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Original article

Neuroprotective activity of macroalgal fucofuroeckols against amyloid β peptide-induced cell death and oxidative stress

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Summary Phlorotannins are polyphenolic compounds predominantly found in brown seaweeds tentatively identified as having neuroprotective bioactivity; however, the effects of individual constituent phlorotannins against amyloid β neurotoxicity, the main hallmark neurotoxic protein in Alzheimer's disease (AD), is yet to be fully characterised. In this study, four phlorotannins, namely eckol, dieckol, phlorofucofuroeckol-A (PFFA) and 974-A sourced from the brown seaweed *Ecklonia* species were assessed for their ability to protect against the toxic effects of H_2O_2, lipid peroxidation via tert-butyl hydroperoxide (*t*-BHP) and $A\beta_{1-42}$ in neuronal PC12 cells. All compounds significantly scavenged reactive oxygen species (ROS). However, only PFFA and 974-A protected PC12 cells from oxidative stress-evoked neurotoxicity, providing significant increases in cell viability in response to both cytosolic (H_2O_2) and lipid peroxidation-evoked (*t*-BHP) cell stress. None of the phlorotannins tested inhibited $A\beta_{1-42}$ aggregate morphology, which suggested that their neuroprotective activity was unrelated to direct interactions with $A\beta_{1-42}$ protein. Our results indicate that while all phlorotannins tested exhibited ROS scavenging activity, only fucofuroeckol-type phlorotannins such as PFFA and 974-A afforded broader neuroprotective activity in response to both oxidative stress and amyloid β exposure. The additional amyloid-protective capacity of fucofuroeckols reveals the potential importance of the benzofuran moiety in neuroprotection and further studies are encouraged to investigate the chemico-biological basis of this distinction in the search for neuroprotective therapies in dementia and other neurodegenerative conditions.

Keywords Antioxidant, eckols, fucofuroeckols, neuroprotection, phlorotannin, β amyloid.

Introduction

Alzheimer's disease (AD) is an age-dependent progressive and irreversible neurodegenerative disorder characterised by extracellular deposition of the amyloid β peptide, occurring as senile plaques in addition to abnormal hyperphosphorylation of tau protein resulting in accumulation of intracellular neurofibrillary tangles (Francis *et al.*, 1999, 2005). The various and complex interactions amongst several contributing factors including genetics, oxidative stress, inflammatory and environmental factors are believed to be the underlying cause of neurodegeneration in AD. Studies reveal that the presence of excessive misfolding amyloid β (Aβ) protein leads to oxidative stress that induces neuroinflammation and apoptotic neurodegeneration, which ultimately leads to cognitive decline and clinical progression (Li *et al.*, 2014; Ahmad *et al.*, 2017). Reactive oxygen species (ROS), including superoxide radical anions and hydroxyl radicals are known to cause oxidative stress and this represents an early event in the pathogenesis of AD (Nunomura *et al.*, 2001). Current treatments such as cholinesterase inhibitors and memantine are limiting and not considered disease-modifying (Vaz & Silvestre, 2020), while the recently approved IgG1 anti-amyloid-β antibody targeting Aβ aggregates, Aducanumab, has questionable efficacy regarding clinical improvement in patients receiving treatment (Hooker, 2021). Recently, sodium oligomannate, a brown seaweed-derived (*Ecklonia kurome*) oral oligosaccharide has been approved in China

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for the treatment of mild to moderate AD (Lu *et al.*, 2021). While further clinical evidence of its efficacy as a dementia treatment awaits, this discovery has revealed potential opportunities for the development of a therapeutic drug from marine natural products for the treatment of AD.

Brown seaweed are considered versatile and can be sustainably used as a potential source for various applications worldwide including biomass feedstock, conversion into green biofuels, aqua and animal feed, active ingredients in the pharmaceutical and nutraceutical products along with integration in the human food chain (Chia *et al.*, 2018; Ong *et al.*, 2019; Biris-Dorhoi *et al.*, 2020). Brown seaweed has been used as a dietary and functional food for centuries, especially in Asian countries where consumption can reach 1 kg of dry weight per person annually (Tamama, 2021). These brown algae (Phaeophyta) synthesise a variety of phloroglucinol-based polyphenols as phlorotannins. Phlorotannins have been previously demonstrated to have neuroprotective activity via various modes of action including inhibition of acetylcholinesterase, butyrylcholinesterase, monoamine oxidase and beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1) activity (Barbosa *et al.*, 2020). Phlorotannins can also modulate neuronal receptors and regulate signalling pathways linked to neuroinflammation, oxidative stress and neuronal cell death (Barbosa *et al.*, 2020; Shrestha *et al.*, 2021b). Previous studies have shown that eckol, dieckol and phlorofucofuroeckol A (PFFA) decreased Aβ-induced cell death, inhibited intracellular ROS generation and calcium generation (Ahn *et al.*, 2012), while Lee *et al.* (2019) demonstrated that eckol and dieckol were ascribed anti-neuroinflammatory properties in $A\beta_{25-35}$-treated neuronal PC12 cells mediated by the downregulation of NF-κB and pro-inflammatory enzymes iNOS and COX-2 (Lee *et al.*, 2019). Additionally, we also recently reported the neuroprotective actions of dibenzodioxin-fucodiphloroethol (Shrestha *et al.*, 2021a), thus collectively demonstrating support for a neuroprotective role for phlorotannins through multiple pathways. However, more research is needed to clarify the contributions of specific phlorotannin classes towards this neuroprotection and some of its potentially operant protective pathways, such as antioxidant capacity and direct modulation of amyloid-aggregating properties.

