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Antioxidant Activity in Hard and Soft Shell Crabs of *Charybdis lucifera* (Fabricius, 1798)

Soundarapandian P*, Shyamalendu Roy and Varadharajan D

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India

Abstract

The crab fishery in India is fast developing and there is a vast scope for the crab meat due to its delicacy and nutritional richness. Studies on antioxidant activity of soft shell crabs are very much need of the hour to stop the wastage of soft shell crabs and also possible utilization. Hence in the present investigation antioxidant activity is studied in soft shelled crabs which are compared with hard shell crabs of *Charybdis lucifera*. The soft shelled crabs showed maximum phenolic content of 48% which is tentatively higher than that of hard shelled. The total antioxidant potential of soft shelled crab exhibited maximum antioxidant potential of 49% and minimum effect of 32% was recorded in hard shelled crab. In the reducing power assay the maximum reducing ability of 59% was noticed in soft shelled crab. The lease reducing capability of 42% was recorded in hard shelled crab. A meager scavenging potential of 28% and 29% was recorded in both the crabs. In deoxyribose radical scavenging activity both the crab tissues exhibited a confined range of 30% scavenging role where the reference drug of Vitamin E records 86% of scavenging potential. In soft shelled crab was characterized with 59% DPPH free radical scavenging ability whereas the hard shelled with 48% was recorded. The results suggest that soft shelled crabs of *Charybdis lucifera* show antioxidant property than hard shell crabs. So the soft shelled crab may be used for the preparation of antioxidant and this will prevent the wastage of useful soft shelled crabs from the landing centers in some extend. To confirm this further extensive study is needed in this regard.

Keywords: Antioxidant activity; Soft; Hard shelled; Haemolymph; *Charybdis lucifera*

Introduction

The crabs rank third after shrimps and lobsters for their esteemed seafood delicacy and also the value of fishery they support. The crab fishery in India is fast developing and there is a vast scope for the crab meat due to its delicacy and nutritional richness. The crab meat contains rich amount of protein, vitamins A and D, minerals, glycogen and free amino acids. Most of the marine crabs occurring along the Indian coasts are belonging to the family portunidae. The commercially important portunid crabs found along Parangipettai coast are *Scylla serrata, S. tranquebarica, Portunus sanguinolentus, P. pelagicus, Podophthalamus vigil, C. feriata, C. lucifera, C. natator, C. granulata* and *C. truncata* [1-3]. The oxidative deterioration of fats and oils in foods are responsible for rancid odours and flavours, with a consequent decrease in nutritional quality and safety caused by the formation of secondary, potentially toxic compounds. The additions of antioxidants are required to preserve flavour and colour and to avoid vitamin destruction. Among the synthetic types, the most frequently used food preservatives are Butyrate Hydroxyanisole (BHA), Butyrate Hydroxytoluene (BHT), Propyl Gallate (PG) and Tertbutyl Hydroquinone (TBHQ). Reports revealing that BHA and BHT could be toxic and the higher manufacturing costs and lower efficiency of natural antioxidants such tocopherols together with the increasing consciousness of consumers with regard to food additive safety, created a need identifying alternative and probably safer sources of food antioxidants [4,5]. Naturally occurring antioxidant substances also need safety testing. Cautions regarding an assumption of safety of natural antioxidants have been repeatedly advised, since the fact an antioxidant comes from a natural source does not prove its assumed safety [6]. Oxidative stress is involved in the pathology of cancer, arteriosclerosis, malaria and rheumatoid arthritis and could be play a role in neurodegenerative disease and ageing processes [7-9]. The role of antioxidants has received increased attention during the past decade. However, the use of such antioxidants have potential health hazard [10]. Therefore, in recent years, interests have been developed for searching effective natural antioxidants, since they can protect human body from free radical and retard the progress of many chronic diseases. The hemolymph, in most crustaceans is a colourless fluid containing large number of cells or hemocytes and various organic and inorganic constituents. About 90% of hemolymph is water, which determines its total volume. The hemolymph serves as a bathing medium for various tissues and organs as they lack an epithelial lining of a true coelom. Therefore, the hemolymph forms the meeting place of both the raw materials required and the products of various physiological activities of the body. Since the hemolymph is not directly connected with the external environment any change in it can be taken as a measure of the physiological state of the internal environment of the intact animal.

