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Original Research Article

α-Glucosidase Inhibition and Antioxidant Activities of Respiratory Tree, Gonad, and Body Wall Extracts of Two Species of Sea Cucumbers (*Holothuria leucospilota, Stichopus hermanni*) from Persian Gulf

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HIGHLIGHTS

- Most of extracts had inhibitory properties on α-glucosidase.
- In both sea cucumbers, the extract of respiration tree showed maximum inhibition of α-glucosidase.
- The respiration tree extract showed the highest activity in antioxidant activity compared to other extracts.

ABSTRACT

In the recent years, the morbidity and death rates of diabetes have quickly increased. The enzyme inhibitors and antioxidants are very important as they can treat or reduce the complications of diabetes type II. The sea cucumbers are potential sources for finding bioactive compounds such as antioxidant and enzymatic-inhibitor compounds; hence, the present study was focused on the screening two species of sea cucumbers extracts included (*Holothuria leucospilota*, and *Stichopus hermanni*) from Persian Gulf on α-glucosidase inhibition and antioxidant activities using two methods of DPPH radical scavenging and reducing power. In enzymatic inhibition assay, the properties of the extracts are divided into three categories, included inhibitory, activatory properties and without any effects. More extracts had inhibitory properties, some of which were activator and some did not have any effects. In both sea cucumbers, the extract of respiration tree showed maximum inhibition of 34% and 40% for *S. hermani* and *H. leucospilota*, respectively, on α-glucosidase activity. The respiration tree extract showed the highest activity in both enzyme inhibition and antioxidant activity compared to methanol and dichloromethane extracts of other parts.

Introduction

Beginning with multitudinous degrees of lack of insulin secretion, insulin resistance, and impaired insulin secretion, diabetes is the most common metabolic disease whose first characteristic is increase of glucose concentration in the blood, named hyperglycemia (Shane-McWhorter, 2005). In recent years, the morbidity and death rates caused by diabetes have quickly increased (Zarei and Poursharifi, 2015). One method to treat diabetes is the elimination of

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postprandial hyperglycemia by the inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase (Chen et al., 2013). Biguanides, sulfonylureas, thiazolidinedione and α -glucosidase inhibitors are the most important drugs to control blood glucose levels. However, these drugs have adverse complications (e.g. abdominal enlargement, flatulence, diarrhea) and many patients are resistant to them (Bolen et al., 2007; Wiener et al., 2008; Chen et al., 2013). Many studies have been conducted to find new inhibitors that have a higher activity, without the side effects of approved drugs (Yoshikawa et al., 2002). While, in search for some inhibitors without side effects, the researchers are attending to the natural sources (Muthuvel et al., 2013)

There is intense interest in discovering natural products of marine for their biomedical potential (Kumar et al., 2008). Natural products of marine animals are rich sources of compounds with nutritional, pharmaceutical, and medical applications (Pomory, 2000).

Sea cucumber is a marine invertebrate and its traditional and medicinal use has antiquity (Shakouri et al., 2009; Revathy et al., 2013). The studies on the extracts of sea cucumber showed their cytotoxic (Althunibat et al., 2009), antioxidant (Ding et al., 2003; Althunibat et al., 2009), anti-microbial, anti-cancer and anti-inflammatory (Chen, 2003; Farouk et al., 2007) properties. Therefore, it is not unlikely that they have antidiabetic potentials. However, the main objective of this study is to examine the antioxidants potentials by evaluating DPPH radical scavenging and reducing power and determine the antidiabetic potentials of different organs of sea cucumber species by evaluating their inhibitory activity on α-glucosidase.

Materials and Methods

Materials

PNPG (4-Nitrophenyl α-D-glucopyranoside) and α-glucosidase of *Saccharomyces cerevisiae* were purchased from Sigma-Aldrich. All the other reagents were obtained from Merck Chemical Co.

Samples collection

In June 2012, sea cucumber samples (*Holothuria leucospilota, Stichopus hermanni*) were collected from the coast of Persian Gulf (Larak Island) by diving in depths of 10–30 meter. The samples were dissected to separate internal organs, and bundled promptly with ice prior to be sent to the laboratory and kept at -20°C until extraction was performed. Identification of the species was based on the identification key.

