ACADEMIA JOURNAL OF BIOLOGY 2022, 44(4): 33–45
DOI: 10.15625/2615-9023/17512

SCREENING OF NEUROPROTECTIVE SUBSTANCES RELEVANT TO ALZHEIMER'S DISEASE FROM SEAWEED SPECIES COLLECTED IN NINH THUAN AND KHANH HOA PROVINCES, VIETNAM

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Received 14 September 2022; accepted 16 December 2022

ABSTRACT

Currently, nearly 36.5 million people live with Alzheimer's disease worldwide, and acetylcholinesterase inhibition is considered the main treatment strategy against it. Seaweeds (or macroalgae) are a natural source of high-value bioactive compounds and have great potential in the production of health foods/foods, pharmaceuticals, cosmetics, animal feeds, biofertilizers, and biofuels. The most studied and applied seaweed species include red seaweed (Rhodophyta), green seaweed (Chlorophyta) and brown seaweed (Phaeophyta). This study aimed to evaluate the antioxidant and neuroprotective activities of extracts/substances isolated with solvents including ethanol 75^o and 96^o, water, n-hexane, ethyl acetate under normal and ultrasonic conditions (power 80W, frequency 4.7 kHz and for 1 hour) from 5 economically important seaweed species belonging to genera *Caulerpa*, *Sargassum*, *Gracilaria*, *Ulva* and *Kappaphycus* collected in Ninh Thuan and Khanh Hoa provinces, Vietnam. The results have shown that 05/30 extracts and substances (including SaE96S, SaH, SaHW, SaEA and fucosterol) isolated from five seaweed species exhibited high antioxidant activity through the free radical screening method using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (with EC_{50} values < 5 mg/mL for extracts and EC_{50} value < 2 mM for substance) in comparison with positive control ascorbic acid (EC_{50} = 0.015 mg/mL); acetylcholinesterase inhibitory activity using acetylcholinesterase inhibitor screening KIT (with IC_{50} values of < 200 µg/mL for both extracts and substances), compared with positive control galantamine (with IC_{50} value of 52,8 µg/mL). The extracts/substances were able to protect the cell against cytotoxity in the C6 alzheimer's disease cell model induced by amyloid beta-protein fragment ($A\beta_{25-35}$). The results achieved from this research have proven that the extracts/substances isolated from seaweed species were a potential source of medicinal agents for the prevention and treatment of alzheimer's disease.

Keywords: Alzheimer, β amyloid ($A\beta_{25-35}$), antioxidant, seaweed, acetylcholinesterase inhibitor.

Citation: Luu Thi Tam, Hoang Thi Minh Hien, Le Thi Thom, Nguyen Cam Ha, Ngo Thi Hoai Thu, Nguyen Van Tru, Tran Mai Duc, Nguyen Thi Minh Hang, Dang Diem Hong, 2022. Screening of neuroprotective substances relevant to Alzheimer's disease from seaweed species collected in Ninh Thuan and Khanh Hoa provinces, Vietnam. *Academia Journal of Biology*, 44(4): 33–45. https://doi.org/10.15625/2615-9023/17512

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INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder that affects millions of elderly people worldwide (Li et al., 2018). According to the report, across the globe, it is estimated that around 35 million people live with Alzheimer's disease and approximately 65 million develop dementia by 2030 (Prince et al., 2013). AD is the most common age-related neurodegenerative disorder that is characterized by redox imbalance in neurons, cholinergic impairment, aggregation of senile plaques, and neurofibrillary tangles due to the presence of β- amyloid (Aβ) peptide and hyperphosphorylation of tau protein (Tau is the major microtubule-associated protein (MAP) of a normal mature neuron). The increased phosphorylation of tau reduces their biological activity. Neurodegenerative diseases with abnormal tau inclusions are referred to as tauopathies (including Alzheimer's disease (AD) other tauopathies) in which the brain is from three to four-fold more hyperphosphorylated than the normal adult's brain, enhancing glutamate stimulation may induce the neuroinflammation and neuronal degeneration, resulting in learning, cognitive decline and memory loss (Feng & Wang, 2012; Mendiola- Precoma et al., 2016). Until now, there is no cure completely for AD due to the complexity of neuropathological mechanisms involved in the disease's development and progression (Graham et al., 2017). AD treatment has mainly focused on the early detection and prevention of the disease. Brand-name drugs used in the treatment and management of AD (including rasagiline, rivastigmine, and donepezil) are primarily associated with the inhibition of cholinesterase and β-secretase (BACE-1) enzymes (Cortés et al., 2015; Vassar, 2011). However, they may have side effects, such as nausea, vomiting, hepatotoxicity, digestive diorders (Ali et al., 2015). Therefore, the potential future direction for AD treatment is to use nutrient-rich nature products with neuroprotective activity, which have few or no side-effects compared to synthetic drugs (Olasehinde et al., 2017).

