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Research Article

Evaluation of nutritional profile and anti-oxidant activity of *Meretrix meretrix* Asiatic hard clam along the Parangipettai coast of Tamilnadu

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Abstract

The current study was conducted to determine the nutritional profile and antioxidant activity of the marine clam *Meretrix meretrix*. The proximate composition, vitamins, and mineral content of the sample were determined by standard biochemical methods. Amino acid and fatty acid profile were analyzed by High performance liquid chromatography (HPLC) and Gas Chromatography (GC) methods. The results showed that *M. meretrix* possessed prominent protein content with rich in essential amino acids (60.96%). Subsequently, the bioactive materials from *M. meretrix* using Acetone & n-hexane (1:3) extract ((AHMME) exhibited potential activity against the total antioxidant activity, superoxide radical scavenging assay, hydroxyl radical scavenging assay, DPH (1, 1- diphenyl – 2 – picrylhydrazyl hydrate) radical scavenging activity, reducing power assay, and ferrous ion chelating assay. Thus, the results of the present study suggest that the *M. meretrix* can be used as a potential food source for providing the cheapest animal protein and powerful natural antioxidants.

Keywords: *Meretrix meretrix;* Antioxidant; Nutrition; Fatty acid; Amino acid.

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Introduction

The marine environments occupied approximately 70% of the earth's surface. However, the study of marine natural products is very limited when compared to natural products reported from the terrestrial environment (Kamboj et al., 1999). Being highly competitive, the marine environment was quite able to produce several bioactive compounds for human benefits. Especially, the marine molluscs represent a promising group of organism in the search of new bioactive compounds. The majority (35%) of the 20 marine natural products currently been approved as drugs or clinical trials have their origin in molluscs (Gerwick et al., 2012). At the same time many bioactive compounds which are reported to have anticancer (Wang et al., 2005), antioxidant (Nazar et al., 2022) and antimicrobial properties (Sugesh et al., 2013) are from the bivalves origin.

The bioactive compounds isolated from marine animals are secondary metabolites which are responsible for various pharmacological activities. These may well separate into alkaloid, phenol, flavonoid, glycoside, steroids, terpenoids, isoprenoids, carotenoids, non-isoprenoids, quinones, brominated compounds, nitrogen heterocyclic and nitrogen sulphur heterocyclic, which were the key players of defense mechanism in the marine species. Bioactive materials produced by marine organisms for instance protozoan's and invertebrates viz. poriferans, cnidarians, annelids, arthropods, molluscs and echinoderms have got a great attention due to the presence of antiviral, antimicrobial, antiprotozoal, antifungal, antihelminthic and anticancer activities (Zapata et al., 2000).

Over the past few decades, marine organisms and their extracts have been recorded that they have many biological activities of potential medicine value (Xie et al., 2012). Therefore, innovative attention has been developed to search bioactive and safe anti-oxidative agents from marine organisms which are considered as a source of bioactive compounds as are able to produce a great variety of secondary metabolites and are characterized by a broad spectrum of biological activities, anticancer (Barone et al., 2014), energy, medicine, cosmetics, etc.

Among various marine molluscs, the *Meretrix meretrix* frequently known as Asiatic hard clam is a historical marine food and it has many valuable medicinal properties. *M. meretrix* prefers the estuarine environment and coastal ecosystems and they broadly distributed over the coastal regions of South and Southeast Asia, including China, Korea, Japan and India (Jeyabal et al., 1986). It has been used extensively as food in people near Cuddalore district, Tamilnadu, India and it employs as medicine in Chinese traditional medicine. Especially various extracts of *M. meretrix* have been proved scientifically for their functional effects including antihypertensive, hypolipidemic, antineoplastic and antioxidant (Xie et al., 2012).

Generally, *M. meretrix* was used as a medicine to reduce inflammation, typhoid fever, hangover and also used as a pain killer. But in the recent decades, natural bioactive components are purified and identified from *M. meretrix* like peptides, proteins, enzyme inhibitors and their chemical functional effects as well as antihypertensive, hypolipidemic, antineoplastic and antioxidant effects (Zhang et al., 2014). But the problem is the availability

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of the sufficient material for drug development which has dogged marine natural products chemistry since its earliest days.

Among various compounds, the antioxidants were significant and playing an important role in trapping the free radical and reducing the risk of chronic disease like melanoma, cardiac disorder, diabetes mellitus, inflammatory and neurogenerative diseases (Ananthi et al., 2010). These antioxidant molecules are present in various molluscs species including *M. meretrix* to prevent cell damage from oxidation reaction (Nagash et al., 2010). Further, these mussels providing resistance capacity to various kind of oxyradicals and it has dietary antioxidants such as phenolic content (Fang et al., 2002; Gorinstein et al., 2004). However the recent reports also stated that *M. meretrix* has rich source of antioxidant compounds (Xie et al., 2012).

Hence, this current study was planned to explore the scientific evidence at *M. meretrix* for its in-vivo anti-oxidant activity of acetone n-hexane extract as well as to investigate its nutritional profile.

Materials And Methods

Collection of samples. The marine clam *Meretrix meretrix* was collected during the low tide period from Vellar estuary, Parangipettai coast (Lat.11 º29'N: and Long. 79 º46'E) which was then immediately transferred to laboratory and washed with fresh water to remove the associated materials. Briefly, whole body (200 g) tissues were dissected and the whole body tissues were blot dried on tissue paper to remove extraneous water content of the tissues.

Extraction of the biological material. The whole body tissues (10% w/v) were homogenized; the clam species after homogenization were extracted with Acetone & n-hexane (1:3) and then agitated for 15 minutes by using a magnetic agitator. The extract was filtered through filter paper made up of cellulose under vacuum. The residue was repeatedly extracted and final extracts were vacuumed dried up to 3 ml and stored in deep freezer at 20 °C for further analysis (Lodeiros et al., 2001).

Proximate composition analysis. Proximate composition of the tissue samples of *M. meretrix* was studied. Briefly, protein concentration was estimated by the standard method (Bradford, 1976) using Bovine serum albumin as reference standard. Total carbohydrate was estimated by phenol-sulfuric acid method (Dubois et al., 1956) using glucose as a reference standard. The amount of lipid was determined (Folch et al., 1957) by the standard method. The moisture content of tissue was estimated gravimetrically. Ash content was determined gravimetrically by incinerating 1 gram dried sample in Muffle furnace at about 550°C for 6 hours until the residues become white (AOAC, 1990).