Isolation and extraction of the samples

Extraction was conducted with a few modifications based on the method proposed by Mohammadizadeh et al. (2013) and Althunibat et al. (2013). The samples of gonad (G), respiration tree (RT), and body wall (BW) were melted before using; then, the recuperated species was cut into small pieces. The samples were homogenized via a blender and suspended after the extraction based on the increasing polarity, with dichloromethane (DM), ethyl acetate (EA), and methanol (M), using percolation method at room temperature. 500 cc of each solvents were added to the samples of sea cucumbers (50 g BW, 4 g G, 4 g RT and). Each samples extraction lasted about 72 hours. After filtration through Whatman filter paper No. 4 and centrifugation (at 6500 rpm for 15 min), extracts were evaporated under vacuum by a rotary evaporator at 45°C. The powdered extract of each sample was obtained by freeze dryer and stored at -20°C (Mohammadizadeh et al., 2013; Althunibat et al., 2013).

Enzyme inhibitory assay procedure

Inhibition of enzyme was conducted with a few modifications based on the method proposed by Rouzbehan et al. (2017). In this method, solution of enzyme contained 5 µL of α-glucosidase (25 unit/mL) and 125 µL of phosphate buffer (pH 6.9, 0.1 M). Substrate solution contained P-Nitrophenyl-α-D glucopyranoside (11 mM) in the mentioned buffer (pH 6.9); then, 20 µL of test extracts at different concentrations were blended with enzyme in microplate wells and incubated for 15 min at 37°C. The reaction was initiated by adding 20 µL of substrate and incubated it for an extra 15 min. The reaction was stopped by adding 80 µL of 0.2 M sodium carbonate solution. Absorbance of the wells was determined using a micro plate reader at 405 nm, and the reaction system without extracts was considered as control. The system without enzyme was blank, and acarbose was the positive control. All determinations were performed in triplicate (Rouzbehan et al., 2017). The enzyme inhibitions by the samples were calculated as follows:

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

Antioxidant activity determination using DPPH radical scavenging method

The antioxidant activity was evaluated according to the method described by Yang et al. (2006). 0.1 mL of extracts solution with different concentrations (2.5, 5, 10 mg/mL) was mixed with 0.1 mL of DPPH solution (0.5 mM). The reaction mixture was incubated in the dark for 30 min; then, the absorption was measured by a UV-Vis spectrophotometer (Cecil instruments- Cambridge England, CE 2501) at a wavelength of 517 nm. (Yang et al., 2006).

The radical scavenging of the extract against the stable DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = ([A_{blank} - A_{sample}]/ A_{blank}) \times 100$$

A blank is the absorbance of DPPH and A sample is the absorbance of extracts. Then the value of IC_{50} was calculated. The butylated hydroxytoluene (BHT) was used as a positive control.

Antioxidant activity determination of reducing power

Reducing power was evaluated to the method described by Oyaizu et al. (1986). At first, 200 µL of different concentrations of the samples (2.5, 5, 10 mg/mL) were blended with phosphate buffer (5 mL, 0.2 M, pH 6.6) and 5 mL potassium ferricyanide (1% w/v) was added, and the mixture was put for 20 min at 50°C. Then, 5mL of trichloroacetic acid (10% w/v) was added to the mixture, and centrifuged at 3000 RPM for 10 min. Five mL of the supernatant solution was blended with 1 mL ferric chloride (1% w/v) and 5 mL of distilled water. The absorbance of this mixture was measured at the wavelength of 700 nm using spectrophotometer. The experiments were performed in triplicate. The ascorbic acid was used as the standard.

Statistical analysis

The data were shown as the mean ± SD of three replicates. Analysis was performed using SPSS version 19. One-way analysis of variance (ANOVA) and Duncan′s new multiple-range test were exerted to evaluate the differences among the means.