Seaweeds (or macroalgae) are marine organisms and have been employed as a natural source of highly bioactive compounds. Among them, a few seaweed species could grow in harsh habitats and release some valuable secondary metabolites. Seaweed extracts have been proven to possess strong antioxidant properties (Corsetto et al., 2020), hepatoprotective effects (Hira et al., 2017) and inhibitory activities on Hela cell proliferation (Lee et al., 2021), antibacterial activities (Fayzi et al., 2020) and antiviral activities (Lomartire et al., 2022). Therefore, seaweed has long been utilized as a source of foods (such as vegetables, and salads), herbs to treat tumors, urinary disorders, gastrointestinal problems, coughs, boils, hemorrhoids, tumors, asthma, goiter and headache (Dang et al., 2007; Dang & Hien, 2004; Niazi et al., 2011).

To obtain highly bioactive seaweed extracts, it is necessary to select suitable separation solvents for each species. The extraction yield of the targeted bioactive compounds is solvent-dependent and different solvents can be used according to the polarity and location of those compounds. An aqueous solvent is suitable for the separation of polysaccharides, while phenolics and carotenoids are usually extracted with organic solvents. In most cases, the extracts obtained with organic solvents appear more bioactive, while it has been confirmed that extracts derived from polar solvents have higher antibacterial activity. When the bioactive extracts are targeted for neutral pharmaceutical, and food industries, the solvents are limited, and must also be non-toxic. Ethanol, water, and their mixtures are ideal solvents for the production of extracts with high antioxidant capacity (Pappou et al., 2017).

The neuroprotective effects of bioactive substances from some seaweeds against neurodegenerative disorders have been reported by Alghazwi et al. (2016). Olasehinde et al. (2019b) have also indicated that crude extracts and substances isolated from seaweeds have neuroprotective effects

against AD through cholinesterase inhibition pathways. The ethanol/water extracts rich in phlorotannins, phenolic acids, and flavonoids isolated from *Ecklonia maxima, Gelidium pristoides, Gracilaria gracilis* and *Ulva lactuca* had acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities. Furthermore, sulfated polysaccharides found in *Ulva rigida* and the aforementioned seaweeds also exhibited strong inhibitory effects toward BChE and AchE *in vitro* models (Olasehinde et al., 2019a). Pang et al. (2022) also reported that ethanol extract of *Gracilaria manilaensis* inhibited cholinesterase enzyme with an EC_{50} value of 3.77 ± 0.20 mg/mL. This suggested that seaweeds are an important marine organism source of highly bioactive substances/compounds with neuroprotective properties against Alzheimer's disease-associated pathology.

Currently, in Vietnam, a total of 827 species of seaweed including 412 species of red seaweed (Rhodophyta), 180 species of green seaweed (Chlorophyta), 147 species of brown seaweed (Ochrophyta-Phaeophytaceae) and 88 species of cyanobacteria (Cyanophyta) have been identified (Phang et al., 2016; Dang et al., 2019, Dang & Ha, 2022). Among them, species of *Caulerpa, Sargassum, Gracilaria, Ulva* and *Kappaphycus* genera are economically important seaweed species of Vietnam with significant reserves, currently being cultivated and exploited on large scale. Therefore, the extraction and screening of the compounds/substances which was capable of neuroprotective activities have practical significance and have been considered to be highly feasible in exploiting substances with neuroprotective properties and memory enhancement from the seaweeds. In this study, we evaluated the neuroprotective properties of the extracts/substances isolated from species of Vietnam seaweed through antioxidant activity, inhibition of enzyme acetylcholinesterase (AchE) and protective effect against cytotoxicity in alzheimer's disease cell model induced by $A\beta_{25-35}$ on the C6 neuronal cell line.

MATERIALS AND METHODS

Materials, chemicals

Seaweeds: The seaweed species of the genera *Sargassum, Caulerpa, Kappaphycus, Ulva* and *Gracilaria* were collected in the sea waters of Ninh Thuan and Khanh Hoa provinces, Vietnam from February to April 2022. After collection, the species of seaweed were transferred to the laboratory, then removed from dirt, and sandy soil, gently dried at room temperature and under low light and stored in a refrigerator at -20 ^{o}C until use. The cell morphology and scientific name of the seaweed species used in this study were shown in Figure 1.

Cell line: C6 glioma cell line was obtained from the American Type Culture Collection (ATCC®; cat. no. CCL-107).

Chemicals: DPPH (2,2-diphenyl-1-picrylhydrazyl, purity 95%, Alfa Aesar, Japan); acetylthiocholine iodide (ACTI) (SigmaAldrich, Singapore); 5-5'-dithiobis-2-nitrobenzoic acid (DTNB) (SigmaAldrich, Singapore), 3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT, Invitrogen, USA), $A\beta_{25-35}$ (purity ≥ 97%, No. A.4559; Sigma, USA), Galantamine hydrobromide (SoPharma AD, Bulgaria). Chemicals used for cell culture: Dulbecco's Modified Eagle Medium (DMEM)/high glucose, Fetal bovine serum (FBS), penicillin and streptomycin (Invitrogen, USA) and other common chemicals were used in this study.

