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Antidiabetic and Antioxidant Properties of Sea Urchin *Echinometra mathaei* from the Persian Gulf

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Abstract

Background: The aim of the present study was to evaluate the inhibition of α-glucosidase and antioxidant properties of different tissues of sea urchin *Echinometra mathaei*.

Methods: α-glucosidase inhibition was determined using p-Nitrophenyl-a-D-glucopyranoside as a substrate, and the antioxidant properties were evaluated by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS),1,1-diphenyl-2-picrylhydrazyl (DPPH), and nitric oxide radicals scavenging. Also, antioxidant potential was evaluated by the ferric reducing antioxidant power (FRAP) method.

Results: Among the studied tissues, the highest α-glucosidase inhibition was revealed by the ethyl acetate extract of the shell and Aristotle's lantern (IC_{50} = 3.7 and 4 mg/mL, respectively). Shell had the highest ABTS (IC_{50} = 183) and DPPH (IC_{50} = 208 µg/mL) radicals scavenging, respectively. And, gonad had the highest antioxidant potential by the FRAP method (1140 µg ASA/mg) and NO radical scavenging (70.68%), respectively.

Conclusion: Antidiabetic potentials of the ethyl acetate extracts of sea urchin tissues suggest that these extracts can be used as antidiabetic drugs.

Introduction

Epidemiological studies have indicated that daily intake of antioxidant substances is inversely correlated with mortality from coronary heart diseases. Although most antioxidants, such as vitamin E, vitamin C, polyphenols, β-carotene, α-carotene, lutein, and zeaxanthin, are from familiar plant sources, there are sea sources (animals and plants) that are generally less well-known (1).

Electron acceptors, such as molecular oxygen, react easily with free radicals. Then, these electron acceptors became radicals, which are known as reactive oxygen species (ROS). The ROS include superoxide anions ($O^{\bullet-}_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$) (2). There are considerable evidences indicating that free radicals induce oxidative damages to biomolecules such as lipids, proteins, and nucleic acids, which eventually cause atherosclerosis, ageing, arthritis, cancer, diabetes mellitus, inflammation, acquired immunedeficiency syndrome (AIDS), liver, Alzheimer's and Parkinson's diseases in humans (3,4).

Diabetes mellitus is a chronic metabolic disorder, which is identified by high levels of blood glucose. One therapeutic approach for treatment of diabetes is retardation of the absorption of glucose by inhibition of the enzymes, such as α-glucosidase (5,6). α-glucosidase (α-d-glucoside glucohydrolase) is an exo-type carbohydrate widespread in microorganisms, plants, and animal tissues (7), which causes the liberation of α-glucose from the non-reducing end of the substrate. Retardation of this enzyme (with declining absorbance of the reaction) decreases the elevation of blood sugar after a carbohydrate meal (8). This enzyme is a membrane-bound enzyme present in the epithelium of the small intestine, to enable the absorption of glucose by small intestine through hydrolyzing oligosaccharides into absorbable monosaccharides (9).

Nowadays, the occurrence of type 2 diabetes mellitus is rising worldwide. Type 2 diabetes is caused by the interaction between a genetic predisposition and behavioral and environmental risk factors (10). Although the genetic basis of type 2 diabetes has not been recognized yet, there are strong evidences indicating that risk factors such as obesity and physical inactivity are the most important non-genetic determinants of this disease (11,12). Impaired glucose tolerance (IGT) is an intermediate link between normal glucose tolerance and overt diabetes (13,14), which can be recognized by an oral glucose tolerance test (OGTT). Individuals with IGT have an increased risk of type 2 diabetes (15), thus, they form a major target group for preventing diabetes (12,16). The Finnish Diabetes Prevention Study was conducted to evaluate the feasibility and the effects of changes in lifestyle planned to prevent or delay the onset of type 2 diabetes in individuals with IGT.

Sea urchins are marine invertebrates which belongs to the phylum Echinodermata and live on the floor of ocean. Up to now, more than 800 species of sea urchins have been identified and their gonads are used as high-priced food in Japan. However, after removal of the edible gonads, the remaining shell with spines, which comprise 40.7-47.9% of the sea urchins weights, are generally discarded as waste (17,18). Shells with three-dimensional meshwork architectures are porous with single crystal of calcite. Calcium carbonate, the main component of the shell (80-96% of shell weight), can be consumed as a calcium source. Also, the sea urchin shells are composed of polyhydroxylated naphthaquinones pigments, which have antioxidant properties (19). In crude calcium carbonate form, shells have low solubility, without acid treatment previously, but they can be consumed as a calcium source to obtain other calcium salts like lactate. Calcium lactate can be consumed as a drug and human nutritional supplement because of its high solubility and bioavailability (20).