The haemolymph of crustacean have potent antimicrobial peptide showed diverse array against several human pathogens [11] and plays a role in host defense response including self or non-self-recognition, cell to cell communication, superoxide anion activity, melanisation, phagocytosis, cytotoxicity and encapsulation [12]. In recent years, natural products from marine samples have a wide spectrum of biological activities and numerous therapeutic applications include antiviral, antibacterial, and antitumor. Cyclic and linear peptides discovered from marine animals have increased our knowledge about new potent cytotoxic, antimicrobial, ion channels specific blockers,

***Corresponding author:** Soundarapandian P, Faculty of Marine Sciences, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India, Tel: 04144-243223; E-mail: soundsuma@gmail.com

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and many other properties with novel chemical structures associated to original mechanisms of pharmacological activity [13]. There is an increasing interest in antioxidants, particularly in those of free radicals in various diseases. These pathological and clinical backgrounds have prompted to investigate novel and potent antioxidant peptides from crab which are ultimately of therapeutic use. Hence the present study was aimed to identify the antioxidant activity of homolymph from soft and hard shelled crabs of *Charybdis lucifera*.

Materials and Methods

Collection of haemolymph

Healthy crabs at two different stages of moulting (pre-moulting stage i.e. hard shell crab and post-moulting stage i.e. soft shell crab) were collected from the Mudasalodai landing centre (Lat. 11º 29'N and Long. 79º 46'E) near Parangipettai. Haemolymph was collected by cutting each walking legs with a fine sterile scissor. The haemolymph was collected by using a 23-guage needle and 1.0 ml syringe contained 300 µl (4°C) precooled 10% sodium citrate solution as anticoagulant in glass distilled water. To remove haemocytes from the haemolymph it was centrifuged at 2000 rpm for 15 min at 4°C. The supernatant was collected and used for the following experiments.

Antioxidant Activities

Total polyphenolic compound

Phenolic contents of crude extracts were estimated by the method of [14]. Briefly, 100 µl aliquot of sample was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 minutes at room temperature. After incubation, 100 µl of 50% Folin ciocalteou's phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand for 30 minutes at the room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Shimadzu, UV-160, Japan). Phenolic contents are expressed as quercetin equivalent per gram.

Total antioxidant activity

Total antioxidant activity was measured following the method of [15,16] 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2359 g of ammonium molybdate (4 mM solution) were mixed together in 250 ml of distilled water and labeled as a Total Antioxidant Capacity (TAC) reagent. 300 µl of extract was dissolved in 3 ml of TAC reagent. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of gallic acid.

Reducing power

Reducing power of different crude extract was determined by the method prescribed by [17]. 1.0 ml of different solvent extract containing different concentration of samples was mixed with 2.5 ml of phosphate buffer (0.2 M, pH-6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50°C for 20 minutes. After incubation, 2.5 ml of Trichloroacetic acid (10%) was added and centrifuged at 650 rpm for 10 minutes. From the layer, 2.5 ml solution was mixed with 2.5 ml of distilled water at 0.5 ml of ferric chloride (0.1%). Absorbance of all the solution was measured at 700 nm. Increased absorbance is indicated increased reducing power.

Hydrogen peroxide radical scavenging assay

The ability haemolymph to scavenge hydrogen peroxide was determined by following the standard procedure of [17]. Hydrogen peroxide (10 mM) solution was prepared in the phosphate buffer saline (0.1 M, PH-7.4). 1 ml (0.25 mg) of the extract was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer (Shimazdu, UV-160) against a blank (without hydrogen peroxide) after 10 minutes of incubation at 37°C. The percentage of scavenging of hydrogen peroxide was calculated using the following formula.

$$\%\text{scavenging}(H_2O_2) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0-Absorbance of control

