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In vitro protective activity of South Australian marine sponge and macroalgae extracts against amyloid beta ($A\beta_{1-42}$) induced neurotoxicity in PC-12 cells

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ABSTRACT

South Australia is a biodiversity hotspot of marine sponges and macroalgae. This study aimed to evaluate the potential neuroprotective activity of extracts from these two marine sources by reducing the toxicity of human amyloid beta $A\beta_{1-42}$ in a cell model assay using PC-12 cells. A total of 92 extracts (43, 13, 16, and 20 extracts from sponge of 8 orders and 17 families, green algae of 3 orders and 4 families, brown algae of 6 orders and 8 families, and red algae of 5 orders and 10 families, respectively) were initially screened at three different concentrations (0.25, 2.5 and 25 μg/mL) to evaluate their toxicity using the MTT assay. About half of these extracts (26, 6, 5, and 10 extracts from sponge, green algae, brown algae, and red algae, respectively) showed some cytotoxicity, and were hence excluded from further assays. The rest of extracts (45 extracts in total) at 0.25 and 25 μg/mL were subsequently screened in a neuroprotection assay against $A\beta_{1-42}$ cytotoxicity. A cell viability reduction of 30% was observed in the MTT assay when the cells were treated with 1 μM $A\beta_{1-42}$. 29 extracts (13, 4, 7, and 5 extracts from sponge, green algae, brown algae, and red algae, respectively) reduced the toxicity induced by $A\beta_{1-42}$ ($P < 0.05$), indicating neuroprotective activity. These results demonstrate that marine sponge and macroalgae form a broad spectrum are promising sources of neuroprotective compounds against the hallmark neurotoxic protein in Alzheimer's disease (AD).

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease responsible for 60–80% of dementia cases (Alzheimer'sAssociation, 2014). Current treatment strategies for AD mostly target acetylcholinesterase and the *N*-methyl-D-aspartate (NMDA) receptor. However, these treatments can only mitigate some of the cognitive and memory loss symptoms and are not considered disease-modifying. Hence, the development of new treatments for AD are required (Scarpini et al., 2003).

One of the main hallmarks of AD is the presence of amyloid beta (Aβ) protein that forms plaques in the brain. $A\beta_{1-40}$ and $A\beta_{1-42}$ are major forms generated from the cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase (Hussain et al., 1999). It is suggested that the aggregation and diminished clearance are pathogenic factors of AD (Hardy and Selkoe, 2002). Animal studies demonstrate that amyloid plaques are correlated with memory defects (Hsiao et al., 1996). For that reason, targeting Aβ may be considered an effective approach in the treatment of AD (Hardy and Selkoe, 2002).

Marine sponges, one of the oldest multicellular animals on the planet (Hentschel et al., 2002), are a rich source of natural compounds contributing > 30% of all compounds discovered from marine organisms (Mehbub et al., 2014). These compounds possess a spectrum of biological activities including anti-viral, anti-bacterial, and anti-inflammatory properties (Mayer et al., 2013). A recent review of neuroprotective compounds from marine sponges ascribed a variety of mechanisms to their neuroprotection, including glutamate and serotoninergic receptor activity, kinase inhibition, neuritogenic and anti-oxidant activity (Alghazwi et al., 2016a). Interestingly, seven out of 90 neuroprotective compounds were reported as sourced from Australian species.

Macroalgae (or seaweeds) have been known for their uses in food and as potential drug sources. Macroalgae can be classified based on the pigment colours into different phyla such as Chlorophyta, Ochrophyta (class Phaeophyceae), and Rhodophyta which are commonly named as

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Table 1
The location sites of the different algal and sponges species collected in South Australia.

