CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Antioxidant Properties from Tissue Extract of Cephalopods around Madras Atomic Power Station, Kalpakkam Coast

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ARTICLE INFO

Received: 30 Mar 2016
Accepted: 26 Apr 2016

ABSTRACT

Objective: Molluscs are considered as an important natural source to derive many novel biological active compounds. Discovery of molluscan bioactive potential and their curious roles are still limited. *Experimental approach:* Hence, the present study was evaluated on the antioxidant activity of cephalopods (*Sepia pharaonis*, *S. intermis* and *Octopus vulgaris*) tissue extract at various concentrations (20-100µg/ml). *Findings:* The results of cephalopod methanol tissue extracts exhibited significant total antioxidant activity, total reducing power, DPPH, NO scavenging and hydrogen peroxide scavenging activity which predicted as 74.32%, 62.71%, 81.25%, 84.02% and 82.19%, respectively. *Conclusion:* These results concluded that, the tissue extract of *Sepia pharaonis* has novel antioxidant potential and it has to further characterize to improve the pharmacological active marine natural products.

Keywords: Marine molluscs, Cephalopods, Methanolic extract, Antioxidant activity.

1. INTRODUCTION

Marine organism are having very rich source of food, feed, medicine and energy. They have also proven to be rich source of structurally diverse bioactive compounds with the valuable pharmaceutical and biomedical application[1]. Among them molluscs is one of the most important groups of invertebrates in the animal kingdom. It's divided taxonomically into seven classes, out of these bivalves, gastropods and cephalopods comprise major marine fishery resources worldwide. There are 66,535 species widely distributed throughout the world and have many representation in the marine and estuarine ecosystem. Out of 5,070

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species recorded in India, only 100 have been implicated in poisoning to man[2]. In addition to that, the bioactive compounds from molluscs exhibit antitumor, antimicrobial, anti-inflammatory and antioxidant activities[3].

Generally, antioxidant compounds are playing an important role to trap free radical and reduce the risk of chronic disease (cancer and heart disease) as a health-protecting factor[4-6]. Antioxidants in biological systems have multiple roles, including defending against oxidative damage and participating in the major signalling pathways of cells. One major function of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species[4&5]. These antioxidant molecules are present in various molluscs species to prevent cell damage from oxidation reaction to consumers[7]. Further, these mussels providing resistance capacity to various kind of oxyradicals[8-11] and it has dietary antioxidants such as phenolic content[12-16]. Hence, the present study was focused to understand the antioxidant activity of cephalopods tissue extracted with methanol at various concentrations.

2. MATERIALS AND METHODS

2.1 Reagents

Ammonium molybdate, Phosphate buffer, Ascorbic acid, Hydrogen peroxide solution, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Sodium nitroprusside, Sulfanilic acid, Naphthyl ethylenediamine dihydrochloride, Sulfuric acid, Butylated Hydroxyltoluene, Potassium ferricyanide, TCA, $FeCl_3$, Folin-Ciocalteu reagent, Sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2 Collection of samples and extraction

The cepahlopods samples such as *sepia inermis*, *sepia pharaonis* and *Octopus vulgaris* were collected from the coastal area which located around the nuclear power plant Kalpakkam coast in Tamilnadu. The collected fresh animals were brought to the laboratory. The shell removed fresh tissue samples were washed with sterile distilled water and the tissue was sonicated with equal volume of methanol. After sonication the methanolic extract was centrifuged at 10000rpm for 35min and the supernatant were collected and concentrated using rotary evaporator and freeze dried to give dark brown gummy mass which stored at -20˚C for further analysis.

2.3 Antioxidant activity

The antioxidant activity of the methanolic extract of *sepia inermis*, *sepia pharaonis* and *Octopus vulgaris* were estimated in terms of total antioxidant activity, total reducing power, DPPH, nitrous oxide scavenging activity and hydrogen peroxide scavenging activity followed by the method of Sivaperumal *et al*. [6] with slight modification.