In this study, $A\beta_{1-42}$, H_2O_2 and the lipid peroxidant *tert*-butyl hydroperoxide (*t*-BHP) were used to induce oxidative stress and toxicity in neuronal PC12 cells. Two eckol-type phlorotannins (eckol and dieckol) and two fucofuroeckol-type phlorotannins (PFFA and 974-A) (Fig. 1) were then investigated for their neuroprotective capacity in these settings. In addition, we investigated the direct interaction of these phlorotannins with ROS scavenging activity and anti-aggregatory effects against $A\beta_{1-42}$ fibrillisation, in order to further explore their potential neuroprotective mechanisms.

Materials and methods

Reagents and chemicals

Eckol was obtained as mentioned previously (Shrestha *et al.*, 2020). Briefly, ethyl acetate fraction of the ethanolic extract of *Ecklonia radiata* was subjected to centrifugal partition chromatography with varying ratios of solvents. The fractions were collected and pooled to give four sub-fractions and eckol was found in subfraction-3. Dieckol, phlorofucofuroeckol-A (PFFA) and 974-A isolated from *Ecklonia* species were kindly provided by Prof. Jae Sue Choi (Pukyong National University, Republic of Korea). All other chemicals and reagents were purchased from Sigma-Aldrich (Sydney, NSW, Australia) otherwise stated. Hydrogen peroxide was obtained from Thermo Fisher (Melbourne, Vic., Australia) and the DCFDA/H2DCFDA kit for ROS quantitation was purchased from Abcam (Melbourne, Vic., Australia). Human amyloid β protein ($A\beta_{1-42}$) was obtained from rPeptide (Bogart, GA, USA).

Preparation of $A\beta_{1-42}$ and phlorotannins

Lyophilised $A\beta_{1-42}$ was dissolved in dimethyl sulfoxide (DMSO) to prepare a concentration of 3.8 mM and diluted to 100 μM with sterile phosphate buffered saline (PBS). The aliquots were stored at −70 °C. The phlorotannins were dissolved in DMSO to prepare 20 mM stock.

Cell culture

Rat pheochromocytoma PC12 (Ordway) cells displaying a semi-differentiated neuronal phenotype with neuronal projections donated by Prof. Jacqueline Phillips (Macquarie University, NSW, Australia) were maintained in complete RPMI-1640 media (10% FBS, 1% penicillin/streptomycin and 1% NEAA) at 37 °C with 5% CO_2.

Cytotoxicity of phlorotannins in PC12 cells

Neuronal cell viability was measured as described previously (Shrestha *et al.*, 2020). Initially, the potential toxicity of each phlorotannin was assessed. PC12 cells were seeded at 2×10^4 cells per well in 100 μL media into 96 well tissue culture plates and incubated for 24 h at 37 °C with 5% CO_2. The cells were each treated with eckol, dieckol, PFFA and 974-A from 0 to 100 μM and incubated for 24 h. The media was replaced with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dissolved in serum-free media and incubated for 2 h. The solution was then