The aim of the present study was to evaluate the antioxidant properties of ethyl acetate and methanol extracts of purple sea urchin *E. mathaei* species by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and nitric oxide (NO) radicals scavenging activities, and the ferric reducing antioxidant power (FRAP). Antidiabetic properties of the samples were evaluated by α- glucosidase inhibition of three tissues including gonad, shell, and Aristotle'slantern of purple sea urchin *E. mathaei* species. Two different solvents including methanol (ME) and ethyl acetate (EA) were used for extraction, while examining for the most efficient sea urchin extraction method.

Materials and Methods

Chemistry

Butylated hydroxyl toluene (BHT), α- glucosidase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich. All other reagents were obtained from Merck Chemical company.

Collection and preparation of sea urchin

The purple sea urchin *E. mathaei* species were collected from Olive Park (Qeshm Island, Hormozgan province, Iran) located in the intertidal zone of the Gulf coastline during low tide, in December 2012. Then, the samples were immediately transported to the laboratory and rinsed with filtered seawater. After dissection phase, the shells, gonads, and *Aristotle's* lantern (mouth parts) were collected and freeze-dried (model FD-10V) in darkness. The dried samples were ground thoroughly and used for further experiments.

Preparation of tissues extracts of sea urchin

By increasing the solvents polarity, dried tissues of *E. mathaei* were immersed in ethyl acetate and methanol (1:6 w/v). Initially, for each sample, 200 g of tissues powder was blended with ethyl acetate and hold at room temperate for 48 hours. The mixture was filtered using a Whatman filter paper No. 2. The obtained ethyl acetate extract was concentrated using a rotary evaporator at 40°C, weighted and stored in 5 mL glass vials. Similarly, the tissues' samples remaining on the filter were immersed in methanol, incubated at room temperate for 48 hours under shaking, and were filtered again. The obtained extracts (totally 30 ml) were reserved at 4°C for later experiments.

α- glucosidase inhibition activity

α- glucosidase inhibition of sea urchin samples was examined using the method described by Mccue et al. (21). In this method, the mixture of 5 µL of α-glucosidase (25 unit/mL, 1 unit equals to 10 mg/ml, the enzyme final concentration was equal to 1.25 mg/mL) and 125 µL of phosphate buffer (pH 6.9, 0.1 M) and p-Nitrophenyl- α-D-glucopyranoside (11 mM) in phosphate buffer (pH 6.9) was used as the substrate solution.

Different concentrations of test extracts (20 µL) were mixed with enzyme solution in microplate wells, and then, put at 37°C for 15 min. The reaction was initiated by adding 20 µL of the substrate solution, and then, incubated for another 15 min. By adding 80 µL of 0.2 M sodium carbonate solution, the reaction was stopped.

The absorbance of the wells was measured by a microplate reader at 405 nm, while the reaction system without sample extracts was used as the control. The system without α-glucosidase was used as the blank, and acarbose was used as the positive control. All determinations were performed in triplicate (21). The rates of enzyme inhibition by the samples were calculated as follows:

Inhibition (%) = [(control absorption - sample absorption) / control absorption] × 100

Antioxidant activity

ABTS+ radical scavenging activity

ABTS radical scavenging assay is a method for determining the antioxidant activity by hydrogen donation and chain breaking of oxidants. The hydrogen, donated by antioxidant components of the sea urchin extracts, reacts with ABTS radicals provided in the assay, and the antioxidant

activities can be determined by the absorption of the samples at 734 nm (22).

The ABTS radical scavenging of sea urchin extracts was determined using the method described by Arnao et al. (22). The sea urchin extracts were tested at concentrations of 200, 400, 800, 1600, and 3200 µg/mL except ethyl acetate extract of shell, which was tested at concentrations of 25, 50, 100, 200, and 400 µg/mL. The stock solution was prepared by mixing 7.4 mM ABTS and 2.6 mM potassium persulfate in equal quantities, then, $ABTS^+$ solution was put in darkness for 16 hours at room temperature. The prepared solution was diluted by mixing 1 mL $ABTS^+$ solution and methanol 96% to obtain an absorbance of 1.00 at 734 nm. For the assay, sea urchin extract (200 µL) was allowed to react with 1 mL of fresh $ABTS^+$ solution for 6 min in darkness, and the absorbance was determined at 734 nm. The percentage of inhibition was calculated using the following formula:

Scavenging activity (%) = $[1-(A_a) / (A_c)] \times 100$

Where A_a and A_c are the absorbance of the test (or standard) and the absorbance of the control (without extract), respectively. BHT was used as a standard, and the antioxidant capacities of the samples and the standard were shown as IC_{50} (mg/mL) value, representing the concentration of the samples scavenged 50% of $ABTS^+$ radicals (22).

Ferric reducing antioxidant power (FRAP) activity

Another method for evaluating antioxidant activities is ferric reducing antioxidant power (FRAP) assay, which measures the reducing capacities of antioxidants or the existence of reduced compounds in the samples. These compounds reduce the Fe^{3+}/ferric cyanide complex to its Fe^{2+} form (23).