3. RESULTS AND DISCUSSION

The antioxidant activities of cephalopods (*Sepia pharaonis*, *S. inermis* and *Octopus vulgaris*) methanolic extracts were evaluated in various methods which formulate to have different levels of antioxidant activity at different concentrations (20, 40, 60, 80 and 100 µg/ml). The total antioxidant activity of methanolic extracts of *Sepia pharaonis*, *sepia inermis* and *Octopus vulgaris* was evaluated by the creation of green phosphate complex at acidic ion nature. The total antioxidant activity of the methanolic extracts of cephalopods was 74.32%, 71.31 and 58.93% respectively (Fig.1). This indicates that the *Sepia pharaonis* has a good source of natural antioxidants. Figure 2 showed the reducing capacity of three cephalopods extracts and the higher reducing ability was 62.71%, 18.4 and 31.8% respectively. Among three different cephalopods extract, higher activity was observed from *Sepia pharaonis.*

The free radical scavenging activity of cephalopods extracts was assessed by DPPH assay. The highest

scavenging ability was observed at 100 µg/ml concentration and the activity was obtained from *Sepia pharaonis, S. inermis* and *Octopus vulgaris* (81.25, 73.31 and 54.47% respectively). Destruction of Nitric oxide free radical release might be accredited to direct nitric oxide free radical scavenging influence to decrease the amount of nitrite made from the decomposition of sodium nitroprusside. The results showed that methanolic extract of cephalopods had scavenging ability 84.02%, 74.31 and 51.65% respectively at 100 µg/ml concentrations.

The hydrogen peroxide and total phenolic compound was obtained from *Sepia pharaonis, S. inermis* and *Octopus vulgaris* cephalopods methanolic extracts. The hydrogen peroxide activity was 82.19%, 69.38 and 26.61% respectively and phenolic content 86.34%, 72.34 and 55.24% respectively. Among three economically important cephalopods, *S. pharaonis* showed predominant results at all antioxidant assays. These species could be useful for pharmaceutical application and further antioxidant response compound and their characterization study is necessary.

Total antioxidant activity was estimated by the formation of green coloured phosphate (Mo IV) from phosphomolybdenum (Mo IV) complex at acidic pH. This non specific reduction reaction was incorporating the presence of antioxidants in the samples [4,17].The extract of *S. pharaonis* was changed the colour of DPPH (deep violet) in to yellow which showed methanolic extracts had effective scavenging activity of constant nitrogen centred free radicals and proxyradicals by the addition of hydrogen atom[5,18-19]. Similarly the reductones play a potential role in the breakage of radical chain reaction[20]. Nitrous oxide reacts with oxygen free radicals to form peroxynitrate and nitrate which damages the nucleic acid and induce the inflammatory activity with multiplication of toxicity in the cells[21]. Mollusc might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological circumstances caused by excessive generation of NO and its oxidation product, peroxynitrite. This is accordance with[22] who was indicated that NO radicals play an important role in inducing inflammatory response and their toxicity multiplies single when they react with O2 radicals to form peroxynitrite, which damages biomolecules such as proteins, lipids and nucleic acids.

In the present study, the mollusc from methanol extracts inhibited the nitrite formation by directly competing with oxygen in the reaction with NO. The present result reveals that, the methanol extract of *S. pharaonis* might be potent agent for scavenging of nitric oxide. The week oxidizing agent hydrogen peroxide reacts with ferrous and copper ions to form hydroxyl radicals deactivate the enzyme and causes the toxic effect in the cells [23]. In this study, methanolic extracts of cephalopods has potential hydroxyl radical scavenging activity at various concentration and the higher scavenging activity was noted from the extract of *S. pharaonis*. Generally, natural polyphenols are important groups of metabolites which play a vital role in the natural medicine, that may be reason to improve radical scavenging activity while increasing levels of phenols and phenolic compounds[24-25]. In recent times, there is not much information about the presence of polyphenols from cephalopods.

Among the six assays used, DPPH was found effective assay method as evident by the sensitivity of the assay. The DPPH test provides information on the reactivity of test compounds with a stable free radical. The electron becomes paired off in the presence of a free radical scavenger, the absorption varnishes; therefore the resulting decolourization is stoictiometric with respect to the number of electrons taken up. The scavenging possessions of anti-oxidants are often associated with their ability to form stable radicals. The

present study observed a considerable DPPH radical scavenging activity with commercially important of marine mollusc. It has been reported that activity of methanolic extract from *B. spinosa* against DPPH radical was found as 39.43% at 10 mg/ml; whereas the BHT and ascorbic acid showed 63.67 and 59.8% respectively[26]. Previous study[27] have reported that the methanolic extract of gastropod *Pleuroploca trapezium* was found to show a good scavenger of DPPH radical with an IC50 value of 4021 µg/ml. These results were in unity with other marine natural products were more active to different radicals [28].

