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Original article

Holothuria scabra Jaegar 1833 (Sandfish) extracts and collagens modulate protein-bound N^{ε}-carboxymethyllysine, N^{ε}-carboxyethyllysine and methylglyoxal-derived hydroimidazolone-1 levels

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Summary In this study, sea cucumber (*Holothuria scabra*; Jaegar 1833) extracts and collagens were evaluated for inhibitory properties of protein-bound advanced glycation end products (AGEs). Processed dried sea cucumber with salt extract showed a significant lower IC_{50} value for fluorescent AGEs (9.19 ± 7.68 μg mL^{-1}, $P < 0.05$) and fructosamine (503.47 ± 46.37 μg mL^{-1}, $P < 0.05$), respectively. Processed dried with and without salt extracts significantly reduced the N^{ε}-carboxymethyllysine (CML) and methylglyoxal-derived hydroimidazolone-1 (MG-H1) levels tested at 250 μg mL^{-1}. Smoked dried and fresh-dried extracts significantly reduced the N^{ε}-carboxyethyllysine (CEL) levels tested at 250 μg mL^{-1}. Pepsin-solubilised collagen and the crude collagen fibrils significantly reduced CML and MG-H1 levels whereas CEL levels were unchanged. Pearson's correlation analysis showed that protein-bound AGE and CML inhibition significantly correlated with the total phenolic and antioxidant activities, respectively. This study provides a strong rationale for further investigation aimed at identifying the active compounds responsible for the observed effects on biomarkers relevant to diabetes.

Keywords Advanced glycation end products, methylglyoxal-derived hydroimidazolones, N^{ε}-carboxyethyllysine, N^{ε}-carboxymethyllysine, protein-bound fluorescent AGEs, sea cucumber.

Introduction

In type 2 diabetes mellitus (T2DM), prolonged exposure to elevated glucose induces the production of free radicals, particularly reactive oxygen species (ROS) *via* several mechanisms, including glucose autoxidation and protein glycation (Thorpe & Baynes, 2003; Rains & Jain, 2011). Glycation is a non-enzymatic Maillard browning reaction between the carbonyl groups of reducing sugars and free amino groups of proteins, initiated by the formation of an unstable Schiff base which rearranges to form a more stable Amadori product (Thomas, 2011; Sell & Monnier, 2012). The Amadori product then undergoes a series of reactions including dehydration, condensation and fragmentation to form a heterogeneous group of compounds known as advanced glycation end products (AGEs; Thomas, 2011; Sell & Monnier, 2012). AGEs are either auto-fluorescent, crosslinked (e.g. pentosidine and crosslines), or non-fluorescent, non-crosslinked, [e.g. N^{ε}-carboxymethyllysine (CML), N^{ε}-carboxyethyllysine (CEL) and methylglyoxal-derived hydroimidazolones (MG-H1)], or non-fluorescent, crosslinked structure (e.g. di-carbonyl-lysine dimers) (Thorpe & Baynes, 2003; Deo *et al.*, 2020b).

Advanced glycation end products are biologically active and their accumulation in the body leads to the development of both macrovascular and microvascular diseases, and diabetic complications, including nephropathy, retinopathy, peripheral neuropathy, atherosclerosis

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and cardiomyopathy (Goh & Cooper, 2008; Brings *et al.*, 2017). The mechanisms involved include increased vascular and myocardial stiffening, inflammation and oxidative stress (Luévano-Contreras *et al.*, 2017; Deo *et al.*, 2021). As the complications of T2DM are known to be associated with accumulation of AGEs, strategies to prevent the excessive formation and accumulation of AGEs may reduce the risk of developing diabetic complications. Currently, there is no commercially available therapeutic agent available that inhibits AGE formation hence attention on alternative treatment including medicinal foods as potential inhibitors are explored. In recent years, biologically active novel compounds, including components from marine sources have been isolated for use in pharmaceuticals, functional foods and nutraceuticals (Malve, 2016; Khotimchenko, 2018).