A_1-Absorbance of sample

Deoxyribose radical scavenging activity

Deoxyribose non-site specific hydroxyl radical scavenging activity of solvent extract and fractions were determined according to the method of [18]. Briefly, 2.0 ml of aliquots of sample were added to the test tube containing reaction mixture of 2.0 ml of $FeSO_4.7H_2O$ (10 mm), 0.2 ml EDTA of (10 mM) and 2.0 ml of deoxyribose (10 mM). The volume was made upto 4.8 ml with phosphate buffer (0.1 M, pH 7.4) and to that 0.2 ml of H_2O_2 (10 mM) was added. The mixture was incubated at 37°C under dark for 4 h. After incubation, 1 ml of TCA (2.8%) and TBA (1%) were added to the mixture, and then left to stand under boiling water bath for 10 minutes. After treatment absorbance was measured at 532 nm. If the mixture was turbid, the absorbance was measured after filtration. Scavenging activity (%) was calculated using the equation given by [19].

DPPH free radicals scavenging activity assay

The scavenging activity for DPPH free radicals was measured according to the method of Shimada et al. [20]. DPPH solution was prepared at the concentration of 0.1 ml in ethanol. During the assay, the 1 ml of test solution (concentration of 0.5-3.5 mg/ml) was mixed with 1 ml DPPH solution. The mixture was incubated in dark place for 30 min at 25°C. After standing for 30 minutes, absorbance was recorded at 517 nm by UV Beckman spectrophotometer (Beckman Coulter). The percentage of DPPH free radicals scavenging activity was calculated by following equation:

$$\%\text{Scavenging activity} = \left[100 - \left(A_0 - A_1\right)/A_0\right] \times 100$$

Where, A_0 is the absorbance of sample and A_1 is the absorbance of blank.

Results

Total polyphenolic compound

The soft shelled crabs showed maximum phenolic content of 48% which is tentatively higher than that of hard shelled. However the phenolic content based antioxidant role is imperatively higher in the case of the quercetin which is used as standard drug. The blank value of reagent alone acts as a control group in this assay (Table 1).

Total antioxidant activity l

The total antioxidant potential of soft shelled crab exhibited maximum antioxidant potential of 49% and minimum effect of 32% was recorded in hard shelled crab. Gallic acid reports to have 86% of total antioxidant efficacy (Table 2).

S.No	Sample	%
1	Soft shell	48
2	Hard Shell	31
3	Quercetin	63

Table 1: Total polyphenolic compound in soft and hard shelled crabs of *Charybdis lucifera.*

S.No	Sample	%
1	Soft shell	49
2	Hard Shell	32
3	Gallic acid (mg/equivalent)	86

Table 2: Total Antioxidant activity in soft and hard shelled crabs of *C. lucifera.*

S.No	Sample	%
1	Soft shell	59
2	Hard Shell	42
3	Ascorbic acid (mg/equivalent)	91

Table 3: Reducing Power in soft and hard shelled crabs of *Charybdis lucifera.*

S.No	Sample	%
1	Soft shell	28
2	Hard Shell	29
3	Ascorbic acid (mg/equivalent)	79

Table 4: Hydrogen peroxide radical scavenging assay in soft and hard shelled crabs of *Charybdis lucifera.*

Reducing power

In the reducing power assay the maximum reducing ability of reference drug Ascorbic acid (91%) was a dominating agent which is followed by the 59% reducing ability in soft shelled crab. The lease reducing capability of 42% was recorded in hard shelled crab (Table 3).

Hydrogen peroxide radical scavenging assay

There is no significant difference of scavenging ability of H_2O_2 in the crab tissues. A meager scavenging potential of 28% and 29% was recorded in both the crabs. Ascorbic acid was resulted with 79% scavenging potential (Table 4).

Deoxyribose radical scavenging activity

In Deoxyribose radical scavenging activity both the crab tissues exhibited a confined range of 30% scavenging role where the reference drug of vitamin E records 86% of scavenging potential. The free radical scavenging potential of both the crabs was imperatively very low in accordance with the standard drug (Table 5).

DPPH free radicals scavenging activity assay

In DPPH radical scavenging activity the crab tissues revealed a considerable scavenging role. In soft shelled crab was characterized with 59% DPPH free radical scavenging ability whereas the hard shelled with 48% was recorded. The reference drug of Gallic acid tends to have 90% scavenging ability (Table 6).