The FRAP power of sea urchin extracts were measured using the method described by Benzie and Strain (23). The sea urchin extracts were tested at concentrations of 50, 100, 200, 400, 800, and 1600 µg/mL. the FRAP solution was prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (2, 4, 6 tripyridyl-S-triazine solution in 40 mM HCl), and 20 mM $FeCl_3$ in the ratio of 10:1:1, v/v/v, respectively. The mixture was warmed at 37°C prior to use. For the assay, 150 µL of sea urchin extract was mixed with 2850 µL of fresh FRAP solution, and the solution was put at room temperature for 30 min. The absorbance of the reaction mixture was determined at 595 nm.

Ascorbic acid was used as a standard, and the results of the FRAP method (reducing activity) were expressed as microgram ascorbic acid equivalent per milligram (µg ASA/mg) dried extracts at a concentration of 1 mg/mL. The antioxidant capacities of the samples and the standard were shown as IC_{50} (mg/mL) value, indicating the concentration required for 50% reduction of Fe^{3+}/ferricyanide complex to its Fe^{2+}/ferrocyanide complex (23).

DPPH radical scavenging activity

The free radical scavenging activity of the sea urchin extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Duan et al. (24). The sea urchin extracts were tested at concentrations of 50, 100, 200, 400, 800, 1600, and 3200 mg/mL, but methanol and ethyl acetate extracts of shell were tested at concentrations of 25, 50, 100, 200, 40, and 800 mg/mL. For the assay, the sea urchin extract (100 µL) was permitted to react with 0.1 mL of DPPH solution (0.5 mM). After mixing, the mixture was put in darkness for 30 min, and the absorbance was determined at 490 nm (24).

Control contains methanol instead of the pigment extract and blank contains methanol instead of DPPH solution. The inhibition of DPPH radical by the extracts was calculated according to the following formula:

DPPH scavenging activity (%) = [1- (A_s –A_0 /A] × 100

Where A_s is the absorbance of the reaction mixture, A_0 is the absorbance of the blank, and *A* is the absorbance of the control. BHT was used as a positive control. IC_{50} (mg/mL) value, representing the concentration of the samples required for 50% scavenging of DPPH, was calculated (24).

Nitric oxide radical scavenging activity

Nitric oxide (NO) radical scavenging was measured according to the method which was previously reported (25,26). The sea urchin extracts were tested at a concentration of 0.2 mg/mL. For the assay, 50 µL of 10 mM sodium nitroprusside in phosphate buffer (0.2 M, pH 7.4) was mixed with 50 µL of each sample and put at 27°C for 150 min. Afterwards, 100 µL of Griess reagent was added to each sample, and then, incubated for another 5 min at room temperature. Finally, the absorbance of the mixture was measured at 542 nm (25). Nitric oxide radical scavenging was determined as the following:

Nitric oxide radical scavenging (%) = [($A_{control}$ – A_{sample}) / $A_{control}$] × 100

Control did not contain any sample.

Statistical analyses

All tests were performed in triplicate, and the results of the experiments were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's test were used to compare the possible differences among the means. Statistical significant level was considered at $P<0.05$. All statistical analyses were performed using SPSS version 21 (SPSS Inc., USA).

Results

In vitro α–glucosidase inhibition

Different extracts of gonad, shell, and Aristotle's lantern of sea urchin were tested to determine the α-glucosidase inhibitory property using colorimetric method. The highest α-glucosidase inhibition was presented in 40 mg/mL of gonad ethyl acetate extract (Table 1). Only ethyl acetate extracts could inhibit α-glucosidase, and methanol extracts did not show any α-glucosidase inhibition (Table 1).

Table 1. Percentage of α-glucosidase inhibition by various concentrations of methanol and ethyl acetate extracts of different tissues of sea urchin of E. *mathaei*

Species	Inhibition of Methanol Extracts (%)						Inhibition of Ethyl Acetate Extracts (%)					
	2.5	5	10	20	40	mg/mL	2.5	5	10	20	40	mg/mL
Gonad	0	0	0	0	0		36.08±0.12	48.09±0.43	50.02±0.75	66.24±0.64	81.31±0.85	
Shell	0	0	0	0	0		46.27±0.24	51.44±0.57	59.78±0.91	67.05±0.82	78.18±0.42	
Aristotle's lantern	0	0	0	0	0		36.04±0.48	52.08±0.81	64.06±0.37	79.12±0.47	86.04±0.58	

In this study, the highest α-glucosidase inhibition was shown by the ethyl acetate extract of the shell (IC_{50}= 3.7 ± 0.57 mg/mL, Figure 1) and Aristotle's lantern (IC_{50}= 4 ± 0.1 mg/mL, Figure 1) with α-glucosidase inhibition of 46-78% and 36-86%, respectively, which are higher than the inhibition by acarbose (IC_{50} = 24.2 ± 0.38 mg/mL, Figure 1). The lowest inhibition of α-glucosidase (IC_{50}= 9.8 ± 0.49 mg/mL, Figure 1) was observed by the ethyl acetate extract of *E. mathaei*.