Sample code	Location	Sample code	Location
C9, C12 R17	Third way from Cape Jaffa to Margaret Brock Reef; near Kingston; S.A.	P1 R8, R11	Nore Creina Beach near Robe; South-East S.A.
D15, D19, D26 P2 R4, R5	Marion Reef off Edithburgh; Southern. Yorke Peninsula; S.A.	D27, D41	Kingston Jetty, far end; Kingston; SE S.A.
C2, C3	Jetty Piles; Cape Jaffa; near Kingston; S.A.	D5 P9, P11 R18, R19	D'estree's Bay; Kangaroo Island; South Australia
C11 D24 R1	Horseshoe Reef-0.65 miles; 355 degrees to Margaret Brock Light; near Kingston; S.A.	C6 D2, D6, D11, D32, D35, D36	American River; Green side of channel west of Strawbridge Point; Kangaroo Island; S.A
C5, C13	Coobowie Bay; Southern. Yorke Peninsula; S.A.	D1, D17, D29, D33, D42	Smith Bay; West of Cape D'estaing; Kangaroo Island; S.A.
C7, C10 D16, D31 P12	Margaret Brock Reef just near lighthouse (ne); near Kingston; S.A.	D7, D12, D13, D23 R6	Between Knob Point and Cape Cassini; Kangaroo Island; S.A.
C8	Cape Thomas; half way between Kingston and Robe; S.A.	D10, D21, D37 P7 R16	Point Ellen, Vivonne Bay; Kangaroo Island; S.A.
R12, R13	Port Mcdonnell Breakwater; Southern; S.A.	D3, D25	Cape D'estaing; North of Reef; Emu Bay; Kangaroo Island; S.A.
P13 R2, R3	Old Jetty Piles; Kingston Jetty; Kingston; SE; S.A.	D18, D20, D22	Smith Point; West of D'estaing; Kangaroo Island; S.A.
D40 P5, P15, P16 R10, R14, R15, R20	Godfrey Island; between Kingston and Robe; STHN. S.A.	D30, D34, D43	West of Cape D'estaing; Emu Bay; Kangaroo Island; S.A.
P3	Beachport Jetty; Beachport; S.E; S.A.	C4 P6, P8 R7, R9	Pandalowie Bay; South of lookouts; STHN Yorke Peninsula; S.A.
D14, D28, D39	1KM off Margaret Brock Lighthouse; Cape Jaffa; near Kingston; S.A.	P4, P10	Edithburgh Jetty, North of Jetty; Yorke Peninsula, S.A.
D9	Horseshoe Reef 3 km wnw of Margaret Brock Lighthouse; Cape Jaffa; Kingston; S.A.	D8	Edge of Marine Reserve - Pelican Lagoon; American River; Kangaroo Island; S.A.
D38	Outside Port Mcdonnell - Deep Creek (old dairy factory creek); under bridge; S.A.	C1 P14	Point Turton Jetty; STHN. Yorke Peninsula; S.A.
D4	PORT GILES JETTY; Southern, YORKE PENIN.;S.A.		

the green, brown and red algae, respectively (Lobban and Harrison, 1994; Guiry, 2012). Macroalgae present a range of biological activities such as anti-viral, anti-bacterial, antioxidant, anti-cancer and neuroprotective activity (Wang et al., 2008; Lima-Filho et al., 2002; Kang et al., 2003; Kang et al., 2004; Aisa et al., 2005; Pangestuti and Kim, 2011). A recent review reported a total of 99 compounds isolated from macroalgae demonstrating neuroprotective activities (Alghazwi et al., 2016b). The mechanisms ascribed to these effects included inhibiting Aβ aggregation and acetylcholinesterase inhibition, decreasing oxidative stress and kinase activity, enhancing neurite outgrowth, anti-inflammatory activity and protecting dopaminergic neurons.

South Australian waters have > 1000 different species of sponges that belong to 200 genera (Bergquist and Skinner, 1982). South Australia hosts one of the highest diversity of macroalgae, as it is home to over 1200 species with 62% of them as endemic (Phillips, 2001; Womersley, 1996). Few studies have reported neuroprotective activities of sponges and macroalgae collected in Australian waters, with only seven neuroprotective compounds from sponges. Esmodil was shown to inhibit acetylcholinesterase (Capon et al., 2004), while debromohymenialdisine inhibited CDK5/p25, CK1, and GSK-3β (Zhang et al., 2012c). Four compounds (Lamellarins O1, Ianthellidone F, lamellarins O2 and O) were shown to inhibit β-site amyloid precursor protein cleaving enzyme (BACE) (Zhang et al., 2012a), in addition to Dictyodendrin J (Zhang et al., 2012b). Moreover, only 3 compounds isolated from macroalgae collected in Australia were shown to have demonstrated neuroprotective activity. Spiralisone A, spiralisone B, and chromone 6 showed inhibition of CDK5/p25, CK1δ and GSK3β kinases (Zhang et al., 2012d). Therefore the present study was conducted to evaluate the potential of South Australia marine sponge and macroalgae extracts as a source of neuroprotective compounds, with a focus on reducing the cytotoxicity of Aβ in neuronal PC-12 cells.

2. Materials and methods

2.1. Samples collection

The Australian Institute of Marine Science (AIMS) provided all the samples used in this study. These samples were collected by hand whilst scuba diving or from shallows at low tide. They were frozen after a representative taxonomy sample was taken. All samples were collected in South Australia. The details of collections sites can be found in the Table 1.

The taxonomy information was provided by AIMS. Phylogenetic trees of these samples were conducted according to their class, order, family, genus, and species with a guide from http://www.algaebase.org/ (for algae samples) (Guiry and Guiry, 2014) and http://www.marinespecies.org/porifera/ (for sponge samples) (Van Soest et al., 2017).

The marine samples were divided in four different categories based on their class for sponges (Demospongiae) or phyla for macroalgae (Chlorophyta, Ochrophyta, and Rhodophyceae). For each category a separate phylogenetic distribution was constructed to distribute the class into order, family, genus, and species, respectively.

All the sponge samples were from Demospongiae class with a broad distribution of 8 orders and 17 families (Fig. 1). In Chlorophyta, there were 3 orders and 4 families (Fig. 2). In Phaeophyceae, there were 6 orders and 8 families (Fig. 3). In Rhodophyceae, there were 5 orders and 10 families (Fig. 4).

2.2. Extract preparation

A small subsample was removed and placed in a glass vial. The subsamples were freeze dried. After the samples were dried they were