Sea cucumbers, usually processed and sold as a dried product known as bêche-de-mer, have been used as a dietary delicacy and folk medicine in Asia and Pacific region (Wen *et al.*, 2010). Sea cucumbers have been reported by previous research for numerous therapeutic values, ie, antidiabetic (Khotimchenko, 2018), anti-angiogenic/anticoagulant (Lu & Wang, 2009), anti-hypertension (Zhao *et al.*, 2007), anti-inflammatory (Borsig *et al.*, 2007), antioxidant (Althunibat *et al.*, 2009), anti-cancer/anti-tumor (Lu & Wang, 2009; Janakiram *et al.*, 2015; Wargasetia & Widodo, 2017) and wound healing (Li *et al.*, 2018) properties. Based on the potential therapeutic properties, isolated bioactive compounds from sea cucumbers could be explored as AGE inhibitors. Limited scientific studies have investigated the antidiabetic properties of *Holothuria scabra* (*H. scabra*), a species of sea cucumber commonly found in Indo-Pacific region and consumed widely (Bordbar *et al.*, 2011). Therefore, the present study aimed to investigate the inhibitory effect of *H. scabra*; Jaegar 1833 extracts and collagens on protein-bound fluorescent and nonfluorescent AGE formation in an *in vitro* model. In addition, phytochemical contents and antioxidant activities of sea cucumber extracts were determined for correlations with the inhibitory properties of protein glycation.

Materials and methods

Chemicals and reagents

All chemicals and solvents were of analytical grade and details are in the Appendix S1.

Preparation of *H. scabra* extracts

Holothuria scabra; Jaegar 1833 were collected from Tavua Bay, Fiji Islands and post-harvest processing was performed as previously described (Ram *et al.*, 2017). Briefly, gut-removed sea cucumber were initially cooked (85 °C) and treated as follows: no salt, solar dried (SC1), salt, solar dried, (SC2) and smoked, solar dried (SC3). Untreated samples that were solar dried, that is fresh solar dried (SC4) were also prepared. Powered form of treated and untreated samples were mixed with 80% (v/v) ethanol and ultrasonicated at 30 °C for 15 min. The extraction technique was repeated twice, and the sonicated slurry was centrifuged for 10 min at 5000 ***g***. The combined ethanolic extracts was concentrated using a rotary evaporator under reduced pressure below 40 °C, then freeze dried and stored at −80 °C. Each extract was dissolved in 20% (v/v) methanol before analysis.

Preparation of crude collagen fibril (CCF)

The water-EDTA-water method (Cui *et al.*, 2007; Zhong *et al.*, 2015) was used to extract crude collagen fibril (CCF) with a slight modification. Briefly, fresh solar-dried *H. scabra* samples (SC4) were homogenised in deionised water and centrifuged at 10 000 ***g*** for 10 min. The resulting pellet was stirred with fresh deionised water for 1 h and then collected by centrifugation. Subsequently, 10 volumes of EDTA (4 mM, dissolved in 0.1 M Tris–HCl, pH 8.0) were added to the pellet. After stirring for 48 h, the homogenate was washed several times with deionised water to remove colour. Ten volumes of 0.1 M NaOH was then added and stirred for 72 h. After alkali extraction, the sample was centrifuged at 20 000 ***g*** for 30 min, and the resulting pellet was washed several times with deionised water to neutral. All operations were performed at 4 °C. Finally, the CCF of the upper layer was lyophilised and stored at −80 °C prior to use. Crude collagen was dissolved in 20% (v/v) methanol before analysis.