Methods

Isolation of seaweed extracts

To study the pharmacological effects of seaweed species, the biomass of selected seaweeds was extracted using solvents including warm water 60 ^{o}C (denoted as W), ethanol 75^{o} (denoted as E75) and 96^{o} (denoted as E96); n-hexane (denoted as H), n-hexane-water (denoted as HW) and ethyl acetate (denoted as EA) on a magnetic stirrer with different extraction conditions: extraction time from 2–9 hours; the ratio of biomass to solvent (w/v) from 1:2 to 1:5;

seaweed extraction without or in combination with ultrasound using an ultrasonic bath (BioBase, ultrasonic power 80 w, ultrasonic time 1 hour, ultrasonic frequency 4.7 kHz, denoted as S). After the extraction process, the samples were filtered through filter papers and concentrated by a vacuum evaporator to recover the total extracts. Particularly in the n-hexane fraction of *Sargassum* species, three pure substances were isolated and identified fucosterol ($C_{29}H_{48}O$), saringosterol ($C_{29}H_{48}O_2$) and allitol ($C_6H_{14}O_6$). All extracts/substances obtained were used as raw feedstock for bioactive evaluation.

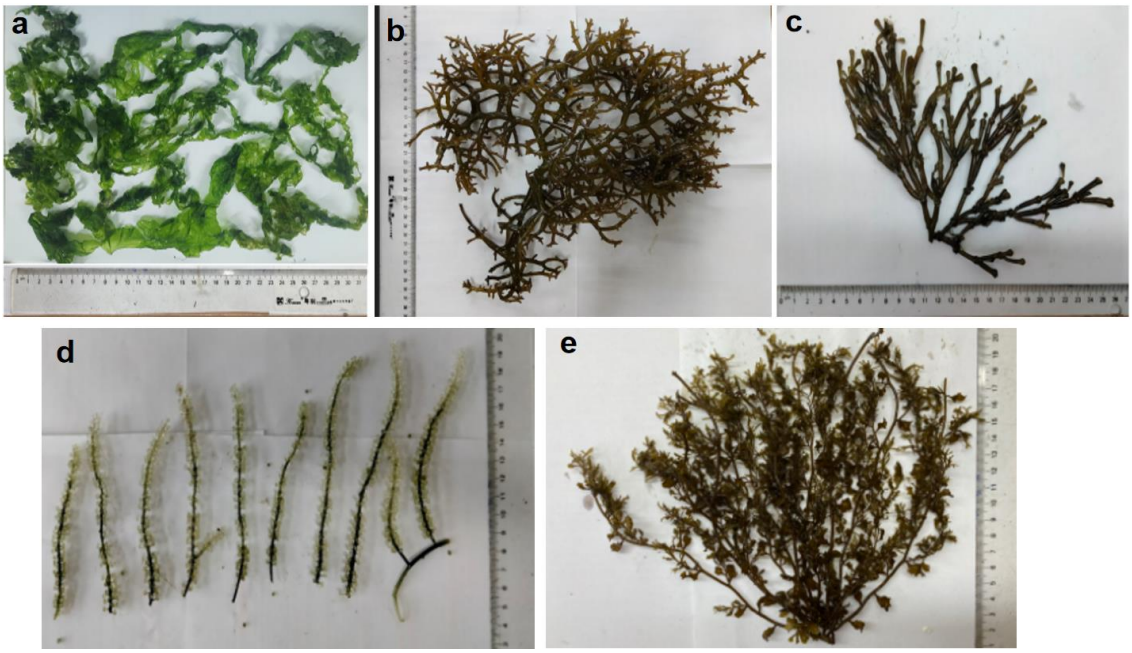

Figure 1. Cell morphology of seaweed species collected from the sea waters of Ninh Thuan and Khanh Hoa provinces in 2022. a. *Ulva reticulata* (Forssk), 1775; b. *Kappaphycus alvarezii* (Doty) Doty bown; c. *Gracilaria salicornia* (C. Agardh) E. Y. Dawson; d. *Caulerpa lentillifera* J. Agardh, 1837; e. *Sargassum mcclurei* Setchell, 1933

Determination of the antioxidant activity of total extracts/substances from seaweed using the DPPH method

The free radical scavenging ability of the extracts/substances was tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as described by Sharma & Bhat (2009). The reaction mixture consisted of 100 µL of DPPH (at a concentration of 0.2 µM) and 100 µL of ethanol/water/n-hexane/ethyl acetate extracts from seaweed species (at concentrations of 0, 0.4, 2 and 10 mg/mL) or substances (fucosterol, saringosterol, allitol) at a concentration of 0.1, 0.5 and 2 mM. The reaction mixture was incubated in the dark at room temperature for 30 min; then the absorbance of the DPPH was measured by spectrophotometer. DPPH produces purple color in methanol solution and fades to shades of yellow color (2,2-diphenyl-1-picrylhydrazine (DPPH-H) in the presence of antioxidants. DPPH radical scavenging capacity of antioxidants is evaluated by the decrease in the absorbance at the wavelength of 517 nm. Ascorbic acid (at the concentrations investigated of 4, 20, and 100 µg/mL) was used as the positive control.