Estimation of Amino acid. The samples for the analysis of free amino acids were prepared by adopting the method (Gratzfeld-Huesgen, 1999). The amino acids were determined by an amino acid analyzer (Shimadzu- High performance liquid chromatography, HITACHI, Detector-SPD 10 AVP, LP pump LC-10AT VP).

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Determination of Fatty acid composition. For the fatty acid analysis, following pre-process were followed such as saponification and methylation for preparation of Fatty Acid Methyl Esters (FAME) extraction, basic wash and FAMEs were separated by gas chromatography GC, finally fatty acids were analyzed by gas chromatography (network gas chromatograph model 6890N, Agilent technologies, USA) (Lepage and Roy, 1986). Samples were injected by split injector with split ratio 100:1, column used was ultra -2 capillary columns, Sherlock version 4.5 with EUKARY database was utilized.

Estimation of vitamins. The fat soluble vitamins A, D, E and K and the water soluble vitamins B_1, B_6, B_{12} and C were analyzed by HPLC (Merk Hitachi L-74000) following the method (Sadasivam and Manickam, 1996). The folic acid was estimated by employing the spectrophotometric. The pyridoxine, pantothenic acids were estimated by following the methods suggested in USP NF 2000 Asian edition.

Estimation of minerals. The minerals were analyzed by following the method (Geccelep et al., 2009). The tissue was washed with double distilled water and was dried in hot air oven at 60 °C for 24 hrs. Dried samples were homogenized in a blender and 1gm of homogenate was digested by microwave digestion system (maximum pressure 800psi, maximum temperature 220 °C). After digestion, residues were diluted to 50mL with ionized water. The metal analysis of samples was carried out by using inductively coupled plasma atomic emission spectroscopy. The metal contents were expressed as mg/kg^{-1} dry weight, (dw).

Total antioxidant activity. The extract of AHMME was used for antioxidant activity. Total antioxidant activity was examined (Lingert et al., 1979). The molluscan extract of AHMME and standard ascorbic acid were taken in the concentration of 25, 50, 75 and 100 µM respectively. These concentrations were mixed with linoleic acid emulsion in sodium phosphate buffer in separate test tubes. The mixture was placed in darkness to accelerate oxidation after incubation at 95 ^oC for 90 min, methanol in de-ionized water was added and the absorbance (OD) of the mixture was measured at 234 nm against blank.

Superoxide radical scavenging assay. The Superoxide Radical Scavenging ability of molluscan extract was assessed (Nishikimi et al., 1972). The reaction mixture containing extract of AHMME and standard ascorbic acid were taken in the concentration of 25, 50, 75 and 100 µM respectively. In this concentration PMS (30 µM), NADH (338 µM) and NBT (72 µM) in phosphate buffer (0.1 m pH 7.4) was incubated at room temperature for 15 min and the absorbance was measured at 560 nm against blank. The ability of scavenging the superoxide radical was calculated by following equation.

Scavenging effect (%) = {1-Sample (560nm)/Control (560nm) ×100}

Hydroxyl radical scavenging assay. Hydroxyl radical scavenging assay was calculated by the standard method (Aruoma and Halliwell, 1988). The reaction mixture containing AHMME and standard ascorbic acid were taken in the concentration of 25, 50, 75 and 100 µM respectively. In this concentration was incubated with deoxyribose (3.75 nm), H_2O_2 (1 mM, pH 7.4) at 37 ^oC. The reaction was terminated by adding TBA (1%, w/v) and TCA (2%,

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w/w) and then test tubes were incubated at 100ºC for 20 min. The contents were cooled and absorbance (OD) of the mixture was measured at 535 nm against reagent blank. Absorbance of reaction mixture was directly proportional to the decreasing rate of oxidation of deoxyribose.

DPH (1, 1- Diphenyl – 2 – Picrylhydrazyl Hydrate) radical scavenging activity. DPH free radical scavenging ability of molluscan extract was assessed (Shimada et al., 1992). AHMME and standard ascorbic acid were taken in the concentration of 25, 50, 75 and 100 µM respectively. In this concentration was mixed with methanol solution containing DPH radicals, resulting in a final concentration of 10 mM/1DPH. The mixture was shaken vigorously and left to stand for 30 minutes in the dark and the absorbance was measured at 517 nm against blank.

Reducing power assay. Reducing power of the AHMME was quantified by the method described earlier (Yen and Chen, 1995). The reaction mixture containing different concentrations of AHMME and standard ascorbic acid were taken in the concentration of 25, 50, 75 and 100 µM respectively. In this concentration, phosphate saline buffer (0.2 M, pH 6.6) was incubated at 50 ºC for 20 min with potassium ferric cyanide (1%, w/v) at 50 ºC. The reaction was terminated by adding TCA solution (10%, w/v) and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

Ferrous-ion-chelating assay. The ferrous ion chelating potential of AHMME extract was investigated according to the method (Decker and Welch, 1990). The Fe^{2+} chelating ability of extract was monitored by measuring ferrozine complex at 562 nm. The reaction mixture containing AHMME and standard EDTA were taken in the concentration of 25, 50, 75 and 100µM respectively. In this concentration, $FeCl_2$ (2 mM) and ferrozine (5 nM) was adjusted to a total volume of 0.8 ml with water, shaken well and incubated for 10 min. The absorbance of the mixture was measured at 562 nm against blank. EDTA was used as positive control and the ability of protein to chelate ferrous ion was calculated by following formula,
Chelating effect (%) = {1-Sample (562nm)/ Control (562nm)×Control) 562nm)}×100.

Statistical analysis. The experimental results were performed in triplicate. The data were recorded as Mean ± SD and analyzed by SPSS and followed by one way ANOVA. The difference was considered to be statistically significant at $P<0.05$ level.

Results

Proximate composition. In this present study the protein, carbohydrate, fat, ash and moisture content of *M. meretrix* were found to be 27.24 %, 11.95 %, 1.18 %, 4.92 % and 6.13 % respectively. Among the proximate compositions, the protein content was the predominant one (Figure 1).

Essential amino acids. In this study, a total number of 10 essential amino acids were recorded. Among the amino acids composition, Lysine 13.87%, Histidine 8.96%, and

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Methionine 9.13% were noted at higher concentrations and the remaining amino acids were observed in trace quantities (Figure 2).

Non-essential amino acids. Totally, nine non-essential amino acids were recorded from clam tissue samples. Among the non-essential amino acids, Alanine 5.63%, Aspartic acid 5.12%, Asparagine 3.83%, Tyrosine 3.59% and Proline 3.25% were noticed as predominant non-essential amino acids (Figure 3). The fatty acid assessment of *M. meretrix* showed six different groups of fatty acids. It includes two saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA). Among the SFAs palmitic acid (C16:0) was recorded with major percentage. The percentage availability of SFA, MUFA and PUFA content were found to be 38.2 %, 12.47 % and 37.45 % respectively (Figure 4).