Isolation of pepsin-solubilised collagen (PSC)

Pepsin-solubilised collagen (PSC) of *H. scabra* was isolated and purified using the method described previously (Saito *et al.*, 2002; Zhong *et al.*, 2015). Briefly, lyophilised CCF was dissolved in 500 volumes (v/w) of 0.5 M acetic acid, mixed with pepsin (3200–4500 units mg^{-1}) and stirred for 48 h in a cold room (4 °C). The supernatant containing PSC was collected by centrifugation at 25 000 ***g*** for 60 min, and PSC was salted out by adding NaCl to a final concentration of 0.8 M with slight stirring for 24 h in a cold room (4 °C). The precipitate containing PSC was collected by centrifugation at 7500 ***g*** for 10 min and dissolved in 0.5 M acetic acid. The sample was dialyzed against 0.02 M Na_2HPO_4 (pH 8.0), 0.1 M acetic acid and deionised water, successively. Each dialysate was stirred slightly for 48 h in the cold room (4 °C) and changed every 6 h. All operations were performed at 4 °C. The purified PSC was

finally lyophilised and stored at −80 °C prior to use. PSC was dissolved in 20% (v/v) methanol before analysis.

In vitro antiglycation assay and determination of fluorescent AGEs

A glucose-BSA glycation model was prepared as previously described (Kim *et al.*, 2020; Deo *et al.*, 2020a). Appropriate controls (BSA only), negative controls (AGE-BSA) and positive control (AGE-BSA-AG) were also prepared. Protein-fluorescent bound AGEs were recorded at 370 nm (excitation) and 440 nm (emission) using a multimode plate reader (EnSpire® Perkin Elmer, Waltham, USA). The antiglycation properties were expressed as follows:

$$\text{Percentage inhibition of fluorescent AGEs (\%)} = \left[\left(F_{\text{negative control}} - F_{\text{sample}}\right)/F_{\text{negative control}}\right] \times 100.$$

Samples that showed greater than 50% inhibition at 1000 μg mL^{-1} were further investigated for the IC_{50} values.

Quantification of CML, CEL and MG-H1 levels

For CML and CEL levels, samples were prepared using acid hydrolysis whereas for MG-H1 samples were prepared using enzymatic hydrolysis levels as previously described (Deo *et al.*, 2020a, 2020b). Analysis of CML, CEL and MG-H1 were conducted using a SCIEX QTRAP 6500 liquid chromatography-mass spectrometer (Sciex, Framingham, MA, USA) and detected in ESI positive multiple reaction monitoring (MRM) mode with the following transitions m/z 147.4 > 83.9 (Lys), 151.2 > 87.9 ([$^4H_2$] Lys), 205.1 > 84 (CML), 207.1 > 129.9 ([$^2H_2$]-CML), 219.2 > 130.2 (CEL), 222.2 > 134.2 ([$^4H_2$] CEL), 229.2 > 116.1 (MG-H1) and 232.2 > 116.1 ([$^3H_2$] MG-H1). Results were expressed as mmol AGEs per mol Lys after BSA adjustment. Quality control parameters are given in the Appendix S1.

Determination of fructosamine formation

The level of fructosamine was measured as detailed elsewhere (Deo *et al.*, 2020a). Samples that showed greater than 50% inhibition at 1000 μg mL^{-1} were further investigated for the IC_{50} values.

Determination of total phytochemical and antioxidant activities

Total phenolic content (TPC) was measured using Folin-Ciocateu's reagent and the results were expressed as μg gallic acid equivalents (GAE) per mg sample as previously described (Deo *et al.*, 2016; Kim *et al.*, 2020). Total flavonoid content (TFC) was estimated using aluminium chloride technique, and results were expressed as μg quercetin equivalents (QE) per mg sample (Deo *et al.*, 2016; Kim *et al.*, 2020).

Ferric reducing antioxidant power (FRAP) assay was carried out as previously described with minor modifications (Kim *et al.*, 2020). FRAP activities were calculated from ascorbic acid standard curve and expressed as μg ascorbic acid equivalents (AAE) per mg sample.