The percentage of DPPH radical scavenging activity (SA) was calculated by the following equation:

$$\%SA = \left\{ \left(OD_{control} - OD_{reagent\ samples} \right) / OD_{control} \right\} * 100$$

In which: $OD_{control}$: Absorbance of the control; $OD_{reagent\ samples}$: Absorbance of the extracts/substances.

EC_{50} value (half maximal effective concentration) is calculated based on the standard curve of y = ax + b. The EC_{50} value is inversely proportional to the free radical scavenging activity/antioxidant property of the sample.

Determination of protective activity against cytotoxicity in alzheimer's disease cell model induced by $A\beta_{25-35}$ on the C6 cell line

This method is performed according to the detailed description in the publication of Yu et al. (2019). 100 µL of C6 neuron cells were seeded in a 96-well plate at an initial cell density of 1 x 10^5 cells/mL and cultured for 24 hours. After that, the old medium was removed, 100 µL of DMEM medium with 1 µL of DMSO solution or 1 µL of extracts/substances (at concentrations of 50 and 100 µg/mL)/galantamine (at a concentration of 100 µg/mL) were added and the cells were continued to culture for 24 hours. To detect the effect of $A\beta_{25-35}$, cells were treated with $A\beta_{25-35}$ at concentrations of 20 µM for 2 hours. The experiment consisted of 3 groups: the Control group (DMSO - $A\beta_{25-35}$); the model group (DMSO + $A\beta_{25-35}$); experimental group (extracts/substances/galantamine + $A\beta_{25-35}$). Each experimental formula was performed at least in triplicate. Then, 5 µL of MTT (5 mg/mL) was added and incubated for 4 hours in the dark. After that, 100 µL of DMSO was added and the plate was shaken on a shaker for 20 min. The absorbance of each well at a wavelength of 570 nm was detected by an automatic microplate analyzer. The cell survival fraction was calculated according to the equation:

$$\%\ \text{of alive cells} = \left[OD_{570\ nm\ experimental} / OD_{570\ nm\ control} \right] \times 100$$

Determination of acetylcholinesterase (AchE) inhibitory activity

The inhibitory effect of AChE (Sigma Aldrich, Singapore) of the sample was carried out using the Acetylcholinesterase Inhibitor Screening Kit (MAK324, Sigma, USA) according to the manufacturer's instructions. This method is based on the following principle: The substrate of acetylthiocholine iodide (ACTI) is hydrolyzed by the catalysis of AChE to produce thiocholin. The product of thiocholin reacts rapidly with a reagent of DTNB and a yellow compound of 5-thio-2-nitro benzoic acid is formed. The amount of this yellow compound is proportional to the AchE activity. Evaluation of AchE activity was based on the measurement of the absorbance of the samples at a wavelength of 412 nm. A detail of the procedure is following: a Reaction mix consisting of 154 µL assay buffer; 1 µL substrate, 0.5 µL DTNB was mixed thoroughly and used within 30 minutes. 5 µL of the test solution at the different concentrations and 45 µL of 0.4 IU/mL AChE reference Enzyme were added to the wells. The control (No Enzyme) consisted of 5 µL DMSO and 45 µL assay buffer, the no inhibitor control consisted of 5 µL of DMSO and 45 L of 0.4 IU/mL AChE reference Enzyme. Galantamine (at a concentration of 100 µg/mL) was used as a positive control. The mixture was mixed well and incubated for 15 minutes at room temperature. Then, 150 µL of the Reaction mixture was added into the corresponding wells and the absorbance at a wavelength of 412 nm was measured at 0 min and 10 mins. Each reagent concentration was repeated 3 times. The results were evaluated through the percentage of AChE inhibition (I%) according to the formula:

$$I\% = \left(1 - \Delta OD_{test} / \Delta OD_{No\ inhibitor}\right) \times 100\%$$

Where: I%: Percentage of AchE inhibition; $\Delta OD_{test:}$: Change in OD_{412nm} value of wells with extracts/substances at 0 min and 10 min; $\Delta OD_{No\ inhibitor}$: Change in OD_{412nm} of wells with no inhibitor control at 0 min and 10 min.

