Persian Gulf Methanolic extract of *Nerita longii* as Efficient Antioxidant Agent

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Abstract

**Background:** Marine organisms are the important key for the development and conception of new drugs with medicine applications. Marine invertebrates produce a high amount of natural products as adaptation to their environmental and as result of their life style. Marine natural compounds with powerful bioactivities were already extracted from marine invertebrates including gastropods, tunicates, sponges, soft corals, sea hares, sea cucumbers and bryozoans. Some of those compounds, have pharmaceutical activity such as antitumor, antimicrobial, anti-inflammatory and antioxidant activities which can be used in clinical and preclinical assays.

**Objectives:** The purpose of this research was to examine the activity of methanolic extract from Persian Gulf gastropod, *Neritalongii* as efficient antioxidant.

**Materials and methods:** Persian Gulf gastropod, *Neritalongii* was washed and macerated with ethyl acetate and methanol for three days. The resulting extracts were dry with freeze dryer and the final residue were consider for antioxidant tests by DPPH, FRAP and FOLIN methods.

**Results:** In DPPH method, the resultant IC50 for methanol and ethyl acetate were 8.07 and 10.13 mg/ml, respectively. In FRAP method, the resultant EC1 for methanol and ethyl acetate were 3.687, 5.35 mg/ml respectively. In FOLIN method, the highest Absorbance in methanolic and ethyl acetate extracts is given 1.626 and 1.592.

**Conclusions:** Muscle tissue of Persian Gulf gastropod, *Neritalongi*, is an important source of natural proteins and peptides. So high antioxidant activity was given from FRAP, DPPH and FOLIN methods of methanolic extract can be related to the ability of methanol to extract corresponding peptides and proteins.

Introduction

The Ocean covers around 70% of the Earth surface. Humans especially depend on marine systems for a high number of their practices such as food resources, ways to travel around, business and more recently as a source of important Metabolites for the cosmetic and pharmaceutical industries. In the last decades the high bioactivity studies of compounds found in marine organisms have turned sea life into a new and prolific source of metabolites, which can be very efficient to improve human health and life quality. Those compounds present a wide range of biological activities...
such as anti-tumor, anti-microbial, anti-inflammatory and anti-oxidant. Therefore, marine organisms are considered to be the key for the development and conception of new drugs with medicine applications [1].

Marine invertebrates produce a high amount of natural products as adaptation to their environmental and as result of their life style. As a result of living in aquatic system, those organisms have face specific biochemical and physiological constrains such as darkness, predation, exposure to ultra-violet radiation, lack of physical defense (soft body), cold temperatures and high pressurized environment’s [2].

Around 10000 marine natural compounds with powerful bioactivities were already extracted from marine invertebrates including gastropods, tunicates, sponges, soft corals, sea hares, sea cucumbers and bryozoans. Some of those compounds, have pharmaceutical activity such as antitumor, antimicrobial, anti-inflammatory and antioxidant activities which can be used in clinical and preclinical assays, leading to the 4 development of new medicines as the example of Ziconitide (Proalt TM), used to treat chronic pains, which was obtained from a mollusk specie, Conus magus [1,3-5].

In our body, oxidation process leads to cell damage, cancer and degenerative diseases; antioxidant molecules present in different mollusks prevent cell damage from oxidation reaction [6]. Compounds isolated from molluscs were also used in the treatment of rheumatoid arthritis and osteoarthritis [7]. Mollus can extracts also exhibited antiviral and antibacterial activity against fish pathogenic bacteria, and the extract also may be applied in aquaculture [8].

In general very fewer studies have been done on the antioxidant activity of gastropods while some of them have been estimated as whole body homogenate which possesses a range of antimicrobial compounds. The gastropod egg and tissue consists of active secondary metabolites that have more antimicrobial activities [9].The antioxidant compounds are essential to trap free radicals and reduce the risk of cancer and heart disorders [10-12]. The large number of works has been done in other organisms, but only a few researches were done on mollusks [13]. The resources of gastropods are not well utilized in the Persian Gulf region. The objective of the present study was to investigate the antioxidant activity of the body tissues of Nerita Longii sp. gastropod around the Persian Gulf region, Bushehr coasts of Iran.