Statistical analysis

All samples were analysed in triplicate and each experiment was repeated three times independently. Results were expressed as mean ± standard deviation (SD). IC_{50} values were evaluated by non-linear regression analysis using GraphPad Prism software (San Diego, CA, USA Version 8.2.1). Statistical differences were determined by one-way ANOVA followed by appropriate *post hoc* test, and $P < 0.05$ was considered significant. Linear regression analysis was used to determine Pearson's correlation coefficient (r_p) to compare relationship between antioxidant activities *versus* protein-bound fluorescent AGEs, CML, CEL and MG-H1 formation.

Results and discussion

Protein-bound fluorescent AGE inhibition by *H. scabra* extracts and collagens

Protein-bound fluorescent AGE inhibition of *H. scabra* extracts (SC1-SC4) and collagens (PSC and CCF) were initially assessed at 1000 μg mL^{-1} ranged from 60.21 ± 14.16% to 81.53 ± 5.10% (Fig. 1). SC2 and SC4 extracts showed significantly higher ($P < 0.05$) AGE inhibition compared to the SC3 extracts (Fig. 1a). SC1 extracts did not show any significant difference when compared to other extracts. PSC collagen showed significantly lower ($P < 0.05$) AGE inhibition when compared to CCF and SC4, respectively (Fig. 1b). No significant difference was observed between the CCF and the original fresh-dried extracts (SC4).

As all the samples tested showed greater than 50% inhibition, the AGE inhibition of the extracts and collagens were further compared for IC_{50} values. The IC_{50} values of fluorescent AGEs ranged from 9.19 ± 7.68 μg mL^{-1} to 324.47 ± 133.40 μg mL^{-1} (Table 1).

SC2 extract showed a strongest protein-bound AGE inhibition when compared to the other extracts and collagens. The results show that processed dried with salt (SC2) extracts has antiglycation properties either by reacting with reducing sugars and other reactive products prior to their interactions with proteins while

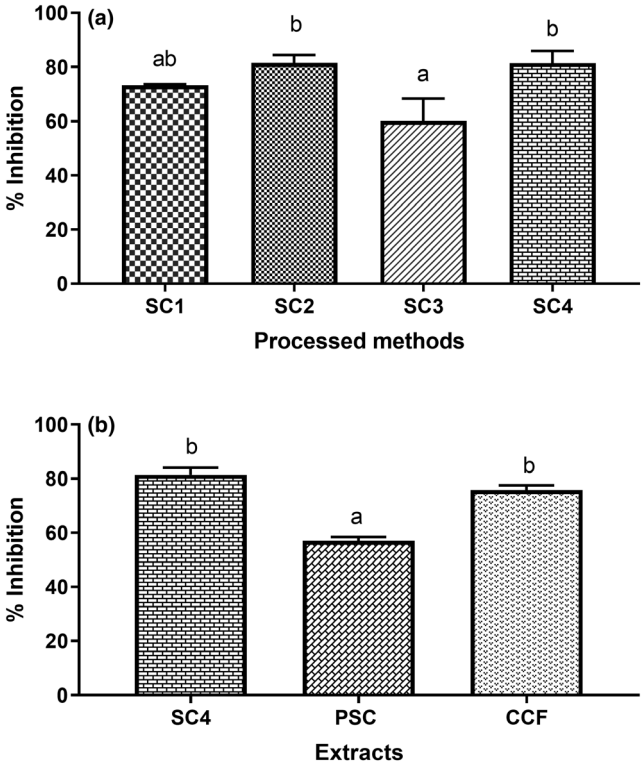

Figure 1 Protein-bound fluorescent advanced glycation end products inhibition in AGE-BSA model with 1000 μg mL^{-1} of *Holothuria scabra* extracts (a) or collagens (b). Values are expressed as mean ± SD, $n = 3$. Data marked with different letters were significantly different ($P < 0.05$). SC1, processed dried without salt; SC2, processed dried with salt; SC3, smoked dried; SC4, fresh dried. CCF, acid extracted crude collagen; PSC, pepsin soluble collagen.