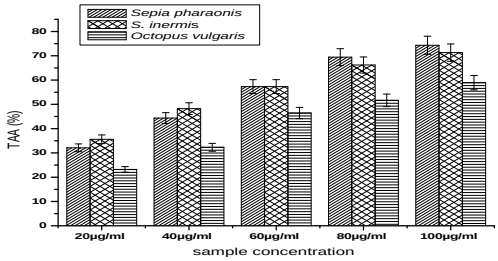

Fig 1: Total antioxidant activity from Cephalopods methanolic extract

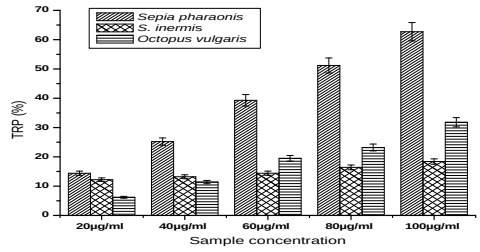

Fig 2: Total Reducing Power from Cephalopods methanolic extract

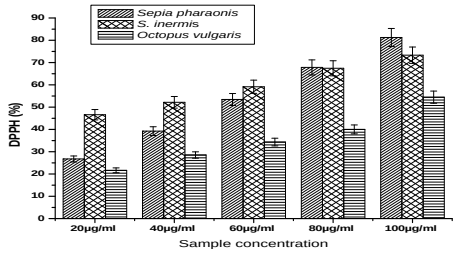

Fig 3: DPPH Assay from Cephalopods methanolic extract

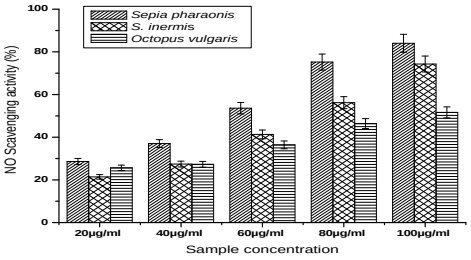

Fig 4: NO Scavenging activity from Cephalopods methanolic extract

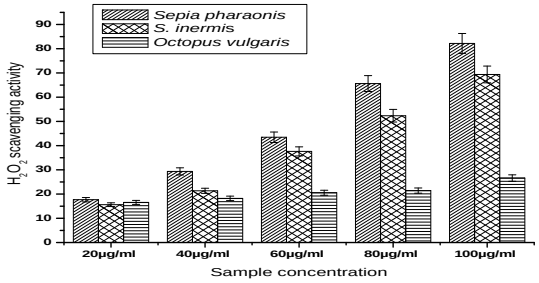

Fig 5: H_2O_2 Scavenging activity from Cephalopods methanolic extract

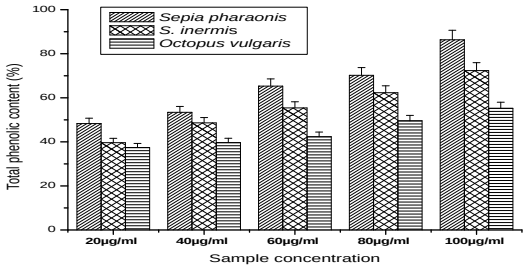

Fig 6: Total Phenolic content from Cephalopods methanolic extract

4. CONCLUSION

The economical important marine molluscs species *Sepia pharaonis* methanol extract was found to possess radical scavenging and antioxidant activities, as determined by scavenging effect on the total antioxidant activity, DPPH, chelating effect on ferrous ions, hydrogen peroxide scavenging activity, nitric oxide scavenging activity and reducing power. Thus it can be concluded that the cephalopods can be used as an accessible source of natural antimicrobial and antioxidants with consequent health benefits. Moreover, further investigations involving characterization and application of the extracts as drug for human administration need more research.

5. ACKNOWLEDGEMENT

Authors are thankful to authorities of ICAR-Central Institute of Fisheries Education, Deemed University, Mumbai and Board of Research in Nuclear Sciences (BRNS), Mumbai.

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Conflict of Interest: None

Source of Funding: Nil