Statistical analysis

Data were expressed by mean ± standard deviation (X ± SD) using Microsoft excel 2013 software. The differences among the sample were assessed using a one-way analysis of variance (ANOVA) at the significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity of extracts/substances from seaweeds

Antioxidant activity of 30 extracts/substances obtained using solvents as aqueous, ethanol, n-hexane, and ethyl acetate from 5 seaweed species including *Sargassum mcclurei* (Sa), *Caulerpa lentillifera* (C), *Ulva reticulata* (U), *Kappaphycus alvarezi* (K) and *Gracilaria salicornia* (G) have been evaluated for their ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals. The antioxidant activity and percentage of free radical neutralization of extracts/substances obtained from selected seaweeds using the DPPH method were shown in Table 1 and Figure 2A.

The results in Table 1 showed that among a total of 30 extracts/substances isolated from 5 seaweed species belonging to the genera *Sargassum, Caulerpa, Ulva, Kappaphycus,* and *Gracilaria*, 9 samples exhibited antioxidant activity at tested concentrations including 4 extracts from *U. reiticulata* (UWS, UE96S, UE75, UE75S) and 5 extracts isolated from *S. mucclurei* (including 4 extracts (SaE96S, SaH, SaEA, SaHW) and 1 substances (fucosterol). Extracts isolated from genera *Caulerpa, Kappaphycus* and *Gracilaria* did not show antioxidant activity at the tested concentrations. The antioxidant capacity of the extracts/substances was demonstrated by the EC_{50} value (Fig. 2B). The lowest values of EC_{50} represent the highest antioxidant capacity and vice versa. The results are in Figure 2B. showed that the EC_{50} values of 9 screened samples ranged from 0.63 to 5.70 mg/mL, of which the *Sargassum* extracted with ethyl acetate (SaEA) and n-hexane/aqueous (SaHW) indicated the best antioxidant capacity, with EC_{50} values of 0.63 mg/mL and 0.68 mg/mL, respectively, followed by fucosterol (with an EC_{50} value of 0.70 mg/mL or 1.7 mM) compared to the positive control, ascorbic acid (EC_{50} = 0.015 mg/mL). Some studies have confirmed that alcoholic solutions and/or hydrophilic solvent mixtures provide extracts with better antioxidant activity. This can be attributed to the selective extraction of polar chemical substances, such as phenolic compounds, that exhibit significant antioxidant capacity (Pappou et al., 2017). Ethanolic and aqueous extracts may contain several components such as saponins, terpenoid, cardiac glycoside, phlobatamin, phenolic, and flavonoids that have been reported to have high antioxidant activity and depend on seaweed species, location as well as seaweed harvest season of the year (Sobuj et al., 2021). In our study, different solvents (water, ethanol, n-hexane, ethyl acetate, and a mixture of ethanol/water with the ratio of 75:25 (v/v) were tested to evaluate antioxidant activity. The alcohol and aqueous extracts of *Sargassum* and *Ulva* exhibited high antioxidant activity possibly due to: water being the best solvent for phenolic compounds and the ethanolic mixture for carotenoid extraction. Using pure ethanol resulted in lower solvation of phenolics, while carotenoids exhibited a better extraction ability in between that of water and the ethanol/water mixture (Pappou et al., 2017).

As published by Prasedya et al. (2019), the ethanol extract of *Ulva lactuca* exhibited relatively high antioxidant activity, with EC_{50} values ranging from 0.50 mg/mL to 0.68 mg/mL. The ethanol extract of

Sargassum policystum exhibited antioxidant activity with an EC_{50} value of 98 ppm (equivalent to 98 µg/mL) as reported by Safitri et al. (2021). Thus, the results on the antioxidant activity of the respective extracts in our study were lower than those reported above. This difference may be due to differences in seaweed species of each region, harvest season, extraction solvent as well as extraction methods used in the study.

Table 1. Antioxidant activity of aqueous/ethanol/n-hexane/ethyl acetate extracts/substances of 5 seaweed species collected from Ninh Thuan and Khanh Hoa provinces, Vietnam using DPPH radical scavenging assay

	Constituents	*Sargassum mcclurei*	*Ulva reticulata*	*Ulva alvarezii*	*Caulerpa lentillifera*	*Gracilaria salicornia*
Extracts	Hot water (W)	-	-	-	nd	nd
	Hot water + sonication (WS)	-	+	-	nd	nd
	Ethanol 75% (E75)	-	+	-	nd	nd
	Ethanol 75% + Sonication (E75S)	-	+	-	-	-
	Ethanol 96% (E96)	-	-	-	nd	nd
	Ethanol 96% + Sonication (E96S)	+	++	-	-	-
	n-hexane (H)	++	nd	nd	nd	nd
	Ethyl acetate (EA)	++	nd	nd	nd	nd
	n-hexane/water (HW)	+++	nd	nd	nd	nd
Substances	Fucosterol	+				
	Saringosterol	-				
	Allitol	-				

Notes: -: Negative; +: weak activity; ++: Moderate activity; +++: strong activity; nd: not determined.