Table 1 IC_{50} values of tested *Holothuria scabra* extracts on fluorescent AGEs and fructosamine

Holothuria scabra	Fluorescent AGEs (IC_{50} μg mL^{-1})	Fructosamine (IC_{50} μg mL^{-1})
Processed dried without salt (SC1)	141.83 ± 12.07^{b}	691.40 ± 32.12^{b}
Processed dried with salt (SC2)	9.19 ± 7.68^{a}	503.47 ± 46.37^{a}
Smoked dried (SC3)	324.47 ± 133.40^{c}	>1000
Fresh dried (SC4)	215.80 ± 84.62^{c}	>1000
Pepsin soluble collagen (PSC)	173.83 ± 66.17^{b}	>1000
Acid Extracted Crude Collagen (CCF)	71.28 ± 22.45^{b}	>1000

Values are expressed as mean ± SD, $n = 3$. Data in the same column marked with different letters were significantly different ($P < 0.05$).

quenching reactive AGE precursors or preventing the advanced stages AGE formation. It is possible that the processing sea cucumber with salt could have generated additional bioactive compounds or additional salt had favoured the antiglycation process. Previous studies have suggested that animal-based foods contain naturally occurring bioactive dipeptides, such as carnosine, which are associated in limiting carbonyl reactions and glycation-induced protein crosslinking (Cararo *et al.*, 2015; Freund *et al.*, 2018). Interestingly, these bioactive compounds are also found in relatively high levels in fish (Li *et al.*, 2019), suggesting the potential of seafood products or extracts to inhibit AGE formation. Sea cucumber has been previously studied for its antidiabetic effects (Khotimchenko, 2018). In a recent study (Hossain *et al.*, 2022), high-pressure processed sea cucumber (*Cucumaria frondosa*) extracts showed inhibitory effects on the formation of fluorescent AGEs; however, there is no studies on the modulation of specific AGEs such as CML, CEL or MG-H1.

Inhibition of CML, CEL and MG-H1 by *H. scabra* extracts and collagens

Among the nonfluorescent AGEs, CML is one of the most studied biomarkers for ageing, diabetes and in protein quality in nutrition evaluation of heat-treated food (Goldin *et al.*, 2006; Deo *et al.*, 2017, 2022). Protein-bound CML formation was significantly inhibited by *H. scabra* extracts (SC1-SC4, 250 μg mL^{-1}) when compared to AGE-BSA treatment (Fig. 2a). Interestingly, SC1 and SC2 extracts showed significantly lower levels of CML when compared to the AG (positive control) tested at the same concentration (Fig. 2a). For the *H. scabra* collagens, CCF showed significant lower ($P < 0.05$) CML formation when compared to PSC and SC4 indicating CCF to be a stronger inhibitor (Fig. 2b).

CEL levels, a homologue of CML, was also modulated by *H. scabra* extracts and collagens. SC1, SC3 and SC4 extracts significantly inhibited ($P < 0.05$) CEL formation when compared to AGE-BSA treatment (Fig. 2c). The CEL inhibition of SC3 and SC4 extracts were comparable with the AG (positive control) treatment (Fig. 2c). No significant difference was observed when the collagens (PSC, CCF) were compared with the SC4 extracts (Fig. 2d).

MG-H1 is one of the most abundant and functionally important AGEs in physiological systems and accounts for >90% of glycation adducts, although it has a relatively long half-life (12 days) compared to most cellular proteins (46 h) indicating that MG-H1 may remain active for long in the body (Rabbani & Thornalley, 2014). All water extracts (SC1–SC4) showed significant ($P < 0.05$) inhibition of MG-H1 levels (Fig. 2e), however, the activities were at different levels. SC1 and SC2 extracts showed greater level of MG-H1 inhibitions compared to SC3 and SC4. Collagens (PSC and CCF) showed significant ($P < 0.05$) inhibition of MG-H1 when compared to SC4.