Vitamin composition. The Vitamin compositions of the present investigation were represented in Figure 5. Totally seven vitamins were recorded, Among which Vitamin A (108.4 IU) was observed at highest level followed by Vitamin C and D, with 23.98 mg/g, 13.82 IU,2.08 µg/g. Vitamin B_{12}, E, K and B_6 were recorded in meager quantities of 1.98, 1.14, 0.59 and 0.31 respectively.

Mineral composition. The mineral composition analysis of study species showed higher and moderates levels of various minerals. The higher concentration of Calcium 314.23 mg/g, Sodium 88.32mg/g, and Magnesium 60.21 mg/g were recorded, while the Potassium, Zinc, Iron and Coper were recorded in inadequate quantities (Figure 6).

Total antioxidant activity. AHMME showed maximum total antioxidant activity (%) of 72.26±0.30 at 100µgml^{-1} and minimum activity was observed in 25 µg/ml^{-1}. Similarly the standard ascorbic acid showed maximum 71.7±0.81 at 100 µM and minimum activity at 25 µM/ml^{-1} was observed. The study of AHMME delivered significant result compared to standard ascorbic acid and the total antioxidant activity was found directly proportional manner (Figure 7).

Superoxide radical scavenging activity. AHMME showed significant superoxide anion radical scavenging activity when compared with the standard ascorbic acid (70.5±0.45). The maximum (71±1.13 at 100 µgml^{-1}) scavenging activity of AHMME was recorded and 100 µgml^{-1} minimum was 39.26±0.25 at 25 µgml^{-1} concentration (Figure 8).

Hydroxyl radical scavenging assay. The hydroxyl radical scavenging assay of molluscan extract showed maximum 50.53±0.50 µg ml^{-1} at the highest concentration of 100 µg ml^{-1} and minimum was 12.53±0.50 µg/ml at 25 µgml^{-1}. The result of present study of AHMME extract was almost similar to standard ascorbic acid (60.15±0.25 at 100 µgml$^{-1)}$ (Figure 9).

DPH (1, 1- diphenyl – 2 – picrylhydrazyl hydrate) radical scavenging activity. The maximum scavenging ability (%) of 35.36±0.40 and 60.4±0.40 at 100 µg/ml were recorded in AHMME and standard ascorbic acid respectively. The minimum scavenging ability (%) of 10.82±0.02 was observed in the molluscan extracts at the concentration of 25 µg/ml. As

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compared to standard, the molluscan extract showed acceptable scavenging activity (Figure 10).

Reducing Power assay. It relies on the concentration of the extract and the standard reference drug. As the concentration increases reducing power ability of the test sample also increases. The reducing power (%) of bivalve extract AHMME showed highest activity 0.83±0.03 at 100 µg ml^{-1} concentration and lowest reducing power was recorded 0.12±0.005 at 25 µgml^{-1} concentration respectively. The standard ascorbic acid used showed maximum reducing activity 1.76±0.02± at 100 µgml^{-1} concentrations (Figure 11).

Ferrous Ion chelating assay. The ferrous ion-chelating assays of AHMME was found to be concentration dependent and displayed as in Figure. 12. The chelating effect (%) of AHMME showed highest activity at 100µgml^{-1} 58.3±0.30 and lowest at 25 µgml^{-1} 7.23±0.25 concentration. The standard showed chelating activity (%) of 83.33±0.30 at 100 µgml^{-1} concentration.

