IJPSR (2021), Volume 12, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 January 2021; received in revised form, 24 April 2021; accepted, 12 October 2021; published 01 December 2021

ANTIOXIDANT POTENTIAL OF MARINE CRAB *PORTUNUS PELAGICUS* FROM GULF OF MANNAR, SOUTHEAST COAST

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Keywords:

Portunus pelagicus, DPPH, Total antioxidant, FRAP assay

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ABSTRACT: Natural products offer great hope in the identification of bioactive compounds and their development into drugs for the treatment of various diseases. The present study aims to evaluate the antioxidant potential of *Portunus pelagicus*. *P. pelagicus* (Station-I) possessed 33.14% of inhibition, the highest DPPH activity, whereas Station II and Station III showed 31.42% and 30.85% of inhibition. *Portunus pelagicus* possessed 3.34% reducing ability at 1000 µg/ml concentration in the present study in station I, station II exhibited 2.62%, and station III shpwed2.55% at 1000 µg/ml concentration. The highest reducing ability was recorded at the station I, compared to stations II and III. The present study revealed that the tissue extract of *Portunus pelagicus* has antioxidant capacity, and it needs further characterization to improve the pharmacologically active marine natural products.

INTRODUCTION: The marine environment has proven to be a source of diverse arrays of bioactive metabolites with great potential for pharmaceutical and other applications. More than 100 pure compounds of known and new structural types have been isolated and characterized. Since ancient times, the marine organism has been used for medicinal purposes in India, China, and Europe. In the recent past, several pharmacological substances of marine origin have been developed [1]. Oxidation is essential to many living organisms for the production of energy to fuel the biological process. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent, which can produce free radicals.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(12).6582-86

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(12).6582-86

These free radicals may oxidize nucleic acids, proteins, lipids, or DNA, which start chain reactions that damage cells and can initiate degenerative diseases such as cancer and other diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease [2]. In addition to that, it blocks the oxidation process by neutralizing the free radicals such as superoxide anion radical (O_2) and hydroxyl radical (OH), which are unavoidable consequences in an aerobic organism. Antioxidants are the first line of defense and are critical for maintaining optimum health and wellbeing.

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by acting as oxygen scavengers [3]. Antioxidants are described as a substance that, when present in low concentrations relative to the oxidizable substrate, significantly delayed or reduced oxidation of the substrate [4]. The need for antioxidants becomes even more critical

with increasing exposure to free radicals. Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. The role of antioxidants has received increased attention during the past decade. However, the use of synthetic antioxidants has a potential health hazard [5].

Therefore, in recent years, interests have been developed for searching for effective natural antioxidants since they can protect the human body from free radicals and retard the progress of many chronic diseases. Natural products from marine samples have a wide spectrum of biological activities and numerous therapeutic applications include antiviral, antibacterial, antitumor, and antioxidants, particularly in those of free radicals in various diseases. These pathological and clinical backgrounds have prompted the investigation of novel and potent antioxidants peptides from crab that are ultimately of therapeutic use. The potential of marine crabs as a source of biologically active products is largely unexplored. Hence, a broad-based screening of marine crabs for the bioactive compound is necessary. A thorough understanding of chemical structure and biological activity will lead to the formulation of novel drugs with specific actions. Hence the present study has been carried out to investigate antioxidant activity of crab.

MATERIALS AND METHODS:

Collection and Preparation of Extract: In the present study, the crab (*Portunus pelagicus*) was collected from three stations *viz.*, Rameswaram, Kanyakumari (ChinnaMuttam), and Thoothukudi by trawl catch kept in ice, and transferred to the laboratory within 24 h. For removing mud, algae, and barnacles stuck to the external skeleton, crabs were washed with fresh seawater. The shells were removed, and the tissues were then dried in a hot air oven at 56 °C for 48 h.