**Materials and Methods**

**Chemicals**

2,2-DiPhenyl-2-Picrylhydrazyl (DPPH), 2,4,6-TriPyridyl-s-TriaZine (TPTZ) and ferulic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Folin-Ciocalteu reagent, ferric chloride (FeCl₃.6H₂O), and methanol and ethyl acetate were from Merck (Darmstadt, Germany)

**Sample collection and extraction**

Live specimens of Nerita longii gastropod was collected from the Bushehr coast of Persian Gulf of Iran and directly brought to the laboratory and identified by scientists from Marine Science and Technology of Khoramshahr, Khuzestan, Iran. The whole body muscle was taken out from the shell, used for extraction by methanol and stored for 4 days.

**Preparation of extracts**

The shells were separated in lab and the whole muscle tissue weighing 80 g was macerated with ethyl acetate and methanol for three days. The supernatant solvent was concentrated using vacuum evaporator (35-55oC) under reduced pressure and the resultant extract was dry with freeze dryer and kept in clean glass vials at -80 oC until use.

**DPPH radical scavenging assay**

The free radical scavenging activity of methanol and ethyl acetate extract of N. Longi was assessed using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical [14]. Due to the poor solubility of methanol and ethyl acetate extract in aqueous medium it was dissolved in 1% DMSO and later on made up to desired concentrations with distilled water. The extract in different concentrations were added to 0.1 ml of 1 M Tris-HCl (pH 7.9), and then mixed with 0.6 ml of DPPH (100 μM) in methanol for 20 min at room temperature under protection from light. Absorbance of the mixture was read on a UV-VIS spectrophotometer (Hitachi, U-2001) at 517nm against the blank. Butyrate Hydroxyl Toluene (BHT) was used as the standard antioxidant and the percentage inhibition of test and the standard were calculated as % Inhibition = ((A

Where A

**Ferric Ion Reducing Antioxidant Power Assay**

The FRAP assay also takes advantage of electron-transfer reactions. Herein a ferric salt, Fe (III) (TPTZ) Cl₂Cl₂, (TPTZ) 2, 4, 6-tripyridyls-triazine), is used as an oxidant [15-17]. The FRAP assay involves the following procedures: The oxidant in the FRAP assay is prepared by mixing TPTZ (2.5 ml, 10 mM in 40 mM HCl), 25 ml of acetate buffer, and 2.5 ml of FeCl₃.6H₂O (20 mM). The conglomerate is referred to as “FRAP reagent”. The final solution has Fe (III) of 1.67 mM and TPTZ of 0.83 mM. Therefore, the

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The FCR is typically made by first boiling (for 10 h) the mixture of sodium tungstate (Na₂WO₄, 2H₂O, and 100 g), sodium molybdate (Na₂MoO₄, 2H₂O, 25 g), concentrated hydrochloric acid (100 mL), 85% phosphoric acid (50 mL), and water (700 mL). After boiling, lithium sulfate (Li₂SO₄, 4H₂O, 150 g) is added to the mixture to give intense yellow solutions the FC reagent. Contamination of reductants leads to a green color, and the addition of oxidants such as bromine can restore the desired yellow color. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotungstates-molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly (PMoW₁₁O₄₀)⁴⁻. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI): Mo (VI) + e → Mo (V).

Obviously, the FC reagent is nonspecific to phenolic compounds as it can be reduced by many non-phenolic compounds [e.g., vitamin C, Cu (I), etc.]. Phenolic compounds react with FCR only under basic conditions (adjusted by a sodium carbonate solution to pH-10). Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing FCR. This supports the notion that the reaction occurs through electron transfer mechanism. The blue compounds formed between phenolate and FCR are independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complexes formed between the metal center and the phenolic compounds.