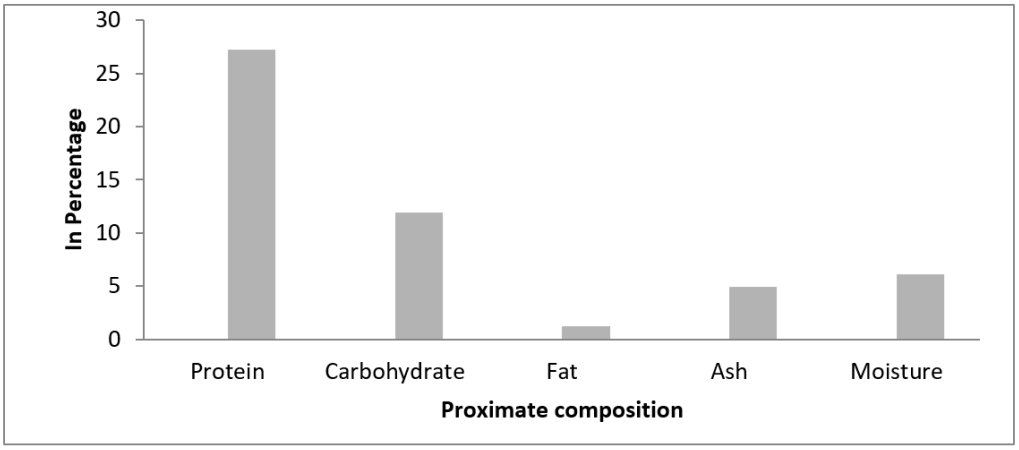

Figure 1: Proximate composition of *M. meretrix.*

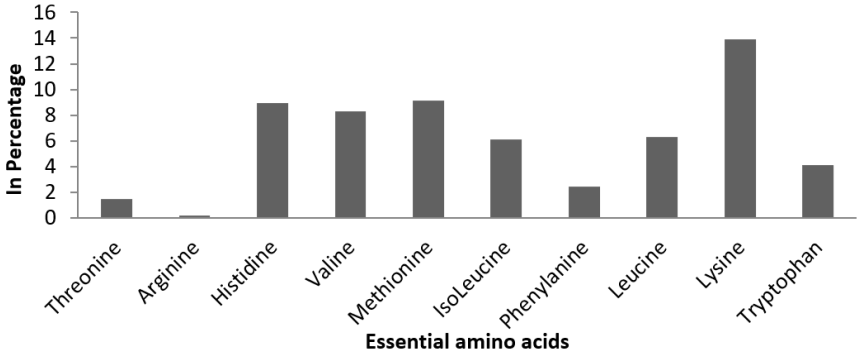

Figure 2: Estimated essential amino acid composition of *M. meretrix.*

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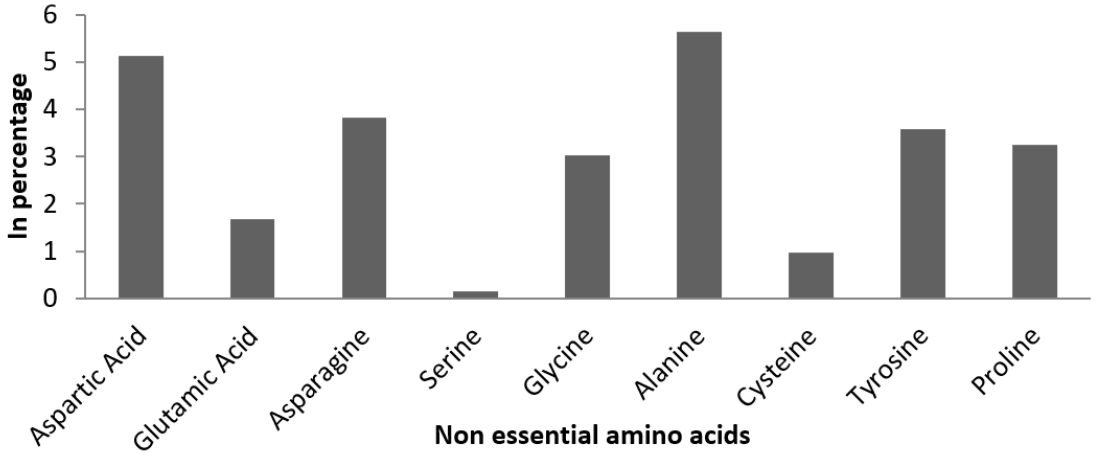

Figure 3: Estimated non-essential amino acid composition of *M. meretrix.*

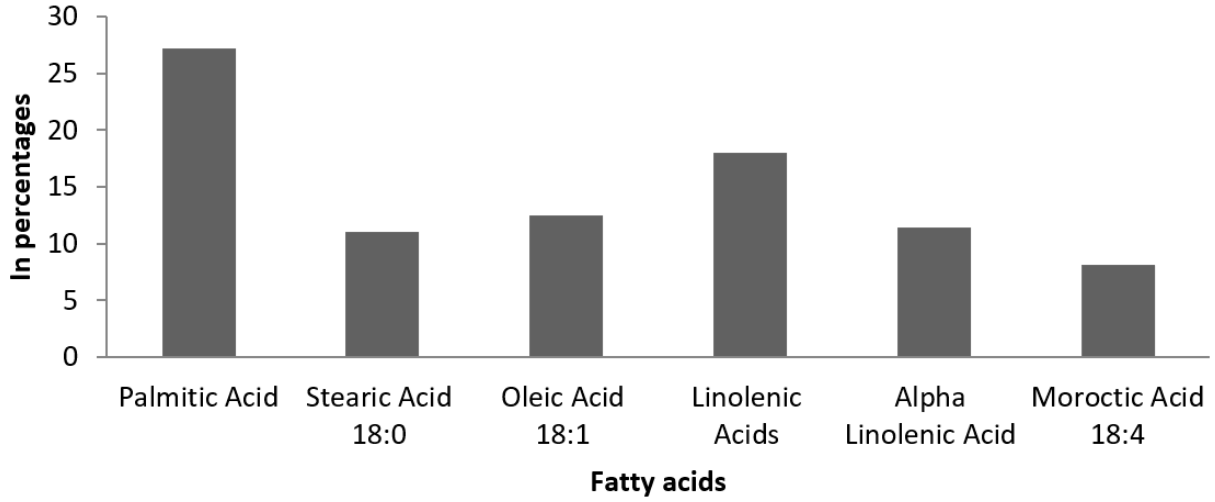

Figure 4: Estimated fatty acids composition of *M. meretrix.*

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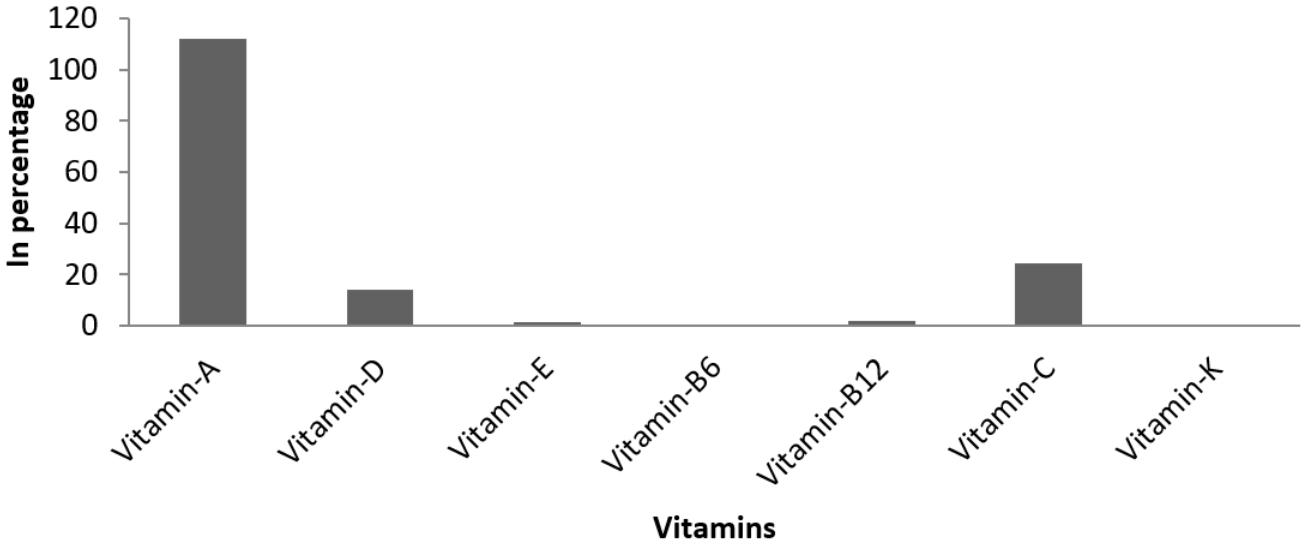