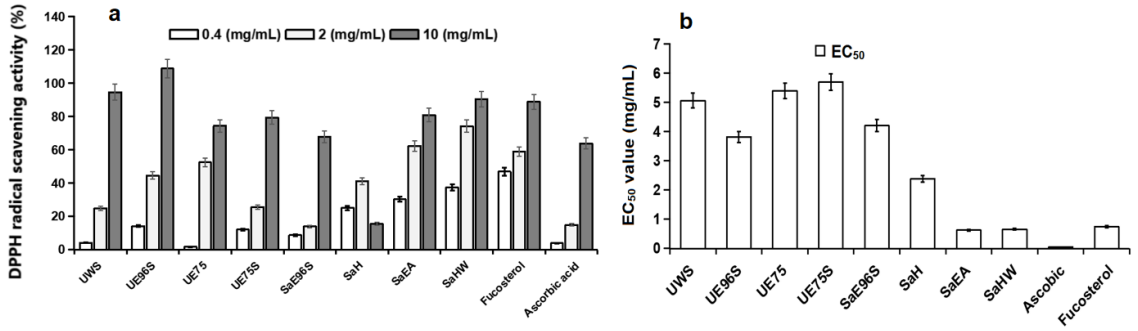

Figure 2. DPPH free radical scavenging ability (%) (a) and EC_{50} values (b) of aqueous/ethanol/n-hexane/ethyl acetate extracts/substances of *Ulva reticulata*, *Sargassum mcclurei*. Ascorbic acid was test at concentration of 4, 20, and 100 µg/mL), fucosterol - at concentration of 0.1, 0.5 and 2 mM

Protective activity against cytotoxicity in alzheimer's disease cell model induced by $A\beta_{25-35}$ on the C6 cell line

In AD patients, deposition of $A\beta_{25-35}$ has been shown to have a direct toxic effect on neurons, it may enhance the sensitivity of neurons to harmful factors such as free radicals and oxidative stress, thus leading to neuronal apoptosis (Ma et al., 2013). Therefore, the inhibition of neuronal apoptosis induced by the $A\beta_{25-35}$ protein provides a feasible method for the prevention and treatment of AD (Yu et al., 2019). In this study, we also evaluated the cytoprotective

effect of 9 potential extracts/substances against the $A\beta_{25-35}$ - induced cytotoxicity on cell line C6. The obtained results were shown in Figure 3.

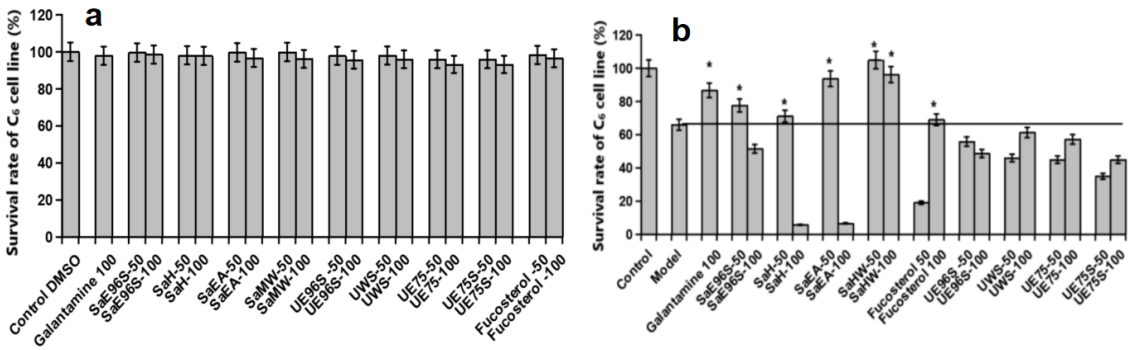

Figure 3. Cytotoxicity (a) and protect cell against cytotoxity in C6 alzheimer's disease cell model induced by amyloid beta - protein fragment ($A\beta_{25-35}$) (b) of extracts/substances from seaweeds. Cells were grouped as follows: the control group (untreated), the model group (cells were treated with 20 μM $A\beta_{25-35}$ for 2 hours to establish the AD cell model), and the test groups (cells were pretreated with extracts/substances from seaweeds and fucosterol at 50 and 100 μg/mL for 24 hours and then with 20 μM $A\beta_{25-35}$ for 2 hours). Cell survival was analyzed with the MTT assay. Note: $*p < 0.05$ comparison of different concentrations of the test groups and the model group

The results in Figure 3A showed that all nine aqueous and alcohol extracts of *U. reticulata* (including UWS, UE96S, UE75, and UE75S) and *S. mcclurei* (including SaE96S, SaH, SaEA, SaHW, and fucosterol) were not cytotoxic at all tested concentrations (50–100 μg/mL). There was no significant difference between the control and the experiment groups in the survival rate of the C6 cell line ($p > 0.05$).