Despite the undefined chemical nature of FCR, the total phenols assay by FCR is convenient, simple, and reproducible.

As a result, a large body of data has been accumulated, and it has become a routine assay in studying phenolic antioxidants.

### Statistical analysis

Statistical analysis was done using SPSS version 14. IC₅₀ values respectively for DPPH were found out by Linear Regression Probit analysis.

### Results and Discussion

#### DPPH radical scavenging activity

DPPH radical scavenging property of N. Longi is shown in Figure 1. The methanolic extract showed a dose dependent pattern in DPPH radical scavenging in the range of concentrations tested (0.312-20 mg/ml). The IC₅₀ value of the methanolic extract was found to be 8.07 mg/ml. The higher concentration of methanol extract showed a percentage inhibition of 82.86 %.
As showed in Figure 2, the EtOAc extract have a dose dependent pattern in DPPH radical scavenging in the range of their concentrations (0.312-20 mg/ml). The IC50 value of the EtOAc extract was found to be 10.13 mg/ml. The higher concentration of EtOAc extract showed a percentage inhibition of 76.04%.

As showed in Figure 3, the resultant IC50 for methanol and ethyl acetate were 8.07 and 10.13 mg/ml, respectively.

As depicted in Figure 4, the methanolic extract showed a dose dependent pattern in FRAP scavenging in the range of their concentrations (0.312-10 mg/ml). The EC1 value of the methanolic extract was found to be 3.687 mg/ml. The higher Absorbance of methanolic extract showed at 1.78.

As depicted in Figure 5, the EtOAc extract showed a dose dependent pattern in DPPH radical scavenging in the range of concentrations tested (0.312 -20 mg/ml). The EC1 value of the EtOAc extract was found to be 5.35 mg/ml. The higher concentration of EtOAc extract showed the absorbance at 1.822.

Ferric reducing activity based on FRAP assay

The ethanolic extract of Sulawesian beans exhibited the highest antioxidant potential among the extracts (Figur 6), based on the FRAP assay. FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripipryldltriazine (Fe3+-TPTZ) complex and producing a colored ferrous tripipryldltriazine (Fe2+-TPTZ) [15]. Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom [19,20]. According to [15], the reduction of Fe3+-TPTZ complex to blue colored of Fe2+-TPTZ occurs at low pH.

Sulawesi an and Malaysian beans are well known to have a low cotyledon pH, while Ghanaian has medium pH, and Ivory Coast has high pH [21]. The highest antioxidant potential of Malaysian beans could be due to the highly acidic (low pH) nature of the bean cotyledon, which many influence the pH of the assay medium.

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As shown in Figure 6, the resultant EC1 for methanol and ethyl acetate were 3.687, 5.35 mg/ml respectively.

**Figure 6:** The comparison of EC1 in methanolic and EtOAc extract of FRAP Value

**Total phenolic content**

Several studies showed a correlation between antioxidant activity and phenolic content [22-24]. As shown in Figure 7, methanolic extract beans had the highest phenolic content. The highest Absorbance in methanolic extract is 1.626.

![Absorbance vs Concentration](image)

**Figure 7:** Effect of methanolic extract on Absorbance of *N. Longi* in FOLIN

As shown in Figure 8 the highest Absorbance in EtOAc is 1.592.

![Absorbance vs Concentration](image)

**Figure 8:** Effect of EtOAc extract on Absorbance of *N. Longi* in FOLIN

**Conclusion**

Muscle tissue of Persian Gulf gastropod, Nerita Longi, is an important source of natural proteins and peptides. So high antioxidant activity was given from FRAP, DPPH and FOLIN methods of methanolic extract can be related to the ability of methanol to extract corresponding peptides and proteins.

