Universidade do Minho
Escola de Ciências

Catarina do Vale Pereira

Antioxidant and Neuroprotective Activities of Algae Extracts

outobro de 2023

Antioxidant and Neuroprotective Activities of Algae Extracts
Catarina do Vale Pereira
UMinho | 2023

Universidade do Minho
Escola de Ciências

Catarina do Vale Pereira

Antioxidant and Neuroprotective Activities of Algae Extracts

Master's dissertation
Master in Applied Biochemistry

Work developed under supervision of
Professor Doctor Rui Pedro Soares Oliveira

outubro de 2023

Despacho RT - 31 /2019

DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

Este é um trabalho académico que pode ser utilizado por terceiros desde que respeitadas as regras e boas práticas internacionalmente aceites, no que concerne aos direitos de autor e direitos conexos.

Assim, o presente trabalho pode ser utilizado nos termos previstos na licença abaixo indicada. Caso o utilizador necessite de permissão para poder fazer um uso do trabalho em condições não previstas no licenciamento indicado, deverá contactar o autor, através do RepositóriUM da Universidade do Minho.

Acknowledgements

I want to express my sincere gratitude to all the individuals and entities who contributed to the success of this academic journey:

To my esteemed supervisor, Dr Rui Oliveira, for his tireless guidance, wisdom, support, and friendship throughout this period. His dedication was crucial for the development of this work.

To my lab colleagues, Adriana, Ana Teixeira, Eva, Mariana, Pedro, and Raquel, for your collaboration and knowledge sharing. Your support and friendship made this experience even more enriching.

To seaExpert company, particularly to the Commercial Director, Artur Oliveira, I would like to express my gratitude for their generosity in providing the algae samples that were essential for this study. Your contribution was fundamental to the success of this research, and I am extremely thankful for your partnership and knowledge sharing.

To the Ph.D. student, Paulo Silva, for your tireless assistance in the transformation. Your dedication, competence, and support was crucial for the success of this stage.

To my family, especially my mother, father, grandmother and Hugo, my gratitude is eternal. Your love, tireless support, and understanding throughout this journey have been my safe haven. Without you, none of this would be possible. Thank you for believing in me and for your constant encouragement.

To my friends, who have always been by my side, sharing joys and difficulties, I thank you from the bottom of my heart. Your presence was a source of comfort in the most challenging moments.

To the technical staff of the Department of Biology for their assistance during this work.

To all those mentioned and to all who, in some way, contributed to the success of this project, my sincere thanks. This work is the result of a collective effort, and without your help and support, it would not have been possible.

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

RESUMO

As doenças neurodegenerativas, caracterizadas pela degeneração progressiva do sistema nervoso representam um desafio crescente para a saúde mundial. Abordar eficazmente essas doenças exige abordagens terapêuticas inovadoras, pois os medicamentos atuais concentram-se principalmente no alívio dos sintomas e, por vezes, têm efeitos colaterais graves. Muitos medicamentos utilizados na medicina são obtidos diretamente de fontes naturais (plantas, algas, fungos e bactérias) ou modificados quimicamente para melhorar as suas propriedades. Estudos recentes revelaram o potencial promissor das algas como recurso natural com compostos bioactivos que podem ajudar na prevenção e tratamento destas doenças debilitantes. A grande diversidade de espécies de algas e seus compostos bioativos únicos, tais como fucoidanos, florotaninos e fucoxantina, oferecem uma via convincente para a neuroprotecção.

Este estudo explorou as possibilidades terapêuticas oferecidas por uma variedade de extratos de macroalgas. Inicialmente, os extratos de macroalgas fornecidos pela Universidade de Vigo foram selecionados com base na sua atividade antiradicalar usando o ensaio 2,2-Difenil-1-picrilhidrazila (DPPH). Entre esses extratos, apenas o de *Ascophyllum nodosum* (AN) exibiu uma atividade antiradicalar significativa (IC_{50} < 500 μg/mL), levando a uma investigação adicional em *Saccharomyces cerevisiae*. Posteriormente, outra fase de triagem envolveu extratos de macroalgas fornecidos pela empresa seaExpert e o extrato de AN. Esta triagem estudou a capacidade destes extratos protegerem contra a perda de viabilidade causada pelo peróxido de hidrogénio (H_2O_2), sendo que os extratos de AN, *Cystoseira humilis* (CH) e *Pterocladiella capillacea* (PC) foram capazes de aumentar a resistência das células de levedura quando expostas ao stresse oxidativo induzido pelo H_2O_2. Além disso, os extratos de AN, CH e PC foram eficazes na redução da oxidação intracelular em *Saccharomyces cerevisiae* quando expostos ao H_2O_2 e diminuíram a agregação de α-sinucleína num modelo de *S. cerevisiae* da doença de Parkinson. Ademais, apenas os extratos de CH e PC apresentaram uma redução na agregação da huntingtina num modelo de *S. cerevisiae* que reproduz a doença de Huntington. Este estudo demonstrou os efeitos promissores das algas no contexto da neurodegeneração e enfatizou a importância da pesquisa dos seus compostos bioativos para a prevenção ou tratamento de doenças neurodegenerativas.

Palavras-chave: Produtos naturais marinhos; Doença de Parkinson; Doença de Huntington; α-sinucleína; Huntingtina; Macroalgas; *Ascophyllum nodosum*; *Cystoseira humilis*; *Pterocladiella capillacea*; *Saccharomyces cerevisiae*.

ABSTRACT

Neurodegenerative diseases, characterized by progressive degeneration of the nervous system, present a growing and substantial global health challenge. Effectively tackling these diseases requires innovative therapeutic approaches, as current medications primarily focus on symptom relief and, at times, come with severe side effects. Many drugs used in medicine are directly obtained from natural sources (plants, algae, fungi and bacteria) or chemically modified to improve their properties. Recent investigations have unveiled algae as a promising natural resource that holds potential for addressing these debilitating conditions. Algae's rich diversity of species and their unique bioactive compounds, such as fucoidans, phlorotannins, and fucoxanthin, offer a compelling avenue for neuroprotection.

This study delved into the therapeutic possibilities offered by a range of macroalgae extracts. Firstly, macroalgae extracts provided by the University of Vigo were selected based on their anti-radical activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Among these extracts, only the *Ascophyllum nodosum* (AN) extract displayed notable anti-radical activity (IC_{50} < 500 µg/mL), prompting further investigation in *Saccharomyces cerevisiae*. Subsequently, another screening phase involved macroalgae extracts supplied by seaExpert company and the AN extract. This screening studied the ability of these extracts to protect against the loss of viability caused by H_2O_2. Extracts from AN, *Cystoseira humilis* (CH), and *Pterocladiella capillacea* (PC) were able to enhance the yeast cells' resistance when exposed to oxidative stress induced by H_2O_2. Furthermore, AN, CH, and PC extracts were effective in reducing intracellular oxidation in *Saccharomyces cerevisiae* when exposed to H_2O_2 and decreased the aggregation of α-synuclein in a *S. cerevisiae* model of Parkinson's disease. It's worth noting that only the CH and PC extracts exhibited a reduction in the aggregation of huntingtin in a *S. cerevisiae* model replicating Huntington's disease. This study demonstrated the promising effects of algae in the context of neurodegeneration and underscored the importance of researching their bioactive compounds for the prevention or treatment of neurodegenerative diseases.

Keywords: Marine natural products; Parkinson's disease; Huntington's disease; α-synuclein; Huntingtin*;* Macroalgae; *Ascophyllum nodosum*; *Cystoseira humilis*; *Pterocladiella capillacea*; *Saccharomyces cerevisiae*.

INDEX

ABBREVIATION LIST

AA	*Asparagopsis armata*
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADC	Antibody Drug Conjugate
ALS	Amyotrophic lateral sclerosis
Amp	Ampicilin
AN	*Ascophyllum nodosum*
APP	Amyloid precursor protein
AT	*Asparagopsis taxiformis*
Aβ	Amyloid-β
BACE1	β-Site amyloid precursor protein cleaving enzyme 1
BB	*Bifurcaria bifurcata*
BChE	Butyrylcholinesterase
BDNF	Brain-derived neurotrophic factor
CC	*Chondrus crispus*
CD	Cluster of Differentiation
CFU's	Colony-forming units
CH	*Cystoseira humilis*
ChE	Cholinesterase enzymes
CNS	Central nervous system
CT	*Codium tomentosum*
DCF	2',7'-dichlorofluorescein
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAM	*Gongolaria abies-marina*
GP	*Gigartina pistillata*
GSH-pX	Glutathione peroxidase
H_2DCFDA	2',7'-dichlorofluorescein diacetate
HD	Huntington's disease

HSP Heat shock protein

HTT Huntingtin

LB Luria-Bertani broth

LO *Laminaria ochroleuca*

MMAE Monomethylauristatin E

MS *Mastocarpus stellatus*

ND Neurodegenerative diseases

OD Optical density

PBS Phosphate buffered saline

PC *Pterocladiella capillacea*

PD Parkinson's disease

PI Propidium iodide

polyQ Polyglutamine

PP *Padina pavonica*

ROS Reactive oxygen species

SCAs Spinocerebellar ataxias

SL *Saccharina latissima*

SNpc Substantia nigra pars. compacta

SOD Superoxide dismutase

SM *Sargassum muticum*

SV *Sargassum vulgare*

TF Tissue Factor

UP *Undaria pinnafitida*

UPS Ubiquitin-proteosome

UR *Ulva rigida*

ZT *Zonaria tournefortii*

FIGURE LIST

TABLE LIST

1. INTRODUCTION

1.1. Natural Products

Since ancient times, natural products obtained from plants and animals have been used by civilizations with the aim of alleviating and curing diseases, resulting in one of the most primitive sources of medicines. Both Eastern and Western civilizations are rich in examples of the use of natural products in medicine and health, with traditional Chinese medicine being an important example of how natural products are efficient in the prevention and treatment of diseases. This effectiveness underscores the importance of scientific research on natural products in order to understand the mechanism of action of their bioactive compounds and thus benefit from the resources offered by nature.

1.1.1. Marine Natural Products

Although bacteria and terrestrial plants have always been the primary source of natural products, it was only in the last 30 years that humans have started to isolate and test invertebrates, marine plants, and marine bacteria, leading to the discovery of many new marine natural products. Over 70% of the Earth's surface is covered by oceans and seas, representing the largest habitat on Earth and an abundant resource of organisms with high biological and chemical diversity (Lu *et al.*, 2021). Of this vast area, only a tiny part (less than 5%) of the seabed has been explored and less than 0.01% has been studied in detail (Ramirez-Llodra *et al.*, 2010).

This exploration escaped the interest of natural product scientists for many years, mainly due to the challenging access to its depths. However, advancements in surveillance gear, harvesting techniques and diving equipment safety have enabled the collection of organisms such as marine algae and invertebrates, which have proven to be potential sources of bioactive compounds with unique chemical structures. Although the majority of pharmaceuticals are derived from terrestrial sources, a significant number of compounds, drug candidates, and other metabolites from marine organisms have been identified in recent years given that some examples of approved medications are present in Table 1.

Table 1. A perspective of pipeline of approved marine drugs (adapted from Malve, 2016)

Approved Marine Drugs					
Compound Name	Commercial Name	Marine Organism	Chemical Class	Molecular Target	Disease Area
Brentuximab vedotin, SGN-35	Adcetris®	Mollusk/ Cyanobacterium	ADC (MMAE)	CD30 & microtubes	Lymphoma, Hodgkin's disease
Cytarabine, ara-C	Cytosar-U®	Sponge	Nucleoside	DNA polymerase	Leukemia
Eribulin mesylate, E7389	Halaven®	Sponge	Macrolide	Microtubes	Breast cancer
Enfortumab vedotin	PADCEV®	Mollusk/ Cyanobaterium	ADC (MMAE)	Nectin-4 & microtubes	Metastatic urothelial cancer
Lurbinectedin	Zepzelca®	Tunicate	Alkaloid	RNA polymerase II	Lung cancer
Omega-3-acid ethyl esters	Lovaza®/ Omacor	Fish	Omega-3 fatty acid	Triglyceride synthesizing enzymes	Hypertriglyceridemia
Polatuzumab vedotin, DCDS 4501A	Polivy®	Mollusk/ Cyanobacterium	ADC (MMAE)	CD79b & microtubes	Diffuse large B-cells lymphoma
Tisotumab vedotin	TIVDAK®	Mollusk/ Cyanobacterium	ADC (MMAE)	TF & microtubes	Metastatic cervical cancer
Trabectedin, ET-743	Yondelis®	Tunicate	Alkaloid		Soft tissue Sarcoma and ovarian cancer
Vidarabine, ara-A	Vira-A®	Sponge	Nucleoside	DNA polymerase	Anti-viral: Herpes Simplex Virus
Ziconotide	Prialt®	Cone snail	Peptide	N-type Ca channel	Severe chronic pain

ADC: Antibody Drug Conjugate; CD: Cluster of Differentiation; MMAE: Monomethylauristatin E; TF: Tissue Factor

Portugal is a country with an extensive coastline, being the third largest maritime area in the European Union and the 20th largest in the world, with a total area of 1,728,718 square kilometers. Therefore, the Portuguese coastline, due to its rich marine biodiversity and vastness, becomes an attractive target for study, exploration, and obtaining various marine organisms and their compounds for the purpose of beneficial application.

1.2. Algae as a Resource

In 1753, the term "algae" was first introduced by Linné (1753). Algae are typically autotrophic and photosynthetic organisms that exist in most aquatic habitats and some terrestrial environments as well. There are various morphological types of algae, ranging from microscopic unicellular forms (microalgae) to large, multicellular complexes (macroalgae). Algae can be divided into prokaryotes, which include Cyanophycota (cyanobacteria) and Prochlorophycota, and eukaryotes, which are further divided into Glaucophyta, Rhodophyta, Heterokontophyta, Haptophyta, Cryptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta, and Chlorophyta (Barsanti & Gualtieri, 2014).

Currently, various species of algae, which have been considered an important natural source of secondary metabolites such as bioactive compounds and, in some cases, nutrients, have been studied. Their biological activities and beneficial effects on health have piqued the researchers' interest. The bioactive compounds found in these organisms, with antioxidant, anti-inflammatory, antibacterial, antiviral, antitumor, and other activities, have intensified the interest of the pharmaceutical industry (Mayer *et al.*, 2009).

1.2.1. Macroalgae

Macroalgae are multicellular, macroscopic photoautotrophic organisms that inhabit coastal areas and are classified into three major groups based on their pigmentation: red algae (Rhodophyta), brown algae (Heterokontophyta), and green algae (Chlorophyta) (Chan *et al.*, 2006).

Because they are sessile organisms, meaning they do not have their own mobility mechanisms, marine algae have evolved to thrive in variable, extreme, and hostile environmental conditions, such as temperature fluctuations, salinity changes, environmental pollutants, or exposure to UV radiation. This has led them to produce a wide range of secondary metabolites, including pigments, vitamins, phenolic compounds, sterols, and other bioactive compounds (Leandro *et al.*, 2020).

Red algae (more common in warm seas) contain chlorophylls a and d, carotenoids, and their coloration is due to the presence of phycoerythrin in their cells. Brown algae have pigments like fucoxanthin, chlorophylls a and c, and carotenoids. They also store oils and polysaccharides as reserve substances. Green algae produce ulvan and contain carotene, xanthophylls, and

chlorophylls a and b, supporting the idea that they are ancestral to plants (Barsanti & Gualtieri, 2014). Due to their production of these molecules, macroalgae have great commercial importance and are already used for various purposes, such as the industrial extraction of phycocolloids or the extraction of bioactive compounds with antioxidant, anti-inflammatory, antiviral, antibacterial, or antitumor activities (Leandro *et al.*, 2020). Additionally, they can also be directly or indirectly used in human and animal nutrition, as well as in agriculture as biofertilizers (Pereira, 2016).

1.2.1.1. *Ascophyllum nodosum*

Ascophyllum nodosum (Linnaeus) Le Jolis 1863 (AN) is a member of the *Fucaceae* family of the Fucales order. This brown seaweed is usually acknowledged as the only species in its genus. Within the *Fucaceae* family, the genus *Ascophyllum* is part of a specific subgroup, characterized by groups of fertile branches that develop from specific points along the margin of the primary shoot. *Ascophyllum nodosum* is found on both coasts of the North Atlantic Ocean. It is commonly attached to rocky shores spanning from Portugal to the White Sea in Europe, as well as along the coasts of Iceland, Greenland, and the stretch from Baffin Island to Delaware in North America. Additionally, this species can also be found in unattached variations, intertwined with marsh plants, situated on mudflats, or floating freely on the ocean's surface.

1.2.1.2. *Pterocladiella capillacea*

Pterocladiella capillacea (PC) is a red alga that belongs to the family *Pterocladiaceae* and is distinguished from other *Pterocladiella* species by its triangular-shaped outline, upright axes without branching at the base, and dioecious gametophyte plants. *Pterocladiella capillacea* is a worldwide distributed species, predominantly resides in tropical and subtropical waters, with a few occurrences reaching temperate latitudes. It is typically found in clusters or sometimes as unattached mats on rocks that are moderately sheltered to moderately exposed to waves, primarily in the lower portion of the upper eulittoral subzone. In the lower intertidal and shallow subtidal zones (down to 15 meters), this species attaches to rocks and is widely distributed in the Azores region. Furthermore, some research indicates that it may contain substances with antibacterial (Hamdona *et al.*, 2018), antifungal (Machado *et al.*, 2014), antihypertensive (Paiva *et al.*, 2017),

antiviral (Santos *et al.*, 1999), anti-inflammatory (Silva *et al.*, 2010) and antioxidant properties (Vega *et al.*, 2020), and possibly other beneficial effects.

1.2.1.3. *Cystoseira humilis*

Cystoseira humilis Schousboe ex Kutzing (CH) is a brown seaweed that falls under the subclass *Fucophycidae,* and the family *Sargassaceae* (Guiry M.D. & Guiry, 2023). One of the distinguishing features of *C. humilis* is the presence bladder-like structures, called pneumatocysts, help the algae stay buoyant in water and provide a means of gas exchange. Among the Fucales order, this seaweed stands out for its wide distribution, being present in the major oceans around the world. The extracts derived from *C. humilis* display various beneficial properties such as antibacterial, antioxidant, and cytotoxic effects (Boujaber *et al.*, 2013; Grina *et al.*, 2020; Grozdanić *et al.*, 2016; Ibtissam *et al.*, 2010). Due to its content of diverse biomolecules, *C. humilis* is highly regarded for potential applications in medicine, cosmetics, and as a source of nourishment (Belattmania *et al.*, 2016).

1.3. Bioactivity of Algae Compounds

Several compounds of diverse chemical classes have been reported from three major groups (brown, red, and green algae) of marine algae (Figures 1). Presently, several lines of studies have provided insight into biological activities and neuroprotective effects of algal metabolites including antioxidant, anti-aggregating, and cholinesterase inhibitory activity. Neuropharmacological properties of these compounds reported in various models are discussed in the following subsections.

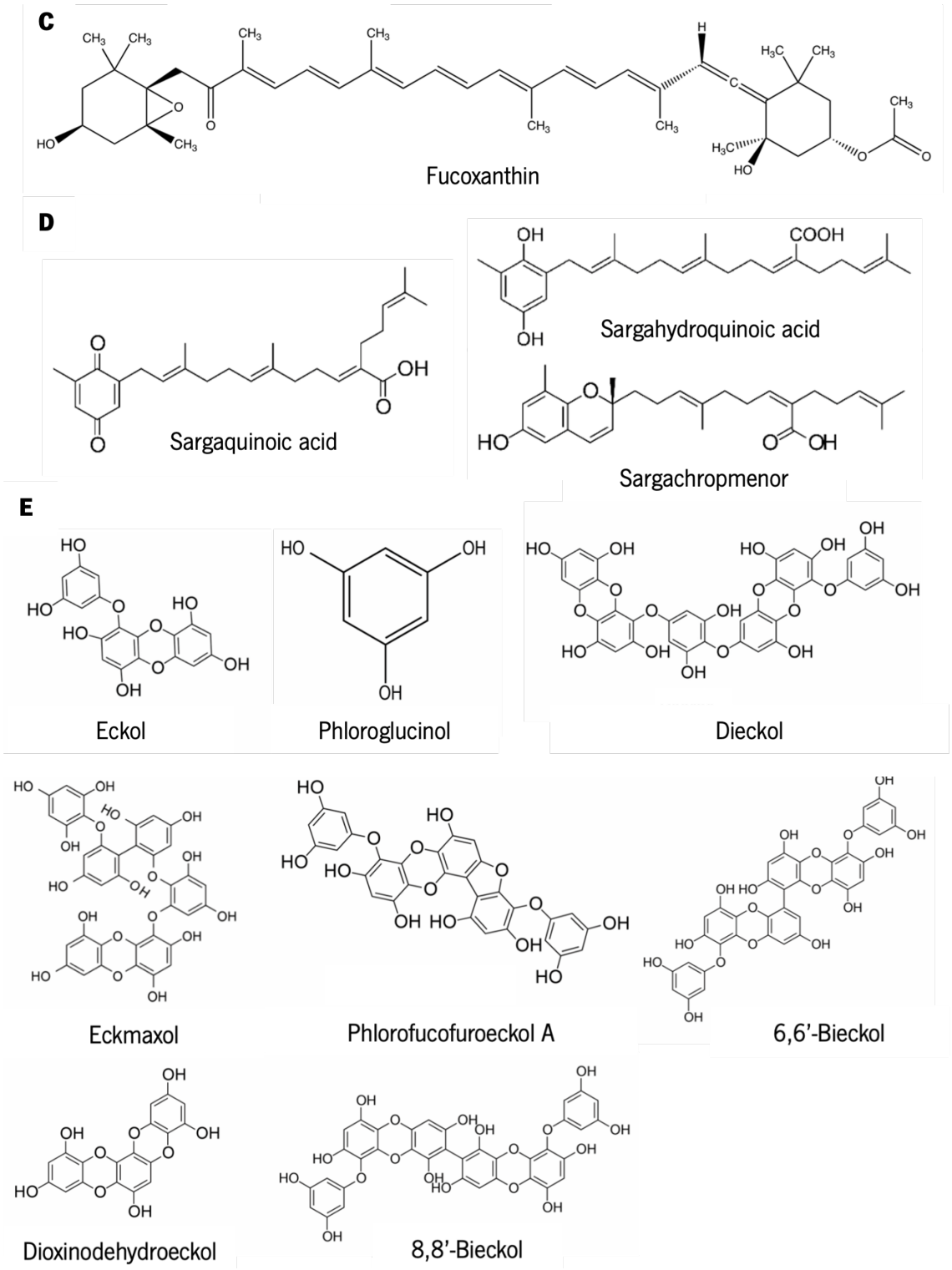


Figure 1. Chemical structure of examples of compounds from different classes: sterols (A), polysaccharides (B), carotenoid (C), meroterpenoids (D) and phlorotannin's of marine algae (E).

1.3.1. Antioxidant activity

Oxidative stress is the result of an imbalance between reactive oxygen species (ROS) and the antioxidant defense mechanisms, a situation where the antioxidants are incapable of neutralizing the ROS effectively. This condition may result from an overabundance of ROS, a deficiency of antioxidant defenses, or a combination of both factors (Barnham *et al.*, 2004). Compared to other parts of the body, the central nervous system (CNS) is more prone to oxidative stress due to its high oxygen consumption and lipid content (Akyol *et al.*, 2002). Oxidative stress in the CNS has been shown to involve excitotoxicity and apoptosis, the two main causes of neuronal death. Furthermore, oxidative stress has been implicated in the progression of Alzheimer's disease (AD), Parkinson's disease (PD), and other neurodegenerative diseases (Behl & Moosmann, 2002; Migliore & Coppedè, 2009).

The most accepted definition for antioxidant refers to any compound that, even present in low concentrations compared to the oxidant substrate, can delay or inhibit the oxidation rates caused by free radicals (Sardas, 2003; Sies, 1995). Thus, antioxidants protect the body against damage caused essentially by ROS, such as hydrogen peroxide, superoxide anion and hydroxyl radicals.

Several compounds derived from algae have been identified and isolated for their antioxidant activity, making them natural antioxidants (Kang *et al.*, 2015). Among the secondary metabolites with antioxidant activity, the following are noteworthy: florotannins (phenolic compounds); fucoxanthin (caratenoid); fucosterol (sterol) and fucoidans (sulfated polysaccharides).

Studies conducted on *Cystoseira trinodis* extracts have shown that its antioxidant activity is directly related to the high concentration of phenolic compounds, especially florotannins (Sathya *et al.*, 2017). Florotannins donate a hydrogen atom to the free radical, resulting in its inactivation, and they also work by breaking the chain reactions of oxidation caused by free radicals (Airanthi *et al.*, 2011). Fucoxanthin is a carotenoid present in brown seaweeds and has been described in macroalgae and microalgae, such as: *Undaria pinnnatifida*, *Laminaria japonica*, *Phaeodactylum tricornutum*, *Cylindrotheca closterium*, *Padina tetrastromatica* and *Sargassum siliquastrum* (Heo *et al.*, 2008; Kim & Pangestuti, 2011; Kim *et al.*, 2012; Kumar *et al.*, 2008; Sangeetha *et al.*, 2009). Its structure is unique and was determined by Englert et al. (Figure 1C). Fucoxanthin, unlike other common carotenoids such as β-carotene, has allenic bonds, 5,6-monoepoxide and acetyl group (Maeda *et al.*, 2018). Several studies have demonstrated the antioxidant activity of this

carotenoid that protects cells from free radical damage (Heo *et al.*, 2008; Heo & Jeon, 2009; Sangeetha *et al.*, 2009).

Fucosterol is the most abundant sterol in brown macroalgae such as *Eisenia bicyclis*, *Ecklonia stolonifera*, *Pelvetia siliquosa*, *Sargassum fusiforme* (Choi *et al.*, 2015; Lee *et al.*, 2003). Its antioxidant activity is related to the fact that fucosterol increases the activity of free radical scavenging enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-px) and negatively regulates the activity of transaminases (Lee *et al.*, 2003).