Discussion

Antioxdant activity is fundamental property and much important for life. Many of the biological functions such as anti-mutagenicity, anti-carcinogenicity and anti-aging, among others originate from this property [21]. Literature survey revealed that shellfish waste is rich source of phenolic compounds. Phenolic compounds play an important role in the antioxidative properties and phenolic substances were also reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and anticancer activity [22]. In the present study the soft shelled crab showed maximum phenolic content of 48% which is tentatively higher than that of hard shelled crab. The soft shelled crab exhibited maximum antioxidant potential of 49% and minimum effect of 32% was recorded in hard shelled crab. Sudhakar [23] recorded the total antioxidant activity ranged from 28.52% to 80.26% at varying concentrations (0.5 to 10 mg/ml) in *P. sanguinolentus* crab shell chitosan sample.

In the reducing power assay the maximum reducing ability of reference drug Ascorbic acid (91%) was a dominating agent which is followed by the 59% reducing ability in soft shelled crab. The lease reducing capability of 42% was recorded in hard shelled crab. The reducing power of chitosan ranged from *P. sanguinolentus* chitosan, 0.20% to 0.42% at varying concentrations (0.5 to 10 mg/ml) [23]. In hydrogen peroxide activity there is no significant difference between hard and soft shelled crabs. A meager scavenging potential of 28% and 29% was recorded in both the crabs. Ascorbic acid was resulted with 79% scavenging potential. Scavenging abilities on hydroxyl radicals were 20.05% at varying 0.5 mg/ml concentration of chitin [23].

In Deoxyribose radical scavenging activity both the crab tissues exhibited a confined range of 30% scavenging role where the reference drug of Vitamin E records 86% of scavenging potential. The free radical scavenging potential of both the crabs was imperatively very low in accordance with the standard drug. In DPPH radical scavenging activity the crab tissues revealed a considerable scavenging role. The soft shelled was characterized with 59% DPPH free radical scavenging ability were of the hard shelled with 48% was recorded. The reference drug of Gallic acid tends to have 90% scavenging ability. DPPH radicals were reported at 41.35% at 10 mg/ml [23].

Lin et al. [24] found that the N-alkylated disaccharide chitosan derivatives with different Degrees of Substitution (DS) of 20-30% exhibited the highest DPPH radical scavenging abilities of 80-95% at 0.1 mg/ml, followed by the derivatives with DS of 40-50% and 60-70%. Apparently, the scavenging ability of chitosan might be reduced after sulfation or might be enhanced after N-alkylation of disaccharide. Oktay et al. [25] suggested that free amino group in the $-CH_2CH_2NH_2$ plays an important role in the free radical scavenging activity. In addition to this, cytotoxic effect of AEC was also assessed using human lung fibroblast (MRC-5) cell line and AEC showed less toxic against MRC-5.

A free radical can be defined as any species containing one or more unpaired electron, includes hydrogen atom, Tricholomethyl, Super oxide, Hydroxyl, Thioyl/perthiyl (RS/RSS), Peroxyl, Alkoxyl

S.No	Sample	%
1	Soft shell	31
2	Hard Shell	30
3	Ascorbic acid (mg/equivalent)	86

Table 5: Deoxyribose radical scavenging activity in soft and hard shelled crabs of *Charybdis lucifera.*

S.No	Sample	%
1	Soft shell	59
2	Hard Shell	48
3	Gallic acid (mg/equivalent)	90

Table 6: DPPH free radicals scavenging activity assay activity in soft and hard shelled crabs of *Charybdis lucifera.*

(RO_2, RO), oxides of nitrogen (NO, NO_2), nitrogen-centered radicals (C_6H_5N=N) and transition metal ions (Fe, Cu etc) [26]. Superoxide anion (O_2^-) is considered as the primary ROS, and further interacts with other molecules to generate secondary ROS, either directly or prevalently through enzyme or metal catalysed processes [27]. Hydroxyl radical can be generated in biologically relevant systems by multiple reactions. A mixture of H_2O_2 with Fe^{2+} salt oxidizes many different organic molecules. Hydroxyl radicals are responsible for a large part of the damage done to cellular DNA, proteins and lipids by ionizing radiation. DNA damage, especially double-strand breaks cannot easily be repaired by the cell. Oxygen normally present in most biological systems aggravates the damage done by ionizing radiation.