The dried tissue was immersed in 10% AR grade methanol for 10 days at room temperature. After filtration with Whatman No.1 paper, the methanol extract was reduced by vacuum evaporation. The extract residue was resuspended in 20 ml of 100% A.R grade methanol. The soluble methanol extracts were dried and solidified in distilled and deionized water. Different concentrations of extracts were prepared and stored at 0 °C for further use.

DPPH Free Radical Scavenging Assay [6]: The ability of the fractions to annihilate the DPPH radical (1, 1 - diphenyl – 2 - picrylhydrazyl) was investigated. Different concentration of the extract of the crab tissue (200 µg/ml, 600 µg/ml and 1000 µg/ml) was added, at an equal volume, to the methanolic solution of DPPH (0.1 mM). The reaction mixture was incubated for 30 min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. The annihilation activity of free radicals was calculated in % inhibition according to the following formula:

% of inhibition = A of control – A of Test / A of control ×100

Total Antioxidant Activity-Ferric Reducing Antioxidant Power (FRAP assay) [7]: The different concentration of methanolic extract of the crab tissue (200, 600 and 1000 µg/ml) was mixed with 300 µl of ferric–TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 Mm HCl and 20 mM FeCl3 6H2O at a ratio of 10:1:1 (v/v/v)). The mixture was incubated at 37oC, and the absorbance readings were taken at 593 nm after 4 min. Results were expressed in mM Fe (II)/g dry mass using a freshly prepared ferrous sulfate solution calibration curve.

A (Sample Final) - A (Sample Initial) × 2 / A (Std Final)-A (Std initial)

A-Absorbance; Std-Standard.

RESULTS:

DPPH Scavenging Activity: The free radical scavenging activity of methanolic extract of the crab tissue was assessed by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Ascorbic acid was used as standard. The DPPH radical scavenging potential of crab tissue extract ranged from 15.57% to 33.14% at varied 200 µg/ml, 600 µg/ml, and 1000 µg/ml. Scavenging activity was increased with the increasing concentration of the extract.

DPPH Scavenging Activity-Station I: The methanolic extract of the crab tissue showed a minimum activity ranging from 18.67% to 33.14%. At a concentration of 200 µg/ml, the extract showed 18.67% of scavenging activity. Maximum activity of 33.14% was observed at a concentration of 1000 µg/ml.

The scavenging activity was increased as the concentration of extract increased **Fig. 1.**

DPPH Scavenging Activity-Station II: The scavenging activity was observed in all concentrations. The methanolic extract showed 17.41% activity at 200 µg/ml concentration followed by 24.64% and 31.42% at a 600 µg/ml concentration and 1000 µg/ml **Fig. 1.**

DPPH Scavenging Activity-Station III: The methanolic extract showed 15.57% of activity at 200 µg/ml concentrations and the activity was increased with the increasing concentration and reached the maximum of 30.85% at 1000 µg/ml concentration. The sample collected from the station I have better scavenging activity when compared to station II and station III **Fig. 1.**

FRAP Total Antioxidant Activity:

FRAP Total Antioxidant Activity-Station I: Extracts showed varied activity ranging from 2.46% to 3.34%. At a concentration of 200 µg/ml, the extract showed 2.46% of reducing activity. The reducing ability was increased as the concentration of extract increased and the activity raised to 3.34% at 1000 µg/ml was subjected. Scavenging activity was higher at a higher concentration of extract **Fig. 2.**

FRAP Total Antioxidant Activity-Station II: Methanolic extract was subjected to total antioxidant assay at varying concentrations of 200, 600, and 1000 µg/ml **Fig. 3**. The activity was observed in all concentrations of extract ranging from 2.03% to 2.62% in which 200 µg/ml concentration of extract showed 2.03% of activity followed by 2.38% (600 µg/ml) and 2.62%, of activity was observed at a concentration of 1000 µg/ml of extract.