The antioxidant activity of fucoidans results from their ability to scavenge free radicals (Pereira, 2018). Several *in vitro* and *in vivo* studies have been conducted to prove this activity. Fucoidans extracted from *Fucus vesiculosus*, have proven free radical scavenging activity (Rocha de Souza *et al.*, 2007) and studies on *Laminaria japonica* Aresch (Laminariales) demonstrated the ability of fucoidans to prevent hydrogen peroxide-induced apoptosis in a cell line derived from a rat adrenal medulla pheochromocytoma (PC12 cells; Gao *et al.*, 2012).

1.3.2. Anti-aggregation activity

Neurodegenerative diseases are generally characterized by the presence of neurotoxic amyloid aggregates underlying progressive neuronal death (Chiti & Dobson, 2006; Currais *et al.*, 2017). Therefore, scientific research is continuously seeking for novel natural molecules that have the ability to block the formation of toxic amyloid fibrils from different proteins. Several metabolites of marine algae have shown anti-amyloidogenic potentials. For example, fucoxanthin has shown considerable effectiveness in preventing the formation of amyloid-β (Aβ) assemblies, likely by engaging in hydrophobic interactions. Furthermore, it has been revealed that fucoxanthin holds strong potential in reversing the negative impacts on learning and memory induced by Aβ oligomers. This effect is achieved through the inhibition of oxidative stress, augmentation of Brain-derived neurotrophic factor (BDNF) expression, and enhancement of the cholinergic system (Xiang *et al.*, 2017). Aβ peptides are produced when the amyloid precursor protein (APP) is broken down through a two-step process involving enzymes called β-secretases and γ-secretases (Zheng & Koo, 2011). One of these enzymes, known as β-Site amyloid precursor protein cleaving enzyme 1 (BACE1), is a type of protease that is embedded in cell membranes (Vassar *et al.*, 1999; Lin *et al.*, 2000). In AD, researchers have found that the amount of BACE1 protein and its activity are increased in the brain (Yang *et al.*, 2003). Since this discovery, researchers have been looking into

using BACE1 as a target for developing drugs. They have found that certain natural compounds, like fucoxanthin and fucosterol from *Ecklonia stolonifera* and *Undaria pinnatifida*, can inhibit BACE1 (Jung *et al.*, 2016). Another compound called α-Bisabolol, which comes from *Padina gymnospora*, not only prevents the clumping together of harmful proteins but also breaks down these protein aggregates (Shanmuganathan *et al.*, 2015). Additionally, some substances found in algae like *Ecklonia cava* and *Sargassum serratifolium*, such as phlorotannins and meroterpenoids, have demonstrated anti-BACE1 activity (Lee & Jun, 2019; Seong *et al.*, 2017). Furthermore, in a study by Olasehinde et al., it was found that four types of seaweed from South Africa - *Gracilaria gracilis*, *Ulva lactuca*, *Ecklonia maxima*, and *Gelidium pristoides* - can inhibit BACE1 and prevent the clumping of harmful proteins (Olasehinde *et al.*, 2019).

1.3.3. Inhibition of acetylcholinesterase

Cholinesterase enzymes (ChE) are a group of esterases capable of hydrolyzing choline esters, producing choline and acetic acid, which are involved in the proper functioning of the nervous system. There are two types: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), also known as pseudocholinesterase. The difference between the two types is related to their respective substrate preferences. AChE hydrolyses the neurotransmitter acetylcholine (ACh) while BChE hydrolyses a wide variety of choline and non-choline esters (de los Ríos, 2012; Mendel & Rudney, 1943).

According to the cholinergic hypothesis, a major loss of cholinergic function in the CNS contributes significantly to the cognitive symptoms associated with AD (Bartus, 2000). In line with this, neuropathological studies have shown that AD is associated with a deficiency of the neurotransmitter ACh (Tabet, 2006). Cholinesterase inhibitors play a role in maintenance acetylcholine neurotransmitter levels by inhibiting the enzymes involved in its degradation, AChE and BChE (de los Ríos, 2012). Therefore, inhibition of the AChE, which specifically catalyzes the degradation of acetylcholine, may be one of the most realistic approaches for the symptomatic treatment of AD (Pangestuti & Kim, 2010).

Recently, several *in vitro* studies conducted on various algal species have shown that several of their secondary metabolites possess AChE inhibitory activity (Table 2).

Table 2. AChE inhibitory activity of algal compounds *in vitro*.

AChE Inhibitory Activity			
Compound (class)	Algal Source	Cellular Effects	Reference
Fucosterol	*Ecklonia stolonifera*	↓BChE activity	(Yoon *et al.*, 2008)
fucoxanthin	Brown seaweed	↓AChE activity	(Lin *et al.*, 2016)
α-Bisabolol	*Padina gymnospora*	↓AChE and BChE activity	(Shanmuganathan *et al.*, 2015)
Glycoprotein	*Undaria pinnatifida*	↓AChE and BChE activity	(Rafiquzzaman *et al.*, 2015)
Fluorotannins (Dieckol and Phlorofucofuroeckol)	*Ecklonia cava*	↓AChE activity	(Lee & Jun, 2019)
Meroterpenoids (Sargaquinoic acid and sargachromenol)	*Sargassum sagamianum*	Sargaquinoic acid shows potent inhibitory activity against BuChE and moderate inhibitory activity against AChE	(Choi *et al.*, 2007)

In addition to the above, some seaweed extracts have exhibited anticholinesterase properties. These include *Asparagopsis taxiformis* (Nunes *et al.*, 2020), *Botryococcus braunii* and *Nannochloropsis oculata* (Custódio *et al.*, 2015), *Cystoseira tamariscifolia* and *Cystoseira nodicaulis* (Custódio *et al.*, 2016), *Halimeda cuneata* (Rengasamy *et al.*, 2015) and *Padina australis* (Murugan *et al.*, 2015).

1.3.4. Neuroprotective Activity

Compounds exhibiting antioxidant, anti-inflammatory, anti-aggregant and anticholinesterase activities, such as those presented in this work, have neuroprotective potential. Fucoxanthin is one such example, and several studies have reported its neuroprotective activity (Alghazwi *et al.*, 2019; Hu *et al.*, 2018; Ikeda *et al.*, 2003; Lin *et al.*, 2017; Mohibbullah *et al.*, 2018; Yu *et al.*, 2017; Zhang *et al.*, 2017). The active compound, α-bisabolol, found in *Padina gymnospora*, is able to protect against neurotoxicity induced by Aβ fragment 25-35 (Aβ25-35; Shanmuganathan *et al.*, 2018, 2019). The neuroprotective role of fucosterol lies in attenuating the neurotoxicity induced by Aβ (Gan *et al.*, 2019; Oh *et al.*, 2018). On the other hand, phlorotannin's have shown significant neuroprotective effects in various neurotoxicity models (Table 3). Therefore,

researchers are currently interested in studying natural bioactive compounds that can act as neuroprotective agents, with algae being a potential candidate.

Table 3. Neuroprotective activity of phlorotannin's in various neurotoxicity models.

Neuroprotective Activity of Phlorotannin's			
Compounds	Algal Source	Cellular Effects	Reference
Eckol, dieckol and 8,8'-bieckol	*Ecklonia cava*	Neuroprotection against Aβ25-35-mediated cytotoxicity in PC12 cells	(Lee *et al.*, 2018)
Phloroglucinol, eckol, triphloroethol A, eckstolonol and dieckol	*Ecklonia cava*	Attenuated H_2O_2-induced oxidative damage in HT22 hippocampus neurons	(Kang *et al.*, 2012)
Phloroglucinol, dioxinodehydroeckol, eckol, phlorofucofuroeckol A, dieckol, and 7-phloroeckol	*Eisenia bicyclis*	Protected against Aβ-induced cytotoxicity	(Ahn *et al.*, 2012)
Phlorofucofuroeckol	Brown algae	Attenuated glutamate-induced cytotoxicity and improved mitochondrial dysfunction in PC12 cells	(Kim *et al.*, 2016)
Eckmaxol	*Ecklonia maxima*	Reduced Aβ-oligomer-induced neuronal apoptosis in SH-SY5Y cells	(Wang *et al.*, 2018)

1.4. Neurodegenerative Diseases

In the last few years, there has been a significant increase in the number of people affected by neurodegenerative diseases (ND) due to the growing world population and the increased life expectancy, with aging being one of the main risk factors for neurodegeneration (Kester & Scheltens, 2009). Neurodegenerative diseases are characterized by the progressive loss of cells of the central and peripheral nervous system (Ross & Poirier, 2004; Taylor *et al.*, 2002). This neuronal loss can affect both body movement, leading to ataxias, and brain function, resulting in dementia. Although several ND have been identified, the most studied ones include AD, spinocerebellar ataxias (SCAs), PD, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), among others. These diseases are typically progressive, late onset and belong to a group of disorders that still remain incurable. Pathologically, they also share common features, often involving abnormal accumulation and aggregation of proteins that, depending on the protein(s) and brain region(s) involved, culminate in different symptomatology (Ross & Poirier, 2004).

1.4.1. Protein Misfolding and Neurodegenerative Diseases

In healthy cells, the process of protein folding is tightly regulated, and misfolded proteins are usually managed through various quality control mechanisms to prevent their accumulation and aggregation. Chaperones are a group of proteins that play a crucial role in assisting protein folding, trafficking, and maintaining protein stability. Chaperones help newly synthesized proteins achieve their correct three-dimensional structures and prevent the formation of non-functional or aggregation-prone conformations. If a protein starts to misfold, chaperones can either facilitate proper refolding or target the misfolded protein for degradation by the cell's protein degradation machinery, such as the proteasome or lysosomes (Hartl & Hayer-Hartl, 2009). A classic example of molecular chaperones is the heat shock proteins (HSP), a group of proteins activated by heat shock. The general purpose of HSPs, therefore, is to combat the denaturing effects of these stress-inducing situations, their activation in response to these conditions helps maintain and restore proper protein folding (De Maio, 1999). Another molecular chaperone is the chaperonins, which have a "box" or "chamber" structure where target proteins can fold in an isolated environment within the cell (Horovitz *et al.*, 2022). In NDs, a specific key protein is repeatedly produced and misfolded into abnormal, toxic forms that serves as a biomarker for that disease. This misfolded protein manages to escape both the cellular machinery responsible for proper protein folding and the mechanisms for cellular degradation. Consequently, it begins to aggregate, serving as a nucleus for the formation of larger fibrillar aggregates (Figure 2; Rochet & Lansbury, 2000). When these fibrils form outside cells, they are referred to as amyloid fibrils. On the other hand, when they form inside cells, they are known as intracellular inclusions. These aggregates can interfere with cellular processes, disrupt intracellular trafficking, and lead to cellular dysfunction and ultimately cell death, since they are often toxic to neurons and neighboring cells, they can induce oxidative stress, trigger inflammation, disrupt cell membranes, and interfere with normal cellular signaling pathways (Chiti & Dobson, 2006).

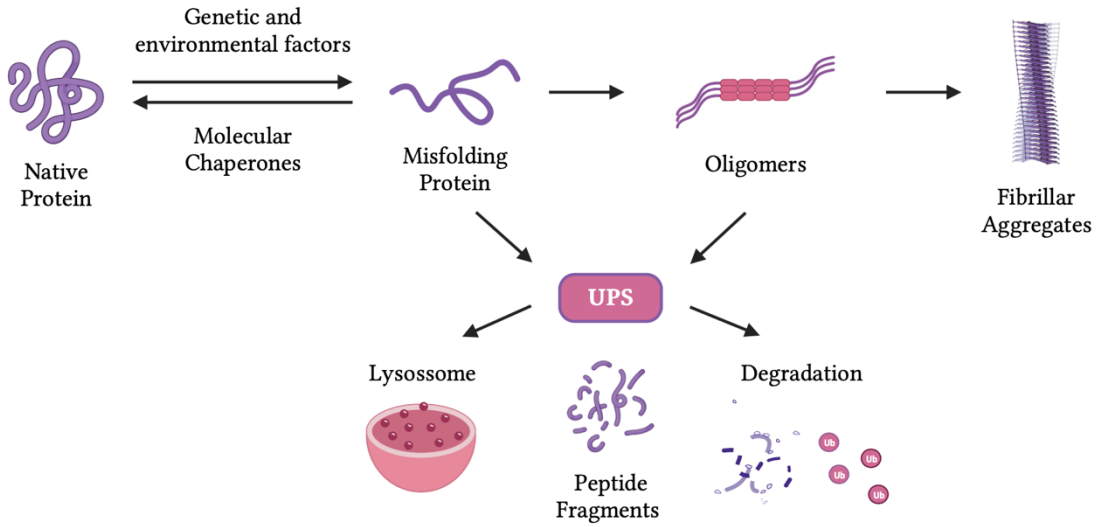


Figure 2. Model of misfolded proteins and fibrillar aggregation that leads to the deposition of protein aggregates (adapted from Saadallah, 2013).

1.4.2. Neurodegenerative diseases and Oxidative Stress

The cellular environment is subject to various forms of stress, including oxidative stress caused by ROS. ROS are highly reactive molecules that contain oxygen and can be generated during the normal metabolism of cells. They play an important role in various cellular processes, such as cell signaling and immune response. However, when there is an imbalance between the generation of ROS and the antioxidant defense mechanisms of cells, oxidative stress occurs. This can result in cellular damage, including damage to protein, lipids and nucleic acids such as DNA. The accumulation of oxidative damage over time is associated with aging and the development of various diseases, such as neurodegenerative disorders (Behl & Moosmann, 2002; Dröge, 2002; Migliore & Coppedè, 2009).

The brain is more susceptible to the harmful effects of oxidative stress due to the combination of several factors such as: abundance of mitochondria (a significant source of ROS during the energy production process); high oxygen consumption, making it more prone to ROS generation during cellular respiration; high content of polyunsaturated fatty acids, which are susceptible to oxidation; lower antioxidant efficiency from antioxidant enzymes and ROS-neutralizing molecules; and its limited capacity for neuronal replication, meaning that damage accumulated over time may be less repairable, leading to an accumulation of damage and dysfunction (Li *et al.*, 2013; Niedzielska *et al.*, 2016).

They play a crucial role in maintaining the redox balance in the body, protecting cells against excessive oxidative damage (Sardas, 2003; Sies, 1995). Given that oxidative stress plays a role in the pathogenesis of neurodegenerative diseases, antioxidants can be useful tools in the treatment and prevention of these diseases (Chen & Zhong, 2014). However, it is important to note that the role of antioxidants in treating neurodegenerative diseases is complex and not fully understood yet.

1.4.3. Parkinson Disease

Parkinson's disease was first described in 1817 by the British physician James Parkinson in his famous monograph "An Essay on the Shaking Palsy" (Parkinson, 1817). It is a slowly progressive neurodegenerative disease with a long and prolonged course, manifested by motor and non-motor symptoms (Gallagher & Schapira, 2009; Kouli *et al.*, 2018).

PD is the second most common neurodegenerative disease, after AD (de Lau & Breteler, 2006). It is a multifactorial disease, whose onset is associated with various contributing factors, including genetic and environmental factors. Age is the major risk factor for PD, with the mean age of onset being 60 years (Lees *et al.*, 2009).

1.4.3.1. Clinical Aspects of PD

The associated features of PD are motor symptoms, namely tremor (Jankovic *et al.*, 1999), rigidity (Riley *et al.*, 1989) and bradykinesia (slow movement; (Berardelli *et al.*, 2001), with postural instability often appearing as the disease progresses (Williams *et al.*, 2006). However, PD is also associated with many non-motor symptoms, which often precede the motor symptoms.

Non-motor symptoms include: reduced olfactory capacity, autonomic dysfunction, pain, fatigue, sleep disturbance, and cognitive and psychiatric disorders (Frucht, 2004; Kouli *et al.*, 2018). These symptoms have a significant impact on the patient's quality of life, as they contribute significantly to their disability (Martinez-Martin *et al.*, 2011).

1.4.3.2. Neuropathology of PD

The neuropathology of PD is characterized by a selective loss of dopaminergic neurons in the susbtancia nigra pars. compacta (SNpc) associated with Lewis pathology.

Macroscopically, the main distinct morphological change in the brain of a patient with PD, is seen in the brainstem, where nearly all cases exhibit depigmentation in the SNpc and locus coeruleus. This loss of pigmentation is directly correlated with the death of dopaminergic neurons containing neuromelanin in the SNpc and of noradrenergic neurons in the locus coeruleus (Dickson, 2012).

Microscopically, the pathological hallmark of PD is the presence of abnormal cytoplasmic inclusions in neuronal cell bodies that are immunoreactive for the α-synuclein protein. These pathological protein aggregates are called Lewis bodies and are often accompanied by Lewis neuritis (Spillantini *et al.*, 1997).

1.4.4. Huntington's Diseases

In 1872, George Huntington documented a description of a genetic movement disorder that is currently recognized as Huntington's disease. HD is an autosomal dominant neurodegenerative disease characterized by a mutated form of the huntingtin (HTT) protein caused by an expanded CAG repeat in the huntingtin gene on chromosome 4 (Bates, 2003; Ross & Tabrizi, 2011). This mutation involves the elongation of a polyglutamine (polyQ) segment within the protein, resulting in its improper folding and the formation of clumps (MacDonald *et al.*, 1993). These aggregates are a hallmark of the disease and contribute to its neurodegenerative effect (DiFiglia *et al.*, 1997; Hackam *et al.*, 1998).

1.4.4.1. Clinical Aspects of HD

The symptoms of Huntington's disease can emerge during any life stage, although they commonly manifest for the first time during a person's 30s or 40s (Harper, 1992). In cases where the disorder emerges prior to the age of 20, it is referred to as juvenile Huntington's disease (Bates *et al.*, 2015). When Huntington's disease starts at an early age, the symptoms exhibit some differences, and the progression of the disease might be swifter.

This condition significantly affects an individual's capacity to carry out everyday tasks and typically and usually results in movement, cognitive function, and psychiatric disorders. The unique movement impairment is the defining feature of this condition. The most notable aspect is chorea, which involves rapid involuntary motions affecting the face, trunk, and limbs. However, chorea is

not the exclusive motor trait (Roos, 2010). Individuals with Huntington's disease also exhibit dystonia, which are gradual twisting movements of the limbs, along with bradykinesia, a deceleration in executing movements, and limb rigidity like what's observed in PD (Hayden, 2012). In cognitive domains the significant alterations primarily affect areas involving psychomotor and executive abilities, memory, the processing of emotions, and social cognition.

1.4.4.2. Neuropathology of HD

Neuropathological studies propose that the disease-causing mechanism in HD might involve the creation of clumps of ubiquitinated proteins, specifically originating from the N-terminal section of the mutated huntingtin protein. This accumulation is believed to be triggered by an increased breakdown and aggregation of the polyglutamine-rich portion within the mutated huntingtin's N-terminus (Gutekunst *et al.*, 1999; Sieradzan *et al.*, 1999; Vonsattel, 2008). These clumps of the mutated protein are commonly found in various brain regions such as the neocortex, entorhinal cortex, subiculum, hippocampal pyramidal neurons, and striatum, especially in more advanced cases or when HD develops in younger individuals. However, these protein aggregates are less prevalent in regions like the globus pallidus, *substantia nigra*, and cerebellum (DiFiglia *et al.*, 1997). Interestingly, some of these protein clusters in HD-affected brains share a structure similar to amyloids, which draws parallels to aggregate formation seen in diseases like Alzheimer's and prion diseases (McGowan *et al.*, 2000). As neurons die, the affected regions of the brain shrink in volume. This progressive atrophy is most pronounced in the striatum but also affects other regions, including the cerebral cortex. Neuroimaging studies, such as MRI scans, can detect and quantify this brain atrophy, and it correlates with disease progression and symptom severity (Figure 3).

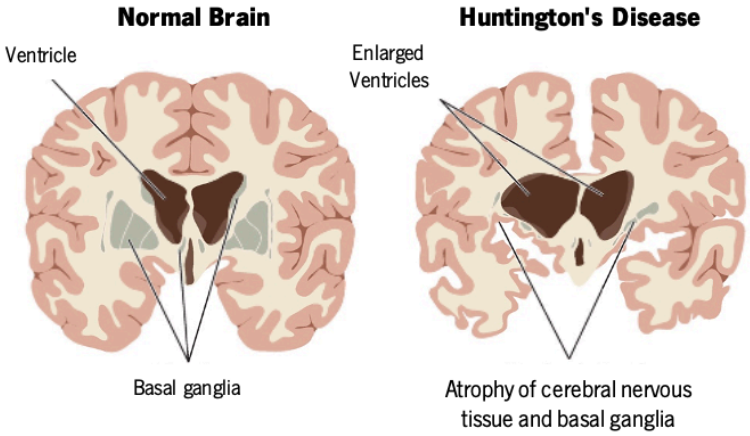


Figure 3. Representation of a healthy brain and one with Huntington's disease.

1.5. Pharmacological Therapies

Thus far, these neurodegenerative diseases remain without any effective treatment despite the increasing number of therapies evaluated in various models (Switonski *et al.*, 2012). Currently, only therapies that provide limited relief of their symptoms are available (Bauer & Nukina, 2009; Saute & Jardim, 2015). However, the identification of new therapeutic strategies for these diseases has been an intense area of study. Often, compounds approved for treatment/application in the context of other diseases are tested in the context of neurodegenerative diseases, in order to accelerate the process of identifying therapies for these diseases.

Many efforts to find new therapeutic strategies have focused on decreasing the expression of the genes and proteins causing these diseases or minimizing the dysfunction of cellular mechanisms that are affected through: activation of autophagy and the ubiquitin-proteosome (UPS) pathway to promote the degradation of these proteins (Yao, 2010); induction of chaperones, which accompany and assist the correct folding of proteins' structures; or even attempting to reverse mitochondrial dysfunction itself (Bauer & Nukina, 2009), among others.

2. THE AIM

Marine organisms have proved to be important producers of bioactive compounds with a wide range of biological activities and therefore of great pharmacological interest. In this context, algae have been little explored, and it is important to study and characterize them in order to select new sources of extracts and compounds to investigate their properties.

Neurodegenerative diseases represent one of the most formidable challenges in the realm of medicine. These conditions, which encompass a range of debilitating disorders share a common thread: the gradual and relentless deterioration of nerve cells in the brain and nervous system. Yet, the path to tackling these diseases is fraught with a multitude of difficulties. Unfortunately, there are no definitive cures for most neurodegenerative diseases. This stark lack of a cure is rooted in the intricate and multifaceted nature of these conditions, involving a complex interplay of biological processes within the brain. Moreover, early diagnosis is paramount for effective treatment, but these diseases are often insidious, evading detection until they have wrought significant damage. Additionally, developing effective therapies involves a delicate balance between potential benefits and side effects.

Therefore, the main goal of this project is to discover natural sources of compounds that can prevent or treat ND, as well as to emphasize the importance of algae as a valuable resource for pharmaceutical development, thereby aiding in the creation of treatments and preventive measures for neurodegenerative diseases. For this purpose, we will identify macroalgae with antioxidant and neuroprotective activity in the biological model *Saccharomyces cerevisiae*. This research also delved into uncovering the targets and pathways activated by the most effective seaweed extract, shedding light on how it works. In doing so, it contributed to two trending areas of interest: the utilization of marine resources and the study of ND.

3. MATERIALS AND METHODS

3.1. Sample Harvesting

The plant material was collected through manual harvesting by hand or with cutting tools, on the Galician coast and in Faial island, in the Azores, by the companies Algamar and seaExpert, respectively (Table 2). The seaweed collected by Algamar was provided by the University of Vigo in extract form, while the samples provided by seaExpert were dehydrated (dark dried or sun dried, depending on the species) and ground (with granulometry of 0.2 mm).

Table 4. Algae tested in this study

Algae			
Species	Origin	Type of drying	Provided by
Ascophyllum nodosum (AN)	Galician coast	Dark	University of Vigo
Asparagopsis armata (AA)	Faial island, Azores	Dark	seaExpert
Asparagopsis taxiformis (AT)	Faial island, Azores	Dark	seaExpert
Bifurcaria bifurcata (BB)	Galician coast	Dark	University of Vigo
Chondrus crispus (CC)	Galician coast	Dark	University of Vigo
Codium tomentosum (CT)	Galician coast	Dark	University of Vigo
Cystoseira humilis (CH)	Faial island, Azores	Sun	seaExpert
Gigartina pistillata (GP)	Galician coast	Dark	University of Vigo
Gongolaria abis-marina (GAM)	Faial island, Azores	Dark	seaExpert
Halopteris scoparia (HS)	Faial island, Azores	Dark	seaExpert
Laminaria ochroleuca (LO)	Galician coast	Dark	University of Vigo
Mastocarpus stellatus (MS)	Galician coast	Dark	University of Vigo
Padina pavonica (PP)	Faial island, Azores	Dark	seaExpert
Pterocladiella capillacea (PC)	Faial island, Azores	Dark	seaExpert
Saccharina latissima (SL)	Galician coast	Dark	University of Vigo
Sargassum muticum (SM)	Galician coast	Dark	University of Vigo
Sargassum vulgare (SV)	Faial island, Azores	Sun	seaExpert
Undaria pinnafitida (UP)	Galician coast	Dark	University of Vigo
Ulva rigida (UR)	Galician coast	Dark	University of Vigo
Zonaria tournefortii (ZT)	Faial island, Azores	Sun	seaExpert

3.1.1. Algae Provided by University of Vigo

The seaweed collected by Algamar was provided by the University of Vigo in extract form. They were thoroughly rinsed with tap water to eliminate salt, sand, and any other debris. Subsequently, the samples were placed in plastic zip bags and stored at -80°C, then freeze-dried using a LyoAlfa10/15 machine (Telstar, Thermo Fisher Scientific). After that, they were ground into a fine powder using a blender and kept at -20 °C until further processing. All the samples underwent a heat-assisted extraction process, where 15 g of algae were mixed with 100 mL of 60% (v/v) methanol for 3 hours at 45 °C while shielded from light. The resulting extracts were later condensed in a rotary evaporator at 40 °C to obtain dry extracts, and stock solutions of 50 mg/mL were prepared using pure dimethyl sulfoxide (DMSO).

3.1.2. Algae Provided by seaExpert

The seaweed provided by seaExpert was harvested off the coast of Faial island and supplied in a dehydrated and ground form right after being harvested in their ideal harvesting season, considering each species' maximum development stage in the wild. The seaweed samples were dried using two different methods: sun-drying, which took 3-5 days at a temperature of 20 ± 5 °C, and dark-drying, which took 2-5 days at 15 ± 5 °C. Both drying processes were carried out in an outdoor setting.