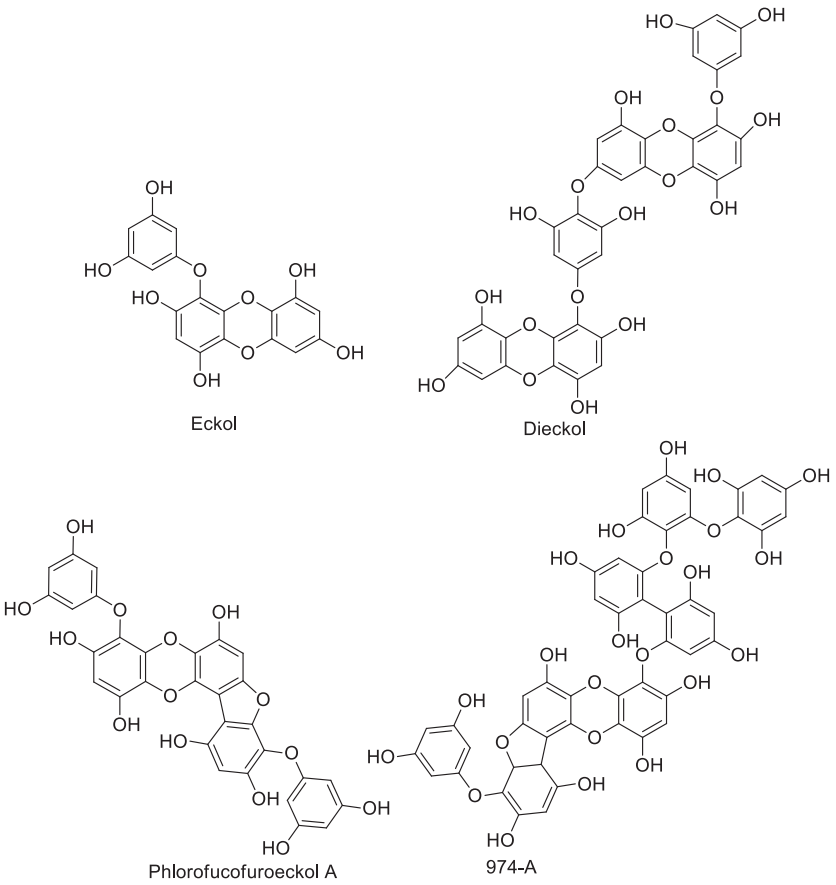

Figure 1 Structure of phlorotannins used in this study; eckol, dieckol, PFFA and 974-A.

replaced with DMSO and was measured at 570 nm using a Synergy MX microplate reader (Bio-Tek, Bedfordshire, UK).

Neuroprotective activity of phlorotannins against $A\beta_{1-42}$

In order to determine the neuroprotective activity of compounds against $A\beta_{1-42}$, PC12 cells were pre-treated with a non-toxic concentration of each phlorotannin (12.5 μM) as determined in the concentration-response profiles for 15 min prior to incubation with $A\beta_{1-42}$ (0–1.5 μM) for 48 h at 37 °C with 5% CO_2. MTT absorbance was measured at 570 nm as described earlier.

Neuroprotective activity of phlorotannins against H_2O_2 and *t*-BHP-evoked toxicity

PC12 cells were seeded at 3×10^4 cells per well into 96 well tissue culture plates and incubated for 24 h at 37 °C with 5% CO_2. The cells were pre-treated with 12.5 μM of compounds prior to treatment with 150 and 200 μM of H_2O_2 and *t*-BHP followed by incubation for 6 and 4 h, respectively. MTT absorbance was measured at 570 nm as described earlier.

Measurement of ROS generation from lipid peroxidation: effects of phlorotannins

A 2′,7′-dichlorofluorescin diacetate (DCFDA) assay was used for the measurement of ROS generation according to the manufacturer's instructions. Briefly, 1×10^5 cells per well in 100 μL phenol red-free media were seeded and stained with DCFDA (20 μM) for 30 min at 37 °C with 5% CO_2. DCFDA was washed out and 100 μL of phenol red-free media added. Cells were pre-treated with 12.5 μM of phlorotannins followed by *t*-BHP (50 μM) for 4 h. The fluorescence intensity was measured using a Synergy MX microplate reader (Bio-Tek) with excitation and emission wavelengths at 485 and 535 nm, respectively.

Transmission electron microscopy of $A\beta_{1-42}$ aggregate morphology

$A\beta_{1-42}$ (10 μM) was incubated alone or with 12.5 μM of each phlorotannin for 48 h at 37 °C in PBS. The interaction was visualised using a FEI Tecnai G2 Spirit Transmission electron microscope (FEI, Milton, Qld, Australia) and representative images were taken

at 18 500× magnification as described earlier (Shrestha *et al.*, 2020).

Statistical analysis

All experiments were performed in quadruplicate with at least 3–4 independent experiments and expressed as mean±SD. GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used for data analysis and graphs. Two-way analysis of variance (ANOVA), with a Bonferroni's post-hoc test was used to determine statistical significance between treatments, with a significance level set at $P < 0.05$.

Results

Effects of phlorotannins on PC12 cell viability

Initially, phlorotannins were tested for their cytotoxicity in PC12 cells up to the concentration of 100 μM as shown in Fig. 2. Each phlorotannin was non-toxic up to the concentration of 12.5 μM. However, when cells were incubated with each phlorotannin from 25 to 100 μM, viability was significantly reduced vs. control (***$P < 0.001$ vs. control). Of the four phlorotannins, dieckol was the most toxic, with 50% cell viability at 50 μM. All phlorotannins reduced cell viability by <32% at 100 μM. Therefore, a non-toxic test concentration of 12.5 μM for all compounds was used for all other interventions.