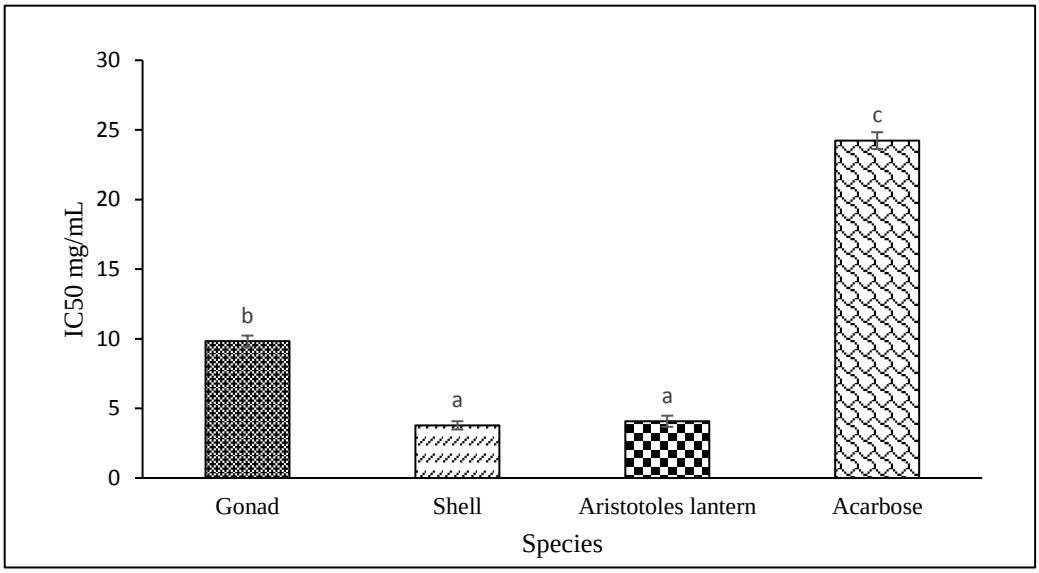

Figure 1. IC50 values of α-glucosidase inhibition of sea urchin ethyl acetate extracts and acarbose. Significant differences between samples are indicated by different letters as determined by Duncan's post-hoc multiple comparison (P<0.05).

ABTS+ radical scavenging activity

ABTS radical scavenging of sea urchin extracts depended on the tested concentrations (200 to 3200 µg/mL). For ABTS radical scavenging of sea urchin ethyl acetate extract, these concentrations were from 25 to 400 µg/mL. The IC_{50} values of the ABTS radical scavenging of the samples depended on the organs and solvents (P<0.05, Figure 2). The methanol and ethyl acetate extracts of shell ($IC5_0$= 183 ± 0.1 and 182 ± 0.3 µg/mL) and methanol extract of gonad (IC_{50} = 2426 ± 0.8 µg/mL) showed the highest and lowest scavenging activities, respectively. The methanol (IC_{50} = 183 ± 0.045µg/mL) and ethyl acetate (IC_{50} = 182 ± 0.06µg/mL) extracts of *E. mathaei* shell, respectively, exhibited higher ABTS radical scavenging than BHT (IC_{50} = 750 ± 0.1µg/mL, P<0.05, Figure 2).

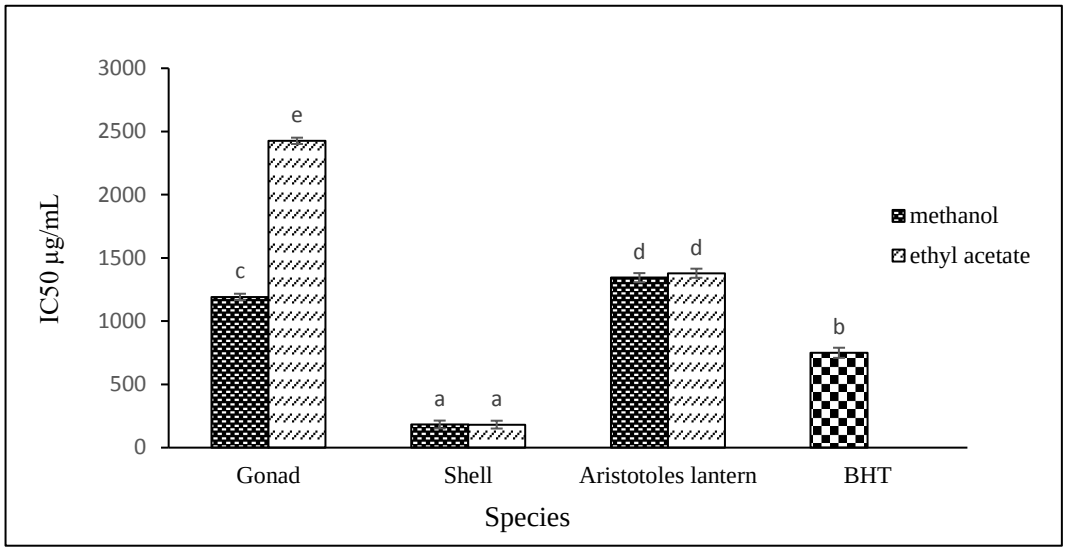

Figure 2. IC50 values of ABTS radical scavenging of sea urchin E. *mathaei* extracts in comparison to BHT as a standard. Significant differences between samples are indicated by Duncan's post-hoc multiple comparison (P<0.05).