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**References**

1. Swathi V, Pratap P, Monila N, Harshini N, RajaSekhar J, et al. (2012) The Oceans- Unlocking the Treasured Drugs. International Journal of Pharmaceutical and Chemical Sciences 9: 2277-5005.
2. Grkovic T, Appleton D, Copp B (2005) Chemistry and Chemical Ecology of some of the Common Opisthobranch Molluscs Found on the Shores of NE New Zealand. Chemistry in New Zealand 12-15.
3. Jha R, Zi-rong x (2004) Biomedical Compounds from Marine organisms. Marine Drugs 2: 123-146.
4. Harvey A (2008) Natural products in drug discovery. Drugs Discovery Today 13: 894-901.
5. Ngo D, Vo T, Ngo D, Wijesekara I, Kim S (2012) Biological activities and potential health benefits of bioactive peptides derived from marine organisms. International Journal of Biological Macromolecules 51: 378-383.
6. Nagash YS, Nazeer RA, Sampath Kumar NS (2010) In vitro antioxidant activity of solvent extracts of mollusks (Loligoduvacellia and Donaxstrateus) from India. World J. Fish. Mar. Sci 2: 240-245.
7. Chellaram C, Edward JKP (2009) Antinociceptive assets of coral associated Gastropod, Drupamargariticola. Int. J. Pharmacol 5: 236-239.
8. Defer D, Bourgnon N, Fleury Y (2009) Screening for antibacterial and antiviral activities in three bivalve and two gastropod marine molluscs. Aquaculture 293: 1-7.
9. Kaviarasani T, Sankar RS, Yogamoorthi A (2011) Studies in ultra-structure of egg capsule wall of snails using scanning electron microscope. J Coast Environ 2: 143-50.
10. Kamala K, Karuppiah V, Sivakumar K (2013) Comparative evaluation of in vitro antioxidant potent of the marine actinobacteria from Gulf of Mannar biosphere reserve. Int J Pharm Biosci 4: 207-216.
11. Sivaperumal P, Kamala K, Natarajan E, Dilipan E (2013) Antimicrobial peptide from crab haemolymph of Ocypodamacroceras (Milne Edwards 1852) with reference to antioxidant: a case study. Int J Pharm Sci 5: 719-727.
12. Sivaperumal P, Kamala K, Rajaram R (2015) Bioactive DOPA melanin isolated and characterized from a marine actinobacterium Streptomyces sp. MVCS6 from Versova coast. Nat Prod Res 29: 2117-2121.
13. Mariappan R, Balasubramanian U (2012) Antibacterial activity of bivalves Meretrix castaand Tridacna maxima from south east coast of India. Int J Pharm Sci Rev Res 13: 137-140.

14. Jayaprakasha GK, Jaganmohan Rao L, Sakariah KK (2004) Antioxidant activities of flavidin in different in vitro model systems. Bioorg. Med. Chem 12: 5141-5146.

15. Benzie IF, Strain JJ (1996) the ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP Assay. Analytical Biochemistry 239: 70-76.

16. Benzie IF, Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods of Enzymology 299: 15-27.

17. Benzie IF, Stezo YT (1999) Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 47: 633-636.

18. Woraratphoku J, Intarapichet KO, Indrapichate A (2007) Phenolic compounds and antioxidative properties of selected Wines from the northeast of Thailand. Food Chemistry 104: 1485-1490.

19. Gordon MH (1990) the mechanism of antioxidant action in-vitro. In B. J. F. Hudson (Ed.), Food antioxidants 1-18.

20. Duh PD, Du PC, Yen GC (1999) Action of methanolic extract of mung hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. Food and Chemical Toxicology 37:1055-1061.

21. Misnawi Jinap S, Nazamid S, Jamilah B (2002) Activation of remaining key enzymes in dried under-fermented cocoa beans and its effect on aroma precursor formation. Food Chemistry 78: 407-417.

22. Nagai T, Reiji I, Hachiro I, Nobutaka S (2003) Preparation and antioxidant properties of water extract of propolis. Food Chemistry 80: 29-33.

23. Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry 46: 4113-4117.

24. Yang JH, Lin HC, Mau JL (2002) Antioxidant properties of several commercial mushrooms. Food Chemistry 77: 229-235.