Effect of phlorotannins on hydrogen peroxide-induced PC12 cell viability

As shown in Fig. 3, PC12 cells treated with 150 and 200 μM of H_2O_2 demonstrated concentration-dependent loss of cell viability, with 56% and 32% of cell viability compared to control (no H_2O_2). Interestingly, a slight increase in cell viability was observed when the cells were treated with PFFA and 974-A only. Pre-treatment of PC12 cells with eckol and dieckol prior to incubation with H_2O_2 did not significantly protect PC12 cells. However, pre-treatment of cells with PFFA and 974-A (12.5 μM each) significantly (***$P < 0.001$ vs. control) inhibited the H_2O_2-induced loss of cell viability compared with vehicle (H_2O_2 only).

Effect of phlorotannins on *t*-BHP-induced PC12 cell viability

As shown in Fig. 4, when PC12 cells were treated with individual phlorotannins (12.5 μM) in the absence of *t*-BHP, the viability of cells was not affected for eckol and dieckol but a slight increase in cell viability was observed for PFFA and 974-A. However, when PC12 cells were treated with 150 and 200 μM of *t*-BHP, cell viability was reduced to 63% and 59% compared to control (no *t*-BHP), respectively. Two phlorotannins, PFFA and 974-A were able to protect PC12 cells significantly compared with the vehicle (*t*-BHP only). PFFA significantly (***$P < 0.001$) increased cell viability when at 150 and 200 μM of *t*-BHP to 94% and 92%, respectively. Similarly, 974-A increased cell viability to 86% and 81% vs. *t*-BHP only. Conversely, eckol and dieckol demonstrated no protective activity against in response to cytosolic oxidative stress evoked by *t*-BHP.

Phlorotannins reduce reactive oxygen species levels from *t*-BHP in PC12 cells

To evaluate the effect of phlorotannins on oxidative stress induced by *t*-BHP in PC12 cells, the level of

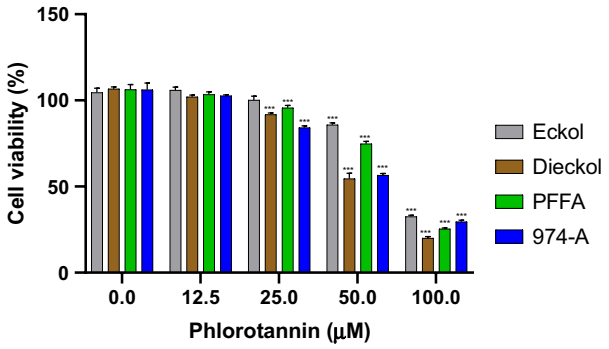

Figure 2 Cell viability following incubation with each of the phlorotannins; eckol, dieckol, PFFA and 974-A (each at 0–100 μM) in PC12 cells. ***$P < 0.001$ vs. control (no treatment).

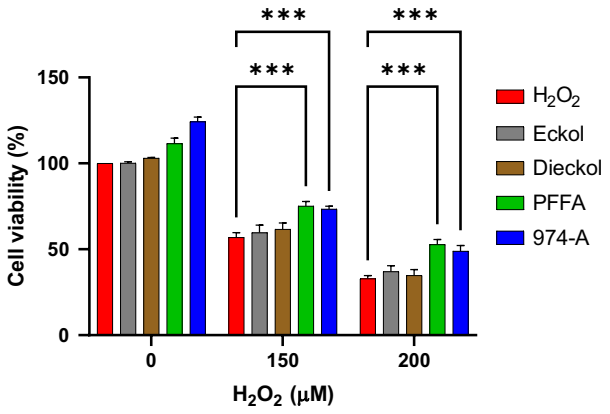

Figure 3 Neuroprotective activity of phlorotannins in H_2O_2 exposed PC12 cells. Cells were pretreated with 12.5 μM of each phlorotannin for 30 min prior to exposure with H_2O_2 (0–200 μM) for 6 h. ***$P < 0.001$ vs. control ($n = 4$).

ROS was measured through the DCFDA fluorescence assay. As shown in Fig. 5, *t*-BHP (50 μM) significantly (***$P < 0.001$) increased ROS levels more than two-fold compared with the untreated control. When PC12 cells were pre-treated with 12.5 μM of phlorotannins before *t*-BHP treatment, the level of ROS was reduced significantly (***$P < 0.001$) and equally (approx. 150%) in all phlorotannin-treated groups.