FRAP Total Antioxidant Activity-Station III: Extract from crab tissue showed varied activity ranging from 1.84% to 2.55%. A concentration of 200 µg/ml of the extract showed a minimum of 1.84% reducing activity. The reducing ability was increased as the concentration of the extract was increased. The activity was raised to 2.55% when 1000 µg/ml of extract was added. Reducing ability was higher at 1000 µg/ml of extract **Fig. 4.**

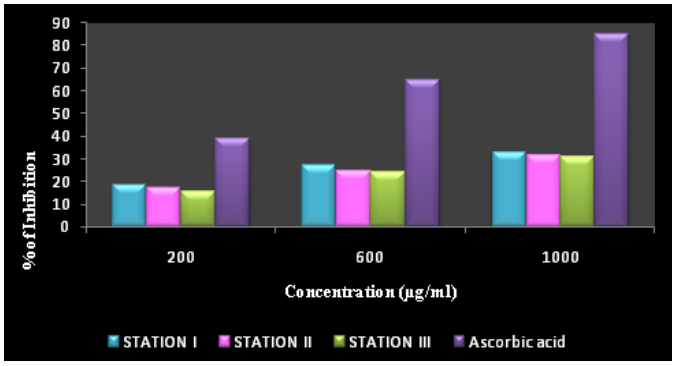

FIG. 1: DPPH SCAVENGING ACTIVITY FOR THREE STATIONS

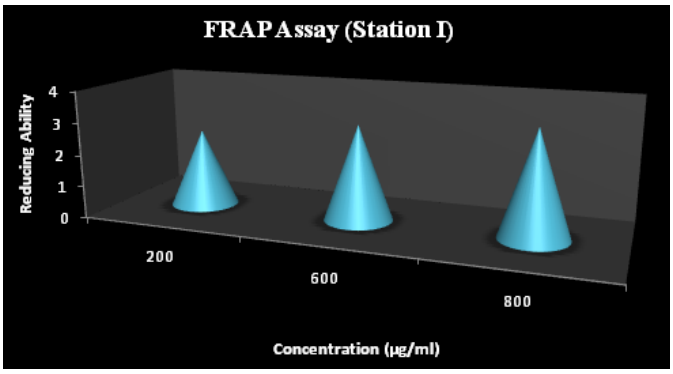

FIG. 2: FRAP TOTAL ANTIOXIDANT ACTIVITY-STATION I

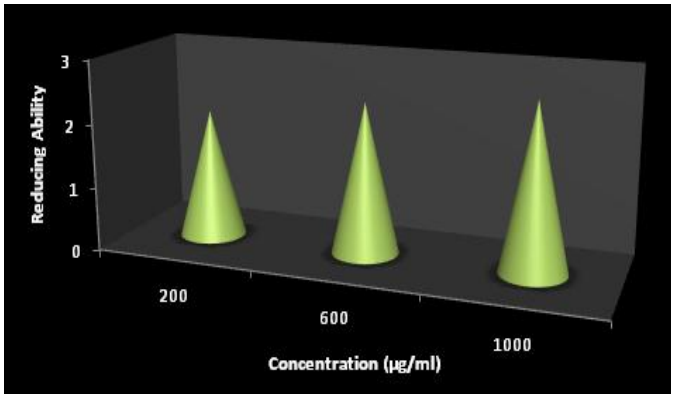

FIG. 3: FRAP TOTAL ANTIOXIDANT ACTIVITY-STATION II

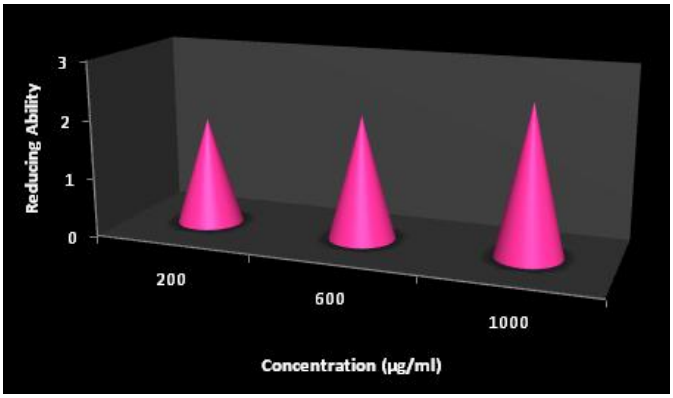

FIG. 4: FRAP TOTAL ANTIOXIDANT ACTIVITY-STATION III

DISCUSSION: Nature has played an instrumental role in providing effective therapeutics entities. Antioxidant activity is the fundamental property and much important for life. Many of the biological functions such as anti-mutagenicity, anti-carcinogenicity, and anti-aging originate from this property [8]. In the present study, antioxidant activity was measured by DPPH scavenging activity. DPPH is one of the stable free radicals used to assay the scavenging capacity of crab tissue.

The DPPH scavenging activity of standard ascorbic acid was found to be 39.12% for (200 µg/ml), 64.39% for 600 µg/ml concentration and 85.07% for 1000 µg/ml concentration respectively. By comparing this percentage with the tissue extract from the crab collected from three different stations, it is noted that *P. pelagicus* (Station-I) possess 33.14% of inhibition, a highest DPPH activity, where as other two crab collected from Station II and Station III showed 31.42% and 30.85% of inhibition. All the samples possessed a good DPPH activity which predicted that DPPH would have picked up the electron in the presence of a free radical scavenger which is reflected as the percentage of DPPH activity. The present study was compared with [9] they reported the scavenging role of 59% and 48% in soft and hard shells of *Callinectes lucifera*. Suganya and Asheeba (2015) evaluated the antioxidant activity of astaxanthin pigment isolated from *P. sanguinolentus* (three spotted crab) *Callinectes sapidus* (Blue crab) and *Paralithodes brevipes* (Spiny King Crab).