FRAP reducing activity

The reduction of Fe^{3+} to its Fe^{2+} form was studied using various sea urchin extracts. All the samples showed a reduction activity at concentrations of 50 to 1600 µg/mL (data not shown). The highest reduction activities were observed in the methanol extract of gonad of *E. mathaei* (1140 ± 0.2µg ASA/mg) and the lowest reduction activities were observed in methanol and ethyl acetate extracts of *E. mathaei* shell (74 ± 0.9 and 68.3 ± 0.3µg ASA/mg), respectively. Statistical analyses showed significant differences in reduction activities of the samples (1 mg/mL) based on the solvents used for extraction (P<0.05, Figure 3).

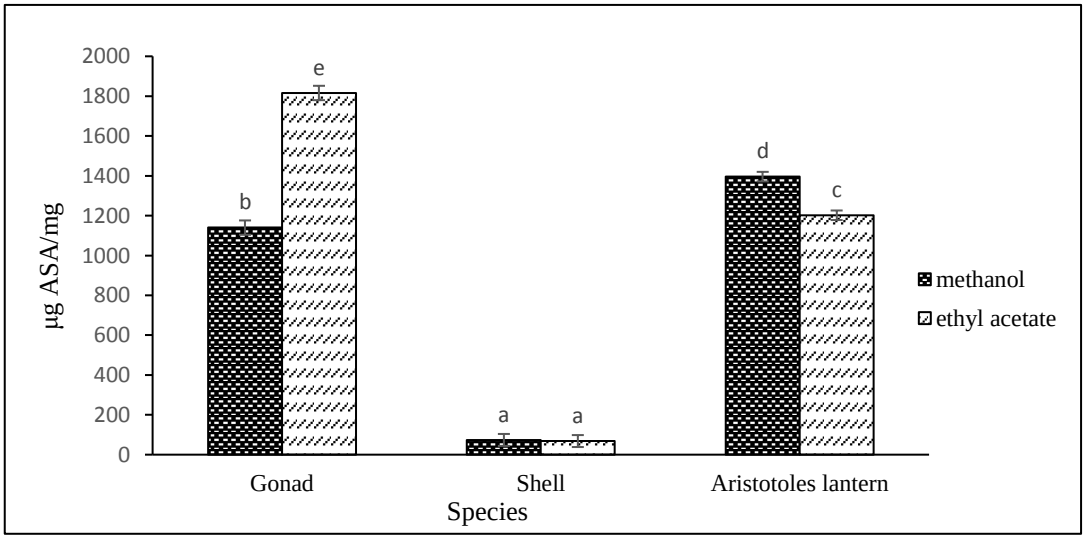

Figure 3. Reduction activities (µg ASA/mg) of sea urchin E. *mathaei* extracts at a concentration of 1 mg/mL by the FRAP method. Significant differences between samples are indicated by Duncan's post-hoc multiple comparison (P<0.05).

DPPH radical scavenging activity (DPPH-RSA)

The DPPH radical scavenging by different concentrations of sea urchin extracts is shown in Figure 3. The reactions of the samples with DPPH led to a change in color from purple to yellow color. DPPH-RSA of sea urchin extracts was performed depending on the tested concentrations of the sea urchin extracts (50 to 3200 µg/mL). These concentrations for methanol and ethyl acetate extracts of shell were 25 to 800 µg/mL.

The methanol extracts of shell (IC_{50} = 208 ± 0.2 µg/mL) and Aristotle's lantern (IC_{50} = 2426 ± 0.1 µg/mL) showed the highest and lowest scavenging activities, respectively. BHT as an antioxidant (IC_{50} = 108 ± 0.13µg/mL), exhibited higher DPPH-RSA than all of the sea urchin *E. mathaei*extracts. The IC_{50} values of the DPPH-RSA of the extracts of sea urchin *E. mathaei* showed significant differences between species ($P<0.05$, Figure 4).