Effect of phlorotannins on $A\beta_{1–42}$-induced PC12 cells

As shown in Fig. 6, incubation of PC12 cells with $A\beta_{1–42}$ over 48 h elicited a concentration-dependent decrease in cell viability to 85%, 79% and 70% at 0.5, 1.0 and 1.5 μM, respectively. Interestingly, only PFFA and 974-A demonstrated a protective effect across all concentrations of Aβ. Specifically, pre-treatment of cells with PFFA and 974-A (12.5 μM) significantly increased cell viability up to 100% (***$P < 0.001$ vs. Aβ-treated cells). In contrast, eckol and dieckol provided no significant neuroprotection.

Effect of phlorotannins on $A\beta_{1–42}$ fibrillisation and aggregate formation

Transmission electron microscopy was used to assess the effects of phlorotannins on Aβ fibril formation and aggregation. $A\beta_{1–42}$ (10 μM) was incubated with each of the four phlorotannins at a concentration of 12.5 μM each for 48 h at 37 °C to enable aggregate formation over an equivalent period to match the cell incubation studies (48 h). Although, PFFA and 974-A provided protection against oxidative stress and Aβ toxicity, none of the four phlorotannins tested altered or diminished the density or morphology of Aβ aggregates (Fig. 7).

Discussion

A growing number of studies have indicated the bioactive potential of phlorotannins in regard to

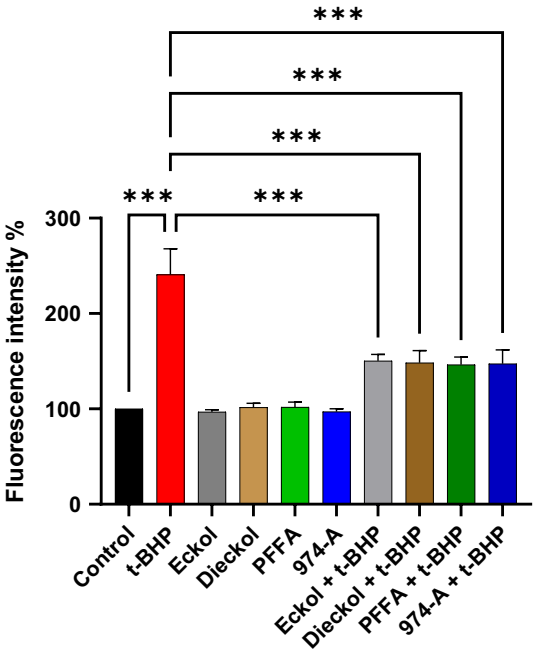

Figure 5 Intracellular radical scavenging activities of phlorotannins in PC-12 cells (as % ROS formation). Cells were labelled with fluorescent dye DCFH-DA (20 μM) and pretreated with 12.5 μM of each phlorotannin followed by *t*-BHP incubation (50 μM) for 4 h. ***$P < 0.001$ vs. *t*-BHP ($n = 4$).

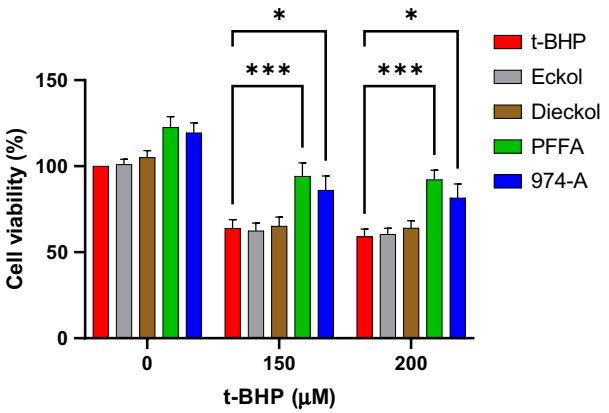

Figure 4 Neuroprotective activity of phlorotannins in *t*-BHP exposed PC-12 cells. Cells were pretreated with 12.5 μM of each phlorotannin for 15 min prior to exposure with *t*-BHP (0–200 μM) for 4 h. ***$P < 0.001$; *$P < 0.05$ vs. control ($n = 5$).

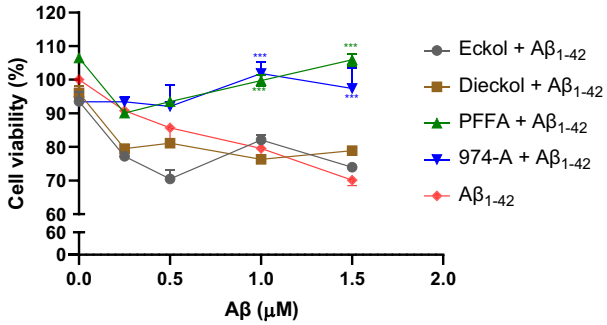

Figure 6 Neuroprotective activity of eckol, dieckol, PFFA and 974-A in $A\beta_{1–42}$ exposed PC-12 cells. Cells were pre-treated with 12.5 μM of each phlorotannin for 15 min prior to exposure with $A\beta_{1–42}$ (0–2.0 μM) for 48 h. ***$P < 0.001$ vs. $A\beta_{1–42}$ ($n = 3$).