Figure 5: Estimated vitamin content of *M. meretrix.*

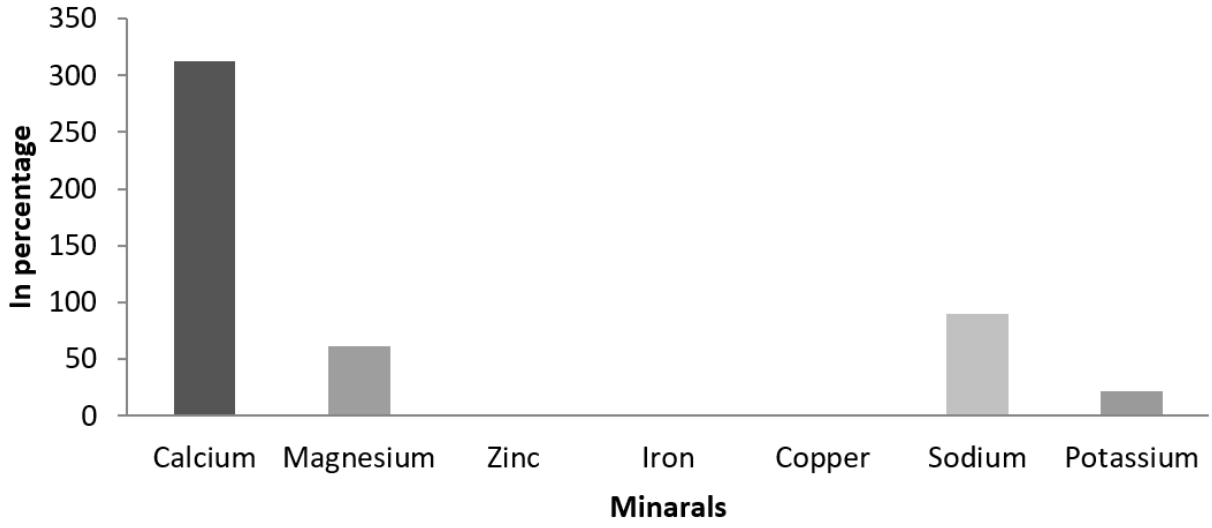

Figure 6: Estimated mineral content of *M. meretrix.*

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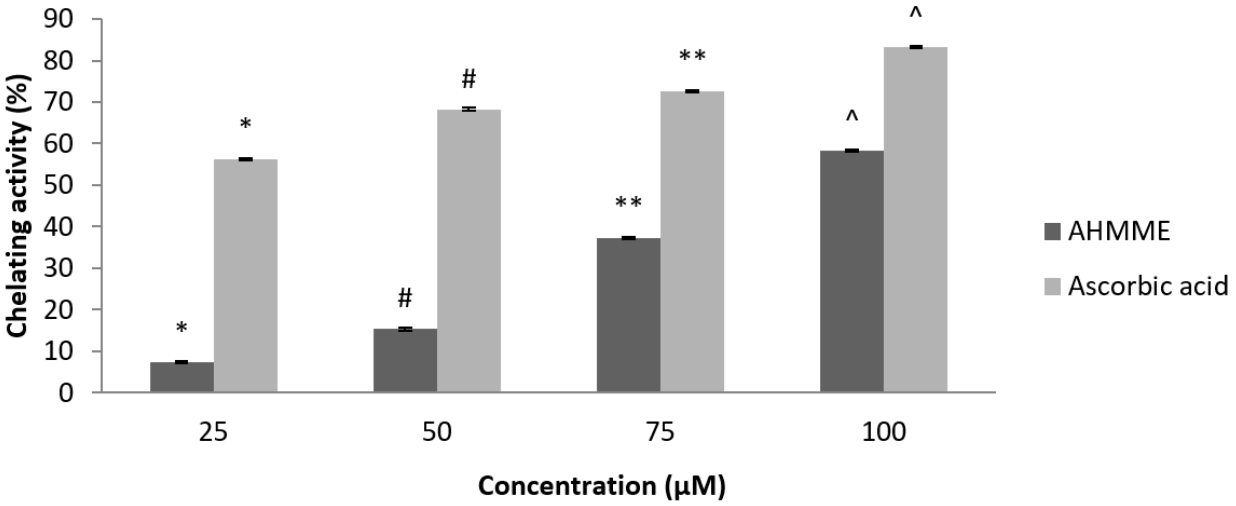

Figure 7: Total antioxidant activity of AHMME.

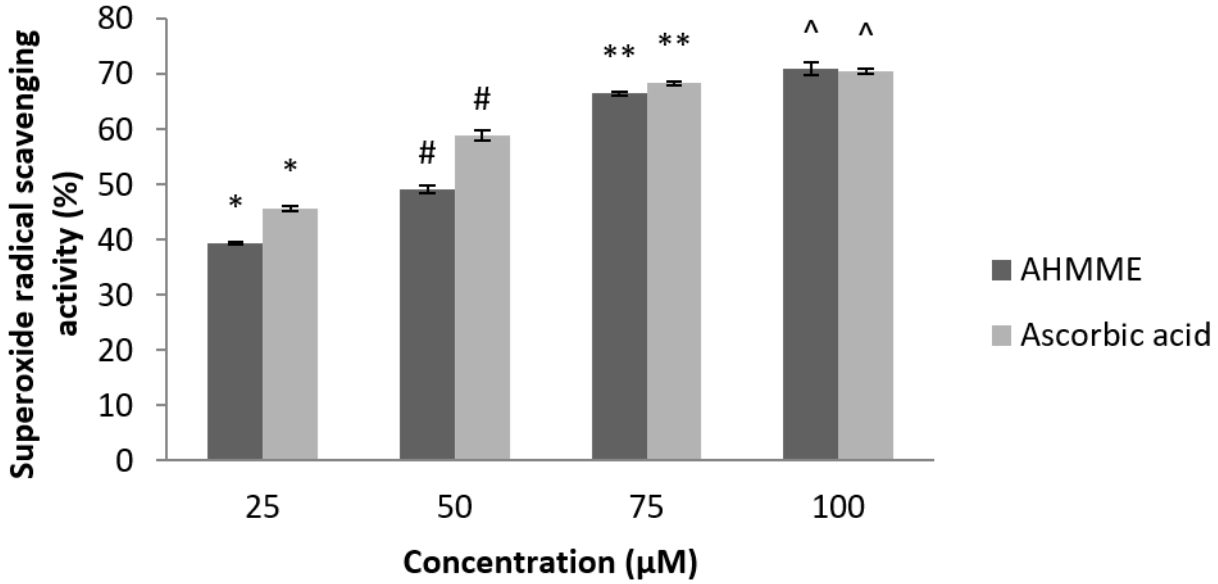

Figure 8: Superoxide radical scavenging activities of AHMME.