3.2. Sample Extraction

Dried algae (1.2 g) were mixed with 40 mL of ethanol (EtOH). The mixture was incubated at 50 °C in an orbital shaker at 150 rpm for 24 h in the dark. The supernatant was removed, and the pellet was re-extracted twice with 20 mL of solvent using the same conditions described before but for one hour. Afterwards, the total volume was collected and centrifuged at 4800 rpm for 8 min to eliminate the remaining solid particles. The extracts were then concentrated in a rotary evaporator at 40 °C to obtain the dry extracts. Dry extracts were then resuspended in 10 mL of distilled water and stored at -80 °C and further lyophilized. The obtained powdered material was stored at −20 ºC until use and 50 mg/mL stock solutions were made in pure DMSO.

3.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

In order to carry out the first analysis of the algae extracts, we conducted a test known as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This assay is based on measuring the absorption capacity of antioxidants, where the unpaired electron of the nitrogen atom in DPPH is reduced when it receives a hydrogen atom from antioxidants, forming hydrazine. When the DPPH solution is mixed with a substance that can donate a hydrogen atom, hydrazine is obtained and simultaneously the color changes from violet to yellow (Kedare & Singh, 2011). Several concentrations of each extract were assessed: 31.25, 62.5; 125; 250; 500; 1000 μg/mL. This experiment was carried out in 96-well plates and in each well the extract was mixed with DPPH (0.04%; EtOH). The plate was incubated for 1 hour at room temperature in the dark, and the absorbance was read at 515 nm with a microplate reader (SpectraMax Plus 384, USA). Each concentration had a blank and a negative control. The blank consisted of mixing the extract with absolute ethanol and the control is DPPH mixed with absolute ethanol. The absorbance values obtained were used to calculate the percentage of antioxidant activity using equation 1, where A_c is the absorbance of the negative control; A_s is the absorbance of the samples for each concentration; A_b is the absorbance of the blank of the samples for each concentration. The efficient concentration (IC_{50}) of each extract was calculated from the percent reduction of the DPPH curve and represents the concentration required to neutralize 50 % of the initial amount of DPPH radical.

$$\% \, DPPHr = \frac{A_c - (A_s - A_b)}{A_C} \times 100 \qquad \text{(Equation 1)}$$

3.4. Strains, Medium and Growth Conditions

Saccharomyces cerevisiae and the mutant strains used in this study are listed in Table 2. Cultures of strains BY4741, W303-1A, SNCA WT, SNCA A53T, and SSB1 were maintained on YPDA medium plates (1 % w/v yeast extract BD BactoTM, 2 % w/v BD BactoTMpeptone, 2 % w/v dextrose and 2 % w/v Labchem agar agar). Strains HTT 25Q, HTT 103Q, SSA2, and HSP104 were kept in minimal medium containing 0.67% (p/v) YNB without aminoacids (Sigma Life Science), 2% (w/v) glucose and supplemented with the nutritional requirements. All yeast cultures were

incubated for 48 h at 30 °C and stored at 4° C. Pre-inoculum of BY4741, W303-1A and derived mutant strains were prepared using an isolated colony from the stock culture and subsequently suspended in the same medium used for yeast culture, but in liquid form (YPDA without agar), and incubated overnight at 30 °C and 200 rpm. Growth was monitored by spectrophotometry at OD_{600}. Following the growth of cell cultures Table 3, the strains that had undergone transformation were subjected to two washes using sterilized 1x PBS at 25 °C and a centrifugation speed of 5000 g. The cells were then suspended again, this time in SCG medium with 0.67% YNB without amino acids, 2% galactose (w/v) and supplemented with the necessary nutrients. All media and solutions used can be found in: Supplementary Table 1.

Table 5. Genotype of *Saccharomyces cerevisiae* strains used in the study.

Yeast Strain				
Description	Name	Genotype	Origin	University
Wild type	BY4741	*MATa his3Δ1leu2Δ0met15Δ0ura3Δ0*	-	-
	W303-1A	*MATa/MATα {leu2-3,11 2trp1-1 can1-100 ura3-1 ade2-1 his3-11,15}*	-	-
Transformed from W303-1A	SNCA WT	W303-1A [pRS306 GALSNCAWT::GFP]	Paula Ludovico and Belém Marques	University of Minho
	SNCA A53T	W303-1A [pRS306 GALSNCAA53T::GFP]		
	HTT 25Q	W3031-A [pRS425 GALHTT25Q::GFP]	Robert Manson	University of Leicester
	HTT 103Q	W303-1A [pRS425 GALHTT103Q::GFP]		
Transformed from BY4741	*SSA2*	BY4741 *SSA2-GFP::HIS3MX6*	Tomas Nyström	University of Gothenburg
	HSP104	BY4741 *HSP104-GFP::HIS*		
	SSB1	BY4741 *SSB1-GFP*	Gunter Kramer	University of Heidelberg

3.5. Viability Assay with *Saccharomyces cerevisiae*

Pre-inoculum was prepared from a colony of BY4741 strain in liquid YPD medium and the culture was incubated at 30 °C and 200 rpm, overnight. The optical density (OD) was measured in the next morning at 600 nm and the culture was diluted to OD_{600}=0.1 using fresh YPD medium. Thereafter, the culture was incubated for 4 h under the same conditions until OD_{600}=0.4. The culture was divided into aliquots and four conditions were prepared: negative control, positive control,

treatments with oxidative stress inducer (2.5 mM H_2O_2) and extract control. Different extracts were tested at different concentrations, for AN: 250, 500 and 1000 μg/ml and for AA, AT, CH, HS, PP, PC, GAM, SV, ZT: 1000 μg/ml. The negative control was prepared only with the cell suspension and the solvent (DMSO) and the extract control was prepared without H_2O_2 in order to see if the extract was toxic to the cells. The suspensions were incubated at 30 °C and 200 rpm for 2 h. At specific time intervals (0, 30, 60, 90 and 120 min), aliquots were harvested and serially diluted in dH_2O until a dilution factor of 10^4. Subsequently 40 μL of the last dilution from each treatment were spread 3 times on YPDA dishes and were incubated at 30 °C for 48 h. Colonies were counted and viability was calculated as percentage of colony-forming units (CFU), assuming 100% viability at time 0 min.

3.6. Intracellular Oxidation

Flow cytometry was used to analyze intracellular oxidation, using the fluorochrome 2',7'-dichlorofluorescein diacetate (H_2DCFDA), and to quantify viable cells and non-viable cells using propidium iodide (PI). H_2DCFDA accumulates intracellularly and is enzymatically deacetylated by esterases, forming the non-fluorescent product H2DCF which is impermeable to the cell. After oxidation, H_2DCF is converted into the fluorescent product 2',7'-dichlorofluorescein (DCF) allowing the cellular oxidation state to be measured, since the increase in fluorescence read at 530 nm is expected to be proportional to the concentration of ROS (Oliveira *et al.*, 2020).

Antioxidant activity was measured in *S. cerevisiae* wild-type strain (BY4741) treated with CH or PC (each at 250, 500 or 1000 μg/mL) or AN (50, 125, 250, 500 or 1000 μg/mL) with 5mM H_2O_2. Controls were prepared by replacing the extract with the respective solvent, DMSO, in the same amount as used in the higher concentration treatment. The cell suspension was incubated overnight at 30°C until OD_{600}=0.4 and the cells were washed twice with phosphate buffered saline (PBS). The cell suspension was centrifuged at 5000 rpm at 4 °C for 3 min. The cell pellet was resuspended in PBS and the OD_{600} was adjusted to 0.04. An autofluorescence sample was taken and the fluorochrome, H2DCFDA (50 μM) and PI (3 μg/mL), were added and the suspensions were incubated at 30 °C in the dark for 1 hour and 10 min, respectively. Hydrogen peroxide (5 mM) was added to the 3 treatments and the positive control. The samples were incubated for 20 min at 30 °C in the dark. Approximately 20,000 cells from each sample were analyzed at a low flow rate (10 μL/min) on an EpicsR XLTM flow cytometer (BeckmanCoulter).

DCF emits green fluorescence in presence of H_2O_2, regardless of whether the cells are alive or dead. To capture this green fluorescence, a specific set of optical components was used: a 488 nm blocking filter to isolate the appropriate wavelength of light, a 550 nm long-pass dichroic filter to separate it from other wavelengths, and a 225 nm band-pass filter to further refine the signal. This fluorescence was then measured and recorded in channel FL1. In addition to DCF, the cells were also exposed to propidium iodide (PI), a fluorescent dye. The purpose of PI was to distinguish between living and non-living cells. Only viable cells, which do not emit fluorescence in response to PI, were selected for the DCF analysis. The red fluorescence emitted by PI-treated cells was captured using a 620 nm long-pass filter and monitored in channel FL4. Concurrently, two control groups were prepared: a positive control and a negative control. These control groups were treated with H2DCFDA under the same conditions as the experimental samples. They served a crucial role in identifying and compensating for any potential overlap between the emission spectra of H2DCFDA and PI, ensuring accurate interpretation of the results. The percentage of non-oxidized and viable cells was quantified using dot-plot graphs after double staining with H2DCFDA and PI through the CytExpert 2.5 software. Non-oxidized and viable cells are in the lower left quadrant, H2DCFDA(-)/PI(-); oxidized and viable cells in the lower right quadrant, H2DCFDA(+)/PI(-); oxidized and non-viable cells in the upper right quadrant, H2DCFDA(+)/PI(+); and non-viable cells in the upper left quadrant, H2DCFDA(-)/PI(+).

3.7. Transformation of pRS425GAL-HTT-GFP Plasmids

The plasmids pRS425GAL-HTT103Q-GFP and pRS425GAL-HTT25Q-GFP were provided by Dr. Robert Manson from the University of Leicester, dried on filter paper. For plasmid assembly, transformations were carried out in *Escherichia coli* XL1-Blue strain, to amplify and store the plasmid on a large scale. The Gene Jet Plasmid Miniprep Kit (Thermo Fisher Scientific, Waltham, MA, USA) for *E. coli* was used to prepare the plasmid DNA. For *E. coli* transformation, 5 μL of plasmid DNA was added to 20 μL competent cells previously prepared according to a standard protocol (Inoue *et al.*, 1990), and then the tubes were gently inverted 4-5 times and placed on ice for 30 min. The tubes were incubated in a water bath at 42°C for 45 s and then placed back on ice for 3 min. We resuspended cells in super optimal broth (SOC). Finally, 100 μL of each cell mixture was spread on LB amp (100 μg/mL ampicillin) plates and incubated overnight at 37°C. The next day, a clone was transferred to liquid LB amp medium and incubated overnight for

plasmid purification using the Gene Jet Plasmid Miniprep Kit (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, yeast transformations were performed on W303-1A strain, as described by the high-efficiency protocol using lithium acetate (LiAc), ssDNA, and polyethylene glycol (PEG) (Figure 4; Gietz & Schiestl, 2007).

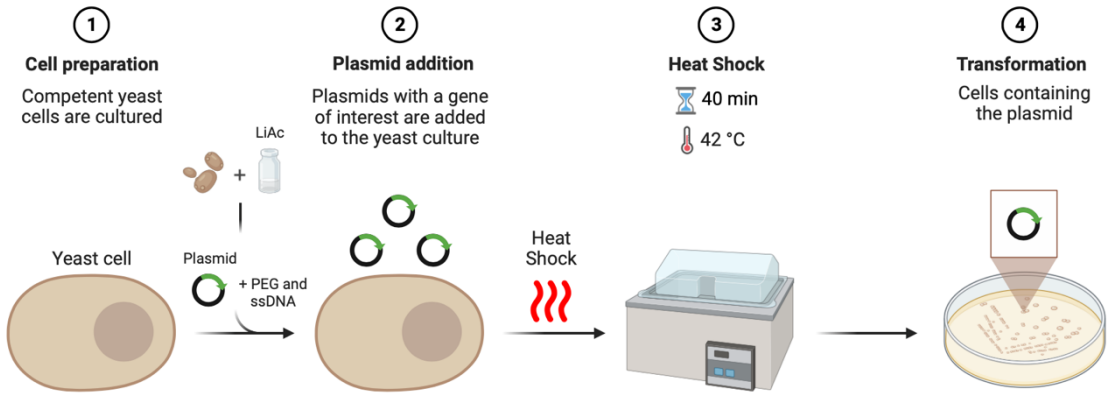


Figure 4. Representation of Yeast Transformation Protocol. Yeast transformations were performed on W303-1A strain, by the high-efficiency protocol using LiAc, ssDNA, and PEG.

3.8. Heat Stress Assay in *Saccharomyces cerevisiae*

The yeast strain W303-1A was grown in YPD medium overnight at 30°C, 200rpm. This initial culture was then diluted with fresh medium, and its optical density at 600 nm (OD_{600}) was adjusted to 0.4. Different treatments were applied: DMSO (used as a control) and each extract at a concentration of 1 mg/mL. The yeast viability was assessed, as described above, by counting CFUs at various time intervals: 0, 2, 4, and 6 hours after treatment. The first measurement was taken before shifting the cultures to a temperature of 46°C. After the temperature shift the number of visible colonies was counted, and the viability of yeast cells was expressed as a percentage of CFUs compared to the mean colony count at different time points, with the viability at 0 h set as 100%.

3.9. Antiaggregant Activity in SNCA and HTT Mutant Strains

The SNCA WT, SNCA A53T, HTT 25Q and HTT 103Q strains were cultured overnight in YPD medium containing 2% (w/v) dextrose. The culture medium was then changed to SCG medium as previously described. These cells were divided into aliquots for the control (treated with DMSO) and the extracts AN, CH and PC (at a concentration of 1 mg/mL). All strains were maintained at a temperature of 30°C and a rotation speed of 200 rpm for a period of 6 h for strains SNCA WT and

SNCA A53T and 18 h for strains HTT 25Q and HTT 103Q. Each sample was observed using a Leica DM5000B fluorescence microscope with a 40x magnification objective. At least 200 cells per experimental condition were counted for quantification of aggregation. Consistent settings for exposure, gain, and intensity were applied to both the control group and the corresponding experimental condition (Figure 5).

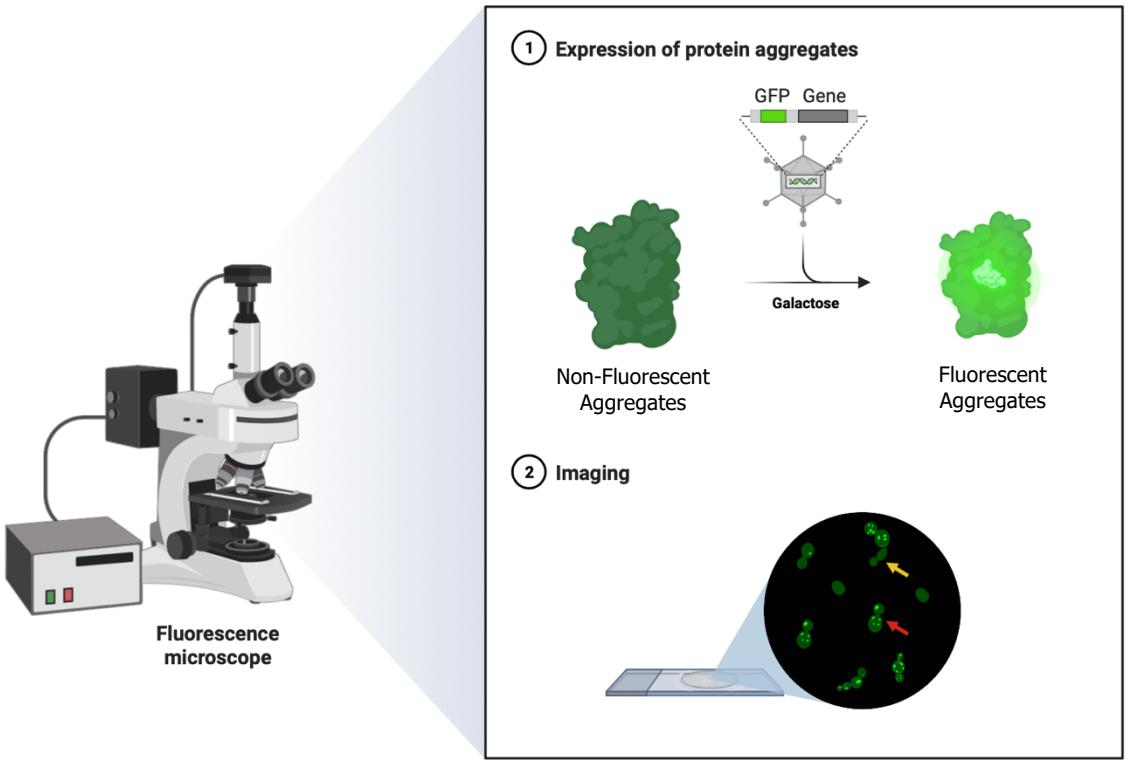


Figure 5. Representation of the activation of protein aggregate expression in genetically modified strains. These strains express human proteins (α-synuclein and huntingtin) fused with GFP. When exposed to galactose, it is possible to visualize the aggregates of these proteins through fluorescence microscopy. Cells that exhibited bright spots were counted as cells with inclusions (red arrow), and those with uniform green color were counted as cells without inclusions (yellow arrow).

The anti-aggregation activity was assessed by calculating the percentage of cells without inclusions for the strains treated with extracts compared to their respective controls. This percentage was obtained by equation 2, where C_{wi} is the number of cells without inclusions; C_T is the total number of cells. Cells that exhibited at least one fluorescent spot in the cytoplasm were considered to contain aggregates.

$$\%\ Cells\ without\ inclusions = \frac{C_{WI}}{C_T} \times 100 \qquad \text{(Equation 2)}$$

3.10. Expression of chaperones in *Saccharomyces cerevisiae*

Flow cytometry was used to analyze the expression of chaperones fused with GFP. Expression of chaperones was measured in *S. cerevisiae* strains (SSA2, SSB1 and HSP104) treated with AN, CH, PC at concentration 1000 μg/mL. Control were prepared by replacing the extract with the respective solvent, DMSO, in the same amount as used in the treatment. The cell suspension was incubated overnight at 30 °C and 200 rpm. The optical density (OD) was measured in the next morning at 600 nm and the culture was diluted to OD_{600}=0.1 using fresh YPD medium and was incubated until OD_{600}=0.4. The cells were washed twice with phosphate buffered saline (PBS). The cell suspension was centrifuged at 5000 *g* at 4°C for 3 min. The cell pellet was resuspended in PBS and the OD_{600} was adjusted to 0.04. The samples were incubated for 20 h at 30 °C in the dark. Approximately 20,000 cells from each sample were analysed at a low flow rate (10 μL/min) on an EpicsR XLTM flow cytometer (BeckmanCoulter).

3.11. Statistical Analysis

All statistical analyses were performed using GraphPad Prism 9 software. After inputting the collected data into the software, statistical analysis was conducted. To assess the IC_{50} of the extracts in the DPPH assay, linear regression analysis was performed for all extracts. Furthermore, to compare the viability of *S. cerevisiae* cells in the presence of the extract and H_2O_2, the percentage of cells without aggregates in *S. cerevisiae* and for flow cytometry assays, one-way ANOVA test followed by the two-sided Dunnett's test was used. The results were expressed as mean ± standard error (SEM) from at least three independent assays. A statistically significant difference was defined as $p < 0.05$ and is indicated in the figures with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), ****($p < 0.0001$).

4. RESULTS AND DISCUSSION

4.1. Out of the 11 algae extracts, only the AN extract showed radical scavenging against DPPH

The DPPH assay was conducted as an initial evaluation prior to experiments in biological models. This step was taken because oxidative stress is known to be associated with the onset and progression of numerous neurodegenerative diseases. In this way, using colorimetric analysis, one can assess whether an extract exhibits anti-radical properties and the potential to offer neuroprotective effects. The DPPH assay relies on the concept that radical DPPH receives a hydrogen atom from a scavenger molecule, typically an antioxidant. This reduction converts DPPH into $DPPH_2$, leading to a visible change in the solution's color from purple to yellow. As a result of this reaction, the absorbance values measured at 515 nm decrease, leading to an increase in the percentage of DPPH reduction (% DPPHr). Samples containing different concentration of AN, BB, CC, CT, GP, LO, MS, SL, SM, UP, UR (31.25, 62.5, 125, 250, 500, 1000 μg/mL) were incubated with DPPH and the absorbance values obtained were used to calculate the percentage of DPPHr, as described in the section 3.3. From the extracts tested, only AN, BB and SM exhibited activity (IC_{50}=61.09 μg/mL; IC_{50}=685.33 μg/mL; IC_{50}=613,12 μg/mL, respectively; Table 6). Only extracts with an IC_{50} below 500 μg/mL were chosen to proceed with the biological assays, as is the case with AN.

Table 6. DPPH antiradical activity of macroalgae extracts. Three replicates were performed just for extracts with activity

DPPH antiradical activity	
Macroalgae extract	IC_{50} (μg/mL)
Ascophyllum nodosum	61.09
Bifurcaria bifurcata	685.33
Chondrus crispus	No activity
Codium tomentosum	No activity
Gigartina pistillata	No activity
Laminaria ochroleuca	No activity
Mastocarpus stellatus	No activity
Saccharina latissima	No activity
Sargassum muticum	613.12
Undaria pinnafitida	No activity
Ulva rigida	No activity

Argregán *et al.* (2018) conducted a study that analyzed the phenolic compounds and antioxidant capacity of three types of macroalgae. It was found that two of these macroalgae exhibited stronger antioxidant activity than what was observed in our study. In the case of the AN extract, the authors reported IC_{50} values of approximately 50 μg/mL, while our extract displayed an IC_{50} of approximately 61 μg/mL. As for the BB extract, they reported an IC_{50} was around 143 μg/mL, whereas our extract showed 685.33 μg/mL. In another study by Sabeena Farvin & Jacobsen (2013), where they also compared phenolic compounds and antioxidant capacity of 16 algae extracts, it was observed that the SM algae displayed higher radical activity compared to our results. In this case, the authors reported IC_{50} values around 457 μg/mL, while our extract displayed an IC_{50} of 613.12 μg/mL. This discrepancy in results can be explained by the type of solvent and the harvesting location. In our case, we used 60% methanol (v/v) as the solvent, while Argregán *et al.* (2018) used 50% ethanol (v/v) for the AN and BB algae, and in the study by Sabeena Farvin & Jacobsen (2013), 60% ethanol (v/v) was used for the SM algae. Our algae were harvested off the coast of Galicia, whereas in these two studies, the AN and BB algae were collected in the Canary Islands, and the SM algae in Denmark. All of these factors can result in varying quantities of secondary metabolites, leading to bioactivities that may be more or less pronounced. Therefore, these results emphasize the importance of considering these factors when evaluating the antioxidant activity of algae extracts.

4.2. Extracts from AN, PC and CH protected viability loss caused by H_2O_2

Viability protection by AA, AN, AT, CH, HS, PP, PC, GAM, SV and ZT was examined through evaluation of *S. cerevisiae* BY4741 strain after exposure to H_2O_2 (oxidative stress inducer). These experiments were performed as complementary assays in the screening of the extracts to select the samples with higher antioxidant activity. To assess the protective effect of this extracts against toxicity induced by H_2O_2, viability was measured, as described in the section 3.4. It was found that the viability of BY4741 treated with AN, PC and CH was significantly increased compared with H_2O_2-treated cells (positive control; Figure 6B and 6D). In the negative controls, an increase in viability over time is observed, indicating that the cells proliferated normally (Figure 6A, 6C and 6E). In the AN extract, we can see that all three tested concentrations promoted higher viability than the positive control, showing a dose-response effect (Figure 6A). However, it was only at the highest concentration (1000 μg/ml) and after 120 min of incubation that the extract was able to

significantly reduce the cytotoxicity caused by H_2O_2 (Figure 6B). Similarly, to AN, 1000 μg/ml CH or PC extracts demonstrated significant protection of cell viability when exposed to H_2O_2 after 60 min incubation. The remaining extracts (AA, AT, GAM, HS, PP, SV, and ZT), did not display protection at the tested concentration (Figure 6C and 6D).

