waste (*Penaeus monodon* and *Penaeus indicus*) shows the DPPH scavenging activities which increased linearly with increasing concentration of protein hydrolysate upto 5 mg/mL. (Dey *et al*., 2014) study supports our findings *Penaeus monodon* (cutured) and *Penaeus indicus* (wild) with maximum inhibition activity.

Carotenoid rich seafood has now become one of the important criteria in determining the quality of the product to be exported. Astaxanthin (3,3'--dihydroxy-β, β-carotene-4,4'- dione) being an important category of carotenoid which are red-orange pigment (Miao,F *et al.,* 2006) present in shrimps fillets (Ushakumari and Ramanujan, 2013; Mondal *et al.,* 2015). Astaxanthin helps in scavenging the singlet oxygen ($^1O_2$) and the peroxyl radicals (H_2O_2) from the system compared to other antioxidants like β-carotene, canthaxanthin, zeaxanthin and Vitamins C and E (Suh *et al*., 2006). The potential of shrimp carotenoid extract as a natural antioxidant for possible use in food and biomedical applications (Sowmya, R. and Sachindra. N, 2012).

Shrimp astaxanthin would be a promising dietary supplement for skin health applications (Chintong *et al.,* 2019). Shabna *et al*. (2015) reported that antioxidant potential of chitin and chitosan isolated from cultured *Penaeus monodon* maximum inhibitory activity at 89.56% inhibition in 1000 µg/mL. The ethyl acetate extract of *P. vannamei* shells possess good scavenging activity and significant inhibitory effect with IC50 value 15.03 µg/mL (Muniyappan *et al*., 2019).

The present study reveals both cultured and wild catch shrimps shows significant inhibitory activity against the free radical DPPH. Only slight variations recorded in percentage of inhibition the P.*vannamei* (cultured) shows 97% the maximum inhibition followed by P.monodon (cultured) 96%. The IC50 value of wild shrimp, *P. indicus*, *P. monodon* and *Metapenaeus monoceros* and the maximum inhibition was 92.68% and 91.75%. The wild shrimps show the IC_{50} value at lowest

concentration (2000µg/mL) and leading in the antioxidant activity.

In 2019 overall exports of shrimps were valued as US$19.1 billion based on the report of Central Intelligence Agency, The World Fact book *Field Listing: Exports – Commodities* (May 2020), In addition to export market, farmed shrimps are also being sold in domestic markets and reaches the revenue as US$1.64 billion. As per India, MPEDA data (2018 – 2019), was around 13,92,559 metric ton of shrimp were exported from India to all over the world and estimated with a net value of 6728.50 (US$ Million). In 2019, shrimp exports generated revenues of (U.S.) $5 billion (Ministry of Commerce and Industry, Government of India).

The quality of the shrimp depends on the niche it grows and physical parameters, feed and medicines. Hence further research needed to provoke the quality assurance.

Conclusion

According to the present study, shrimp fillets have noticeable effect on the scavenging of free radical. This study reveals that both wild and cultured shrimps are rich in antioxidant property observed by DPPH radical scavenging activity. Shrimp could be a unique source of the antioxidant and prevent the reduction of oxidative stress. Shrimp sea foods almost embedded all nutrition which is a superfood supplement for malnutrition.

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