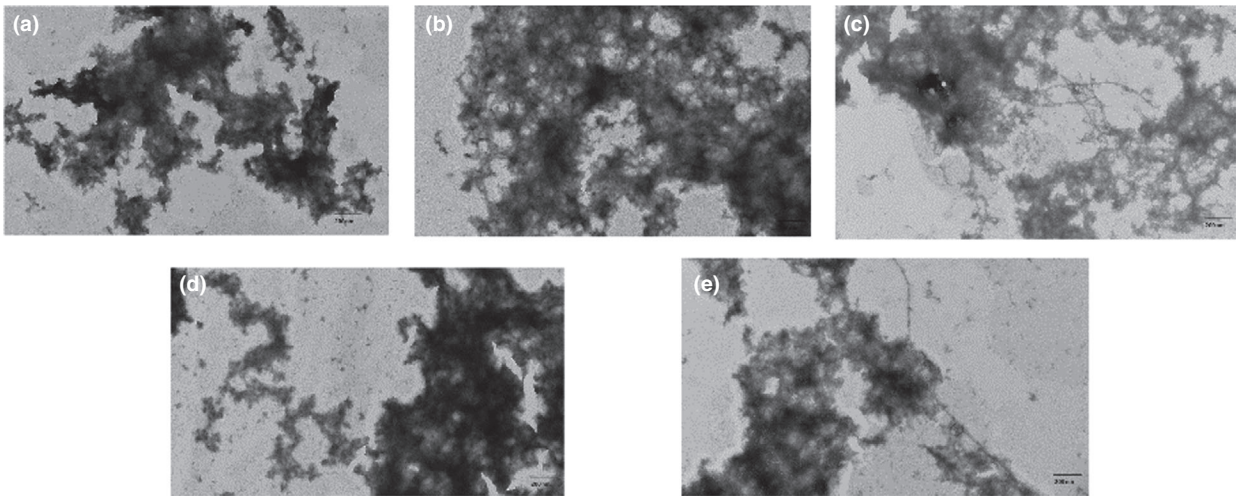

Figure 7 Representative transmission electron microscopic images of $A\beta_{1-42}$ fibril and aggregate formation alone (a) and following 48 h incubation with eckol (b), dieckol (c), PFFA (d) and 974-A (e). Each 12.5 μM concentration was used. Scale bar = 200 nm.

neuroprotection via antioxidant and anti-neuroinflammatory properties, in addition to some reported anti-aggregatory activity vs. amyloid β (Ahn *et al*., 2012; Wang *et al*., 2018; Lee *et al*., 2019; Barbosa *et al*., 2020; Shrestha *et al*., 2021b). Overall, this study suggests that while diverse phlorotannins more broadly share free radical scavenging and antioxidant neuroprotective properties, not all phlorotannin classes provide discreet protection against amyloid β-evoked neurotoxicity. Notably though, selected fucofuroeckols such as PFFA and 974-A have pronounced neuroprotective properties against oxidative stress and amyloid β, despite limited direct influence on amyloid β aggregation.

The brain is more vulnerable than other organs to oxidative stress due to the high consumption of oxygen, relatively low levels of endogenous antioxidant capacity and generation of superoxides by the action of various oxidases and nitric oxide synthase (Yavin *et al*., 2002). Oxidative stress can impair redox homeostasis and induce mitochondrial dysfunction, leading to damage in neuronal cells (Li *et al*., 2020) and H_2O_2 has previously been used to effectively generate cytosolic oxidative stress (Cai *et al*., 2008; Shin *et al*., 2021). Our results demonstrated that the fucofuroeckols PFFA and 974-A were able to prevent H_2O_2-induced death in PC12 cells, while no protective effects were observed with eckol and dieckol (Fig. 3). Our results also demonstrated that the phlorotannins were cytotoxic beyond 12.5 μM, which was consistent with previous studies (Ahn *et al*., 2012; Kim *et al*., 2016). By contrast, Lee *et al*. (2019) reported that eckol and dieckol were not toxic up to a concentration of 100 μM. Additionally, Shin *et al*. (2021) reported that dieckol at a concentration of 50 μg mL^{-1} (approx. 67 μ M) protected PC12 cells against H_2O_2 (200 μM) (Shin *et al*., 2021). This variability could be due to the difference in phlorotannin concentrations and/or the PC12 cells, where PC12 cells displaying a semi-differentiated phenotype with neuronal projections (Ordway subclone) (Dixon *et al*., 2005) were used in the present study, whereas these previous studies used undifferentiated cells (Lee *et al*., 2019; Shin *et al*., 2021).