Results and Discussion

α-Glucosidase is a significant enzyme in human digestive tract by which polysaccharides of food can hydrolyze and convert to more simple and restorable sugars (Shai et al., 2010).

The inhibition of this enzyme has an important role in the reduction of sugar absorption in the digestive tract (Yee and Fong, 1996). Its inhibitors have been used as anti-obesity drugs, fungi-static compounds, antiviral and immune modulators (El-Ashry et al., 2000). Mechanism of this enzyme has not been well- understood but it has been reported that the antioxidant compounds can cause inhibition of α-glucosidase and exert a protection against diabetes development (Feskens et al., 1995; Jo et al., 2009). The combination of radical scavenging and α-glucosidase inhibition make antioxidants as a potent antidiabetic drug (Revathy et al., 2013). Therefore in the present study, α-glucosidase inhibition and antioxidant properties of two species of sea cucumbers extracts were determined considering DPPH radical scavenging and reducing power.

The ability of two species of sea cucumber extracts for α-glucosidase inhibition were categorized into 3 groups. More extracts had inhibitory effects on α-glucosidase, some of which were activators of α-glucosidase such as G (EA) and RT (DM) extracts of *S. hermanni* and G (M) and BW (M) extracts of *H. leucospilota*. While, G (DM) and BW (EA) extracts of *S. hermanni* and RT (EA) and BW (EA) extracts of *H. leucospilota* had not effects on the inhibition of α-glucosidase .

In enzyme inhibitory assay, the methanol extract of the respiration tree of *S. hermanni* showed maximum inhibition of nearly 34±0.04% at the concentration of 10 mg/mL; while, the dichloromethane and ethyl acetate extracts of the gonad did not reveal inhibitory effects. In addition, some extracts were activator of α-glucosidase (Fig. 1). All of the extracts, in comparison to acarbose as the positive control (63±0.08% at 10 mg/mL, p=0.001) had less inhibitory effects on α-glucosidase .

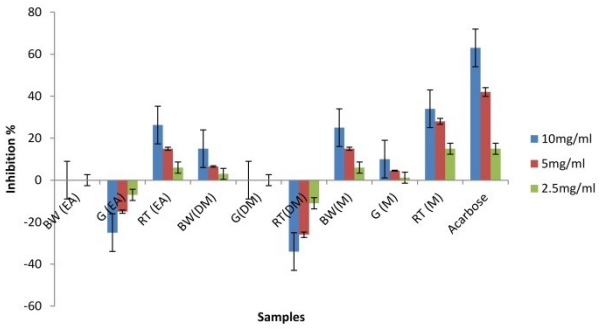

Figure 1. The percentage inhibition of α-glucosidase by three extracts of *S. hermanni*. The samples are of gonad (G), respiration tree (RT), and body wall (BW) extracted with dichloromethane (DM), ethyl acetate (EA), and methanol (M).

Dichloromethane extract of *H. leucospilota* respiration tree showed maximum inhibition on α-glucosidase, nearly 40±0.09% inhibition at 10 mg/mL; while, ethyl acetate extracts of respiration tree and body wall did not show any inhibitory effects (Fig. 2). All of the extracts, in comparison to acarbose (as a positive control) had less inhibitory effects (63±0.01% at 10 mg/mL, p=0.001) on α-glucosidase activity. As can be seen in Fig. 1 and 2, in both sea cucumbers, the extracts of respiration tree showed the highest inhibition on α-glucosidase; in addition, the ethyl acetate extract of body walls in both sea cucumbers did not show any inhibitory effects on α-glucosidase.

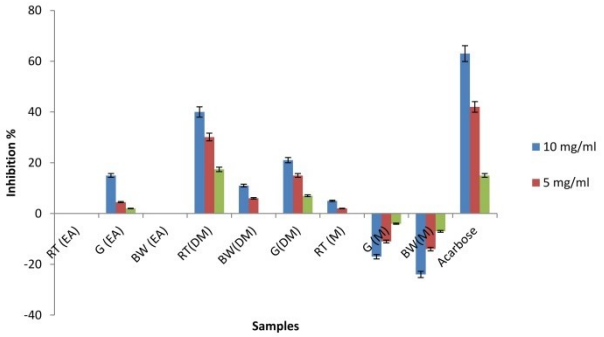

Figure 2. The percentage inhibition of α-glucosidase by three extracts of *H. leucospilota*. The samples are of gonad (G), respiration tree (RT), and body wall (BW) extracted with dichloromethane (DM), ethyl acetate (EA), and methanol (M).