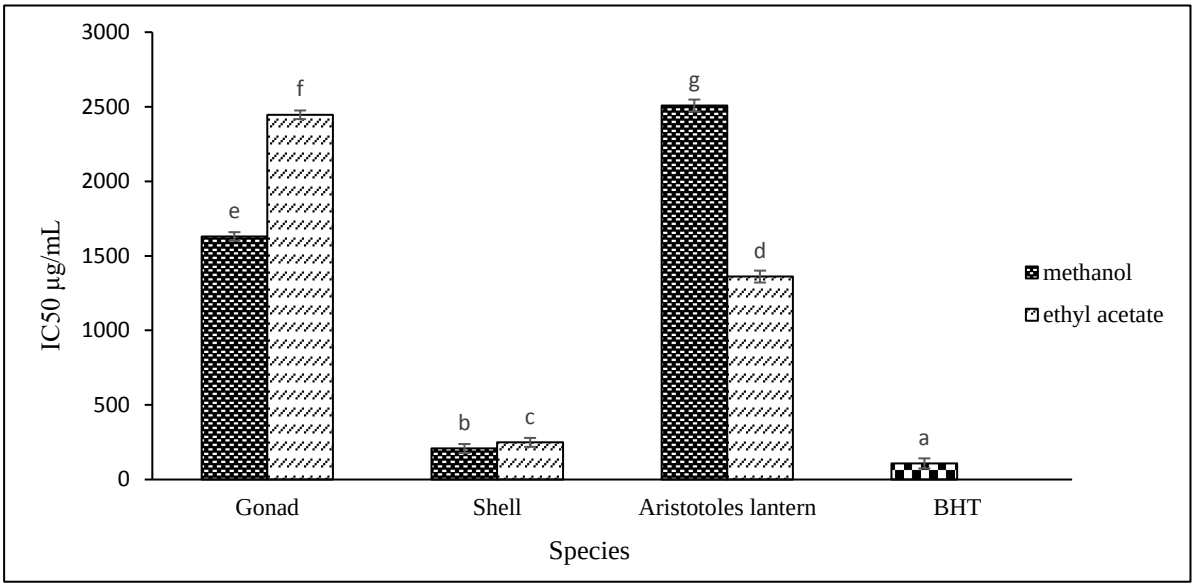

Figure 4. IC50 values of DPPH radical scavenging of sea urchin E. *mathaei* extracts in comparison to BHT as a standard. Significant differences between samples are indicated by Duncan's post-hoc multiple comparison ($P<0.05$).

Nitric oxide radical scavenging activity

The highest nitric oxide scavenging was observed in methanol extract of gonad (70.68 ± 0.21%), which was higher than BHT (42%). The lowest nitric oxide scavenging was observed in ethyl acetate extract of Aristotle's lantern of *E. mathaei* (30.52 ± 0.28%). Statistical analyses showed significant differences in nitric oxide scavenging between different samples at a concentration of 200 µg/mL ($P<0.05$, Figure 5).

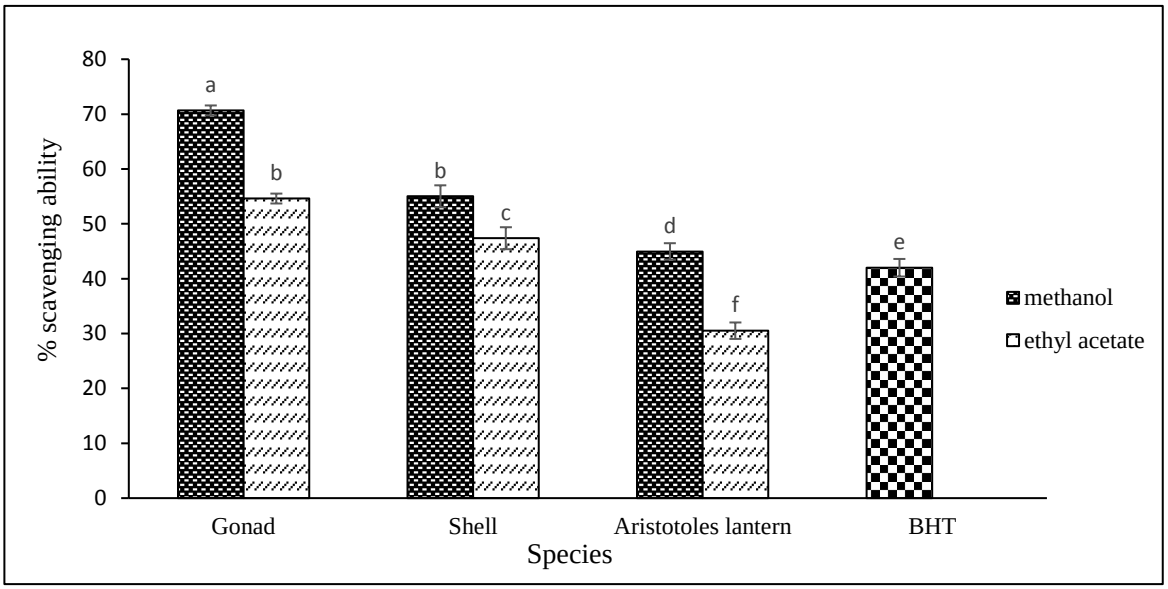

Figure 5. Nitric oxide radical scavenging of sea urchin E. *mathaei* extracts at a concentration of 200 µg/mL. Significant differences between samples are indicated by Duncan's post-hoc multiple comparison (P<0.05).