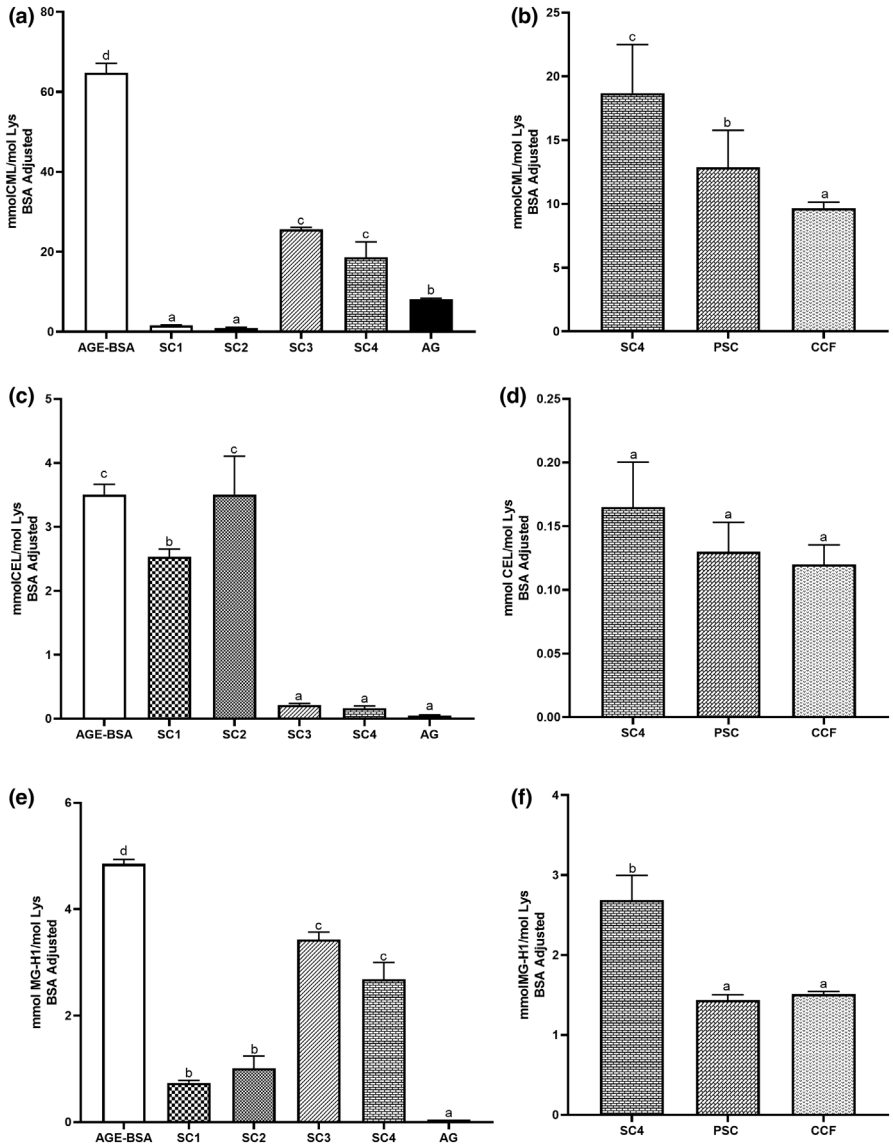

Figure 2 Advanced glycation end product levels in AGE-BSA model with 250 μg mL^{-1} of *Holothuria scabra* extracts or collagens. (a, b) N^{ϵ}-carboxymethyllysine levels; (c, d) N^{ϵ}-carboxyethyllysine levels, (e, f) Methylglyoxal-derived hydroimidazolone-1 levels. Values are expressed as mean ± SD, $n = 3$. Data marked with different letters were significantly different ($P < 0.05$). SC1, processed dried without salt; SC2, processed dried with salt; SC3, smoked dried; SC4, fresh dried. CCF, acid extracted crude collagen; PSC, pepsin soluble collagen.