Superoxide is far less reactive with non-radical species in aqueous solution. It does not react quickly with some other radicals such as NO; certain iron-sulphur clusters in enzymes and certain phenoxyl radicals. Peroxyl (RO_2) and alkoxyl (RO) radicals are good oxidizing agents. Radicals (RO) formed in biological systems often undergo rapid molecular rearrangement to other radicals species. For example: RO_2, Radicals oxidize ascorbate and NADH, later leading to O_2^- formation in the presence of O_2. *In-vitro*, thiols (especially reduced glutathions GSH) are often regarded as antioxidant agents, since them potent protein-SH groups against oxidation and can scavenge oxygen radicals and some other reactive species such as hypochlorous acid and themselves generate free radicals [26]. Kobayashi et al. [28] suggest that low molecular chitosans may be absorbed well from the gastrointestinal tract and inhibit neutrophil activation and oxidation of serum albumin that is frequently observed in patient's plasma undergoing hemodialysis, resulting in a reduction in oxidative stress associated with uremia. Dai et al. [29] concluded that antioxidative activates were found in the culture supernatant of *Serratia italica* TKU013 with squid pen as the sole carbon/ nitrogen source and the 4th day supernatant showed the strongest antioxidant activities and the highest total phenolic content. Dharmishtha et al. [30] concluded that chito oligosaccharides low molecular weight can be used as antioxidant in biological systems. All the tested compounds reduced either the hemolytic and DNA damage by inhibiting H_2O_2 and AAPH- radicals. Park et al. [10] reported that the scavenging activity was dependent on the number of amino groups available (degree of acetylation). Dai et al. [29] tested the efficacy of chito oligosaccharides low molecular weight as inhibitors of H_2O_2 and AAPH induced RBC hemolysis. Hydrogen peroxide crosses the RBC membrane and is known to react with Hb, generating highly reactive radical species including hydroxyl radicals. It has been reported that shrimp shell waste contains natural antioxidants; mainly phenolic compounds. The reducing power could be attributed mainly to the bioactive compounds associated with antioxidant activity [19,24,31]. The data provide a useful example of utilizing squid pen bio-waste material as a valuable functional ingredient. The findings of the current report appear useful for further research aiming to isolate and identify the specific compounds responsible for the antioxidant activity of TKU013-fermented supernatant. It was reported that the antioxidant activity is concomitant with the reducing power, which may serve as a significant indicator of the potential antioxidant activity for a compound [32]. Vitamin C has a protective function against oxidative damage and a powerful quencher of singlet oxygen (O_2), hydroxyl ($^{\cdot}OH$) and peroxyl (RO_2) radicals. The occurrences of carotenoids in crustaceans are mainly due to the absorption of pigments from the diet, which they deposit as such or transfer metabolically to keto or hydroxyl derivatives [33,34].

One of the important characteristic of carotenoids is their ability to act as antioxidants, thus protecting cells and tissues from the damaging effect of free radicals and singlet oxygen [35]. Among the carotenoids, astaxanthin was found to be more effective than β-carotene as an antioxidant. Hydroxyl radicals were generated by direct addition of Fe^{2+} to a reaction mixture containing phosphate buffer under normal conditions [36]. Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells [37]. This oxidative stress may be related to many disorders such as cancer, atherosclerosis, diabetes and liver cirrhosis [36,38].