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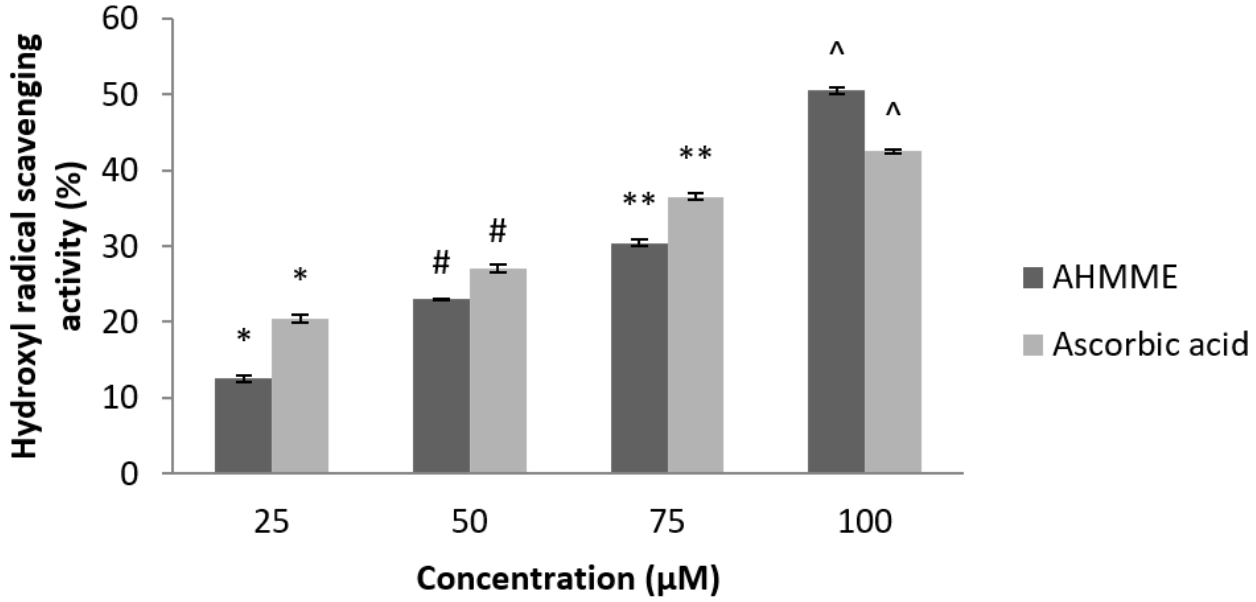

Figure 9: Hydroxyl radical scavenging activities of molluscan extract of AHMME.

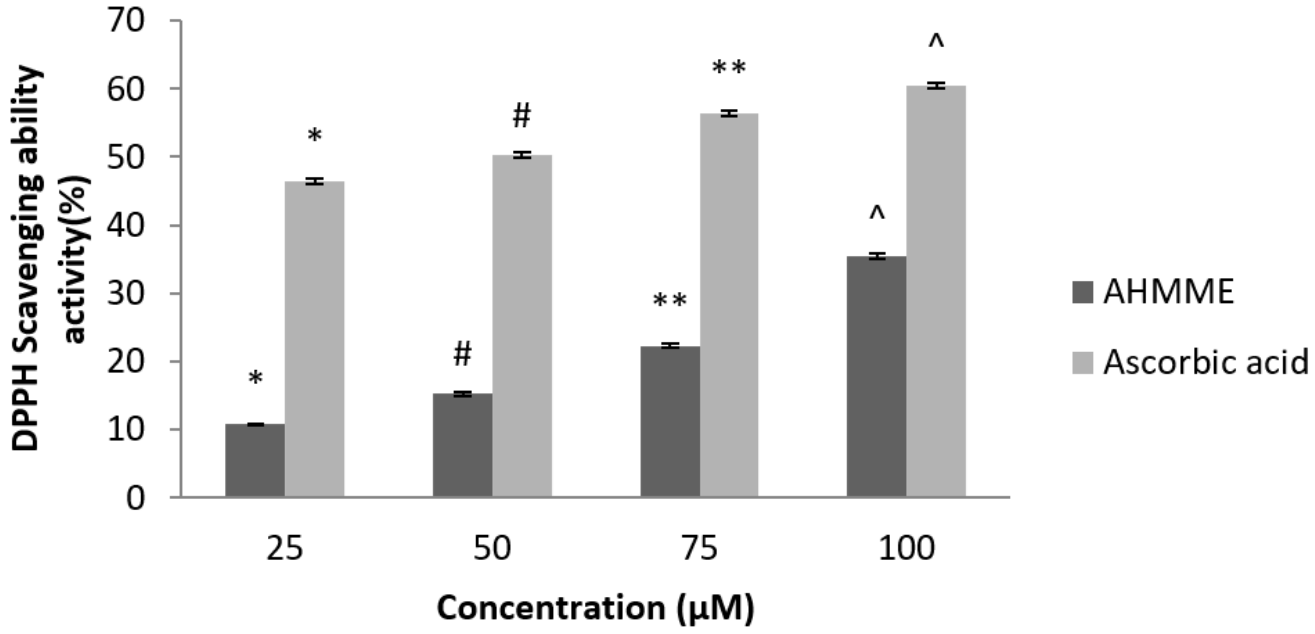

Figure 10: Scavenging ability of the AHMME on DPH.

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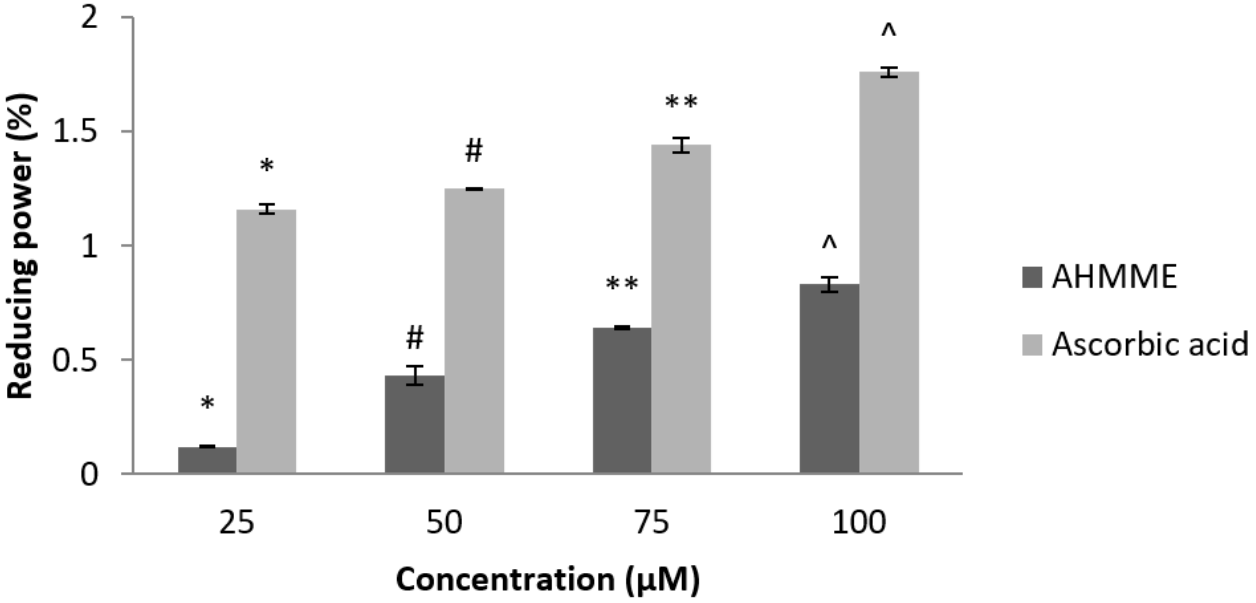