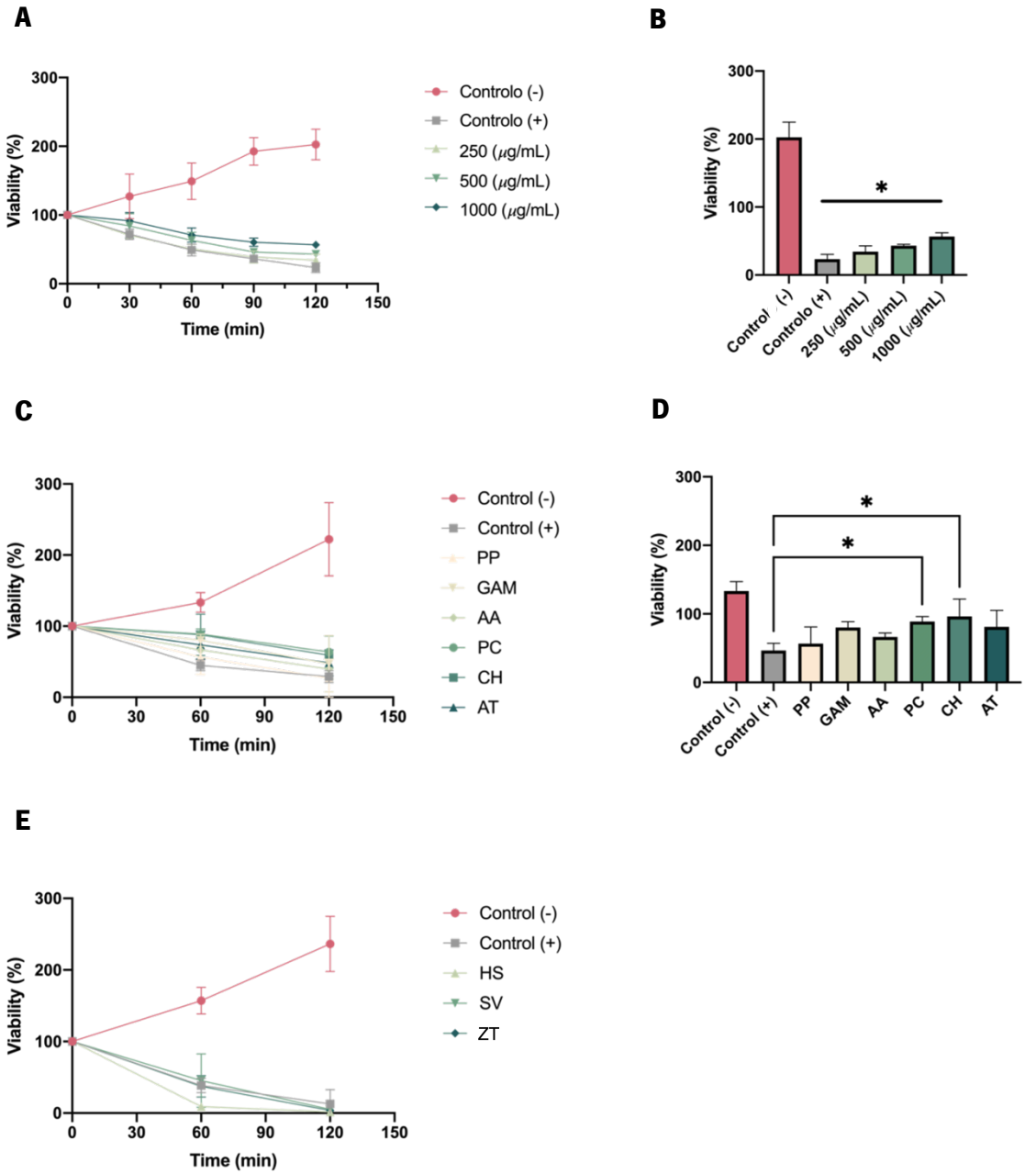


Figure 6. Effect of algae extracts on loss viability in *Saccharomyces cerevisiae* BY4741 strain, induced by H_2O_2. Yeast cells, strain BY4741, were incubated with *Ascophyllum nodosum* (AN; A) at concentrations of 250, 500, or 1000 μg/mL, or *Asparagopsis armata* (AA; C), *Asparagopsis taxiformis* (AT; C), *Cystoseira humilis* (CH; C), *Gongolaria abies-marina* (GAM; C), *Halopteris scoparia* (HS; E), *Pterocladiella capillacea* (PC; C), *Padina pavonica* (PP; C), *Sargassum vulgare* (SV; E), and *Zonaria tournefortii* (ZT, E) at a concentration of 1000 μg/mL. Aliquots were taken at different time intervals and serially diluted 10^{-4}, from this dilution, 100 μL were spread on YPDA medium, the plates were incubated at 30°C for 48 hours and the colonies were counted. DMSO was used as a negative control at the maximum volume of extract used. Viability was calculated as percentage of CFU's, assuming 0 min incubation as 100% viability. Pannel A, C and E represents viability along time and B and D represent viability at 120 min and 60 min incubation, respectively. The results are presented as Mean ±SEM (standard error of the mean) from three independent replicates, and statistical analysis was performed using a one-way ANOVA, $*p<0.05$.

The findings from this study underscore the protective potential of *Ascophyllum nodosum*, *Cystoseira humilis*, and *Pterocladiella capillacea* extracts against viability loss triggered by H_2O_2 exposure. This protective capacity aligns seamlessly with established antioxidant properties of AN (Agregán *et al.*, 2018; Apostolidis & Lee, 2010; Gisbert *et al.*, 2023; Yuan & Macquarrie, 2015), CH (Belattmania *et al.*, 2016; Grina *et al.*, 2020) and PC (Alencar *et al.*, 2016; Heo *et al.*, 2006; Kelman *et al.*, 2012; Paiva *et al.*, 2012, 2016). The capacity of these extracts to shield cells from H_2O_2-induced viability loss is a testament to their ability to counteract oxidative stress. These extracts are known to harbor bioactive compounds, including antioxidants like polyphenols and carotenoids, which are adept at neutralizing ROS and mitigating oxidative damage (D'Archivio *et al.*, 2010; Holdt & Kraan, 2011). This ability is consistent with their antioxidant properties. In summary, these findings have important implications in the context of neurodegenerative diseases, where oxidative stress-induced cell damage is a well-recognized contributor to disease pathogenesis (Butterfield & Halliwell, 2019; Uttara *et al.*, 2009).

4.3. AN, CH and PC decreased intracellular oxidation imposed by H_2O_2

Flow cytometry is a technique that allows us to examine the physical and fluorescent characteristics of individual cells as they flow through a beam of light. It helps us analyze and distinguish between different types of cells within a heterogeneous population based on their size, texture, and fluorescence properties. This is achieved through the interaction of light with the cells as they move. Light scattering in flow cytometry can be divided into two components: forward scatter (FSC), which is related to the size of the cell, and side scatter (SSC), which reflects the granularity of the cell. These parameters are influenced by various factors like the cell's membrane, nucleus, granularity, and shape. Fluorescence emission is associated with fluorescence probe, which is proportional to the number of fluorescent probe molecules in the cell.

In this study, intracellular oxidation in *S. cerevisiae* cells treated with AN, CH or PC and exposed to H_2O_2 was evaluated, as an indicator of antioxidant activity. The initial observation was the cell suspensions only treated with AN, CH or PC emitted fluorescence similar to the negative control (no treatment; data not shown; Figure A1), which suggests that the extracts did not promote significant effect on intracellular oxidation. Additionally, the percentage of negative events for PI was consistently high across all tested concentrations, suggesting that the extract did not affect cell viability neither (detailed data not provided; Figure A2). However, when *S. cerevisiae* cells exposed to H_2O_2 were simultaneously treated with AN fluorescence was lower than the positive

control and decreased progressively along with increasing extract concentractions (up to 500 μg/mL), approaching those seen in the negative control. This decrease in fluorescence indicates that as the AN concentration increased, intracellular oxidation decreased in direct dependence of the dose until 500 μg/mL (Figure 7A). In the case of cells treated with CH or PC and exposed to oxidative stress, they consistently revealed lower fluorescence and, consequently, lower intracellular oxidation compared to the positive control (Figure 7C and 7E). However, at the highest concentration (1000 μg/mL), both CH and PC extracts displayed higher fluorescence than at lower concentrations (250 and 500 μg/mL) although without statistical significance.

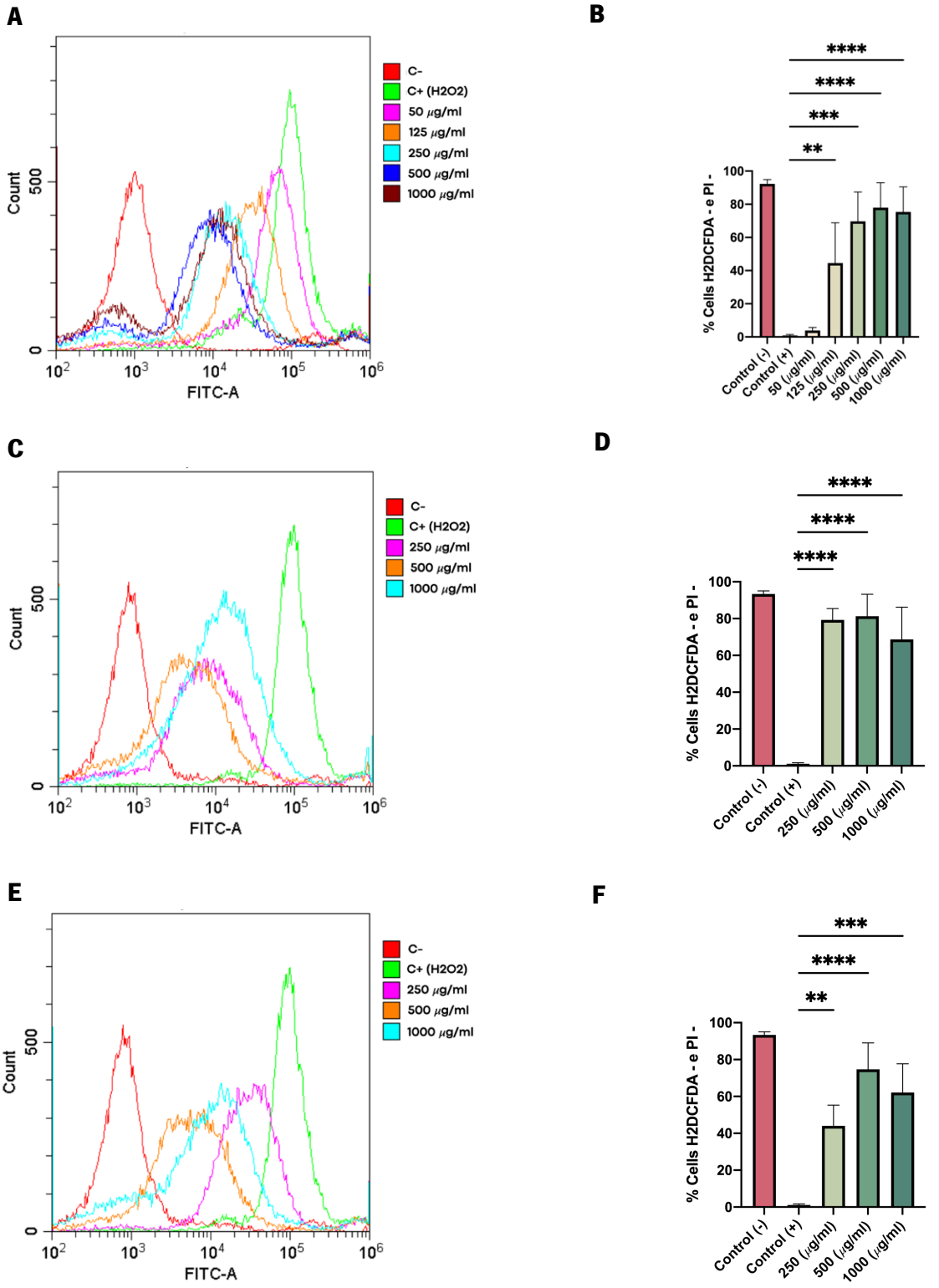


Figure 7. *Ascophyllum nodosum* (AN; A and B), *Cystoseira humilis* (CH; C and D) and *Pterocladiella capillacea* (PC; E and F) extracts attenuates intracellular oxidation induced by H_2O_2 in *Saccharomyces cerevisiae* strain BY4741. Cells from exponentially growing cultures were treated with 250, 500 or 1000 µg/mL of each extract and 5 mM H_2O_2 (pink, line, orange line and blue line, respectively; A, C and E), incubated for 20 min at 30 °C, 200 rpm, labelled with 2',7'-dichlorofluorescein diacetate (50 µM; H2DCFDA) and propidium iodide (3 µg/ml) in the dark, at 30 °C for 1h or 10 min, respectively and the fluorescence was measured by flow cytometry. Negative control was prepared with cells treated only with DMSO (C-, red line; A, C and E) and positive control was prepared with cells treated only with 5mM of H_2O_2 (C+, green line; A, C and E). All histograms (A, C and E) were made with PI-negative cells and bar charts were prepared with PI- and H2DCFDA- negative cells. Results are representative of at least three independent experiments are presented as Mean ±SEM (Standard Error of the Mean), and statistical analysis was performed using a one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

The results of the study demonstrate that extracts from three different types of seaweed, *Ascophyllum nodosum*, *Cystoseira humilis*, and *Pterocladiella capillacea*, exhibit antioxidant effects by effectively reducing intracellular oxidation when cells were exposed to H_2O_2. The antioxidant properties of seaweed extracts have been well-documented in previous studies. Seaweeds are rich in bioactive compounds such as polyphenols (e.g., phlorotannins), flavonoids, carotenoids, and vitamins, which are known for their ability to scavenge ROS and protect cells from oxidative damage in various biological systems (D'Archivio *et al.*, 2010; Holdt & Kraan, 2011; Lomartire & Gonçalves, 2022; Tung *et al.*, 2022) For example, the phenolic compounds in seaweed extracts have been shown to have strong free radical scavenging activities (Rajauria *et al.*, 2017). The observed reduction in intracellular oxidation when cells were exposed to H_2O_2 in this study is consistent with the expected antioxidant effects of these seaweed extracts. The use of the *S. cerevisiae* assay, which mirrors the cellular redox environment more faithfully, underscores the pronounced antioxidant effect of these extracts. The reduction in intracellular oxidation in the presence of H_2O_2 indicates that these extracts have the potential to protect cells from oxidative stress. Oxidative stress is implicated in aging and various health conditions (Liguori *et al.*, 2018). In summary, these findings have significant relevance to ND. There is accumulating evidence indicating that oxidative stress plays a crucial role in these conditions, leading to neuron loss and cognitive decline (Butterfield & Halliwell, 2019; Uttara *et al.*, 2009). Therefore, interventions that reduce oxidative stress, such as using seaweed extracts with antioxidant properties, hold promise for mitigating the progression of these diseases. In conclusion, this study provides valuable scientific evidence supporting the antioxidant effects of seaweed extracts from AN, CH, and PC.

4.4. Integration of pRS425GAL-HTT-GFP plasmids in the yeast genome

Plasmid pRS425GAL-HTT-GFP (25Q and 103Q polyglutamine variants) provided in dried form in filter paper disks were recovered by solubilization by soaking in TE buffer. Agarose gel electrophoresis analysis did not reveal the presence of plasmid DNA (Figure 8A), while the quantification indicated that concentration was very low: 30.4 ng/µL and 20.2 ng/µL for pRS425GAL-HTT25Q-GFP and pRS425GAL-HTT103Q-GFP, respectively. For the integration of the plasmids into the genome of the W303-1A strain, a transformation was performed in *E. coli* to amplify the plasmid to increase yeast transformation efficiency. *Escherichia coli* is a bacterium known for its rapid growth and reproduction. This means that a small number of initially

transformed cells can quickly multiply into a large population, each containing the plasmid of interest. As *E. coli* cells grow and divide, the plasmids replicate along with the genomic DNA. This results in an increase in the number of plasmid copies per cell with each generation. The more generations that pass, the higher the plasmid yield. After the transformation in *E. coli*, the plasmid DNA was isolated and quantified again, and this time the agarose gel electrophoresis analysis revealed the presence of plasmid DNA (Figure 8B). Accordingly, quantification confirmed amplification of the plasmids with 865.5 ng/µL and 512.3 ng/µL for pRS425GAL-HTT25Q-GFP and pRS425GAL-HTT103Q-GFP, respectively.

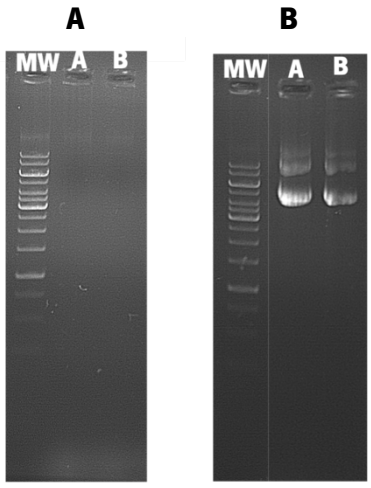


Figure 8. 1% Agarose gel electrophoresis of plasmid DNA before and after transformation in *Escherichia coli.* Run done at approximately 6 V/cm and DNA stained with Midori Green. MW represents the DNA Molecular Weight marker (GeneRuler 1 kb SM0313). Lane A corresponds to pRS425GAL-HTT25Q-GFP and Lane B to pRS425GAL-HTT103Q-GFP.

Subsequently, the integration of pRS425GAL-HTT-GFP plasmids (25Q and 103Q) was established in the laboratory W303-1A strain, which were then denominated HTT 25Q and HTT 103Q. The transformed cells were transferred to a selective medium. Since the pRS425 plasmid contains the prototrophic marker gene *LEU2*, after genomic integration, the W303-1A cells reverted from a Leu- phenotype to a Leu+ phenotype, becoming independent of leucine. Therefore, only the transformed cells were capable of growing in the absence of leucine in the medium, as the W303-1A yeast strain is unable to synthesize this amino acid.

4.5. Extracts did not protect *Saccharomyces cerevisiae* cells from thermal stress

One possible reason for the anti-aggregation effect of the extracts could be their ability to trigger the induction of Heat Shock Proteins (Hsps). When the temperature reaches 46 °C, the proteostasis is unbalanced, leading to an increase in the presence of misfolded proteins. In response to this stress, the cell activates mechanisms such as upregulating chaperone proteins that assist in the degradation of misfolded proteins or activating transcription factors that stimulate the production of protective proteins. If this is their mechanism, the extracts would be capable of promoting the protection of proteins against thermal denaturation, which would be an indicator of the anti-aggregating activity mechanism of alpha-synuclein and huntingtin. Therefore, we decided to perform a viability assay where cells were exposed to heat stress, and we evaluated their viability in presence and absence of the extracts. The cell cultures were exposed to a temperature of 46 °C for a duration of 6 hours, with each extract or DMSO (used as a control) present, and we monitored viability throughout this period. As expected, in the control group, cell viability progressively declined during the experiment, reaching approximately 40% after 6 hours (Figure 9A). In the cultures with AA, AN, CH, GAM, PC, PP, SV and ZT viability was similar to the control, however in culture with HS, after 2 h incubation, viability was significantly lower than the control (Figure 9B). After 4 h, HS still promoted increasing loss of viability as compared to the control and additionally, AT significantly had a similar effect. These results suggest that HS and AT potentiate the denaturing effect caused by heat.

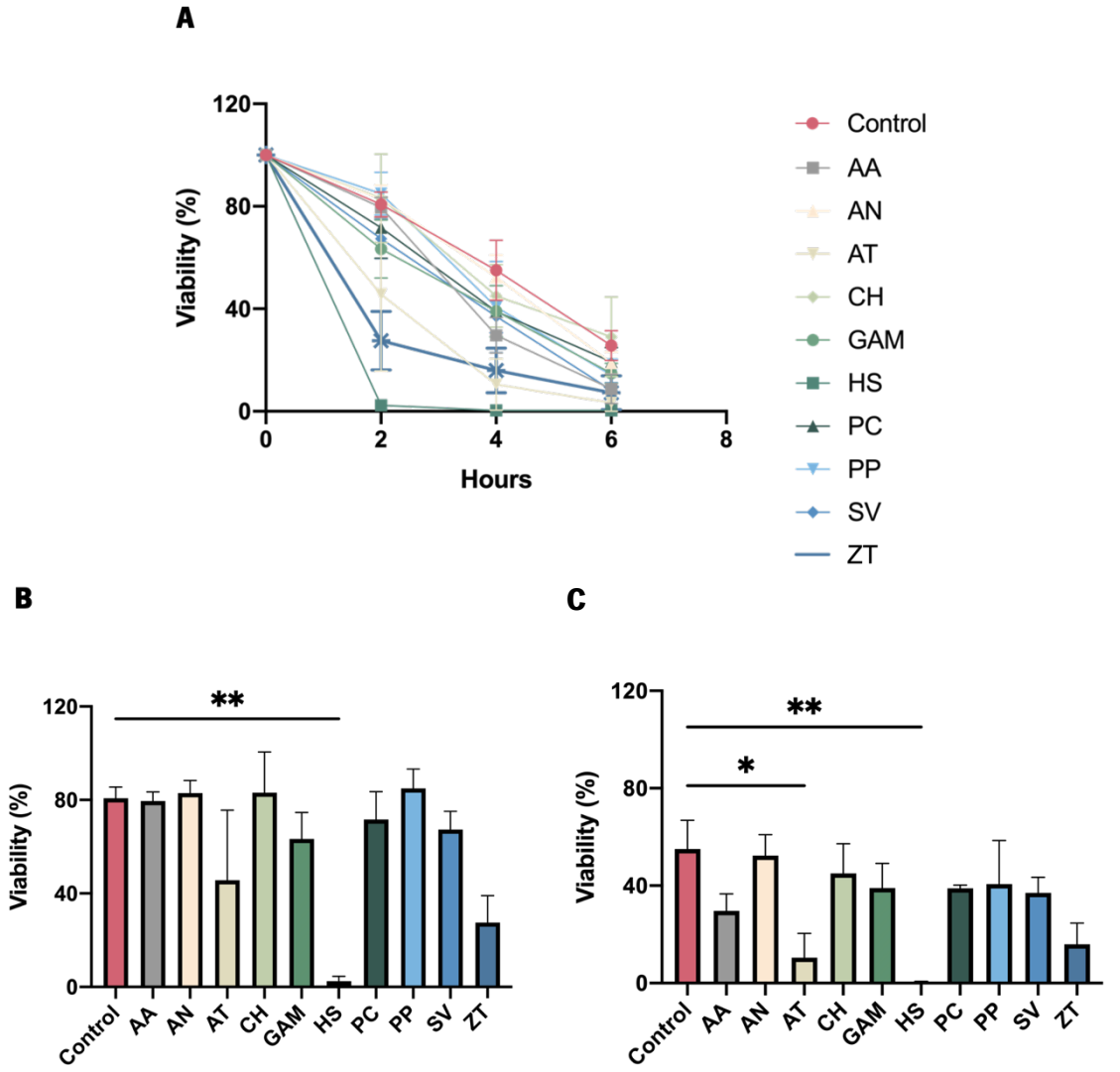


Figure 9. The effect of the extracts of *Asparagopsis armata* (AA), *Ascophyllum nodosum* (AN), *Asparagopsis taxiformis* (AT), *Cystoseira humilis* (CH), *Gongolaria abies-marina* (GAM), *Halopteris scoparia* (HS), *Pterocladiella capillacea* (PC), *Padina pavonica* (PP), *Sargassum vulgare* (SV) and *Zonaria tournefortii* (ZT) on protection against thermal stress in *Saccharomyces cerevisiae* strain W303-1A. Cells in the presence of 1 mg/mL extract were incubated at 46°C, aliquots were removed along time and spread on YPDA plates after serial dilution. After incubation at 30 °C for 48h, colonies were counted and viability was calculated as percentage of CFU's, assuming time 0 min as 100% viability. The negative control (control) was prepared with the same volume of DMSO as the extracts. Pannel A represents viability along time and B and C represent viability at 2h and 4 h incubation, respectively. Results are presented as Mean ±SEM (Standard Error of the Mean) from three independent replicates, and statistical analysis was performed using a one-way ANOVA, *p<0.05, **p<0.01.

Proteostasis, the state in which the proteome of a living organism is in functional balance, must be meticulously regulated within individual cells, tissues, and organs. An important role of these systems is to prevent the accumulation of protein species that are improperly folded and can potentially form toxic aggregates. At a temperature of 42°C, proteostasis, is disrupted, resulting in an increase in the quantity of misfolded proteins or proteins with incorrect conformations. This cellular response mainly involves the activation of protein quality control systems, which function

to recognize and target misfolded proteins for degradation or, alternatively, activate transcription factors capable of inducing the synthesis of protective proteins (Balch *et al.*, 2008; Hartl *et al.*, 2011). A series of neurodegenerative diseases are associated with the deposition of aggregates, such as AD, HD, and PD (Knowles *et al.*, 2014). The accumulation of aberrant protein species in these pathological states, in turn, overwhelms the proteostasis mechanism (Balch *et al.*, 2008; Gidalevitz *et al.*, 2006; Hipp *et al.*, 2014; Olzscha *et al.*, 2011). Elevated temperatures or other forms of cellular stress can lead to an increased production of misfolded proteins or accelerate the aggregation of existing ones. This exacerbates the burden on the proteostasis machinery and contributes to disease progression. In response to thermal stress, cells deploy a protective mechanism involving HSPs. Heat shock proteins are molecular chaperones that help proteins maintain their proper conformation, prevent aggregation, and facilitate their refolding or degradation when necessary (Hartl & Hayer-Hartl, 2009).

The yeast gene *SSQ1* corresponds to the mammalian HSP70 and is essential for the survival of *S. cerevisiae* cells. In the absence of this gene, yeast becomes more sensitive to thermal stress (Nwaka *et al.*, 1996; Verghese *et al.*, 2012). In a study conducted by Bayliak *et al.*, (2014), it was demonstrated that when a *Rhodiola rosea* extract is used at low concentrations, it has the ability to provide protection to the yeast *S. cerevisiae* from both heat stress and oxidative stress, while also extending its lifespan. The researchers propose that this extract has the capacity to activate specific transcription factors, notably Yap1 and Msn2/Msn4 (Bayliak *et al.*, 2014). In *S. cerevisiae*, Yap1 is primarily associated with oxidative stress response. When cells experience oxidative stress, they activate Yap1 to regulate the expression of genes involved in protecting the cell from damage caused by ROS (Ma & Liu, 2010). Msn2 and Msn4 (multicopy suppressor of SNF1 mutation proteins 2 and 4) are master regulators of stress-responsive gene expression. Part of their function involves the regulation of chaperone genes, ensuring that chaperones are produced to help cells maintain protein homeostasis (Estruch & Carlson, 1993).

The hypothesis is that the mechanism of action of the extracts may involve the ability to promote the preservation of proteins against thermal denaturation, which could indicate a potential anti-aggregative activity of these extracts. Contrary to expectations, none of the extracts protected *S. cerevisiae* cells from thermal stress. Consequently, we proceeded with anti-aggregation activity assays using only the algae that exhibited antioxidant activity.

4.6. AN, CH and PC extracts prevented the aggregation of proteins associated with neurodegenerative diseases.

To evaluate the ability of AN, CH and PC extracts to prevent the aggregation of proteins associated with neurodegenerative diseases (Parkinson and Huntington diseases), we used genetically modified yeast strains. These yeast strains were engineered to produce human α-synuclein or huntingtin proteins fused with GFP, a green fluorescent marker. In the cells, the presence of α-synuclein or huntingtin aggregates appears as distinct bright green spots when viewed under a fluorescence microscope. The genetic construction of α-synuclein and huntingtin fused with GFP are under the transcriptional regulation of the GAL promoter, which is activated when galactose is the sole carbon and energy source. Therefore, to induce the expression of α-synuclein or huntingtin proteins fused with GFP, we cultured the cells in medium where galactose was the source of carbon and energy. This experimental setup allowed us to investigate whether the tested extracts had the capacity to prevent the formation of α-synuclein and huntingtin aggregates within the cells.

4.6.1. AN, CH and PC treatment increased the number of cells without α-synuclein inclusions

SNCA A53T is a genetically modified strain in which a specific mutation has been introduced into the SNCA gene. In this case, the A53T mutation replaces the amino acid alanine with threonine at position 53 of the α-synuclein protein. Notably, this A53T mutation is associated with hereditary forms of PD and is known to lead to the formation of toxic α-synuclein aggregates in the brain.