Discussion

The present study investigated the antioxidant properties and α-glucosidase inhibition of methanol and ethyl acetate extracts of gonad, shell, and Aristotle's lantern of sea urchin.

Deleterious free radicals-mediated oxidation occurs in aerobic organisms (27). As a general definition, an antioxidant is any substance which is capable of preventing oxidation. However, as the use of synthetic antioxidants is restricted, the interest in finding natural sources for therapeutic approaches has been increased (28,29).

In this study, different tissues extracted with different solvents showed a range of antioxidant capacities.

Antioxidant properties of different samples were determined by ABTS, DPPH, and NO radicals scavenging, and antioxidant properties were also determined by the FRAP method. Methanol and ethyl acetate extracts of shell revealed the highest ABTS radical scavenging (IC_{50} = 183 ± 0.1 µg/mL, Figure 2). Ethyl acetate extract of gonad showed the lowest ABTS radical scavenging (IC_{50} = 2426 ± 0.8 µg/mL, Figure 2). Using the FRAP method, the highest reduction capacities were observed in methanol and ethyl acetate extracts of *E. mathaei* shell (74 ± 0.9 and 63.8 ± 0.3 µg ASA/mg), respectively (Figure 3). The methanol extracts of shell (IC_{50}= 208 ± 0.2 µg/mL) and Aristotle's Lantern (IC_{50}= 2426 ± 0.1 µg/mL) revealed the highest and lowest DPPH radical scavenging, respectively (Figure 4).

Based on the other studies, the highest DPPH radical scavenging of sea urchin was found in *Stomopneustes variolaris* (IC_{50} = 57.81 ± 0.07 µg/mL) (36). The highest and lowest NO radical scavenging were observed in methanol extract of gonad (70.68 ± 0.21%) and ethyl acetate of Aristotle's lantern (30.52 ± 0.28%), respectively (Figure 5).

The control of postprandial hyperglycemia is one of the approaches to treat diabetes (30, 31). It can be performed by decreasing the absorption of glucose through the inhibition of α-amylase and α-glucosidase, as carbohydrate hydrolyzing enzymes in the digestive system (32). Approximately, all of the

ethyl acetate extracts revealed inhibitory effects on α-glucosidase.

Among echinoids, the activity of α-glucosidase was different (33). The ability of α-glucosidase to cleave linkages in carbohydrates, would seem to be of nutritional value to echinoids (34).

The low levels of α-galactosidase activity are consistent with the lack of α-galactans in natural foods. Disaccharides such as melibiose and raffinose are found at low levels and are distributed randomly through the territory of the plant. An ability to digest these carbohydrates would be of little value to echinoids.

Ethyl acetate extracts of Aristotle's lantern and gonad of sea urchin revealed the highest inhibition of α-glucosidase (86.04 ± 0.58% and 81.3 ± 0.85%) at 40 mg/ml, respectively (Table 1). Methanol extracts of the samples could not inhibit α-glucosidase (Table 1).

Conclusion

The results of this study could suggest a basis for future investigation on the isolation of antidiabetic and antioxidant compounds from the sea urchin extracts.

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Conflict of interests

Financial support was given by Shiraz University of Medical Sciences, Shiraz, Iran. The authors declare that they have no conflict of interests.

References

1. Moon JK, Shibamoto T. Antioxidant assays for plant and food components. J Agr Food Chem 2009; 57(5):1655-66.
2. Grisham M. Chemistry and cytotoxicily of reactive oxygen metabolites. Dig Dis Sci. 1988; 33:150-5.
3. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, orconsequence?Lancet 1994;344(8924):721-4.
4. Maxwell SR. Anti-oxidant therapy: does it have a role in the treatment of human disease? Expert Opin Investig Drugs 1997;6(3):211-36.
5. Holman RR, Cull CA, Turner RC. A randomized double-blind trial of acarbose in type 2 diabetes shows improved glycemic control over 3 years (UK Prospective Diabetes Study 44). Diabetes Care 1999;22(6):960-4.
6. Kim KY, Nam KA, Kurihara H, Kim SM. Potent α-glucosidase inhibitors purified from the red alga Grateloupia elliptica. Phytochemistry 2008;69(16):2820-5.
7. Kimura A, Lee JH, Lee IS, Lee HS, Park KH, Chiba S, et al. Two potent competitive inhibitors discriminating α-glucosidase family I from family II. Carbohydr Res 2004;339(6):1035-40.
8. Ciotta L, Calogero AE, Farina M, De Leo V, La Marca A, Cianci A. Clinical, endocrine and metabolic effects of acarbose, an α-glucosidase inhibitor, in PCOS patients with increased insulin response and normal glucose tolerance. Hum Reprod 2001;16(10):2066-72.
9. Baig I. Phytochemical studies on Ferula mongolica and other mongolian medicinal plants

[dissertation].Karachi: University of Karachi; 2002.