Figure 11: Reducing power of bivalve extract AHMME.

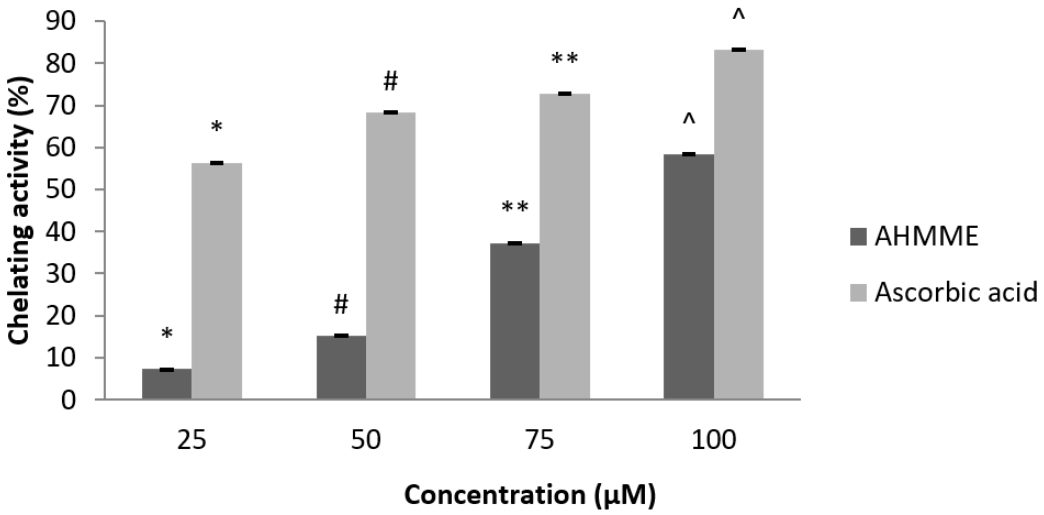

Figure 12: Ferrous ion chelating ability of AHMME

Discussion

Marine foods are the most essential food source with low cost and higher nutritional values. Marine bioactive natural compounds have drawn the attention of many researchers in recent years because of their pharmacological values. Protein is an indispensable for the nourishment of life and exists in major quantity of all nutrients as a component of human body (Okuzum and Fujii, 2000). Present investigation revealed that the protein content is prominent when it is compared with carbohydrate and lipids contents on *M. meretrix*.

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Therefore, the results suggest that the *M. meretrix* can be considered as another potential food source for providing cheapest animal protein. Some researchers have reported 11.9% of protein has been observed in surf clam *Mactra violacea* (Laxmilatha, 2009). Beside clams the extract of Gonggong sea snails was found to be an excellent source of antimicrobial peptide (Viruly et al., 2022). Moreover, the protein level of *Bursa spinosa* was varied from 18.71 to 29.81% at Parangipettai coast (Subhapradha et al., 2013). The protein 23.51% was on marine bivalve *Donax incarnates* at Cuddalore coast (Periyasamy et al., 2013).

In the present study *M. meretrix* showed carbohydrate, fat, ash and moisture content at the range of 11.95%, 1.18%, 4.92% and 6.13% respectively. Carbohydrates comprise of sugars, starches and fiber, and acts as a major source of energy for animals. The carbohydrate level at Parangipettai coast on Catfish, *Arius maculatus*, *Plotosus lineatus* and puffer fish of *Lagocephalus inermis* and *Lagocephalus lunaris* was analyzed (Manikandarajan et al., 2014) and the carbohydrate levels varied from 2.15 gm to 1.98 gm head and body part of the cat fish, 1.87 % and 1.96 % at whole body tissue of Puffer fish. Maximum carbohydrate level of 9.2 % and minimum level of 8.3 % was recorded on *Babylonia spirata* in the gonad and digestive glands (Shanmugam et al., 2006). When compared with fish, the molluscan species are very cheap and economically less valued species that can substantiate the fish nutritional supplements.

The lipids are highly well-organized source of energy, in that they contain more than twice the energy of carbohydrate and proteins. The lipid content 1.18% was noticed on an investigated clam sample. In male and female species of *Rapana rapiformis,* the lipid content was recorded to be 0.85-2.12% and 0.95- 2.96% respectively (Rajkumar, 1995). In *Babylonia zeylanica* and *Pleuroploca trapezium* species 10.38% and 1.97% of highest lipid content were noticed (Nirmal, 1995)

Protein values are obviously reflected in the essential amino acids concentrations. Present study found total of 19 amino acids in the *M. meretrix*. This study reveals that the clams were rich in essential amino acids (60.96%) than that of non-essential amino acids (27.24%). The result reveals that the meat of *M. meretrix* can be very good source for human diet due to an elevated level of quality protein, as well as balanced essential amino acids. The amino acid concentrations on different marine molluscs in Southeast Coast of India, was observed (Babu et al., 2010). They recorded that the total amino acid level in *Perna viridis* was 95.76%, *Crassostrea madrasensis* was 98.4% and in *Meretrix casta* was 65.17%. *Strombus canarium* was comprised of 80.97% of essential and 15.07% of non-essential amino acids, *Bursa spinosa* was comprised of 50.01% of essential amino acids and 46.79% of non-essential amino acids. In this context, current study clearly demonstrates that these marine mollusks might be a good potential source of amino acid.