After inducing the SNCA A53T strain for 6 hours in medium with galactose as carbon and energy source, photographs were taken of the control culture treated with DMSO (Figure 9A), as well as cultures treated with AN (Figure 10B), CH (Figure 10C), and PC (Figure 10D). Subsequently, the percentage of cells without protein inclusions was calculated, as detailed in Popova *et al.*, (2021). In the micrographs it is clear that fluorescence is more intense and the number of cells with fluorescent inclusions is higher in the control with the extract's solvent (DMSO; Figure 10A) than in the treatments with AN, CH or PC (Figure 10B, 10C and 10D). The quantitative analysis revealed significant differences in the SNCA A53T strain when treated with AN, CH, and PC extracts

compared to the control group treated with DMSO (Figure 10E). Notably, the percentage of cells without protein inclusions was nearly double in the CH extract and almost triple in the AN and PC extract, in comparison with DMSO control. In summary, the presence of 1 mg/mL AN, CH, or PC extracts significantly reduced the number of protein aggregates in the SNCA A53T strain, suggesting their potential as effective agents to mitigate α-synuclein aggregates formation. Simultaneously, a viability assay was conducted to assess whether the extracts were toxic to the cells. All the extracts exhibited a similar profile to that of the control, suggesting that they are non-toxic to the cells and the decrease in alpha-synuclein aggregation is not a result of cell toxicity (Figure 10F).

When we replicated the same experiment using the SNCA WT strain, which expresses αSynWT-GFP. SNCA WT represents the normal or wild type form of the SNCA gene and thus encodes normal α-synuclein without mutations. This strain is used as a reference point to understand how normal α-synuclein functions in cells. We observed different results to those seen in the control group of the SNCA A53T strain (Figure 11A). In the case of the SNCA WT strain, the percentage of cells without protein inclusions in the DMSO control group was considerably higher (nearly 70%), compared to the SNCA A53T strain (nearly 20%). Notably, significant differences were only observed when the AN (Figure 11B) and PC (Figure 11D) extracts were used. However, treatment with the CH extract (Figure 11C) did not show significant differences in terms of the percentage of cells without protein inclusions (Figure 11E). Just as it occurred for the SNCA A53T strain, all the extracts exhibited a similar profile of viability assessment to that of the control in the case of the SNCA WT strain. This indicates that these extracts are not toxic to these cells. The results suggest that these extracts indeed reduce the aggregation of α-synuclein, and this reduction in aggregation is not due to cell toxicity or loss of viability (Figure 11F).

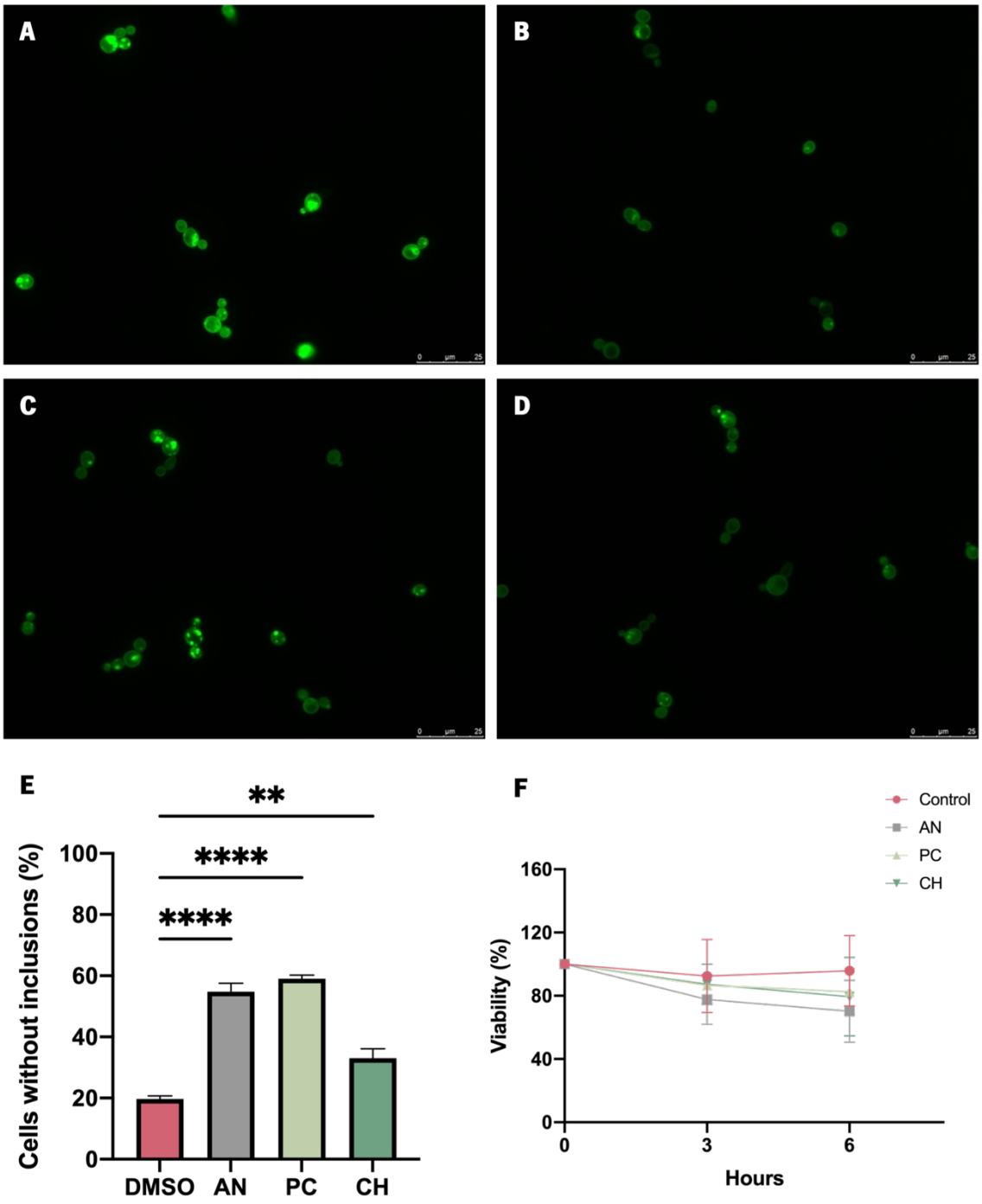


Figure 10. *Ascophylum nodosum* (AN), *Cystoseira humilis* (CH) and *Pterocladiella capillacea* (PC) treatment reduces αSynA53T-GFP aggregation in *Saccharomyces cerevisiae* SNCA A53T stain. Cells were incubated in SCG medium to induce the expression of αSynA53T-GFP, and they were treated as follows: a control group with DMSO (A), 1 mg/mL AN (B), 1 mg/mL CH (C), and 1mg/mL PC (D). After 6 hours, the cells were examined for the presence of green fluorescent spots. Cells lacking these intracellular fluorescent spots were counted, and the percentage was calculated and depicted in graph (E). The images were captured using a fluorescent microscope at 40x magnification, with the scale bar representing 25 μm. The results are presented as the Mean ± SEM of three independent replicates, and statistical analysis was performed using one-way ANOVA, with indicating statistical significance at $^{**}p<0.01$ and $^{****}p<0.0001$. Viability (F) was monitored over time through CFUs as described above.

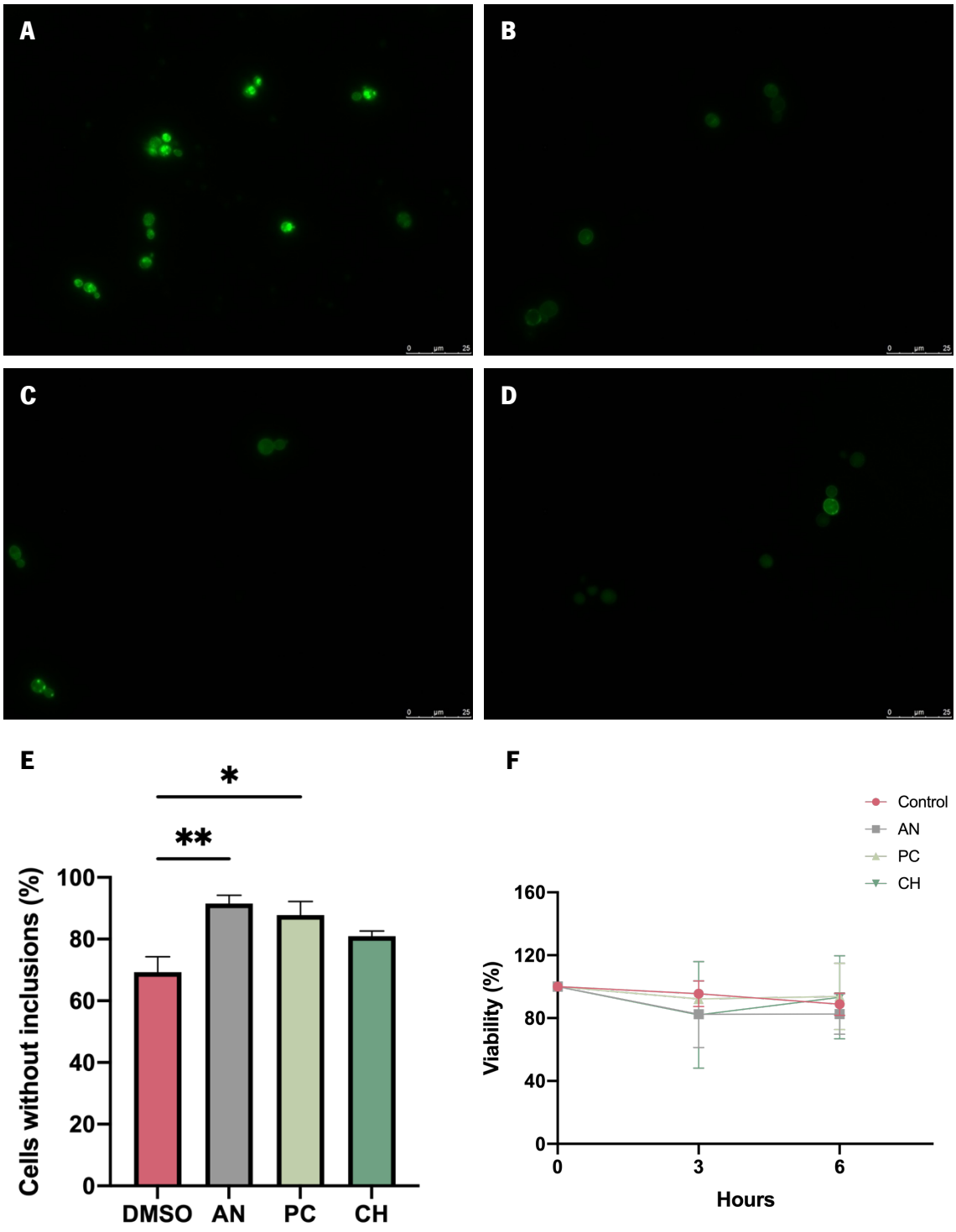


Figure 11. *Ascophylum nodosum* (AN), *Cystoseira humilis* (CH) and *Pterocladiella capillacea* (PC) treatment reduces αSynWT-GFP aggregation in *Saccharomyces cerevisiae* SNCA WT stain. Cells were incubated in SCG medium to induce the expression of αSynA53T-GFP, and they were treated as follows: a control group with DMSO (A), 1 mg/mL AN (B), 1 mg/mL CH (C), and 1mg/mL PC (D). After 6 hours, the cells were examined for the presence of green fluorescent spots. Cells lacking these intracellular fluorescent spots were counted, and the percentage was calculated and depicted in graph (E). The images were captured using a fluorescent microscope at 40x magnification, with the scale bar representing 25 µm. The results are presented as the Mean ± SEM of three independent replicates, and statistical analysis was performed using one-way ANOVA, with indicating statistical significance at $^{**}p<0.01$ and $^{****}p<0.0001$. Viability (F) was monitored over time through CFUs as described above.

In line with our findings, previous research by Popova *et al.* (2021) demonstrated a similar proportion of α-synuclein aggregates in the control treatment. SNCA A53T demonstrates a heightened tendency to form aggregates when compared to SNCA WT, closely mirroring the pathogenicity typically observed in familial PD. This disparity can be attributed to the presence of the A53T mutation, which renders SNCA more susceptible to misfolding and subsequent aggregation (Tenreiro & Outeiro, 2010). Our experimental findings corroborate this insight, as we observed that within the control group, the SNCA WT strain exhibited a greater percentage of cells devoid of inclusions compared to the SNCA A53T strain.

Several studies have investigated strategies aimed at counteracting protein aggregation, revealing the central role of Hsp70, Hsp90 in regulating α-synuclein protein aggregates in PD. Hsp70 has emerged as a potent force in the battle against α-synuclein-related toxicity, acting effectively to prevent, decrease, and eliminate α-synuclein oligomers within cells (Klucken *et al.*, 2004; Luk *et al.*, 2008; McLean *et al.*, 2004). Furthermore, under conditions of heat shock stress, experiments conducted in yeast cells have illustrated that Hsp70 expression serves as a protective shield, effectively preventing α-synuclein-induced cell death, a phenomenon recognized as apoptosis (Flower *et al.*, 2005). Hsp90 has also been found to suppress α-synuclein toxicity in a yeast model (Liang *et al.*, 2008) Considering the impact of Hsps, it is plausible that the anti-aggregating properties of macroalgae extracts may arise from their ability to kickstart a protective mechanism against protein damage during the initial stages. This early intervention could help deter the heightened toxicity caused by α-synuclein, subsequently leading to a reduction in the number of cells containing protein aggregates. In essence, macroalgae extracts may activate a defense mechanism that shields cells from the harmful effects of α-synuclein and curbs the formation of aggregates.

4.6.2. CH and PC treatment increased the number of cells without huntingtin inclusions

The genetic construction of HTT fused with GFP was also under the transcriptional regulation of the GAL promoter. Therefore, the conditions of induction of the expression were similar to those of the strains with α-synuclein-GFP except for the time of induction. In the case of the HTT 25Q strain, the huntingtin protein encompasses a polyglutamine (polyQ) tract comprising 25 consecutive glutamine amino acids (Q). This particular strain is characterized by the presence of a relatively normal and stable polyQ tract length within the huntingtin protein. Consequently, it frequently serves as a control or benchmark strain in experimental investigations and research endeavors focused on understanding Huntington's disease. Conversely, the HTT 103Q strain presents a huntingtin protein featuring an elongated polyQ tract, encompassing 103 consecutive glutamine amino acids (Q). This specific strain is designed to replicate the genetic mutation responsible for Huntington's disease in humans, wherein an anomalous expansion of the polyQ tract gives rise to the disease's distinctive pathological manifestations.

In this case, induction was extended to 16 h after studies of optimization of time of incubation and fluorescence of cells (data not shown). After subjecting the HTT 103Q strain to a 16-hour treatment with DMSO (as the control; Figure 12A), AN (Figure 12B), CH (Figure 12C), or PC (Figure 12D), we captured images and proceeded to calculate the percentage of cells without protein inclusions. Our analysis uncovered significant disparities in the HTT 103Q strain when exposed to AN, CH, and PC extracts in comparison to the control group. Notably, all three extracts demonstrated a nearly 20 percentage point increase in the proportion of cells without protein inclusions when compared to the DMSO control (Figure 12E). In summary, the presence of 1 mg/mL of AN, CH, or PC extracts had a notable effect in reducing the formation of protein aggregates in the HTT 103Q strain, indicating their potential as effective agents for mitigating huntingtin aggregates formation. Additionally, we conducted a viability assay to determine whether these extracts had any toxic effects on the cells. The results indicated that CH and PC extracts exhibited a similar viability profile to that of the control, suggesting that these extracts are non-toxic to the cells, and the reduction in alpha-synuclein aggregation is not attributable to toxicity or loss viability. Nonetheless, it is important to note that the AN extract showed a significant drop in cell viability when compared to the control. This strongly implies that the AN extract is harmful to the cells. Consequently, the substantial increase in cells without inclusions observed in microscopy

compared to the control may be attributed to cell loss of viability rather than an actual reduction in aggregate formation caused by this extract (Figure 12F).

When we conducted the identical experiment with the HTT 25Q strain, which contains the HTT25Q-GFP fusion, we noticed outcomes akin to what was observed in the control group of the HTT 103Q strain (Figure 13A). Notably, we detected significant distinctions when we used the AN extract (Figure 13B), as well as the CH (Figure 13C) and PC (Figure 13D) extracts with significant differences in the increase of the number of cells without inclusions (Figure 13E). Unlike what was observed with the HTT 103Q strain, the CH and PC extracts showed a significantly higher viability profile compared to the control group when applied to the HTT 25Q strain. These results indicate that not only do these extracts not exhibit toxicity to these cells, but they are also capable of protecting against HTT25Q-GFP toxicity. However, the toxicity observed in the HTT 103Q strain cells due to the AN extract was also observed in the HTT 25Q strain. These results suggest that in these cells as well, the reduction in the number of cells with inclusions may be attributed to the toxicity of this extract towards these cells rather than an anti-aggregating effect (Figure 13F).

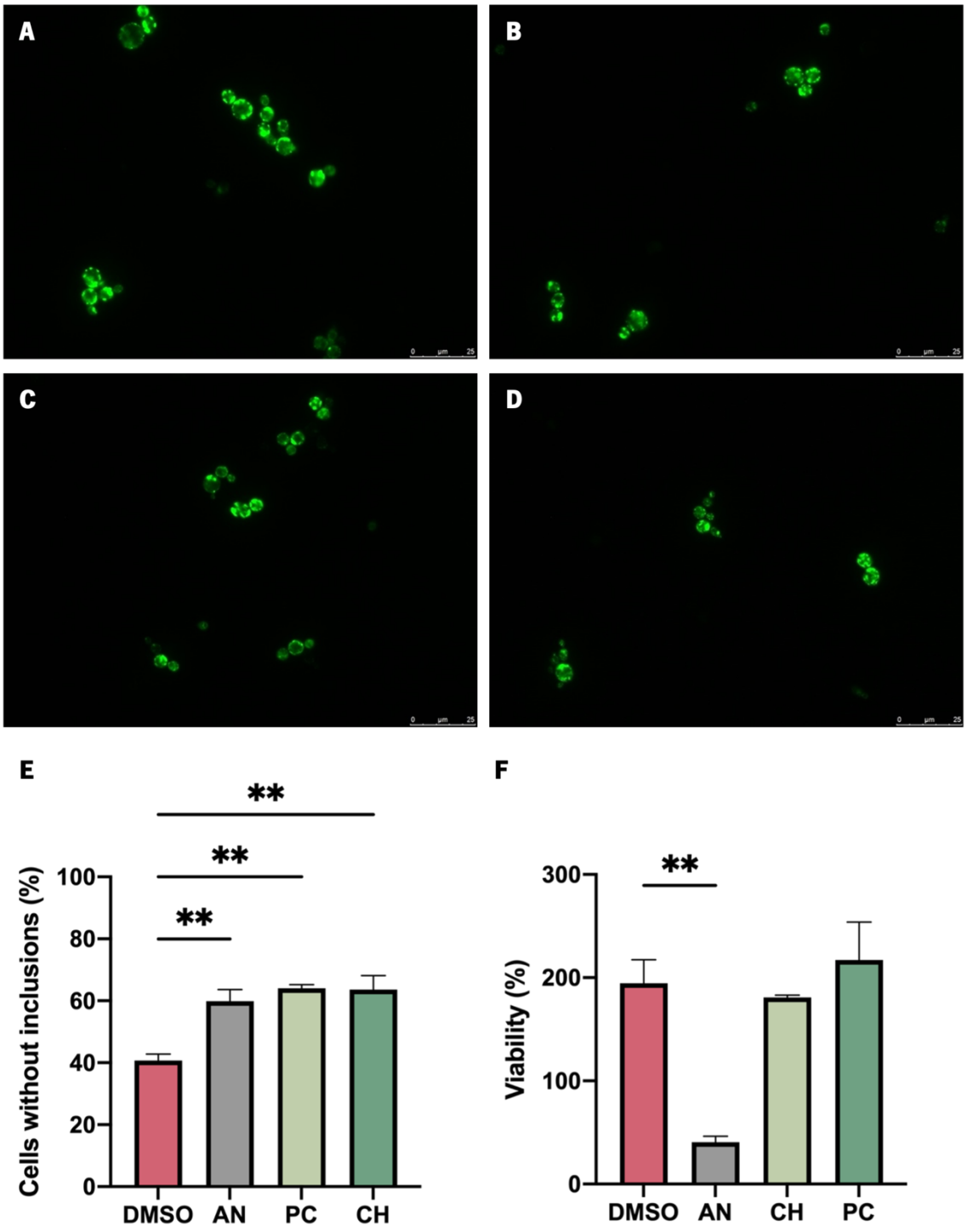


Figure 12. *Cystoseira humilis* (CH) and *Pterocladiella capillacea* (PC) treatment reduces HTT103Q-GFP aggregation in *Saccharomyces cerevisiae* HTT 103Q stain. Cells were incubated in SCG medium to induce the expression of HTT103Q-GFP, and they were treated as follows: a control group with DMSO (A), 1 mg/mL AN (B), 1 mg/mL CH (C), and 1mg/mL PC (D). After 16 hours, the cells were examined for the presence of green fluorescent spots. Cells lacking these intracellular fluorescent spots were counted, and the percentage was calculated and depicted in graph (E). The images were captured using a fluorescent microscope at 40x magnification, with the scale bar representing 25 µm. The results are presented as the Mean ± SEM of three independent replicates, and statistical analysis was performed using one-way ANOVA, with indicating statistical significance at $^{**}p<0.01$ and $^{****}p<0.0001$. Viability (F) was monitored at 16 hours through CFUs as described above.

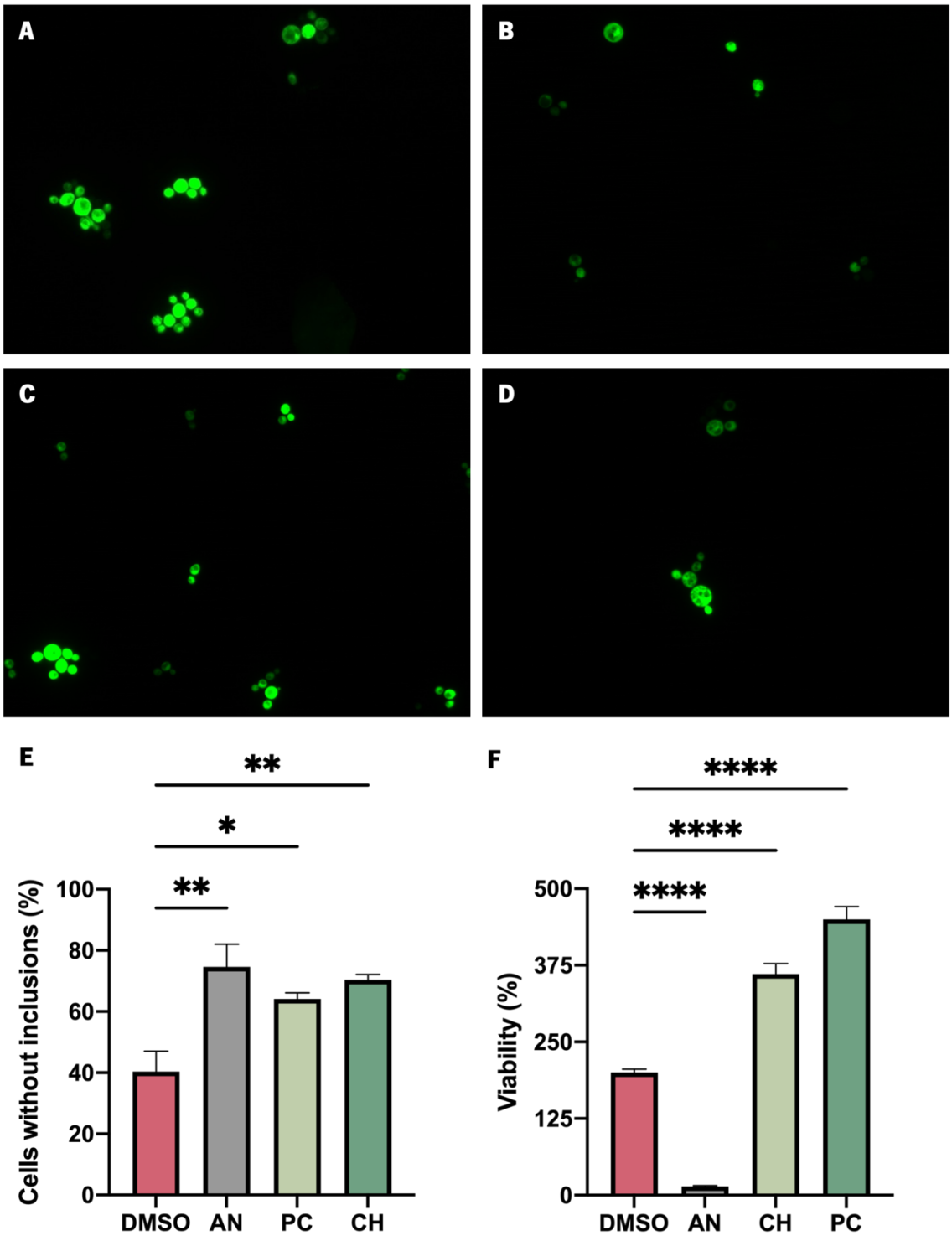


Figure 13. *Cystoseira humilis* (CH) and *Pterocladiella capillacea* (PC) treatment reduces HTT25Q-GFP aggregation in *Saccharomyces cerevisiae* HTT 25Q stain. Cells were incubated in SCG medium to induce the expression of HTT25Q-GFP, and they were treated as follows: a control group with DMSO (A), 1 mg/mL AN (B), 1 mg/mL CH (C), and 1mg/mL PC (D). After 16 hours, the cells were examined for the presence of green fluorescent spots. Cells lacking these intracellular fluorescent spots were counted, and the percentage was calculated and depicted in graph (E). The images were captured using a fluorescent microscope at 40x magnification, with the scale bar representing 25 μm. The results are presented as the Mean ± SEM of three independent replicates, and statistical analysis was performed using one-way ANOVA, with indicating statistical significance at $^{**}p<0.01$ and $^{****}p<0.0001$. Viability (F) was monitored at 16 hours through CFUs as described above.