Compared to obtained results in this study, a study had reported the maximum inhibition (93%) of α-glucosidase in the methanol extract of nudibranch which is a marine invertebrate (Muthuvel et al., 2013). Other studies on sea cucumber revealed the inhibitory effects of their extracts on α-glucosidase. Nguyenab and Kim (2011) had reported that n-hexane fraction of *Stichopus japonicas* showed the most inhibition effects on α-glucosidase (98% at 10 µg/mL). Also, it was reported, n-hexane fraction of sea cucumber (IC_{50} = 14.87 µM) inhibited α-glucosidase (Nguyen and Kim, 2015).

As mentioned before, some extracts were activators of α-glucosidase. The activation of α-glucosidase can improve the mobility of sperm (Viljoen et al., 1990; Mahmoud et al., 1998). This enzyme also has therapeutic effects on glycogen storage disease in which deficiency of α-glucosidase may occur (Fernandez-Hojas et al., 2002; Kishnani and Howell, 2004). FAD in 2006 approved the consumption of α-glucosidase recombinant human enzymes (rhGAA) for the treatment of glycogen storage disease (Schoser et al., 2008). The extracts of two species of sea cucumber which activate α-glucosidase can be used for the mentioned purposes.

Phenolic compounds inhibit α-glucosidase activity (Franco et al., 2002), these components are significant part of our diets. These components have antioxidant and anticancer potentials (Dai and Mumper, 2010). Sea cucumbers are daily consumed phytoplankton and micro-algae which are rich of phenolic components (Ridzwan, 2007); hence, these components are found in the tissues of sea cucumber (Mamelona et al., 2007).

Moreover, the extracts of sea cucumbers revealed poor antioxidant activities. The value of the IC_{50} of sea cucumber extracts (body wall, gonad and respiration tree) were showed in Fig. 3, in comparison with BHT as a standard antioxidant (IC_{50} = 76 µg/mL). In the method of DPPH radical scavenging, methanol extract of *S. hermanni* gonad (IC_{50} value =369±1.5 µg/mL) and ethyl acetate extract of *H. leucospilota* respiratory tree (IC_{50} value =491±3.5 µg/mL) showed the highest antioxidant activities.

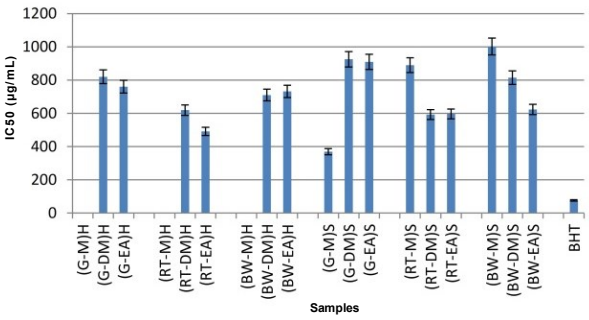

Figure 3. The IC_{50} value (µg/mL) of DPPH scavenging potential of different species of sea cucumbers extracts. The samples are of gonad (G), respiration tree (RT), and body wall (BW) extracted with dichloromethane (DM), ethyl acetate (EA), and methanol (M). H presents *H. leucospilota* and S presents *Stichopus hermanni*.

Zhong et al. also studied the antioxidant activity of sea cucumber (*Cucumaria frondosa*). The results of the radical scavenging tests revealed that sea cucumber contained some active antioxidant substances. Samples of internal organ had a significantly higher DPPH scavenging capacity than body wall (5.86 ±0.34 µmol of trolox equivalent/g of dry sample) (Zhong et al., 2007).