Moreover, 28.13 % of palmitic acid and 18.74 % of Linolenic acid in *D. incarnates* from Cuddalore, Southeast coast of India was reported (Periyasamy et al., 1971). And *Donax cuneatus* contain saturated, mono and polyunsaturated fatty acids at the range of 35.28%, 12.71%, 11.72% respectively (Annaian et al., 2007). The results of the present study also showed that the marine animals are richest source of PUFA.

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Molluscs contain a variety of minerals, vitamins, essential and non-essential amino acids and high quality protein (Periyasamy et al., 1971). The flesh of fish and shell fish are imperative sources of vitamin A (Pigott, 1990). In the present study, the Clam tissue showed the major source of vitamin A and vitamin C which constituted 108.4 IU and 23.98 mg/g. The vitamin levels on three different mollusc species green mussels (*P. viridis)*, true oyster (*C. madrasensis)* and yellow clam species (*M. casta)* was studied (Ajaya Bhaskar, 2002). These species contain a significant level of Vitamins like B_1 (0.11), B_2 (031) and B_6 (0.31). Shellfish enclosed in the present study, showed complete range of vitamins. The Vitamins content on Clam *M. casta* from Parangipettai and Cuddalore coast was estimated (Srilatha et al., 2014). According to the estimation, the composition is as follow: vitamin A (14.40IU, 8.200IU), vitamin D (200IU, 150IU), vitamin E (1.18 mg/g., 1.06 mg/g) and Vitamin K (0.62 mg/g, 0.18 mg/g). Minerals constitute important components of enzymes, hormones and enzyme activators (Khan et al., 2007). Mineral components such as sodium, potassium, magnesium, calcium, iron, phosphorus and sulphur are imperative nutrients for human diet (Erkan and Ozden, 2007).

The above discussion revealed that diminutive variations carotenoids of proximate contents were noticed when compared to the present data. The variation in concentration of clam nutrition might be due to the feeding behavior, environment, ecosystem and migratory event within the same region. In addition, the chemical composition of fish meat was found to vary with sex, season, size, age and geographical locality of catch (Zenebe et al., 1998).

Moreover, in the last three decades about 1000 research papers have been published on molluscs secondary metabolites (Avila, 2006), with a total of 729 compounds were reported from 199 species (Faulkner, 2009). Dietary antioxidants, for instance carotenoids aids in preventing several human diseases and potential antioxidants absorbs the excited energy from the singlet oxygen in to the carotenoid chain, that leads to degradation of carotenoid molecule thereby preventing the damages of other molecules and tissues (Buhl-Mortensen and Hoisaeter, 1993). They also prevent the production of free radicals induced by the poly-unsaturated fatty acids degradation. Moreover, Carotenoids prevents the peroxidation of membranous phospholipids and lipids (Naguib, 2000). Therefore, therapeutic interventions having antioxidants or free radicals scavenging activity may be useful against oxidative stress associated with various cardiovascular diseases including myocardial infarction (Rajadurai and Prince, 2007). AHMME was involved to evaluate the total antioxidant nature of it. It showed some promising results at high concentrations and the values were significantly similar when compared with the standard reference drug.

Superoxide anion is very harmful to cellular components. Numerous biological reactions generate superoxide anions which are highly toxic in nature. In PMS/NADH-NBT system, the superoxide anion derived from PMS/NADH coupling reaction reduces NBT. The decrease of absorption at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture (Alagumanivasagam et al., 2012). In the present investigation, the superoxide radical scavenging activity of AHMME was comparable to standard reference compound ascorbic acid. In the above observation it is concluded that AHMME potent scavenging capacity of superoxide anion. Likewise, the cuttlefish extract

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GAG scavenge the superoxide in a concentration dependent manner (Vino et al., 2012). Similarly, the superoxide anion radical scavenging ability of marine species were also dose dependent (Maximas et al., 2014).

Hydroxyl radical is one of the more potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause damage to cell (Gutteridge et al., 1981). Hydroxyl radical causes severe damage in bio-molecules (Yan et al., 2005) and also plays a major role in lipid peroxidation. In the present analysis the hydroxyl radical scavenging effect of AHMME showed some promising hydroxyl radical scavenging activity and results are comparable to the standard ascorbic acid. Previously, chitosan from cuttlefish shows high levels of hydroxyl scavenging activity of 72.1% (Vino et al., 2012). Present results were lined with the previous findings AHMME reaction mixture scavenged the hydroxyl radicals from the sugar and prevented the reaction. It indicates that AHMME was proved as better hydroxyl radical scavenger.

DPH possess proton free radicals with characteristics absorbance which decrease significantly on exposure to proton radical scavengers. Further it is accepted that the DPH free radical scavenging by antioxidant is due to their hydrogen donating ability. Thus scavenging of DPH free radicals was directly affected by the amount of attractable hydrogen atoms in a protein molecule. The free radical scavenging ability using the DPH method was accessed to identify the antioxidant potential of marine species (Hong-Yu et al., 2010). In the present investigation AHMME reported strong DPH scavenging activity and it clearly indicates the hydrogen donating ability of Mollusca extract. Similar results were observed by several researchers on different marine species. The effect of antioxidant on DPH radical scavenging is thought to be due to their hydrogen donating ability (Conforti et al., 2009).

Reducing power assay has been used to evaluate the ability of natural antioxidants to donate electrons. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Dorman et al., 2003). The present data also showed excellent reducing capacity when compared to the standard drug. And the reducing power assay of *Berberis tinctoria* was also studied and it also showed dose dependent mannered activity which could be due to the free radical chain by denoting hydrogen atom (Sasikumar et al., 2012). Further suport that, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In addition, the antioxidant activity of antioxidants has been attributed to various mechanisms, including the prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Yildirim et al., 2000).

Metal chelating capacity was significant, since it reduced the concentration of the catalyzing transition metal in lipid peroxidation. Previous report support that, chelating agents are effective as secondary metabolites, because they reduce redox potential, thereby stabilizing the oxidized form of metal ion (Gulcin et al., 2007). Present investigation was in agreement with the previous research on *Meretrix casta* extract, which also explained the antioxidant potential by carrying out DPH, Iron, chelating and reducing (Pachaiyapan et al., 2014). And

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Babylonia zeylanica produce remarkable antioxidant effect on iron chelating study by stabilizing the oxidized from of metal-ions (Velayutham et al., 2014).

Conclusion

The present study AHMME showed significant in-vitro free radical scavenging effects. And the results of the study strongly suggest that the free radical scavenging potential of AHMME noticed in dose dependent manner. Further the outputs of the nutritional profile showed that, *M. meretrix* have been recommended as a good source of food for human consumption in future. Further AHMME extract will be purified and characterized to evaluate their biological activity in future.

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