To better study and understand HD, scientists have used a variety of organisms as research models, including the yeast *S. cerevisiae* (Krobitsch & Lindquist, 2000). These yeast models can replicate many of the cellular problems observed in HD patients. This includes disruptions in mitochondrial function, an increase in the production of reactive oxygen species, damage to the cytoskeleton, issues with proteasome function, and alterations in tryptophan metabolism, particularly in the quinurenine pathway (Mason & Giorgini, 2011). In the study of mutant HTT's impact on yeast cells, various yeast models have been employed (Duennwald *et al.*, 2006; Krobitsch & Lindquist, 2000; Meriin *et al.*, 2002).

These models have demonstrated that the aggregation and harmful effects of mutant HTT in yeast are influenced by the presence of Rnq1p in its prion-like [PIN+] conformation (Meriin *et al.*, 2002). Notably, removing RNQ1 or reversing [PIN+] has been shown to improve both aggregation and toxicity of polyQ. Yeast cells have a remarkable ability to deal with heat-damaged proteins, and they employ a chaperone complex when challenged with heat stress. This complex comprises key proteins like Hsp104, Hsp70, and Hsp40 (Struhl & Moqtaderi, 1998). Interestingly, various strategies have been observed to disrupt the aggregation of poly-Q proteins within yeast cells. These include increasing the production of specific Hsp70 and Hsp40 proteins (Muchowski *et al.*, 2000), simultaneously reducing the levels of Ssa1 and Ssa2 proteins from the Hsp70 family, or introducing a specific point mutation in the Hsp40 protein known as Ydj1 (Meriin *et al.*, 2002). All of these approaches have been found to effectively counteract the formation of poly-Q aggregates in yeast cells. In line with our findings, a study conducted by Kantcheva *et al.* (2014), showed a similar proportion of huntingtin aggregates compared to our control treatment. This study found that an interchangeable group of aggregation-prone proteins can affect how mutant HTT aggregates in yeast cells. This discovery might also apply to how mutant HTT behaves in human cells (Kantcheva *et al.*, 2014). Considering the influence of the presence of Rnq1q in its [PIN+] conformation on HTT aggregation, it is plausible that the anti-aggregation properties of macroalgae extracts may arise from their ability to express chaperones. They can interact with Rnq1q and other proteins involved in aggregate formation, assisting in proper protein folding or aggregate dissolution. This intervention could potentially help counteract the increased toxicity caused by huntingtin, subsequently leading to a reduction in the number of cells containing protein aggregates.

4.7. Unfolded protein response by activation of chaperone expression with treatment with AN, CH or PC

To investigate the mechanisms responsible for the neuroprotective effects of AN, CH, and PC extracts, we used mutant strains of *S. cerevisiae* in which GFP was used to replace genes that express chaperones involved in unfolded protein response (UPR). These mutant strains were designed to express fluorescent proteins like GFP, controlled by promoters of genes involved in UPR (Ssa2, Ssb1 and HSP104). This setup allowed researchers to measure the extent to which the expression of these genes is induced at the transcriptional level. The expression of these chaperones is low under normal conditions, so if the mechanism of action of these extracts involves the activation of any of these chaperones, we will observe an overexpression of them, with fluorescence being proportional to their expression.

4.7.1. Expression of HSP70

SSA2 and *SSB1* encode chaperone proteins that comprise the yeast cytosolic HSP70 proteins (Werner-Washburne *et al.*, 1987). HSP70 proteins were originally classified based on their response to heat shock. These protein's main function is as molecular chaperones, binding newly-synthesized proteins to facilitate their proper folding and prevent aggregation or misfolding (Becker & Craig, 1994; Bukau & Horwich, 1998). In yeast, HSP70s also play roles in breaking down clumps of incorrectly folded proteins, transporting specific proteins to the mitochondria and endoplasmic reticulum, disposing of abnormal proteins, and regulating the expression of other heat shock proteins (Deshaies *et al.*, 1988; Glover & Lindquist, 1998; Nishikawa *et al.*, 2001; Stone & Craig, 1990). Notably, *SSA2* stands out in the SSA subfamily because its transcription is not influenced by heat or stress (López-Ribot & Chaffin, 1996). Unlike *SSA2*, *SSB1* expression is repressed, as opposed to induced, upon heat shock (Craig & Jacobsen, 1985). Instead, *SSB1* transcription is coregulated with ribosomal protein genes (Lopez *et al.*, 1999). HSP70 has been studied in various animal models, and its effects on polyglutamine toxicity differ among them.

We conducted an assay to explore the possibility of AN, CH and PC triggering their antiaggregant activity through silencing the *SSA2* gene or overexpressing the *SSB1* gene. As expected, in *SSA2*, we detected fluorescence when these chaperones were exposed to DMSO (control; Figure 14A), since the *SSA2* gene is constitutively expressed at high levels (López-Ribot & Chaffin, 1996).

However, treatment with AN (Figure 14B), CH (Figure 14C) or PC (Figure 14D) extracts was not able to repress an activation of *SSA2* promoter since the fluorescence of cells seemed similar to the fluorescence of the control group (Figure 14A) and the analysis by flow cytometry did not display any shift of fluorescence of the population to higher values (Figure 14E).

In contrast to the majority of *HSP70* genes, like *SSA2*, *SSB1* expression is repressed, as opposed to induced (Craig & Jacobsen, 1985; Figure 15A). In similar experiments as with *SSA2* promoter, the *SSB1* promoter was not activated upon exposure to AN (Figure 15B), CH (Figure 15C), or PC (Figure15D) compared to the control group treated with DMSO. These results were corroborated by flow cytometry analysis with similar levels of fluorescence intensity (Figure 15E).

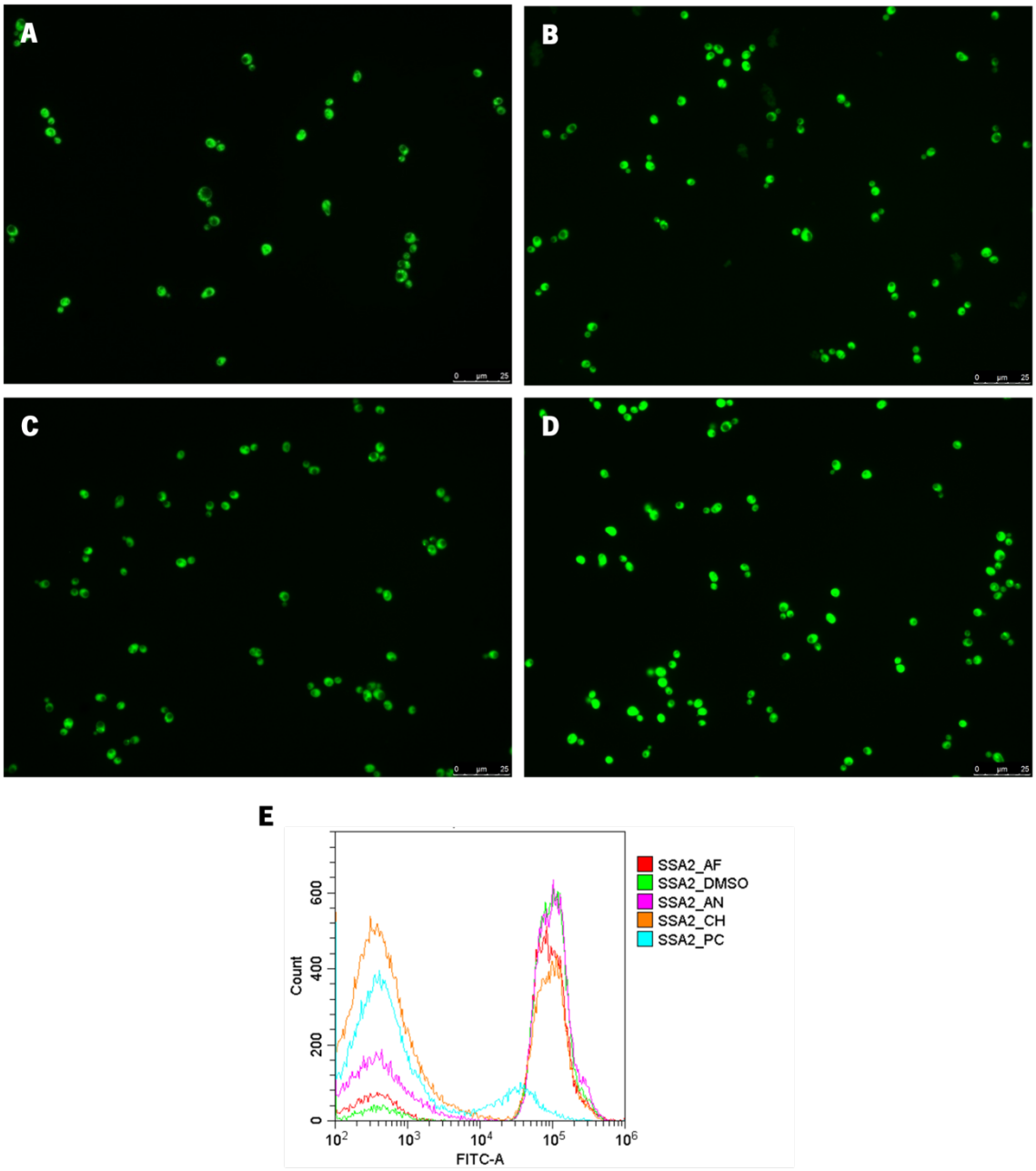


Figure 14. *Ascophylum nodosum* (AN), *Cystoseira humilis* (CH) and *Pterocladiella capillacea* (PC) on the activation of the SSA2 promoter of *Saccharomyces cerevisiae*. Representative microphotographs of a strain expressing GFP under the regulation of the SSA2 promoter (A-D). GFP fluorescence images were acquired in yeast treated with DMSO (A), 1 mg/mL AN (B), 1 mg/mL CH (C) or 1 mg/mL PC (D) for 20h. Representation of GFP fluorescence intensity by flow cytometry (E). The results are representative of three independent replicates. SSA2_AF: autofluorescence of yeast cells; SSA2_DMSO: negative control; SSA2_AN: cells treated with AN; SSA2_CH: cells treated with CH; SSA2_PC: cells treated with PC.

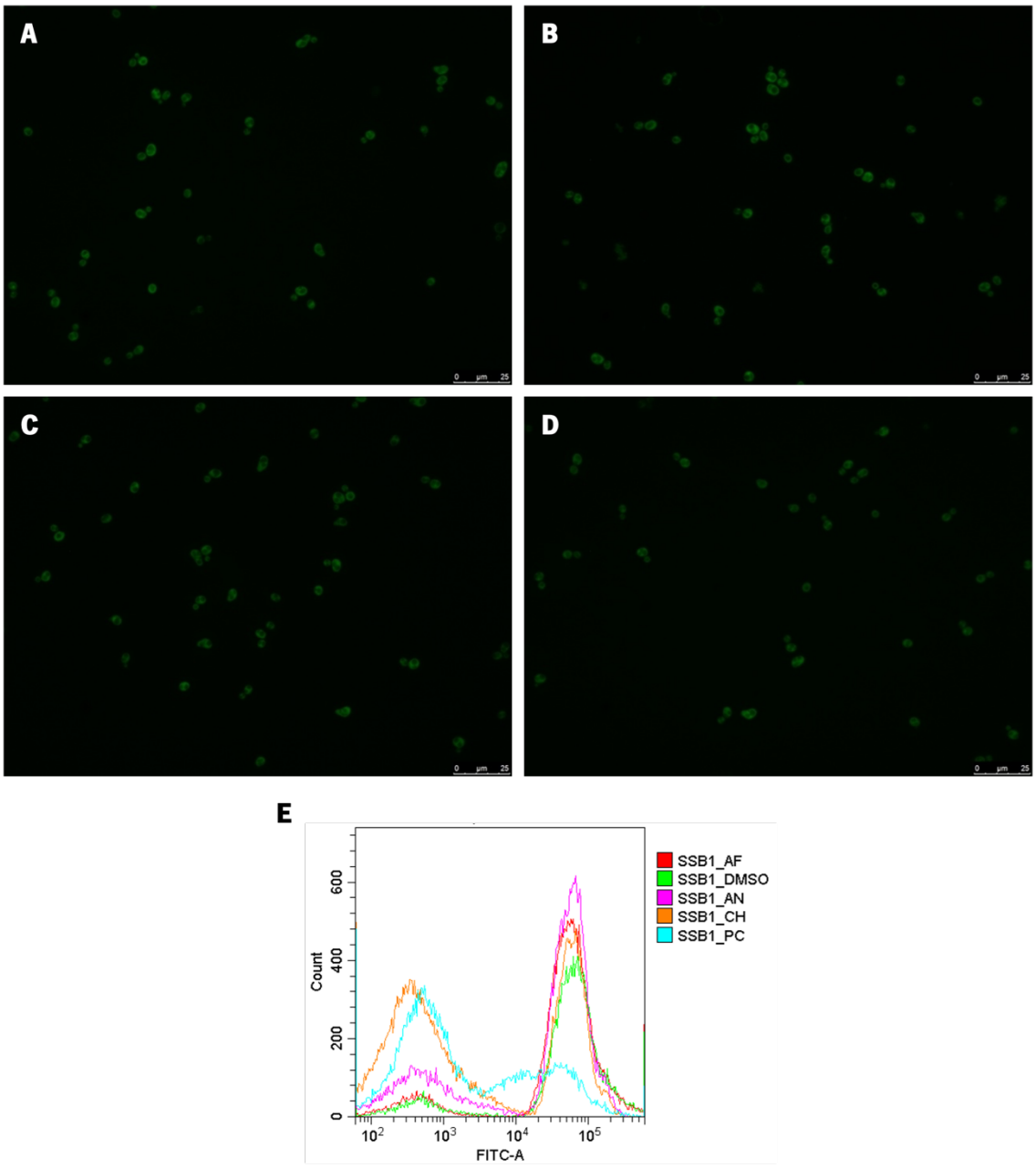


Figure 15. *Ascophylum nodosum* (AN), *Cystoseira humilis* (CH) and *Pterocladiella capillacea* (PC) on the activation of the SSB1 promoter of *Saccharomyces cerevisiae*. Representative microphotographs of a strain expressing GFP under the regulation of the SSB1 promoter (A-D). GFP fluorescence images were acquired in yeast treated with DMSO (A), 1 mg/mL AN (B), 1 mg/mL CH (C) or 1 mg/mL PC (D) for 20h. Representation of GFP fluorescence intensity by flow cytometry (E). The results are representative of three independent replicates. SSB1_AF: autofluorescence of yeast cells; SSB1_DMSO: negative control; SSB1_AN: cells treated with AN; SSB1_CH: cells treated with CH; SSB1_PC: cells treated with PC

In yeast, the aggregation and toxicity of the expanded polyglutamine fragment of human huntingtin strictly depend on the presence of self-perpetuating endogenous aggregated proteins (prions) that contain glutamine/asparagine-rich domains (Meriin *et al.*, 2002). Some chaperones from the Hsp100/70/40 complex, which modulate the propagation of yeast prions, have also been

reported to influence polyglutamine aggregation in yeast (Chernoff, 2001; Chernoff, 2004). However, it has not been clear whether they do so directly or through affecting the prions.

In cells with the [PIN+] prion, the absence of the *SSA2* gene, which encodes a heat shock protein involved in protein quality control, has a notable effect. The [PIN+] can promote the formation of polyglutamine aggregates, which are toxic to the cells. However, the absence of Ssa2 affects the growth of prion aggregates. This means that proteins that would typically aggregate may remain soluble, thereby reducing the expansion and toxicity of polyglutamine aggregates (KC *et al.*, 2005). Another study involving the [PSI+] prion, a variant of the Sup35 protein in yeast that induces abnormal protein states, also demonstrated that the overexpression of Ssa2 proteins promotes [PSI+] formation. Additionally, Ssa2 overexpression interferes with the action of Hsp104p, which normally eliminates [PSI+], allowing it to persist in the cells (Allen *et al.*, 2005; Jones & Tuite, 2005). This information is in line with the previously proposed role of Ssa in prion aggregate growth in yeast (Chernoff, 2001). On the other hand, it has been demonstrated that the overproduction of Ssb1 cures cells that propagate the prion form of Sup35p, [PSI+] (Chacinska *et al.*, 2001). Ssb1 is a yeast protein that assists in protein folding and quality control during translation, the process by which cells synthesize proteins (Nelson *et al.*, 1992). In the context of [PSI+], it can facilitate the conversion of misfolded Sup35p back to its regular and functional form, non-prionic (Chacinska *et al.*, 2001). That said, our results suggest that the mechanism through which these extracts exert their anti-aggregating effects does not involve the shutdown or activation of chaperones Ssa2 and Ssb1, respectively.

4.7.2. Expression of HSP104

HSP104 belongs to the HSP100 gene family and encodes a broad-spectrum anti-stress chaperones (Bösl *et al.*, 2006). Unlike typical chaperones that aim to prevent protein aggregation, Hsp104p, in collaboration with the chaperone Ssa1 and co-chaperone Ydj1, can help break down protein clumps that may form when proteins denature or misfold due to stress (Glover & Lindquist, 1998; Parsell *et al.*, 1994). This disaggregation activity is crucial for cell survival under stress. Normally, *HSP104* is expressed at minimal levels under ordinary conditions, but its expression increases significantly in response to stress (Sanchez & Lindquist, 1990). The effect of *HSP104* expression has been studied in yeast models of human disease, such as HD. These studies demonstrated that the overexpression of *HSP104* reduced the aggregation and toxicity of huntingtin

in mammalian cells and to extend the lifespan of transgenic Huntington mice (Lum *et al.*, 2004; Vacher *et al.*, 2005).

We aimed to test whether AN, CH and PC extracts promote the overexpression of the Hsp104 molecular chaperone (that does not have anti-apoptotic effects) and thus understand if this upregulation plays a role in reducing the formation of α-synuclein and huntingtin aggregates seen in Parkinson's and Huntington´s diseases. As in previous experiments, we used a strain where the coding region of *HSP104* was replaced by the GFP gene thus becoming under the transcriptional regulation of the HSP104 promoter. Therefore, GFP was a reporter of the activity of activation of the *HSP104* gene upon incubation with extracts. As expected, in the DMSO-treated control, *HSP104* was expressed at minimal levels (Figure 16A). As observed with Ssa2 and Ssb1, treatments with AN (Figure16B), CH (Figure16C) or PC (Figure16D) did not increase green fluorescence when compared to the control group treated with DMSO (Figure 16E), suggesting that the extracts did not promote expression of the Hsp104 chaperone. These results are in line with results obtained with analysis of expression of *SSA2* and *SSB1*, indicating the antiaggregant activity of AN, CH and PC does not involve activation of the unfolded protein response.

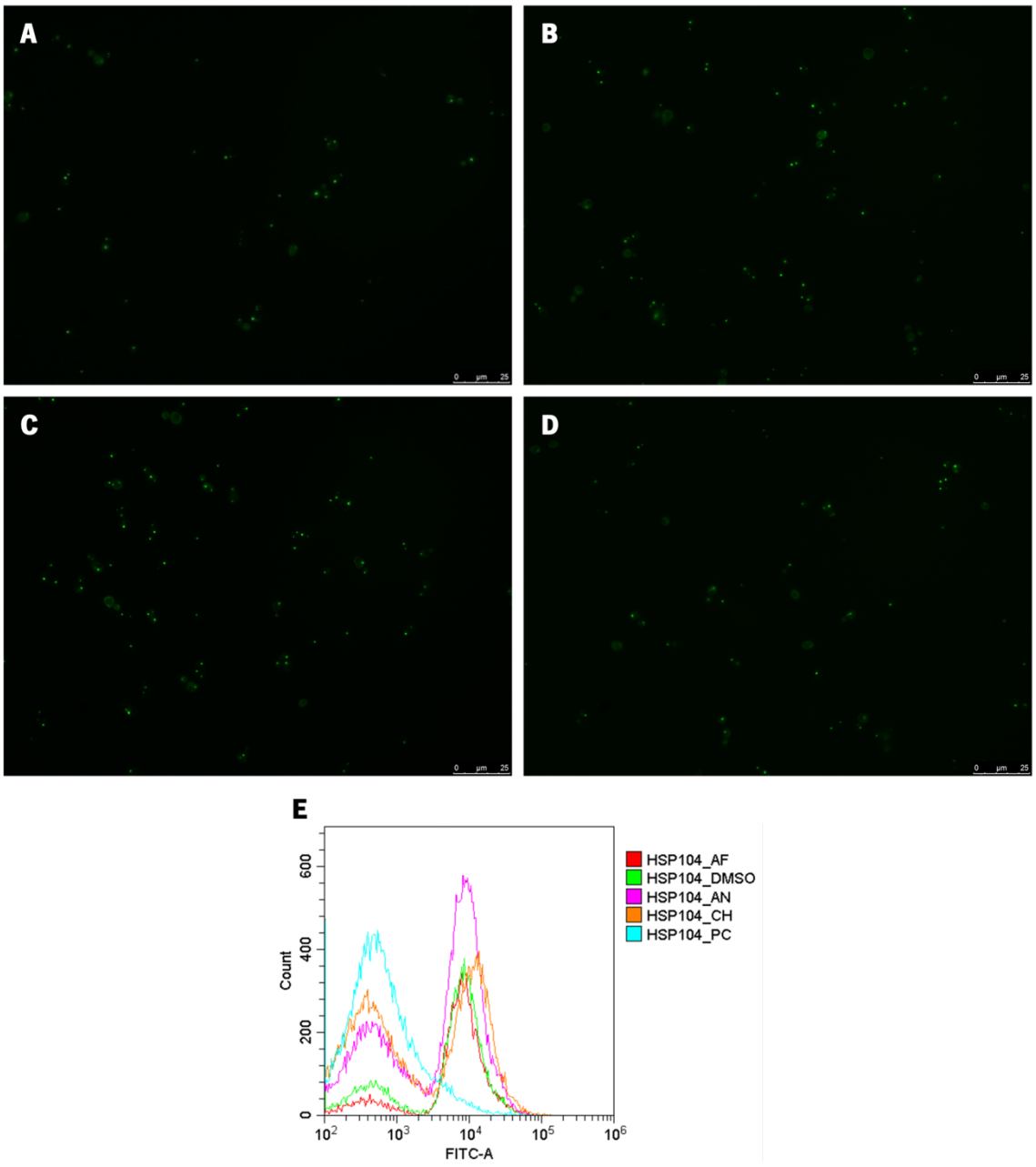


Figure 16. ***Ascophylum nodosum*** **(AN),** ***Cystoseira humilis*** **(CH) and** ***Pterocladiella capillacea*** **(PC) on the activation of the HSP104 promoter of** ***Saccharomyces cerevisiae*****.** Representative microphotographs of a strain expressing GFP under the regulation of the HSP104 promoter (A-D). GFP fluorescence images were acquired in yeast treated with DMSO (A), 1 mg/mL AN (B), 1 mg/mL CH (C) or 1 mg/mL PC (D) for 20h. Representation of GFP fluorescence intensity by flow cytometry (E). The results are representative of three independent replicates. HSP104_AF: autofluorescence of yeast cells; HSP104_DMSO: negative control; HSP104_AN: cells treated with AN; HSP104_CH: cells treated with CH; HSP104_PC: cells treated with PC.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Currently, there is still no effective treatment or cure for ND. This fact has led to a growing number of researchers in recent years focusing on the development of new, more selective, specific and effective therapeutic agents. Over the past few decades, numerous studies have highlighted the positive health effects of incorporating seaweed into one's diet (Burtin, 2003; Smit, 2004). It is worth noting that the occurrence of neurodegenerative diseases is lower in Southeast Asian countries compared to Europe (Jorm & Jolley, 1998; Mishra & Palanivelu, 2008), and this trend may be linked to the significant consumption of both fish and seaweed by some Asian populations, mainly Japan, Indonesia, China, Vietnam, South Korea, among others.

The wide variety of seaweed species and their many unique bioactive compounds make them an intriguing resource for potentially developing new treatments for ND. This research showcased how treatments involving macroalgae have promising prospects for addressing ND. Specifically, a set of extracts with established anti-radical properties bolstered the resilience of yeast cells when subjected to oxidative stress triggered by H_2O_2. Furthermore, these extracts mitigated intracellular oxidation in yeast exposed to H_2O_2 and demonstrated their effectiveness in decrease aggregation when applied to yeast models simulating PD and HD. Additional studies, particularly focused on the ability of chronic treatment with the AN, CH, and PC extracts revealed that while these extracts had a significant anti-aggregating effect, their mechanism of action does not involve the protection of proteins against thermal denaturation or the activation of UPR^{MT} triggered by the expression of chaperone proteins Ssa2, Ssb1 and Hsp104. In light of these results, additional experiments can be conducted to enhance our understanding of how these extracts work. One possible approach would involve evaluating mitochondrial performance. Dysfunctional mitochondria can increase oxidative stress, contributing to the formation of improperly folded and aggregated proteins (Yeger-Lotem *et al.*, 2009). Improving mitochondrial function may help reduce this stress, thereby decreasing the toxicity of aggregated proteins (Su *et al.*, 2010). Furthermore, a chronological life span (CLS) assay can be conducted simultaneously to determine if these extracts can extend it. Given that neurodegenerative diseases are inherently linked to the aging process, utilizing models that enable the investigation of proteotoxicity within the aging framework will offer a more appropriate approach for enhancing comprehension of these conditions and for examining alterations in mitochondrial function associated with aging and age-related diseases (Jarolim *et al.*, 2004; Ruetenik & Barrientos, 2018).

Additionally, in the absence of a chemical analysis of these extracts, it is not possible to determine which specific molecule or molecules are directly responsible for the neuroprotective effects. Therefore, a comprehensive examination of the chemical composition of these AN, CH, and PC extracts will be conducted in the future. It is possible that one or more of their bioactive components, such as fucoidans, phlorotannins, fucoxanthin, fatty acids, and others, might play a crucial role in the neuroprotective properties exhibited by these extracts. Research involving the isolated and purified forms of these compounds should be carried out, both in the present model and other superior models. Upon confirming their bioactivity, various administration methods, such as microencapsulation for enhanced bioavailability, can be explored. Another possibility is utilizing these compounds in dietary supplements.