In foods, antioxidants are added to minimize changes in flavour, aroma, color or nutritional value. Antioxidants can protect the body against damages caused by free radicals and degenerative diseases. Synthetic antioxidants usually employed in industry are effective and stable, but their use is limited in many countries because they are not considered complete safe for human health [39]. Incorporation of antioxidants into packaging materials have become popular since oxidation is a major problem affecting the food quality. Currently, the most frequently used antioxidants in active packaging are Butylated Hydroxysanisol (BHA) and Butylated Hydroxytoluene (BHT). Although these synthetic antioxidants can effectively be used in active food packing because of their high stability, low cost and efficiency, there are significant concerns related to their toxicological aspects. Moreover, uses of synthetic antioxidants are under strict regulation due to the potential health risk caused by such compounds. Therefore, extensive research has been conducted to employ some natural antioxidants such as phenolic compounds as alternatives to synthetic antioxidants [40,41]. The results suggest that soft shelled crabs of *C. lucifera* show antioxidant property than hard shell crabs. So the soft shelled crab may be used for the preparation of antioxidant and this will prevent the wastage of useful soft shelled crabs from the landing centers in some extend. To confirm this further extensive study is needed in this regard.

References

1. John Samuel N, Thirunavukkarasu N, Soundarapandian P, Shanmugam A, Kannupandi T (2004) Fishery potential of commercially important portunid crabs along Parangipettai coast. Proceedings of International conference and exposition on marine living resources of India for food and medicine, Aquaculture Foundation of India, Chennai 165-173.
2. Varadharajan D, Soundarapandian P, Dinakaran GK, Vijakumar G (2009) Crab Fishery resources from Arukkattuthurai to Aiyammpattinam, South east coast of India. Cur Res Biol Sci 1: 118-122.
3. Varadharajan D, Soundarapandian P (2012) Commercially Important Crab Fishery Resources from Arukkattuthurai to Pasipattinam, South East Coast of India. J Marine Sci Res Dev 2: 1-6.
4. Sherwin ER (1990) Antioxidants. In: Branen R. (ed.), Food Additives, New York, Marcel Dekker.
5. Wanasundra NU, Shahidi F (1998) Antioxidant and pro-oxidant activity of green tea extracts in marine oils. Food Chem 63: 335-342.
6. Hattori M, Yamaji-Tsukamoto K, Kumagai H, Feng Y, Takahashi K (1998) Antioxidative activity of soluble elastin peptides. J Agri Food Chem 46: 2167-2170.
7. Nakagami T, Tamura N, Nakamura T (1995) Plant phenol compounds as anticomplement agents and therapeutics for complement-associated diseases and health foods containingthem. Jpn Kokai Tokkyo Koho 7 JP 07223941.
8. Hollman PCH, Gaag MVD, Mengelers MJB, Trijp JMP, Vries JHM, et al. (1996) Absorption and disposition kinetics of the dietary antioxidant quercetin in man. Free Rad Biol Med 21: 703-707.
9. Aruoma OI (1997) Scavenging of hypochlorous acid by carvedilol and ebselen in vitro. Gen Pharmacol 28: 269-272.
10. Park PJ, Je JY, Kim SW (2004) Free radical scavenging activities of different

deacetylated chitosans using an ESR spectrometer. Carbohydrate Polymer 55: 17-22.