In this study, both PSC and CCF showed lower CML and MG-H1 levels when compared to SC4, indicating that collagens may have greater potential in inhibiting specific AGE levels. To our knowledge no previous studies assessed the antiglycation potential of sea cucumber extracts in terms of inhibition of AGEs formation, including CML, CEL and MG-H1. However, previous studies reported antiglycation properties of extracts from other marine animals, such as a fish species *Misgurnus anguillicaudatus* (Zhang *et al.*, 2011) and yellowfin tuna *Thunnus albacares* (Nurilmala *et al.*, 2020), attributed to the polysaccharides and collagens, further supporting the antiglycation potential of extracts from marine sources.

Fructosamine inhibition by *H. scabra* extracts and collagens

Fructosamine inhibition of *H. scabra* extracts (SC1-SC4) and collagens (PSC and CCF) were initially

assessed at 1000 μg mL^{-1}. The SC1 and SC2 extracts showed greater than 50% inhibition thus were tested for IC_{50} values. The IC_{50} values of fructosamine of SC1 extract and SC2 extract were 503.47 ± 46.37 μg mL^{-1} and 691.40 ± 32.12 μg mL^{-1}, respectively (Table 1). This indicates that SC1 and SC2 extracts are associated with preventing the early stages of glycation by reducing the formation of Amadori products. In a previous study, carnosine, reported its antiglycation properties *via* prevention of early stages of glycation (Cararo *et al.*, 2015; Freund *et al.*, 2018). PSC and CCF showed weak inhibitors of fructosamine formation.

Pearson correlations analysis

Phytochemicals and antioxidant potential of food extracts have been previously reported for its antiglycation activity which are attributed to the multiple stage targets in the AGE pathway (Li *et al.*, 2014; Wang *et al.*, 2016; Kim *et al.*, 2020). In this study, total TPC, TFC and FRAP levels were measured in *H. scabra* extracts and correlated with AGE biomarkers. The TPC of *H. scabra* extracts (SC1-SC4) ranged from 14.82 ± 1.70 to 18.52 ± 1.95 μg GAE mg^{-1}, however, were non-significant when compared to each other (Table S1). TFC levels ranged from 7.34 ± 1.93 to 15.58 ± 1.59 μg QE mg^{-1} (Table S1) with SC1 and SC3 showing significantly ($P < 0.05$) higher levels when compared to other extracts. The FRAP values ranged from 5.25 ± 0.38 to 11.91 ± 0.58 μg AAE mg^{-1} (Table S1) with SC3 extracts showing significantly ($P < 0.05$) higher levels when compared to other extracts. AGE inhibition was moderately correlated with the TPC ($r_p = 0.549$, $P < 0.05$) and FRAP ($r_p = 0.563$, $P < 0.05$) (Table 2). The IC_{50} values for protein-bound AGEs was negatively correlated with TPC ($r_p = -0.537$, $P < 0.05$) and FRAP values ($r_p = -0.707$, $P < 0.01$). For non-fluorescent AGEs, only CML formation was negatively correlated with TPC ($r_p = -0.575$, $P < 0.01$) and FRAP ($r_p = -0.505$, $P < 0.05$). However, frutosamine, CEL and MG-H1 did not correlate with the TPC, TFC and FRAP, respectively (Table 2). These data suggest that the antiglycation potential of sea cucumber are associated with phenolic content and the antioxidant activities.