10. Neel JV. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? Am J Hum Genet 1962;14(4):353-62.
11. Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, et al. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet 1991;338(8770):774-8.
12. Tuomilehto J, Wolf E. Primary prevention of diabetes mellitus. Diabetes Care 1987;10(2):238-48.
13. Harris MI. Impaired glucose tolerance in the US population. Diabetes Care 1989;12(7):464-74.
14. Lüder W. Prevention of diabetes mellitus. Report of a WHO study group. WHO technical report series 844. 100 Seiten, 8 Abb., 6 Tab. World Health Organisation. Geneva 1994, Preis: 15,–Sw. fr.; 13, 50 US$. Mol Nutr Food Res 1995;39(3):252-3.
15. Borch-Johnsen K, Neil A, Balkau B, Larsen S,Borch-Johnsen K,Nissinen A, et al. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. Lancet 1999;354:617.
16. Hamman RF. Genetic and environmental determinants of non-insulin-dependent diabetes mellitus (NIDDM). Diabetes Metab Rev 1992;8(4):287-338.
17. Amarowicz R, Synowiecki J, Shahidi F. Chemical composition of shells from red (Strongylocentrotus franciscanus) and green (Strongylocentrotus droebachiensis) sea urchin. Food Chem 2012;133(3):822-6.
18. La Cruz-García D, López-Hernández J, González-Castro MJ, Rodríguez-Bernaldo De Quirós AI, Simal-Lozano J. Protein, amino acid and fatty acid contents in raw and canned sea urchin (Paracentrotus lividus) harvested in Galicia (NW Spain). J Sci Food Agri 2000;80(8):1189-92.
19. Pozharitskaya ON, Ivanova SA, Shikov AN, Makarov VG. Evaluation of free radical-scavenging activity of sea urchin pigments using HPTLC with post-chromatographic derivatization. Chromatographia 2013;76(19-20):1353-8.
20. Krishtopina AS, Urakova IN, Terekhina YA, Pozharitskaya ON, Shikov AN. Seaurchin shell waste is a rich source of calcium. Reviews of Clinical pharmacology and Drug Therapy 2017;15(Suppl 1):35.
21. Mccue P, Kwon YI, Shetty K. Anti-amylase, anti-glucosidase and anti-angiotensin i-converting enzyme potential of selected foods. Journal ofFood Biochemistry 2005;29(3):278-94.
22. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 2001;73(2):239-44.
23. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem 1996;239(1):70-6.
24. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphonia urceolata. Food Chem 2006;95(1):37-43.
25. Lee SH, Lee JB, Lee KW, Jeon YJ. Antioxidant properties of tidal pool microalgae, Halochlorococcum porphyrae and Oltamannsiellopsis unicellularis from Jeju Island, Korea. ALGAE 2010;25(1):45-56.
26. Olabinri BM, Odedire OO, Olaleye MT, Adekunle AS, Ehigie LO, Olabinri PF. *In vitro* Evaluation of Hydroxyl and Nitric Oxide Radical Scavenging Activities of Artemether. Research Journal of Biological Sciences 2010;5(1):102-5.

27. Fraga CG, Oteiza PI. Iron toxicity and antioxidant nutrients. Toxicology 2002;180(1):23-32.

28. Husni A, Wijayanti R. Inhibitory Activity of [alpha]-Amylase and [alpha]-Glucosidase by Padina pavonica Extracts. Journal of Biological Sciences 2014;14(8):515.

29. Senthil SL, Kumar TV, Geetharamani D, Maruthupandi T. Screening of seaweeds collected from Southeast Coastal area of India for α-amylase inhibitory activity, antioxidant activity and biocompatibility. Int J Phar Pharm Sci 2013;5(1):240-4.

30. Kwon YI, Apostolidis E, Shetty K. In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresour Technol 2008;99(8):2981-8.

31. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 2009;5(1):54-63.

32. DeFronzo RA, FerranniniE, Zimmet P, Alberti KG. International Textbook of Diabetes Mellitus.4th ed.UK: John Wiley & Sons; 2015.

33. Klinger TS, Watts SA, Forcucci D. Effect of short-term feeding and starvation on storage and synthetic capacities of gut tissues of *Lytechinus variegatus* (Lamarck)(Echinodermata: Echinoidea). Journal of Experimental Marine Biology and Ecology 1988;117(3):187-95.

34. Cao Y, Ashline DJ, Ficko-Blean E, Klein AS. Trehalose and (iso) floridoside production under desiccation stress in red alga *Porphyra umbilicalis* and the genes involved in their synthesis. Journal of Phycology 2020; 56(6):1468-1480.

35. Klinger TS, Diehl WJ. Activities and kinetics of digestive α-and β-glucosidase and β-galactosidase of *Luidia clathrata* (Say)(Echinodermata: Asteroidea). Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 1985;81(2):401-3.

36. Archana A, Babu KR. Nutrient composition and antioxidant activity of gonads of sea urchin *Stomopneustes variolaris*. Food Chem 2016;197(Part A):597-602.