The results in Figure 3B showed that when C6 cells were induced by $A\beta_{25-35}$, the cell survival rate decreased to 66% compared with the control formula (cells were left untreated, and the survival rate was 100%). The addition of extracts/substances from genera *Ulva* and *Sargassum* at different concentrations positively affected the survival rate of C6 cells. Among the 9 samples tested, 5/9 samples had the effect of protecting C6 cells against toxicity when cells were induced by $A\beta_{25-35}$, including *Sargassum* extracted with ethanol 96^o combined with ultrasound (SaE96S), n-hexane (SaH), ethyl acetate (SaEA), n-hexane/aqueous (SaHW) and fucosterol. The extracts from *Ulva* did not show protective activity on C6 cells against $A\beta_{25-35}$ - induced toxicity at the tested concentrations. Two samples of SaEA and SaHW showed very good cytoprotective activity with the survival rate of C6 cells of 93.7 ± 1.85% and 104.9 ± 0.97% respectively which were higher than 27.7% and 38.9% compared with the model group (DMSO + $A\beta_{25-35}$), respectively. Furthermore, the cytoprotective effect on C6 cells in these two formulations was even higher than that of galantamine (used as a positive control) at a concentration of 100 μg/mL) in an AD model. The SaH, SaE96S extracts and fucosterol substance showed lower protective activity, with C6 cell survival rates of 71.13 ± 0.36%; 77.61 ± 1.24% and 69 ± 0.58%, respectively. These values are higher than 5.13, 11.61, and 3% compared with the corresponding values in the model group. Research by Oh et al. (2018) showed that fucosterol isolated from *Ecklonia stolonifera* was effective in reducing neurotoxicity induced by oligomers amyloid in neuronal cell line models (Oh et al., 2018). Fucosterol from *Padina australis* was evaluated for its neuroprotective effects in

SH-SY5Y cells treated with $A\beta_{25-35}$; activation of the down-regulation of amyloid precursor protein (APP) expression (Alghazwi et al., 2018). Choi et al. (2017) also reported that ethanol extract of *S. serratifoilum* reduced the accumulation of β-amyloid ($A\beta_{1-42}$) in the cell line CHO-751 (Chinese hamster ovary cells). Our obtained results were also similar to the above statement.

AchE inhibitory activity of extracts/substances from seaweeds

AchE inhibitory activities of 9 screened potential extracts/substances were shown in Figure 4. According to Vinutha et al. (2017), plant extracts/substances with strong or weak AchE inhibitory effects were classified based on their percentage of AchE inhibition. Potential inhibitors (AchE inhibition ≥ 50%), moderate inhibitors (AchE inhibition of 30–50%) and weak inhibitors (AchE inhibition ≤ 30%) have been proposed and recognized. On that basis, the percentage of AchE inhibition of the seaweed extracts/substances and the positive control galantamine at the tested concentration of 100 µg/mL shown in Figure 4. indicated that 4/9 seaweed extracts were moderate AchE inhibitors, including UE96S (44.12%); SaE96S (41.88%), SaHW (41.19%) and SaEA (36.21%). UWS, UE75, UE75S, SaH, and fucosterol were weak AchE inhibitors, with AchE inhibition percentages of only 8.3; 12.7; 14.6; 29.97 and 22.3%, respectively. AchE inhibitory activity of the positive control galantamine reached 76.4% (with an IC_{50} value of 52.8 µg/mL). Compared with the positive control galantamine, IC_{50} values of seaweed extracts as UE96S; SaE96S; SaHW; SaEA; UWS, UE75, UE75S, SaH and fucosterol were lower, reaching 161.01; 157.22; 156.68; 180.33; 1034.22; 586.98; 478.58; 283.61 µg/mL and 250 µM, respectively.

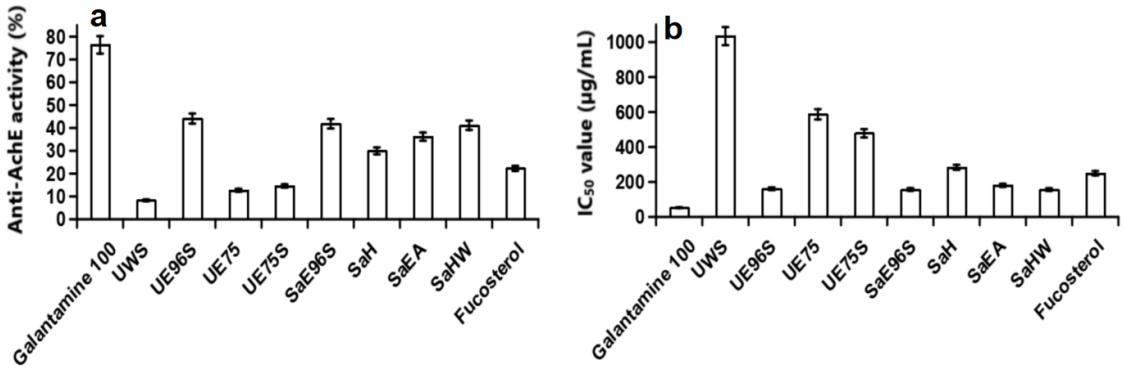

Figure 4. AchE inhibitory activities (a) and IC_{50} value (b) of seaweed extracts. AChE inhibition activity of 9 extracts/substance (fucosterol) from seaweeds Ulva and Sargassum at 100 µg/mL with galantamine (100 µg/mL) as the positive control. Data are expressed as the mean ± SD (n = 3)