Based on the results in our study, a plausible mechanism for the antiglycation potential of sea cucumber extracts could be possible due to their ability to scavenge free radicals. Generation of free radicals through various mechanism in protein glycation model plays key role in the formation of Schiff base and the Amadori rearrangement that undergo subsequent dehydration and rearrangement to generate AGEs (Thorpe & Baynes, 2003; Rains & Jain, 2011; Sell & Monnier, 2012). In this study, SC2 extracts showed significantly ($P < 0.05$) lower IC_{50} values for fructosamine and protein-bound fluorescent AGEs, respectively. In addition SC2 extracts also showed significantly ($P < 0.05$) lower CML and MG-H1 levels.

Table 2 Pearson's correlation between total phytochemical and antioxidants activities of extracts *versus* advanced glycation end products

	Total phytochemical and antioxidant activities		
Pearson's correlation (r_p)	**TPC**	**TFC**	**FRAP**
Protein-bound fluorescent AGE inhibition	0.549*	0.358	0.563*
Fructosamine inhibition	−0.342	0.391	−0.220
CML formation	−0.575**	−0.191	−0.505*
CEL formation	−0.430	0.091	−0.393
MG-H1 formation	0.528	−0.313	0.427
Protein-bound AGEs (IC_{50})	−0.537*	−0.358	−0.707**

AGE, advanced glycation end product; CEL, N^{ε}-carboxyethyllysine levels; CML, N^{ε}-carboxymethyllysine levels; MG-H1, Methylglyoxal-derived hydroimidazolone-1 levels.
*, ** indicate significant correlations at $P < 0.05$ and $P < 0.01$, respectively.

These suggest that the SC2 extracts are associated with preventing the early and later stages of glycation process. Processing sea cucumber with salt had favoured the antiglycation process either due to additional bioactive compounds other than antioxidants or additional salt in the model system however this needs further investigation. Our study also showed that the fluorescent AGEs inhibition and IC_{50} values for protein-bound AGEs showed a moderate to strong correlation between phenolic contents and antioxidant properties respectively. As for non-fluorescent AGEs, formation of CML is mainly thought to be promoted by the oxidative breakdown of Amadori products, the oxidation of ascorbic acid and the reactions between lysine and dicarbonyls in glycation process (Thorpe & Baynes, 2003; Rains & Jain, 2011). Similarly, in this study, results showed moderate correlation of CML formation with phenolic contents and antioxidant properties, respectively. In previous studies phenolic compounds have shown significant inhibitory activity against protein glycation which are attributed to free radicals scavenging, chelate metal ions or by traping reactive carbonyl species through adduct formation. These results indicate the antiglycation potential of the tested sea cucumber extracts could be at least in part be attributed to the scavenging activities of antioxidants to free radicals formed during early and late states of protein glycation.

Conclusion

Our study showed that *H. scabra* extracts and collagens have antiglycation potential which are attributed to the total phenolic and antioxidant activities.

Processed dried with salt extracts showed the strongest inhibition in formation of protein-bound fluorescent and non-fluorescent AGEs indicating that the processing techniques for sea cucumber is crucial. The results support that *H. scabra* could be a promising source of natural antioxidant and antiglycation agent and there is potential for the isolated bioactive compounds in this species to be developed as a functional food product.

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Author contributions

Hoi Y. Wong: Data curation (equal); formal analysis (equal); methodology (equal); writing – original draft (lead). **Emma L. Jaunay:** Formal analysis (equal); methodology (equal); writing – review and editing (supporting). **Wai C.D Lau:** Formal analysis (equal); methodology (equal). **Brock Peake:** Formal analysis (equal); methodology (equal). **Ravinesh Ram:** Conceptualization (supporting); methodology (equal); writing – review and editing (supporting). **Paul C. Southgate:** Supervision (supporting); writing – review and editing (supporting). **Permal Deo:** Conceptualization (lead); formal analysis (lead); investigation (lead); methodology (lead); supervision (lead); validation (lead); writing – review and editing (lead).

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.16341.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Additional information on methods and materials and data on antioxidant acitivities.

Table S1. Total phytochemical and antioxidant activities of crude extracts of *Holothuria scabra*.