T -BHP is a short-chain, cell permeant lipid hydroperoxide analogue commonly used to generate lipid peroxidation (Hibaoui *et al*., 2009; Kučera *et al*., 2014). *T*-BHP-induced oxidative stress was reported to exert deleterious effects on mitochondria involving ferroptosis and dysfunction leading to cell death (Wu *et al*., 2018). When PC12 cells were pre-treated with phlorotannins and exposed to *t*-BHP, a similar profile of protective effects was observed as in response to H_2O_2. The two fucofuroeckols, PFFA and 974-A significantly protected PC12 cells exposed to 150 and 200 μM of *t*-BHP, while no protection was observed with eckol and dieckol. Interestingly, all tested phlorotannins significantly scavenged intracellular ROS when measured via the DCFDA assay. Previously, Manandhar *et al*. (2019) reported a similar scavenging activity of eckol, PFFA and 974-A in B16F10 melanoma cells treated with 400 μM of *t*-BHP (Manandhar *et al*., 2019). The author points out that the effect is likely related to their phenol rings, which act as electron traps to scavenge peroxynitrite and superoxide anions as well as hydroxyl radicals.

Phlorotannins were then tested for their ability to protect PC12 cells against $A\beta_{1-42}$. A similar pattern (as seen in H_2O_2 and *t*-BHP-stimulated cells) was observed when PC12 cells where pre-treated with

phlorotannins prior to exposure to $A\beta_{1–42}$. PFFA and 974-A protected PC12 cells against $A\beta_{1–42}$. However, eckol and dieckol were not able to similarly rescue cells. Previously, eckol and dieckol were reported to have protected PC12 cells against $A\beta_{25–35}$, which contrasts with our results (Ahn *et al*., 2012; Lee *et al*., 2019). However, in this study we used $A\beta_{1–42}$, which is more pathologically relevant than $A\beta_{25–35}$. Previous studies demonstrated that $A\beta_{25–35}$ did not induce hippocampal damage and neurotoxicity (Malouf, 1992; Stein-Behrens *et al*., 1992) and lacks key neurotoxic residues limiting its comparative use for studies seeking insights into neurotoxicity mechanisms related to $A\beta_{1–42}$ (Butterfield & Sultana, 2011).

We previously reported the neuroprotective activity of an ethyl acetate fraction of *E. radiata* and suggested that eckol and eckol-type phlorotannins might be the predominant bioactive agents (Shrestha *et al*., 2020). However, the findings of this study underscore the potential contribution of varying phlorotannin types present in *Ecklonia* species. Previous studies also reported a similar pattern in LPS-induced RAW 264.7 cells (Kim *et al*., 2009). When dieckol and PFFA were assessed for their antioxidant and anti-inflammatory properties, both compounds were able to scavenge intracellular ROS significantly. By contrast, only PFFA was able to significantly reduce the production of nitric oxide and PGE_2 and suppress the expression of iNOS and COX-2 proteins. Additionally, fucofuroeckol-A isolated from *Eisenia bicyclis* was reported to have prevented Aβ42-induced damage in SH-SY5Y cells (Lee & Byun, 2018). Our results aligned with this study, and it can be suggested that the selective activity might be related to the structure–activity relationship (SAR) of these phlorotannins to Aβ42 oligomers, notably to the fucofuroeckols containing the additional benzofuran ring compared with eckols. This is supported by previous studies demonstrating that benzofuran derivatives have neuroprotective properties against Aβ (Rizzo *et al*., 2008; González-Ramírez *et al*., 2018; Lee & Byun, 2018; Cabrera-Pardo *et al*., 2020). González-Ramírez *et al*. (2018) reported a natural fungal-derived benzofuran with potent neuroprotective activity and suggested the direct effect on neuronal function without interfering with the Aβ aggregation process (González-Ramírez *et al*., 2018), which is consistent with the findings of this study. Additionally, Cabrera-Pardo *et al*. (2020) explored the multi-target neuroprotective potential of benzofuran scaffolds and suggested that privileged oxygen-containing heterocycles such as benzofurans exert neuroprotection by inhibiting several important events involved in the AD process including cholinesterase, ROS stress and Aβ-cell membrane binding (Rizzo *et al*., 2008, 2012; Cabrera-Pardo *et al*., 2020). However, further in-depth investigations with individual phlorotannins are required to confirm neuroprotective specificity and the chemico-biological basis conferring neuroprotection.