In summary, this study demonstrates that ocean resources can be harnessed as a source of new bioactive agents that may prove effective in the treatment of various ND.

6. BIBLIOGRAPHY

Agregán, R., Munekata, P. E. S., Franco, D., Carballo, J., Barba, F. J., & Lorenzo, J. M. (2018). Antioxidant Potential of Extracts Obtained from Macro- (*Ascophyllum nodosum*, *Fucus vesiculosus* and *Bifurcaria bifurcata*) and Micro-Algae (*Chlorella vulgaris* and *Spirulina platensis*) Assisted by Ultrasound. *Medicines (Basel, Switzerland)*, *5*(2). https://doi.org/10.3390/medicines5020033

Ahn, B. R., Moon, H. E., Kim, H. R., Jung, H. A., & Choi, J. S. (2012). Neuroprotective effect of edible brown alga *Eisenia bicyclis* on amyloid beta peptide-induced toxicity in PC12 cells. *Archives of Pharmacal Research*, *35*(11), 1989–1998. https://doi.org/10.1007/s12272-012-1116-5

Airanthi, M. K. W.-A., Hosokawa, M., & Miyashita, K. (2011). Comparative antioxidant activity of edible Japanese brown seaweeds. *Journal of Food Science*, *76*(1), C104-11. https://doi.org/10.1111/j.1750-3841.2010.01915.x

Akyol, A., Herken, H., Uz, E., Fadıllıoğlu, E., Ünal, S., Söğüt, S., Özyurt, H., & Savaş, H. A. (2002). The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients: The possible role of oxidant/antioxidant imbalance. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *26*(5), 995–1005. https://doi.org/10.1016/S0278-5846(02)00220-8

Alencar, D., Carvalho, F., Rebouças, R., Santos, D., Pires-Cavalcante, K., Lima, R., Baracho, B., Bezerra, R., Viana, F., Vieira, R., Sampaio, A., Sousa, O., & Saker-Sampaio, S. (2016). Bioactive extracts of red seaweeds *Pterocladiella capillacea* and *Osmundaria obtusiloba* (Floridophyceae: Rhodophyta) with antioxidant and bacterial agglutination potential. *Asian Pacific Journal of Tropical Medicine*, *9*. https://doi.org/10.1016/j.apjtm.2016.03.015

Alghazwi, M., Smid, S., Musgrave, I., & Zhang, W. (2019). In vitro studies of the neuroprotective activities of astaxanthin and fucoxanthin against amyloid beta (Aβ1-42) toxicity and aggregation. *Neurochemistry International*, *124*, 215–224. https://doi.org/https://doi.org/10.1016/j.neuint.2019.01.010

Allen, K. D., Wegrzyn, R. D., Chernova, T. A., Müller, S., Newnam, G. P., Winslett, P. A., Wittich, K. B., Wilkinson, K. D., & Chernoff, Y. O. (2005). Hsp70 chaperones as modulators of prion life cycle: novel effects of Ssa and Ssb on the Saccharomyces cerevisiae prion [PSI+]. *Genetics*, *169*(3), 1227–1242. https://doi.org/10.1534/genetics.104.037168

Apostolidis, E., & Lee, C. M. (2010). *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated alpha-glucosidase and alpha-amylase inhibition. *Journal of Food Science*, *75*(3), H97-102. https://doi.org/10.1111/j.1750-3841.2010.01544.x

Balch, W. E., Morimoto, R. I., Dillin, A., & Kelly, J. W. (2008). Adapting proteostasis for disease intervention. *Science (New York, N.Y.)*, *319*(5865), 916–919. https://doi.org/10.1126/science.1141448

Barnham, K. J., Masters, C. L., & Bush, A. I. (2004). Neurodegenerative diseases and oxidatives stress. *Nature Reviews Drug Discovery*, *3*(3), 205–214. https://doi.org/10.1038/nrd1330

Barsanti, L., & Gualtieri, P. (2014). *Algae: Anatomy, Biochemistry, and Biotechnology*,.

Bartus, R. T. (2000). On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Experimental Neurology*, *163*(2), 495–529. https://doi.org/10.1006/exnr.2000.7397

Bates, G. (2003). Huntingtin aggregation and toxicity in Huntington's disease. *Lancet (London, England)*, *361*(9369), 1642–1644. https://doi.org/10.1016/S0140-6736(03)13304-1

Bates, G. P., Dorsey, R., Gusella, J. F., Hayden, M. R., Kay, C., Leavitt, B. R., Nance, M., Ross, C. A., Scahill, R. I., Wetzel, R., Wild, E. J., & Tabrizi, S. J. (2015). Huntington disease. *Nature Reviews Disease Primers*, *1*(1), 15005. https://doi.org/10.1038/nrdp.2015.5

Bauer, P. O., & Nukina, N. (2009). The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. In *Journal of Neurochemistry*. https://doi.org/10.1111/j.1471-4159.2009.06302.x

Bayliak, M., Burdyliuk, N., Izers'ka, L., & Lushchak, V. (2014). Concentration-Dependent Effects of *Rhodiola Rosea* on Long-Term Survival and Stress Resistance of Yeast *Saccharomyces cerevisiae*: The Involvement of YAP 1 and MSN2/4 Regulatory Proteins. *Dose-Response : A Publication of International Hormesis Society*, *12*, 93–109. https://doi.org/10.2203/dose-response.13-013.Bayliak

Becker, J., & Craig, E. A. (1994). Heat-shock proteins as molecular chaperones. *European Journal of Biochemistry*, *219*(1–2), 11–23. https://doi.org/10.1007/978-3-642-79502-2_2

Behl, C., & Moosmann, B. (2002). Antioxidant neuroprotection in Alzheimer's disease as preventive and therapeutic approach. This article is part of a series of reviews on "Causes and Consequences of Oxidative Stress in Alzheimer's Disease." The full list of papers may be found on the ho. *Free Radical Biology and Medicine*, *33*(2), 182–191. https://doi.org/10.1016/s0891-5849(02)00883-3

Belattmania, Z., Engelen, A., Pereira, H., Serrao, E., Mustapha, B., Samir, E. A., Zrid, R., Bentiss, F., Chahboun, N., Reani, A., & Sabour, B. (2016). Potential uses of the brown seaweed *Cystoseira humilis* biomass: 2- Fatty acid composition, antioxidant and antibacterial activities. *Journal of Materials and Environmental Science*, *7*, 2074–2081.

Berardelli, A., Rothwell, J. C., Thompson, P. D., & Hallett, M. (2001). Pathophysiology of bradykinesia in Parkinson's disease. *Brain : A Journal of Neurology*, *124*(Pt 11), 2131–2146. https://doi.org/10.1093/brain/124.11.2131

Bösl, B., Grimminger, V., & Walter, S. (2006). The molecular chaperone Hsp104–a molecular machine for protein disaggregation. *Journal of Structural Biology*, *156*(1), 139–148. https://doi.org/10.1016/j.jsb.2006.02.004

Boujaber, N., Oumaskour, K., Etahiri, S., & Assobhei, O. (2013). Cytotoxic activity of some marine algae collected from the coast of Sidi Bouzid (El Jadida-Morocco). *INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACEUTICAL RESEARCH*, *4*, 2542 – 2547.

Bukau, B., & Horwich, A. L. (1998). The Hsp70 and Hsp60 chaperone machines. *Cell*, *92*(3), 351–366. https://doi.org/10.1016/s0092-8674(00)80928-9

Burtin, P. (2003). Nutritional value of seaweeds. *Electron. J. Environ. Agric. Food Chem*, *2*.

Butterfield, D. A., & Halliwell, B. (2019). Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nature Reviews. Neuroscience*, *20*(3), 148–160. https://doi.org/10.1038/s41583-019-0132-6

Chacinska, A., Szczesniak, B., Kochneva-Pervukhova, N. V, Kushnirov, V. V, Ter-Avanesyan, M. D., & Boguta, M. (2001). Ssb1 chaperone is a [PSI+] prion-curing factor. *Current Genetics*, *39*(2), 62–67. https://doi.org/10.1007/s002940000180

Chan, C. X., Ho, C. L., & Phang, S. M. (2006). Trends in seaweed research. *Trends in Plant Science*, *11*(4), 165–166. https://doi.org/10.1016/j.tplants.2006.02.003

Chen, Z., & Zhong, C. (2014). Oxidative stress in Alzheimer's disease. *Neuroscience Bulletin*, *30*(2), 271–281. https://doi.org/10.1007/s12264-013-1423-y

Chernoff, Y O. (2001). Mutation processes at the protein level: is Lamarck back? *Mutation Research*, *488*(1), 39–64. https://doi.org/10.1016/s1383-5742(00)00060-0

Chernoff, Yury O. (2004). Replication vehicles of protein-based inheritance. *Trends in Biotechnology*, *22*(11), 549–552. https://doi.org/10.1016/j.tibtech.2004.09.001

Chiti, F., & Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annual Review of Biochemistry*, *75*, 333–366.

https://doi.org/10.1146/annurev.biochem.75.101304.123901

Choi, B. W., Ryu, G., Park, S. H., Kim, E. S., Shin, J., Roh, S. S., Shin, H. C., & Lee, B. H. (2007). Anticholinesterase activity of plastoquinones from *Sargassum sagamianum*: lead compounds for Alzheimer's disease therapy. *Phytotherapy Research : PTR*, *21*(5), 423–426. https://doi.org/10.1002/ptr.2090

Choi, J. S., Han, Y. R., Byeon, J. S., Choung, S.-Y., Sohn, H. S., & Jung, H. A. (2015). Protective effect of fucosterol isolated from the edible brown algae, *Ecklonia stolonifera* and *Eisenia bicyclis*, on tert-butyl hydroperoxide- and tacrine-induced HepG2 cell injury. *Journal of Pharmacy and Pharmacology*, *67*(8), 1170–1178. https://doi.org/10.1111/jphp.12404

Craig, E., & Jacobsen, K. (1985). Mutations in cognate genes of *Saccharomyces cerevisiae* hsp70 result in reduced growth rates at low temperatures. *Molecular and Cellular Biology*, *5*(12), 3517–3524.

Currais, A., Fischer, W., Maher, P., & Schubert, D. (2017). Intraneuronal protein aggregation as a trigger for inflammation and neurodegeneration in the aging brain. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *31*(1), 5–10. https://doi.org/10.1096/fj.201601184

Custódio, L., Silvestre, L., Rocha, M. I., Rodrigues, M. J., Vizetto-Duarte, C., Pereira, H., Barreira, L., & Varela, J. (2016). Methanol extracts from *Cystoseira tamariscifolia* and *Cystoseira nodicaulis* are able to inhibit cholinesterases and protect a human dopaminergic cell line from hydrogen peroxide-induced cytotoxicity. *Pharmaceutical Biology*, *54*(9), 1687–1696. https://doi.org/10.3109/13880209.2015.1123278

Custódio, L., Soares, F., Pereira, H., Rodrigues, M. J., Barreira, L., Rauter, A. P., Alberício, F., & Varela, J. (2015). Botryococcus braunii and Nannochloropsis oculata extracts inhibit cholinesterases and protect human dopaminergic SH-SY5Y cells from H2O2-induced cytotoxicity. *Journal of Applied Phycology*, *27*(2), 839–848. https://doi.org/10.1007/s10811-014-0369-4

D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B., & Masella, R. (2010). Bioavailability of the polyphenols: status and controversies. *International Journal of Molecular Sciences*, *11*(4), 1321–1342. https://doi.org/10.3390/ijms11041321

de Lau, L. M. L., & Breteler, M. M. B. (2006). Epidemiology of Parkinson's disease. *The Lancet. Neurology*, *5*(6), 525–535. https://doi.org/10.1016/S1474-4422(06)70471-9

de los Ríos, C. (2012). Cholinesterase inhibitors: a patent review (2007 - 2011). *Expert Opinion on*

Therapeutic Patents, *22*(8), 853–869. https://doi.org/10.1517/13543776.2012.701619

De Maio, A. (1999). Heat shock proteins: facts, thoughts, and dreams. *Shock (Augusta, Ga.)*, *11*(1), 1–12. https://doi.org/10.1097/00024382-199901000-00001

Decreto-Lei nº 62/2020. (2021). Presidência do Conselho de Ministros. Resolução do Conselho de Ministros 59/2001. *Diário Da República - I Série-B*, *108*(2), 23–62. https://dre.pt/application/conteudo/496727

Deshaies, R. J., Koch, B. D., Werner-Washburne, M., Craig, E. A., & Schekman, R. (1988). A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. *Nature*, *332*(6167), 800–805. https://doi.org/10.1038/332800a0

Dickson, D. W. (2012). Parkinson's disease and parkinsonism: neuropathology. *Cold Spring Harbor Perspectives in Medicine*, *2*(8). https://doi.org/10.1101/cshperspect.a009258

DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., & Aronin, N. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science (New York, N.Y.)*, *277*(5334), 1990–1993. https://doi.org/10.1126/science.277.5334.1990

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, *82*(1), 47–95. https://doi.org/10.1152/physrev.00018.2001

Duennwald, M. L., Jagadish, S., Muchowski, P. J., & Lindquist, S. (2006). Flanking sequences profoundly alter polyglutamine toxicity in yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(29), 11045–11050. https://doi.org/10.1073/pnas.0604547103

Estruch, F., & Carlson, M. (1993). Two homologous zinc finger genes identified by multicopy suppression in a SNF1 protein kinase mutant of *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, *13*(7), 3872–3881. https://doi.org/10.1128/mcb.13.7.3872-3881.1993

Flower, T. R., Chesnokova, L. S., Froelich, C. A., Dixon, C., & Witt, S. N. (2005). Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. *Journal of Molecular Biology*, *351*(5), 1081–1100. https://doi.org/10.1016/j.jmb.2005.06.060

Frucht, S. J. (2004). Parkinson disease: an update. *The Neurologist*, *10*(4), 185–194. https://doi.org/10.1097/01.nrl.0000131146.08278.a5

Gallagher, D. A., & Schapira, A. H. V. (2009). Etiopathogenesis and treatment of Parkinson's disease. *Current Topics in Medicinal Chemistry*, *9*(10), 860–868.

Gan, S. Y., Wong, L. Z., Wong, J. W., & Tan, E. L. (2019). Fucosterol exerts protection against

amyloid β-induced neurotoxicity, reduces intracellular levels of amyloid β and enhances the mRNA expression of neuroglobin in amyloid β-induced SH-SY5Y cells. *International Journal of Biological Macromolecules*, *121*, 207–213. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2018.10.021

Gao, Y., Dong, C., Yin, J., Shen, J., Tian, J., & Li, C. (2012). Neuroprotective effect of fucoidan on H2O2-induced apoptosis in PC12 cells via activation of PI3K/Akt pathway. *Cellular and Molecular Neurobiology*, *32*(4), 523–529. https://doi.org/10.1007/s10571-011-9792-0

Gidalevitz, T., Ben-Zvi, A., Ho, K. H., Brignull, H. R., & Morimoto, R. I. (2006). Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science*. https://doi.org/10.1126/science.1124514

Gietz, R. D., & Schiestl, R. H. (2007). High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nature Protocols*, *2*(1), 31–34. https://doi.org/10.1038/nprot.2007.13

Gisbert, M., Franco, D., Sineiro, J., & Moreira, R. (2023). Antioxidant and Antidiabetic Properties of Phlorotannins from *Ascophyllum nodosum* Seaweed Extracts. *Molecules (Basel, Switzerland)*, *28*(13). https://doi.org/10.3390/molecules28134937

Glover, J. R., & Lindquist, S. (1998). Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell*, *94*(1), 73–82. https://doi.org/10.1016/s0092-8674(00)81223-4

Grina, F., Ullah, Z., Kaplaner, E., Moujahid, A., Eddoha, R., Nasser, B., Terzioğlu, P., Yilmaz, M. A., Ertaş, A., Öztürk, M., & Essamadi, A. (2020). *In vitro* enzyme inhibitory properties, antioxidant activities, and phytochemical fingerprints of five Moroccan seaweeds. *South African Journal of Botany*, *128*, 152–160. https://doi.org/https://doi.org/10.1016/j.sajb.2019.10.021

Grozdanić, N., Kosanić, M., Etahiri, S., Assobhei, O., Kljajic, Z., & Stanojkovic, T. (2016). An insight into cytotoxic and antimicrobial effects of *Cystoseira humilis* crude extract. *Studia Marina*, *29*, 21–30.

Guiry M.D. & Guiry, G. M. (2023). AlgaeBase. *World-Wide Electronic Publication, National University of Ireland, Galway*. http://www.algaebase.org

Gutekunst, C. A., Li, S. H., Yi, H., Mulroy, J. S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R. J., Hersch, S. M., & Li, X. J. (1999). Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(7), 2522–2534. https://doi.org/10.1523/JNEUROSCI.19-07-

02522.1999

Hackam, A. S., Singaraja, R., Wellington, C. L., Metzler, M., McCutcheon, K., Zhang, T., Kalchman, M., & Hayden, M. R. (1998). The influence of huntingtin protein size on nuclear localization and cellular toxicity. *The Journal of Cell Biology*, *141*(5), 1097–1105. https://doi.org/10.1083/jcb.141.5.1097

Hamdona, S., Negm, M., Ibrahim, H., Shaltout, N., Shawky, H., & Abdel-Mottaleb, M. (2018). *Green Synthesis of Silver nanoparticles Using Marine Algae Extract and Their Antibacterial Activity*.

Harper, P. S. (1992). The epidemiology of Huntington's disease. *Human Genetics*, *89*(4), 365–376. https://doi.org/10.1007/BF00194305

Hartl, F. U., Bracher, A., & Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature*, *475*(7356), 324–332. https://doi.org/10.1038/nature10317

Hartl, F. U., & Hayer-Hartl, M. (2009). Converging concepts of protein folding *in vitro* and *in vivo*. *Nature Structural & Molecular Biology*, *16*(6), 574–581. https://doi.org/10.1038/nsmb.1591

Hayden, M. R. (2012). *Huntington's chorea*. Springer Science & Business Media.

Heo, S.-J., Cha, S.-H., Lee, K.-W., & Jeon, Y.-J. (2006). Antioxidant Activities of Red Algae from Jeju Island. *Algae*, *21*, 149–156. https://doi.org/10.4490/ALGAE.2006.21.1.149

Heo, S.-J., & Jeon, Y.-J. (2009). Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage. *Journal of Photochemistry and Photobiology. B, Biology*, *95*(2), 101–107. https://doi.org/10.1016/j.jphotobiol.2008.11.011

Heo, S.-J., Ko, S.-C., Kang, S.-M., Kang, H.-S., Kim, J.-P., Kim, S.-H., Lee, K.-W., Cho, M.-G., & Jeon, Y.-J. (2008). Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H2O2-induced cell damage. *European Food Research and Technology*, *228*(1), 145–151. https://doi.org/10.1007/s00217-008-0918-7

Hipp, M. S., Park, S.-H., & Hartl, F. U. (2014). Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends in Cell Biology*, *24*(9), 506–514. https://doi.org/10.1016/j.tcb.2014.05.003

Holdt, S., & Kraan, S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology*, *23*, 543–597. https://doi.org/10.1007/s10811-010-9632-5

Horovitz, A., Reingewertz, T. H., Cuéllar, J., & Valpuesta, J. M. (2022). Chaperonin Mechanisms:

Multiple and (Mis)Understood? *Annual Review of Biophysics*, *51*, 115–133. https://doi.org/10.1146/annurev-biophys-082521-113418

Hu, L., Chen, W., Tian, F., Yuan, C., Wang, H., & Yue, H. (2018). Neuroprotective role of fucoxanthin against cerebral ischemic/reperfusion injury through activation of Nrf2/HO-1 signaling. *Biomedicine & Pharmacotherapy*, *106*, 1484–1489. https://doi.org/https://doi.org/10.1016/j.biopha.2018.07.088

Ibtissam, C., Hassane, R., José, M.-L., Seglar, D., Francisco, J., Vidal, G., Antonio, J., Bouziane, H., & Kadiri, M. (2010). *Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco*.

Ikeda, K., Kitamura, A., Machida, H., Watanabe, M., Negishi, H., Hiraoka, J., & Nakano, T. (2003). Effect of *Undaria pinnatifida* (Wakame) on the development of cerebrovascular diseases in stroke-prone spontaneously hypertensive rats. *Clinical and Experimental Pharmacology & Physiology*, *30*(1–2), 44–48. https://doi.org/10.1046/j.1440-1681.2003.03786.x

Inoue, H., Nojima, H., & Okayama, H. (1990). High efficiency transformation of *Escherichia coli* with plasmids. *Gene*, *96*(1), 23–28. https://doi.org/https://doi.org/10.1016/0378-1119(90)90336-P

Jankovic, J., Schwartz, K. S., & Ondo, W. (1999). Re-emergent tremor of Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *67*(5), 646–650. https://doi.org/10.1136/jnnp.67.5.646

Jarolim, S., Millen, J., Heeren, G., Laun, P., Goldfarb, D. S., & Breitenbach, M. (2004). A novel assay for replicative lifespan in *Saccharomyces cerevisiae*. *FEMS Yeast Research*, *5*(2), 169–177. https://doi.org/10.1016/j.femsyr.2004.06.015

Jones, G. W., & Tuite, M. F. (2005). Chaperoning prions: the cellular machinery for propagating an infectious protein? *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology*, *27*(8), 823–832. https://doi.org/10.1002/bies.20267

Jorm, A. F., & Jolley, D. (1998). The incidence of dementia: a meta-analysis. *Neurology*, *51*(3), 728–733. https://doi.org/10.1212/wnl.51.3.728

Jung, H. A., Ali, M. Y., Choi, R. J., Jeong, H. O., Chung, H. Y., & Choi, J. S. (2016). Kinetics and molecular docking studies of fucosterol and fucoxanthin, BACE1 inhibitors from brown algae *Undaria pinnatifida* and *Ecklonia stolonifera*. *Food and Chemical Toxicology*, *89*, 104–111. https://doi.org/https://doi.org/10.1016/j.fct.2016.01.014

Kang, H.-K., Seo, C. H., & Park, Y. (2015). The effects of marine carbohydrates and glycosylated

compounds on human health. *International Journal of Molecular Sciences*, *16*(3), 6018–6056. https://doi.org/10.3390/ijms16036018

Kang, S. M., Cha, S. H., & Ko, J. Y. (2012). Neuroprotective effects of phlorotannins isolated from a brown alga, *Ecklonia cava*, against H2O2-induced oxidative stress in murine hippocampal HT22 cells. *Environ. Toxicol. Pharmacol.*, *34*, 96.

Kantcheva, R. B., Mason, R., & Giorgini, F. (2014). Aggregation-prone proteins modulate huntingtin inclusion body formation in yeast. *PLoS Currents*, *6*. https://doi.org/10.1371/currents.hd.501008f3051342c9a5c0cd0f3a5bf3a4

KC, G., GP, N., MY, S., & YO, C. (2005). Modulation of prion-dependent polyglutamine aggregation and toxicity by chaperone proteins in the yeast model. *The Journal of Biological Chemistry*, *280*(24), 22809–22818.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*(4), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Kelman, D., Posner, E., McDermid, K., Tabandera, N., Wright, P., & Wright, A. (2012). Antioxidant Activity of Hawaiian Marine Algae. *Marine Drugs*, *10*, 403–416. https://doi.org/10.3390/md10020403

Kester, M. I., & Scheltens, P. (2009). Dementia: THE BARE ESSENTIALS. *Practical Neurology*. https://doi.org/10.1136/jnnp.2009.182477

Kim, J.-J., Kang, Y.-J., Shin, S.-A., Bak, D.-H., Lee, J. W., Lee, K. B., Yoo, Y. C., Kim, D.-K., Lee, B. H., Kim, D. W., Lee, J., Jo, E.-K., & Yuk, J.-M. (2016). Phlorofucofuroeckol Improves Glutamate-Induced Neurotoxicity through Modulation of Oxidative Stress-Mediated Mitochondrial Dysfunction in PC12 Cells. *PloS One*, *11*(9), e0163433. https://doi.org/10.1371/journal.pone.0163433

Kim, S. K., & Pangestuti, R. (2011). Biological activities and potential health benefits of fucoxanthin derived from marine brown algae. In *Advances in Food and Nutrition Research* (1st ed., Vol. 64). Elsevier Inc. https://doi.org/10.1016/B978-0-12-387669-0.00009-0

Kim, S. M., Jung, Y. J., Kwon, O. N., Cha, K. H., Um, B. H., Chung, D., & Pan, C. H. (2012). A potential commercial source of fucoxanthin extracted from the microalga *Phaeodactylum tricornutum*. *Applied Biochemistry and Biotechnology*, *166*(7), 1843–1855. https://doi.org/10.1007/s12010-012-9602-2

Klucken, J., Shin, Y., Masliah, E., Hyman, B. T., & McLean, P. J. (2004). Hsp70 Reduces alpha-

Synuclein Aggregation and Toxicity. *The Journal of Biological Chemistry*, *279*(24), 25497–25502. https://doi.org/10.1074/jbc.M400255200

Kouli, A., Torsney, K. M., & Kuan, W.-L. (2018). *Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis.* (T. B. Stoker & J. C. Greenland (eds.)). https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch1

Krobitsch, S., & Lindquist, S. (2000). Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(4), 1589–1594. https://doi.org/10.1073/pnas.97.4.1589

Leandro, A., Pereira, L., & Gonçalves, A. M. M. (2020). Diverse applications of marine macroalgae. *Marine Drugs*, *18*(1), 1–15. https://doi.org/10.3390/md18010017

Lee, J., & Jun, M. (2019). Dual BACE1 and Cholinesterase Inhibitory Effects of Phlorotannins from *Ecklonia cava*—An *In Vitro* and *in Silico* Study. In *Marine Drugs* (Vol. 17, Issue 2). https://doi.org/10.3390/md17020091

Lee, Sanghyun, Yeon, S. L., Sang, H. J., Sam, S. K., & Kuk, H. S. (2003). Anti-oxidant activities of fucosterol from the marine algae *Pelvetia siliquosa*. *Archives of Pharmacal Research*, *26*(9), 719–722. https://doi.org/10.1007/BF02976680

Lee, Seungeun, Youn, K., Kim, D. H., Ahn, M.-R., Yoon, E., Kim, O.-Y., & Jun, M. (2018). Anti-Neuroinflammatory Property of Phlorotannins from *Ecklonia cava* on Aβ25-35-Induced Damage in PC12 Cells. *Marine Drugs*, *17*(1), E7. https://doi.org/10.3390/md17010007

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *Lancet (London, England)*, *373*(9680), 2055–2066. https://doi.org/10.1016/S0140-6736(09)60492-X

Li, J., O, W., Li, W., Jiang, Z.-G., & Ghanbari, H. A. (2013). Oxidative stress and neurodegenerative disorders. *International Journal of Molecular Sciences*, *14*(12), 24438–24475. https://doi.org/10.3390/ijms141224438

Liang, J., Clark-Dixon, C., Wang, S., Flower, T. R., Williams-Hart, T., Zweig, R., Robinson, L. C., Tatchell, K., & Witt, S. N. (2008). Novel suppressors of alpha-synuclein toxicity identified using yeast. *Human Molecular Genetics*, *17*(23), 3784–3795. https://doi.org/10.1093/hmg/ddn276

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *13*, 757–772. https://doi.org/10.2147/CIA.S158513

Lin, J., Huang, L., Yu, J., Xiang, S., Wang, J., Zhang, J., Yan, X., Cui, W., He, S., & Wang, Q. (2016). Fucoxanthin, a Marine Carotenoid, Reverses Scopolamine-Induced Cognitive Impairments in Mice and Inhibits Acetylcholinesterase in Vitro. In *Marine Drugs* (Vol. 14, Issue 4). https://doi.org/10.3390/md14040067

Lin, J. J., Mak, S. H., & Zheng, J. C. (2017). Assessment of Neuronal Viability Using Fluorescein Diacetate-Propidium Iodide Double Staining in Cerebellar Granule Neuron Culture. *J. Visualized Exp.*, *123*, e55442.