They found that all the samples of astaxanthin possessed a good DPPH activity. *C. Sapidus* possess 79%, and the other two crabs possess 67%. In the present study, all the samples possessed a good DPPH activity. The highest DPPH scavenging activity was recorded at the station I, compared to stations II and III [10]. Sudhakar (2011) recorded the total antioxidant activity ranged from 28.52% to 80.26% at varying concentrations (0.5 to 10 mg/ml) in *Portunus sanguinolentus* crab shell chitosan sample. In the present study *Portunus pelagicus* possess 3.34% reducing ability at 1000 µg/ml concentration in the station I, when compared to the other two stations II (2.62%) and station III (2.55%) at 1000 µg/ml concentration. The highest reducing ability was recorded at the station I, compared to station II and III [11]. The available results clearly show the potential of the marine ecosystem in cancer therapy. Identifying new targets for therapeutic intervention in cancer is instrumental in improving the natural history of cancer patients. However, an unfavorable balance between discovery and the very small number of candidates incorporated for clinical evaluation exists. So, it appears that a better and more pragmatic approach is urgently needed to translate innovative discoveries into active clinical therapeutics. The available data demonstrates that the marine ecosystem is productive to discover anticancer entities and is also a tool to identify new cellular targets for therapeutic intervention. The results from the study show that *P. pelagicusis* promising to act as cancer curative.

CONCLUSION: The methanolic extract of marine crab *Portunus pelagicus* was found to possess good radical scavenging and antioxidant activities, as determined by the scavenging effect on the DPPH and reducing power. Thus it could be concluded that the crustaceans can be used as an accessible source of natural antioxidants with consequent health benefits. Moreover, further investigations involving characterization and application of the extracts as a drug for human administration need more research.

ACKNOWLEDGEMENT: The authors are grateful to the Principal of St. Mary's College (Autonomous), Thoothukudi, for providing the necessary facilities to carry out this work successfully.

CONFLICTS OF INTEREST: No conflicts of interest.

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How to cite this article:
Shibana C and Subavathy P: Antioxidant potential of marine crab *Portunus pelagicus* from Gulf of Mannar, Southeast coast. Int J Pharm Sci & Res 2021; 12(12): 6582-86. doi: 10.13040/IJPSR.0975-8232.12(12).6582-86.