11. Arulprakash A, Balasubramanium S, Gunasekaran G, Prakash K, Snthil Raja P (2011) Control of clinical pathogens by the haemolymph of *Paratelphusa hydrodromous*, a Freshwater Crab. ISRN pharmacology: 642768.
12. Sahoo B, Sethi S, Mishra BK, Das BK (2005) Effects Elecitors on Prophenonoloxidase and Superoxide Anion Activities of Freshwater Prawn, *Macrobrachium malcolmsonii*. Asian Fish Sci 18: 345-353.
13. Zheng LH, Wang YJ, Sheng J, Wang F, Zheng Y, et al. (2011) Antitumor peptide from marine organisms. Mar Drugs 9: 1840-1859.
14. Taga MS, Miller EE, Pratt DE (1984) Chia seeds as a source of natural lipid antioxidants. J American Oil Chem Soc 61: 928-931.
15. Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex, specific application to the determination of vitamin E. Analy Biochem 269: 337-341.
16. Khan MA, Shahwar D, Ahmad N, Khan Z, Ajaib M (2009) Chemical constituents of *Carissa opaca* extracts and their evaluation as antioxidant and preservative in Edible Oils.
17. Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AKS (2003) Studies on the Antioxidant Activities of *Desmodium gangeticum.* Biol Pharma Bull 26: 1424-1427.
18. Chung SK, Osawa T, Kawakishi S (1997) Hydroxyl radical-scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). Biosci Biotechnol Biochem 61: 118-123.
19. Heo SJ, Park EJ, Park PJ, Jeon YJ (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. Biores Technol 96: 1613-1623.
20. Shimada K, Fujikawa K, Yahara K, Nakamura T (1992) Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agricult Food Chem 40: 945-948.
21. Cook NC, Samman S (1996) Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Nutriti Biochem 7: 66- 76.
22. Ismail N, Siti Faezah S, Dzulkiply SK, Muhamad Fauzi M, Luqman AB (2011) Protein profiling of the crab, *Tachpleus gigas*. Empowering Science. Technol Innovat Towards Bett Tomor 609-614.
23. Sudhakar M (2011) Biodiversity, resources, nutritional status and shell utilization of crabs from Cuddalore coast, Ph.D thesis, Annamalai University 1-170.
24. Lin HY, Chou CC (2004) Antioxidant activities of water-soluble disaccharide chitosan derivatives. Food Res Inter 37: 883-889.
25. Oktay M, Gulcin I, Kufrevioglu OI (2003) Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts, Lebensmittel-Wissenchaft und Technologie 36: 263-271.
26. Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine. Oxford University Press, New York.
27. Farkas V (1995) Fungal cell walls, Their structure, biosynthesis and biotechnological aspects. Acta Biotechnol 10: 225-238.
28. Kobayashi M, Watanabe T, Suzuki S, Suzuki M (1990) Effect of N-acetylchitogexaose against Candida albicans infection of tumor-bearing mice. Microbiol Immunol 34: 413-426.
29. Dai HP, Shan CJ, Wei AZ, Yang TX (2012) Leaf enescence and photosynthesis in foxtail millets (*Setaria italica* (L.) *P.beauv*) leave under drought condition. Aust J Crop Sci 62: 232-237.
30. Dharmishtha M, Shrihari M, Falguni G (2010) Free radical scavenging activity of *Randia dumetorum* lamk fruits. IRJP 11: 122-126.
31. Xing R, Yu H, Liu S, Zhang W, Zhang Q, et al. (2005) Antioxidative activity of differently regioselective chitosan sulfates in vitro. Bioorgan Medi Chem 13: 1387-1392.
32. Tokoro A, Kobayashi M, Tatewaki N, Suzuki K, Okawa Y, et al. (1989) Protective effect of N-acetylchitohexaose on Listeria monocytogenes infection in mice. Microbiol Immunol 33: 357-367.
33. Castillo R, Negre-Sadargues G, Lenel R (1982) General survey of the carotenoids in crustacea. In: Castillo R, Negre-Sadargues G, Lenel R (edn). Carotenoid Chem Biochem, Pergamon Press, Oxford 211-224.
34. Davies BH (1985) Carotenoid metabolism in animals: A biochemist's view. Pure Appl Chem 57: 679.
35. Hirayama O, Nakamura K, Hamada S, Kobayashi Y (1994) Singlet oxygen quenching ability of naturally occurring carotenoids. Lipids 29: 149-150.
36. Halliwell B, Gutteridge JMC (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219: 3-16.
37. Mau JL, Chao GR, Wu KT (2001) Antioxidant properties of methanolic extracts from several ear mushrooms. J Agri Food Chem 495: 461-546.
38. Muramatsu H, Kogawa K, Tanaka M, Okumura K, Koike K, et al. (1995) Superoxide dismutase in SAS human tongue carcinoma cell line is a factor defining invasiveness and cell motility. Cancer Res 55: 6210-6214.
39. Schinella GR, Troiani G, Davila V, De Buschiazzo PM, Tournier HA (2000) Antioxidant effects of an aqueous extract of *Ilex paraguariensis.* Biochem Biophys Res Comm 269: 357-360.
40. Chan EWC, Lim YY, Omar M (2007) Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. Food Chem 104: 1586-1593.
41. Yen MT, Mau JL (2007) Preparation of fungal chitin and chitosan from shiitake stipes. Presented at the 33rd Annu Meet Taiwan Asso Food Sci Technol, Taiwan.