Acetylcholinesterase inhibitors have been identified as an important therapeutic strategy for the management of alzheimer's disease. High acetylcholinesterase activity is associated with cholinergic dysfunction and memory impairment in AD. This is due to the rapid hydrolysis of acetylcholine - an important neurotransmitter responsible for sending nerve impulses from one nerve cell to another. Thus, inhibiting AchE increased the availability of acetylcholine, and improved neuronal transmission and memory function. The AchE inhibitory effect found in *Sargassum* and *Ulva* extracts/substances in our study will be the basis for the application of seaweeds in the control of AD. According to Syad et al. (2013), petroleum ether, n-hexane, benzene, dichloromethane, chloroform, and acetone extracts isolated from *Sargassum wightii* Greville at a concentration

of 100 µg/mL exhibited AchE inhibitory effect of 100 ± 5.9%; 87.21 ± 6.62%; 82.58 ± 1.18%; 43.82 ± 1.2% and 63.68 ± 3.4%, with IC_{50} values of 19.33 ± 0.56; 46.81 ± 1.62; 27.24 ± 0.90; 50.56 ± 0.90; 70.90 ± 1.07 and 69.58 ± 3.55 µg/mL, respectively. Natarajan et al. (2009) also reported that the methanol extract of *Sargassum* had AchE inhibitory activity with an IC_{50} value of 1 mg/mL. Castro-Silva et al. (2019) also reported that fucosterol isolated from *Sargassum horridum* had a strong inhibitory effect on AchE. The polysaccharides from *Ulva lactuca* have been proven effective in AchE inhibition with an IC_{50} value of 106.93 ± 1.45 µg/mL (Olasehinde et al., 2019b). Our results are also similar to the above study. However, the AchE inhibitory effect of the extracts/substances isolated from *Sargassum mcclurei* and *Ulva lentillifera* in this study was lower than that of other publications. The cause may be due to differences in seaweed species and extraction conditions, leading to differences in composition and substances present in the obtained seaweed extracts.

Acetylcholinesterase (AChE) inhibition and antioxidant activity are considered to be highly correlated with alzheimer's disease treatment. Antioxidant compounds might be implicated in AChE inhibition. Recent studies bound alzheimer's disease to an inflammatory process induced by reactive oxygenated substances. The oxidative stress intervenes, for a share, in the physiopathology of neuronal degeneration (Ferreira et al., 2006). The multifactorial nature of AD suggests that a multi-targeted therapeutic approach might be more advantageous than single-target drugs and combination therapies. Some of the AChE inhibitor molecules currently being evaluated in clinical studies, such as memoquin targets different additional pathways of AD by exhibiting antioxidant functions, acting as a β-secretase inhibitor, preventing Aβ aggregation, and influencing tau hyperphosphorylation. Natural drug candidates with anti-amyloidogenic and antioxidant properties in addition to cholinesterase inhibitory activity could be regarded as having special potential and need to focus on research to produce drugs for AD for human use in the short term coming (Mathew & Subramanian, 2014).

From the obtained results, there were 8 total crude extracts and 1 substance having a neuroprotective activity that met 2–3 screening criteria, including UWS, UE96S, UE75, UE75S (isolated from *U. reticulata*) and SaE96S, SaH, SaHW, SaEA and fucosterol (isolated from *S. mcclurei*). Which, there were 04 total crude extracts and 01 potential fucosterol from the *S. mcclurei* that had neuroprotective effects based on all 3 criteria used for screening. These are only preliminary results, we need to continue to elucidate the molecular mechanism of these extracts/substance in *in vitro* models as well as evaluate acute and subchromic oral toxicity *in vivo* experimental animal models. The obtained research will be presented in the further publication by the research team.

CONCLUSION

The results of screening seaweed extracts with neuroprotective activity showed that in total 30 extracts from 5 seaweed species including *S. mcclurei, C. lentillifera, U. reticulata, K. alvarezi* and *G. salicornia* collected in Ninh Thuan and Khanh Hoa provinces, we have selected 5 potential extracts/substances isolated from the seaweed species *S. mcclurei* (such as SaE96S, SaH, SaHW, SaEA and fucosterol) with three good pharmacological properties as antioxidant activity, AchE inhibitory activity and cytoprotective activity against toxicity induced by amyloid β-protein fragment 25–35 on the neuronal C6 cell line. Thus, *S. mcclurei* is potential seaweed with neuroprotective effects against alzheimer's disease.

Acknowledgements: The project was funded by the project "Neuroprotective effects on Alzheimer's disease (AD) of some seaweed species in Vietnam" with code VAST04.10/22–23 for Prof. Dr. Dang Diem Hong.

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