Transmission electron microscopy revealed no difference in amyloid β aggregate density or morphology between any of the phlorotannin treatments compared with control (Fig. 7). Previously, Seong *et al*. (2019) reported that eckol, dieckol and PFFA inhibited $A\beta_{25–35}$ self-aggregation via the thioflavin-T (ThT) fluorescence assay (Seong *et al*., 2019). However, polyphenolic compounds may inhibit ThT fluorescence without necessarily inhibiting fibril formation, directly quenching fluorophores and generating false positives (Hudson *et al*., 2009; Coelho-Cerqueira *et al*., 2014; Das *et al*., 2016). In such cases, transmission electron microscopy can therefore provide qualitative but arguably more definitive evidence of any anti-aggregatory effect of polyphenols, and in this study, we observed no direct anti-fibrillar effect of the selected phlorotannins.

In term of food safety and dosing, several human studies have confirmed that the phlorotannins are safe for consumption as a food supplement (Oh *et al*., 2010; Lee *et al*., 2012; Choi *et al*., 2015). Additionally, the European Food Safety Authority (EFSA) Panel on Dietetic Products recommends an intake level of 3.75 mg per kg body weight per day (163 mg day^{-1} for adolescents from 12 to 14 years of age, 230 mg day^{-1} for adolescents above 14 years of age and 263 mg day^{-1} for adults) (EFSA Panel on Dietetic Products *et al*., 2017).

In terms of the bioavailability of phlorotannins for use as functional food or nutraceutical supplements, various studies indicate that phlorotannins exhibit similar bioavailability to that of plant polyphenols, which are absorbed and metabolised predominantly in the large intestine via gut microbial metabolism (Crozier *et al*., 2010; Corona *et al*., 2016; Li *et al*., 2017; Baldrick *et al*., 2018). Analysis of human plasma and urine after phlorotannin-rich brown seaweed consumption indicate levels of various phlorotannin metabolites such as hydroxytrifuhalol A, 7-hydroxyeckol and phloroglucinol dimers with levels of individual phlorotannins attained up to 8 μg mL^{-1} in human plasma (Corona *et al*., 2016). This would accord to a plasma concentration of approximately 20 μM for a small (<400 g mol^{-1}) phlorotannin such as eckol and hence the concentrations used in our present study (12.5 μM) would be reasonably expected to be attainable *in vivo*.

While polyphenol bioavailability is generally considered low in the gastrointestinal tract, much attention has been recently focussed on prebiotic effects of polyphenols and their beneficial attribution towards the gut microbiome as it impacts neurodegenerative disorders. This is exemplified by the mechanism of oligomannate derived from brown seaweed as a clinically approved treatment for AD in China, whereby

improvements in gut dysbiosis are believed to be the underlying mechanism behind its clinical benefit (Wang *et al*., 2019). Phlorotannin-rich fractions from brown seaweed also modulate human enteric bacterial populations in vitro, suggesting changes in both microbial population and their host-beneficial mediators such as short-chain fatty acids (Charoensiddhi *et al*., 2017; Catarino *et al*., 2021). This recognition of the importance of the gut-brain axis and microbiome health has important implications for dietary interventions that until now were often considered as limited based on low gastrointestinal bioavailability. This also now underscores the importance of further research in dietary polyphenols, including seaweed-derived phlorotannins, in the normalisation of dysbiosis that occurs in neurodegenerative conditions and its applications for brain health.

Conclusions

Specific phlorotannins, in particular the fucofuroeckols PFFA and 974-A, possess protective effects against oxidative stress-induced neuronal cell damage through antioxidant mechanisms as well as preventing $A\beta_{1–42}$-induced neurotoxicity. These results highlight fucofuroeckols as a class of phlorotannin from brown seaweed that can effectively mitigate both oxidative stress and amyloid-evoked toxicity of relevance to neurodegenerative pathways in AD. Future studies are required to differentiate the mechanistic basis for the protection conferred by fucofuroeckols, as well as additional in vivo studies to further establish preclinical efficacy as a guide to informing further clinical trials in nutraceutical or pharmaceutical settings.

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Conflict of interest

The authors have no conflict of interest to declare.

Author contribution

Srijan Shrestha: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Software (lead); Writing – original draft (lead); Writing – review & editing (lead). **Scott D. Smid:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Methodology (equal); Resources (equal); Supervision (equal); Writing – review & editing (equal). **Jae Sue choi:** Methodology (equal); Resources (equal). **Wei Zhang:** Conceptualization (equal); Methodology (equal); Resources (equal); Supervision (equal).

Ethical approval

Ethics approval was not required for this research.

Peer review

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Data availability statement

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

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