Permeh et al. evaluated the antioxidant activities of some extracts of sea cucumbers such as *H. leucospilotas*. They reported that *H. leucospilota* extract scavenged DPPH radical 11.12 % at concentration of 1 mg/mL (Permeh et al., 2013).

The results of a study by Althunibat et al. showed that both aqueous and organic extracts of *Holothuria edulis* were able to scavenge DPH radical (IC_{50} = 2.04 mg/mL and 8.73 mg/mL, respectively) (Althunibat et al., 2013).

The ability of reducing power of the extract has been shown in Fig. 4; moreover, in this method the reduction of Fe^{+3} to Fe^{+2} has been measured, in comparison with ascorbic acid as the standard sample (absorbance value of 0.301±0.9). Furthermore, reducing power of the extracts depended on concentrations of the extracts .

In order to evaluate the antioxidant properties of two species of sea cucumbers, this series of tests was evaluated. Dichloromethane extract of *S. hermanni* tree respiratory (with the absorbance value of 0.268±2.7) and ethyl acetate extract of respiratory tree of *H. leucospilota* (0.281±1.1) possessed higher reducing powers than ascorbic acid as the standard (0.301±0.9) (Fig. 4).

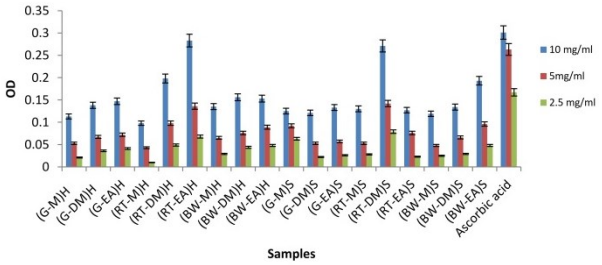

Figure 4. The reducing power of sea cucumbers extracts. The samples are of gonad (G), respiration tree (RT), and body wall (BW) extracted with dichloromethane (DM), ethyl acetate (EA), and methanol (M). H presents *H. leucospilota* and S presents *Stichopus hermanni.*

Accordingly, the respiratory tree showed the highest activity in both reducing powers and enzyme inhibition methods. The combination of radical scavenging and α-glucosidase inhibition make antioxidant as a potent antidiabetic drug (Revathy et al., 2013). It seems that the compounds with antioxidant properties inhibit α-glucosidase enzyme which is what requires more investigation (Franco et al., 2002).

The antioxidant activity of sea cucumber has also been reported by other researchers and compared with the results of the present study. A research group studied the antioxidant activity of dichloromethane extract of *H. leucospilota* by methods of DPPH (51.9 % at a concentration of 1 mg/mL) and reducing power, which showed an increase in the absorbance with increasing the concentration of the extract. The highest activity was at the concentration of 2000 µg/mL (absorbance value of 1.5) and BHA as a standard possessed the absorbance of 2. They showed the antioxidant activities of *H. leucospilota* as a natural antioxidant (Soltani and Baharara, 2014).

Also, Mashjoor et al. studied the antioxidant activity of *H. leucospilota* and *H. parva* sea cucumbers. They revealed that ethyl acetate extract of *H. leucospilota* body wall (absorbance value of 0.66±0.01) and ethyl acetate extract of body wall of *H. parva* (0.48±0.03) possessed the highest reducing powers and ascorbic acid as a standard possessed the absorbance value of 0.52±0.01. Ethyl acetate extracts of body walls in both sea cucumbers showed maximum antioxidant. It was recognized that the difference in ecological condition of various aquatic environments cause differences in physiological properties of sea cucumber which it may be a reason to justify disagreement in biological activities (antioxidants activities) of sea cucumbers (Pazooki and Khakshoor, 2015).

Conclusion

The results of the present study showed that biological activities of respiration tree extract of sea cucumbers were better than other samples. These extracts may possess the most components with antioxidant and α-glucosidase inhibitory effects. Moreover, structural and *in vivo* studies of these components will be helpful to discover new antidiabetic drugs.

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Competing Interests

The authors declare that they have no conflict of interests.

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