Lin, X., Koelsch, G., Wu, S., Downs, D., Dashti, A., & Tang, J. (2000). Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(4), 1456–1460. https://doi.org/10.1073/pnas.97.4.1456

Linné, C. von. (1753). *Species plantarum : exhibentes plantas rite cognitas ad genera relatas, cum diferentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas : Vol. t.1 (1753)*. Junk. https://www.biodiversitylibrary.org/item/84235

Lomartire, S., & Gonçalves, A. M. M. (2022). An Overview of Potential Seaweed-Derived Bioactive Compounds for Pharmaceutical Applications. *Marine Drugs*, *20*(2). https://doi.org/10.3390/md20020141

López-Ribot, J. L., & Chaffin, W. L. (1996). Members of the Hsp70 family of proteins in the cell wall of *Saccharomyces cerevisiae*. *Journal of Bacteriology*, *178*(15), 4724–4726. https://doi.org/10.1128/jb.178.15.4724-4726.1996

Lopez, N., Halladay, J., Walter, W., & Craig, E. A. (1999). SSB, encoding a ribosome-associated chaperone, is coordinately regulated with ribosomal protein genes. *Journal of Bacteriology*, *181*(10), 3136–3143. https://doi.org/10.1128/JB.181.10.3136-3143.1999

Lu, W. Y., Li, H. J., Li, Q. Y., & Wu, Y. C. (2021). Application of marine natural products in drug research. *Bioorganic and Medicinal Chemistry*, *35*(December 2020), 116058. https://doi.org/10.1016/j.bmc.2021.116058

Luk, K. C., Mills, I. P., Trojanowski, J. Q., & Lee, V. M.-Y. (2008). Interactions between Hsp70 and the hydrophobic core of alpha-synuclein inhibit fibril assembly. *Biochemistry*, *47*(47), 12614–12625. https://doi.org/10.1021/bi801475r

Lum, R., Tkach, J. M., Vierling, E., & Glover, J. R. (2004). Evidence for an unfolding/threading mechanism for protein disaggregation by *Saccharomyces cerevisiae* Hsp104. *The Journal*

of Biological Chemistry, *279*(28), 29139–29146. https://doi.org/10.1074/jbc.M403777200

Ma, M., & Liu, Z. L. (2010). Comparative transcriptome profiling analyses during the lag phase uncover YAP1, PDR1, PDR3, RPN4, and HSF1 as key regulatory genes in genomic adaptation to the lignocellulose derived inhibitor HMF for *Saccharomyces cerevisiae*. *BMC Genomics*, *11*, 660. https://doi.org/10.1186/1471-2164-11-660

MacDonald, M. E., Ambrose, C. M., Duyao, M. P., Myers, R. H., Lin, C., Srinidhi, L., Barnes, G., Taylor, S. A., James, M., Groot, N., MacFarlane, H., Jenkins, B., Anderson, M. A., Wexler, N. S., Gusella, J. F., Bates, G. P., Baxendale, S., Hummerich, H., Kirby, S., Harper, P. S. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, *72*(6), 971–983. https://doi.org/https://doi.org/10.1016/0092-8674(93)90585-E

Machado, L. P., Matsumoto, S. T., Jamal, C. M., da Silva, M. B., Centeno, D. da C., Colepicolo Neto, P., de Carvalho, L. R., & Yokoya, N. S. (2014). Chemical analysis and toxicity of seaweed extracts with inhibitory activity against tropical fruit anthracnose fungi. *Journal of the Science of Food and Agriculture*, *94*(9), 1739–1744. https://doi.org/10.1002/jsfa.6483

Maeda, H., Fukuda, S., Izumi, H., & Saga, N. (2018). Anti-oxidant and fucoxanthin contents of brown alga ishimozuku (*Sphaerotrichia divaricata*) from the west coast of aomori, Japan. *Marine Drugs*, *16*(8), 1–10. https://doi.org/10.3390/md16080255

Martinez-Martin, P., Rodriguez-Blazquez, C., Kurtis, M. M., & Chaudhuri, K. R. (2011). The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *26*(3), 399–406. https://doi.org/10.1002/mds.23462

Mason, R. P., & Giorgini, F. (2011). Modeling Huntington disease in yeast: perspectives and future directions. *Prion*, *5*(4), 269–276. https://doi.org/10.4161/pri.18005

Mayer, A., Rodríguez, A., Berlinck, R., & Hamann, M. (2009). Marine pharmacology in 2005-6: Marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal,. *Biochimica et Biophysica*, *1790*(5), 283–308. https://doi.org/10.1016/j.bbagen.2009.03.011.Marine

McGowan, D. P., van Roon-Mom, W., Holloway, H., Bates, G. P., Mangiarini, L., Cooper, G. J., Faull, R. L., & Snell, R. G. (2000). Amyloid-like inclusions in Huntington's disease. *Neuroscience*, *100*(4), 677–680. https://doi.org/10.1016/s0306-4522(00)00391-2

McLean, P. J., Klucken, J., Shin, Y., & Hyman, B. T. (2004). Geldanamycin induces Hsp70 and prevents alpha-synuclein aggregation and toxicity *in vitro*. *Biochemical and Biophysical Research Communications*, *321*(3), 665–669. https://doi.org/10.1016/j.bbrc.2004.07.021

Mendel, B., & Rudney, H. (1943). On the type of cholinesterase present in brain tissue. *Science (New York, N.Y.)*, *98*(2539), 201–202. https://doi.org/10.1126/science.98.2539.201

Meriin, A. B., Zhang, X., He, X., Newnam, G. P., Chernoff, Y. O., & Sherman, M. Y. (2002). Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. *The Journal of Cell Biology*, *157*(6), 997–1004. https://doi.org/10.1083/jcb.200112104

Migliore, L., & Coppedè, F. (2009). Environmental-induced oxidative stress in neurodegenerative disorders and aging. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *674*(1–2), 73–84. https://doi.org/10.1016/j.mrgentox.2008.09.013

Mishra, S., & Palanivelu, K. (2008). The effect of curcumin (turmeric) on Alzheimer's disease: An overview. *Annals of Indian Academy of Neurology*, *11*(1), 13–19. https://doi.org/10.4103/0972-2327.40220

Mohibbullah, M., Haque, M. N., Khan, M. N. A., Park, I.-S., Moon, I. S., & Hong, Y.-K. (2018). Neuroprotective effects of fucoxanthin and its derivative fucoxanthinol from the phaeophyte *Undaria pinnatifida* attenuate oxidative stress in hippocampal neurons. *Journal of Applied Phycology*, *30*(6), 3243–3252. https://doi.org/10.1007/s10811-018-1458-6

Muchowski, P. J., Schaffar, G., Sittler, A., Wanker, E. E., Hayer-Hartl, M. K., & Hartl, F. U. (2000). Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(14), 7841–7846. https://doi.org/10.1073/pnas.140202897

Murugan, A. C., Vallal, D., Karim, M. R., Govindan, N., Yusoff, M. B. M., & Rahman, M. M. (2015). *In vitro* antiradical and neuroprotective activity of polyphenolic extract from marine algae *Padina australis* H. *Journal of Chemical and Pharmaceutical Research*, *7*(8), 355–362.

Nelson, R. J., Ziegelhoffer, T., Nicolet, C., Werner-Washburne, M., & Craig, E. A. (1992). The translation machinery and 70 kd heat shock protein cooperate in protein synthesis. *Cell*, *71*(1), 97–105. https://doi.org/10.1016/0092-8674(92)90269-i

Niedzielska, E., Smaga, I., Gawlik, M., Moniczewski, A., Stankowicz, P., Pera, J., & Filip, M. (2016). Oxidative Stress in Neurodegenerative Diseases. *Molecular Neurobiology*, *53*(6), 4094–

4125. https://doi.org/10.1007/s12035-015-9337-5

Nishikawa, S. I., Fewell, S. W., Kato, Y., Brodsky, J. L., & Endo, T. (2001). Molecular chaperones in the yeast endoplasmic reticulum maintain the solubility of proteins for retrotranslocation and degradation. *The Journal of Cell Biology*, *153*(5), 1061–1070. https://doi.org/10.1083/jcb.153.5.1061

Nunes, N., Rosa, G. P., Ferraz, S., Barreto, M. C., & de Carvalho, M. A. A. P. (2020). Fatty acid composition, TLC screening, ATR-FTIR analysis, anti-cholinesterase activity, and *in vitro* cytotoxicity to A549 tumor cell line of extracts of 3 macroalgae collected in Madeira. *Journal of Applied Phycology*, *32*(2), 759–771. https://doi.org/10.1007/s10811-019-01884-9

Nwaka, S., Mechler, B., von Ahsen, O., & Holzer, H. (1996). The heat shock factor and mitochondrial Hsp70 are necessary for survival of heat shock in *Saccharomyces cerevisiae*. *FEBS Letters*, *399*(3), 259–263. https://doi.org/https://doi.org/10.1016/S0014-5793(96)01336-1

Oh, J. H., Choi, J. S., & Nam, T.-J. (2018). Fucosterol from an Edible Brown Alga *Ecklonia stolonifera* Prevents Soluble Amyloid Beta-Induced Cognitive Dysfunction in Aging Rats. In *Marine Drugs* (Vol. 16, Issue 10). https://doi.org/10.3390/md16100368

Olasehinde, T. A., Olaniran, A. O., & Okoh, A. I. (2019). Macroalgae as a Valuable Source of Naturally Occurring Bioactive Compounds for the Treatment of Alzheimer's Disease. *Marine Drugs*, *17*(11). https://doi.org/10.3390/md17110609

Oliveira, D., Latimer, C., Parpot, P., Gill, C. I. R., & Oliveira, R. (2020). Antioxidant and antigenotoxic activities of *Ginkgo biloba* L. leaf extract are retained after *in vitro* gastrointestinal digestive conditions. *European Journal of Nutrition*, *59*(2), 465–476. https://doi.org/10.1007/s00394-019-01915-8

Olzscha, H., Schermann, S. M., Woerner, A. C., Pinkert, S., Hecht, M. H., Tartaglia, G. G., Vendruscolo, M., Hayer-Hartl, M., Hartl, F. U., & Vabulas, R. M. (2011). Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell*, *144*(1), 67–78. https://doi.org/10.1016/j.cell.2010.11.050

Paiva, L., Lima, E., Neto, A. I., Marcone, M., & Baptista, J. (2016). Health-promoting ingredients from four selected Azorean macroalgae. *Food Research International*, *89*. https://doi.org/10.1016/j.foodres.2016.08.007

Paiva, L., Lima, E., Neto, A. I., Marcone, M., & Baptista, J. (2017). Nutritional and Functional Bioactivity Value of Selected Azorean Macroalgae: *Ulva compressa*, *Ulva rigida*, *Gelidium*

microdon, and *Pterocladiella capillacea*. *Journal of Food Science*, *82*(7), 1757–1764. https://doi.org/10.1111/1750-3841.13778

Paiva, L., Patarra, R., Neto, A. I., EMC, L., & JAB, B. (2012). Antioxidant activity of macroalgae from the Azores. *Arquipelago. Life and Marine Sciences*, *29*, 1–6.

Pangestuti, R., & Kim, S.-K. (2010). Neuroprotective properties of chitosan and its derivatives. *Marine Drugs*, *8*(7), 2117–2128. https://doi.org/10.3390/md8072117

Parkinson, J. (1817). An essay on the shaking palsy. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *14*(2), 223–236; discussion 222. https://doi.org/10.1176/jnp.14.2.223

Parsell, D. A., Kowal, A. S., Singer, M. A., & Lindquist, S. (1994). Protein disaggregation mediated by heat-shock protein Hsp104. *Nature*, *372*(6505), 475–478. https://doi.org/10.1038/372475a0

Pereira, L. (2016). Edible Seaweeds of the World. *Edible Seaweeds of the World*, *January*. https://doi.org/10.1201/b19970

Pereira, L. (2018). Therapeutic and Nutritional Uses of Algae. *Therapeutic and Nutritional Uses of Algae*. https://doi.org/10.1201/9781315152844

Popova, B., Wang, D., Rajavel, A., Dhamotharan, K., Lázaro, D. F., Gerke, J., Uhrig, J. F., Hoppert, M., Outeiro, T. F., & Braus, G. H. (2021). Identification of Two Novel Peptides That Inhibit α-Synuclein Toxicity and Aggregation. *Frontiers in Molecular Neuroscience*, *14*, 659926. https://doi.org/10.3389/fnmol.2021.659926

Rafiquzzaman, S. M., Kim, E. Y., Lee, J. M., Mohibbullah, M., Alam, M. B., Soo Moon, I., Kim, J.-M., & Kong, I.-S. (2015). Anti-Alzheimers and anti-inflammatory activities of a glycoprotein purified from the edible brown alga *Undaria pinnatifida*. *Food Research International*, *77*, 118–124. https://doi.org/https://doi.org/10.1016/j.foodres.2015.08.021

Rajauria, G., Foley, B., & Abu-Ghannam, N. (2017). Characterization of dietary fucoxanthin from *Himanthalia elongata* brown seaweed. *Food Research International (Ottawa, Ont.)*, *99*(Pt 3), 995–1001. https://doi.org/10.1016/j.foodres.2016.09.023

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C. R., Levin, L. A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B. E., Smith, C. R., Tittensor, D. P., Tyler, P. A., Vanreusel, A., & Vecchione, M. (2010). Deep, diverse and definitely different: Unique attributes of the world's largest ecosystem. *Biogeosciences*, *7*(9), 2851–2899. https://doi.org/10.5194/bg-7-2851-2010

Ravi Kumar, S., Narayan, B., & Vallikannan, B. (2008). Fucoxanthin restrains oxidative stress

induced by retinol deficiency through modulation of Na(+)K(+)-ATPase [corrected] and antioxidant enzyme activities in rats. *European Journal of Nutrition*, *47*(8), 432–441. https://doi.org/10.1007/s00394-008-0745-4

Rengasamy, K. R. R., Amoo, S. O., Aremu, A. O., Stirk, W. A., Gruz, J., Šubrtová, M., Doležal, K., & Van Staden, J. (2015). Phenolic profiles, antioxidant capacity, and acetylcholinesterase inhibitory activity of eight South African seaweeds. *Journal of Applied Phycology*, *27*(4), 1599–1605. https://doi.org/10.1007/s10811-014-0438-8

Riley, D., Lang, A. E., Blair, R. D., Birnbaum, A., & Reid, B. (1989). Frozen shoulder and other shoulder disturbances in Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *52*(1), 63–66. https://doi.org/10.1136/jnnp.52.1.63

Rocha de Souza, M. C., Marques, C. T., Guerra Dore, C. M., Ferreira da Silva, F. R., Oliveira Rocha, H. A., & Leite, E. L. (2007). Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. *Journal of Applied Phycology*, *19*(2), 153–160. https://doi.org/10.1007/s10811-006-9121-z

Rochet, J. C., & Lansbury, P. T. J. (2000). Amyloid fibrillogenesis: themes and variations. *Current Opinion in Structural Biology*, *10*(1), 60–68. https://doi.org/10.1016/s0959-440x(99)00049-4

Roos, R. A. C. (2010). Huntington's disease: a clinical review. *Orphanet Journal of Rare Diseases*, *5*(1), 40. https://doi.org/10.1186/1750-1172-5-40

Ross, C. A., & Poirier, M. A. (2004). Protein aggregation and neurodegenerative disease. *Nature Medicine*. https://doi.org/10.1038/nm1066

Ross, C. A., & Tabrizi, S. J. (2011). Huntington's disease: from molecular pathogenesis to clinical treatment. *The Lancet. Neurology*, *10*(1), 83–98. https://doi.org/10.1016/S1474-4422(10)70245-3

Ruetenik, A., & Barrientos, A. (2018). Exploiting Post-mitotic Yeast Cultures to Model Neurodegeneration. *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2018.00400

Sabeena Farvin, K. H., & Jacobsen, C. (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chemistry*, *138*(2), 1670–1681. https://doi.org/https://doi.org/10.1016/j.foodchem.2012.10.078

Sanchez, Y., & Lindquist, S. L. (1990). HSP104 required for induced thermotolerance. *Science (New York, N.Y.)*, *248*(4959), 1112–1115. https://doi.org/10.1126/science.2188365

Sangeetha, R. K., Bhaskar, N., & Baskaran, V. (2009). Comparative effects of beta-carotene and fucoxanthin on retinol deficiency induced oxidative stress in rats. *Molecular and Cellular Biochemistry*, *331*(1–2), 59–67. https://doi.org/10.1007/s11010-009-0145-y

Santos, M. G. M., Lagrota, M. H. C., Miranda, M. M. F. S., Yoneshigue-Valentin, Y., & Wigg, M. D. (1999). A Screening for the Antiviral Effect of Extracts from Brazilian Marine Algae against Acyclovir Resistant Herpes Simplex Virus Type 1. *42*(3), 227–230. https://doi.org/doi:10.1515/BOT.1999.026

Sardas, S. (2003). The Role of Antioxidants in Cancer Prevention and Treatment. *Indoor and Built Environment*, *12*(6), 401–404. https://doi.org/10.1177/1420326X03039009

Sathya, R., Kanaga, N., Sankar, P., & Jeeva, S. (2017). Antioxidant properties of phlorotannins from brown seaweed *Cystoseira trinodis* (Forsskål) C. Agardh. *Arabian Journal of Chemistry*, *10*, S2608–S2614. https://doi.org/10.1016/j.arabjc.2013.09.039

Saute, J. A. M., & Jardim, L. B. (2015). Machado Joseph disease: Clinical and genetic aspects, and current treatment. In *Expert Opinion on Orphan Drugs*. https://doi.org/10.1517/21678707.2015.1025747

Seong, S. H., Ali, M. Y., Kim, H.-R., Jung, H. A., & Choi, J. S. (2017). BACE1 inhibitory activity and molecular docking analysis of meroterpenoids from *Sargassum serratifolium*. *Bioorganic & Medicinal Chemistry*, *25*(15), 3964–3970. https://doi.org/https://doi.org/10.1016/j.bmc.2017.05.033

Shanmuganathan, B., Malar, D. S., Sathya, S., & Devi, K. P. (2015). Antiaggregation potential of *Padina gymnospora* against the toxic Alzheimer's beta-amyloid peptide 25-35 and cholinesterase inhibitory property of its bioactive compounds. *PLoS ONE*, *10*(11), 1–14. https://doi.org/10.1371/journal.pone.0141708

Shanmuganathan, B., Sathya, S., Balasubramaniam, B., Balamurugan, K., & Devi, K. P. (2019). Amyloid-β induced neuropathological actions are suppressed by *Padina gymnospora* (Phaeophyceae) and its active constituent α-bisabolol in Neuro2a cells and transgenic *Caenorhabditis elegans* Alzheimer's model. *Nitric Oxide*, *91*, 52–66. https://doi.org/https://doi.org/10.1016/j.niox.2019.07.009

Shanmuganathan, B., Suryanarayanan, V., Sathya, S., Narenkumar, M., Singh, S. K., Ruckmani, K., & Pandima Devi, K. (2018). Anti-amyloidogenic and anti-apoptotic effect of α-bisabolol against Aβ induced neurotoxicity in PC12 cells. *European Journal of Medicinal Chemistry*, *143*, 1196–1207. https://doi.org/https://doi.org/10.1016/j.ejmech.2017.10.017

Sieradzan, K. A., Mechan, A. O., Jones, L., Wanker, E. E., Nukina, N., & Mann, D. M. (1999). Huntington's disease intranuclear inclusions contain truncated, ubiquitinated huntingtin protein. *Experimental Neurology*, *156*(1), 92–99. https://doi.org/10.1006/exnr.1998.7005

Sies, H. (1995). Strategies of antioxidant defense. *Pharmacological Research*, *31*, 161. https://doi.org/10.1016/1043-6618(95)86904-2

Silva, L. M. C. M., Lima, V., Holanda, M. L., Pinheiro, P. G., Rodrigues, J. A. G., Lima, M. E. P., & Benevides, N. M. B. (2010). Antinociceptive and anti-inflammatory activities of lectin from marine red alga *Pterocladiella capillacea*. *Biological & Pharmaceutical Bulletin*, *33*(5), 830–835. https://doi.org/10.1248/bpb.33.830

Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, *16*(4), 245–262. https://doi.org/10.1023/B:JAPH.0000047783.36600.ef

Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). Alpha-synuclein in Lewy bodies. In *Nature* (Vol. 388, Issue 6645, pp. 839–840). https://doi.org/10.1038/42166

Stone, D. E., & Craig, E. A. (1990). Self-regulation of 70-kilodalton heat shock proteins in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, *10*(4), 1622–1632. https://doi.org/10.1128/mcb.10.4.1622-1632.1990

Struhl, K., & Moqtaderi, Z. (1998). The TAFs in the HAT. *Cell*, *94*(1), 1–4. https://doi.org/10.1016/s0092-8674(00)81213-1

Su, L., Auluck, P., Outeiro, T., Yeger-Lotem, E., Kritzer, J., Tardiff, D., Strathearn, K., Liu, F., Cao, S., Hamamichi, S., Hill, K., Caldwell, K., Bell, G., Fraenkel, E., Cooper, A., Caldwell, G., McCaffery, J., Rochet, J.-C., & Lindquist, S. (2010). Compounds from an Unbiased Chemical Screen Reverse Both Er-to-Golgi Trafficking Defects and Mitochondrial Dysfunction in Parkinson's Disease Models. *Disease Models & Mechanisms*, *3*, 194–208. https://doi.org/10.1242/dmm.004267

Switonski, P. M., Szlachcic, W. J., Gabka, A., Krzyzosiak, W. J., & Figiel, M. (2012). Mouse models of polyglutamine diseases in therapeutic approaches: Review and data table. Part II. In *Molecular Neurobiology*. https://doi.org/10.1007/s12035-012-8316-3

Tabet, N. (2006). Acetylcholinesterase inhibitors for Alzheimer's disease: anti-inflammatories in acetylcholine clothing! *Age and Ageing*, *35*(4), 336–338. https://doi.org/10.1093/ageing/afl027

Taylor, J. P., Hardy, J., & Fischbeck, K. H. (2002). Toxic proteins in neurodegenerative disease. In *Science*. https://doi.org/10.1126/science.1067122

Tenreiro, S., & Outeiro, T. F. (2010). Simple is good: yeast models of neurodegeneration. *FEMS Yeast Research*, *10*(8), 970–979. https://doi.org/10.1111/j.1567-1364.2010.00649.x

Tung, Y.-T., Wu, C.-H., Chen, W.-C., Pan, C.-H., Chen, Y.-W., Tsao, S.-P., Chen, C.-J., & Huang, H.-Y. (2022). *Ascophyllum nodosum* and *Fucus vesiculosus* Extracts Improved Lipid Metabolism and Inflammation in High-Energy Diet-Induced Hyperlipidemia Rats. *Nutrients*, *14*(21). https://doi.org/10.3390/nu14214665

Uttara, B., Singh, A. V, Zamboni, P., & Mahajan, R. T. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*, *7*(1), 65–74. https://doi.org/10.2174/157015909787602823

Vacher, C., Garcia-Oroz, L., & Rubinsztein, D. C. (2005). Overexpression of yeast hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease. *Human Molecular Genetics*, *14*(22), 3425–3433. https://doi.org/10.1093/hmg/ddi372

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., Citron, M. (1999). Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science (New York, N.Y.)*, *286*(5440), 735–741. https://doi.org/10.1126/science.286.5440.735

Vega, J., Álvarez-Gómez, F., Güenaga, L., Figueroa, F. L., & Gómez-Pinchetti, J. L. (2020). Antioxidant activity of extracts from marine macroalgae, wild-collected and cultivated, in an integrated multi-trophic aquaculture system. *Aquaculture*, *522*, 735088. https://doi.org/https://doi.org/10.1016/j.aquaculture.2020.735088

Verghese, J., Abrams, J., Wang, Y., & Morano, K. A. (2012). Biology of the heat shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. *Microbiology and Molecular Biology Reviews : MMBR*, *76*(2), 115–158. https://doi.org/10.1128/MMBR.05018-11

Vonsattel, J. P. G. (2008). Huntington disease models and human neuropathology: similarities and differences. *Acta Neuropathologica*, *115*(1), 55–69. https://doi.org/10.1007/s00401-007-

0306-6

Wang, J., Zheng, J., Huang, C., Zhao, J., Lin, J., Zhou, X., Naman, C. B., Wang, N., Gerwick, W. H., Wang, Q., Yan, X., Cui, W., & He, S. (2018). Eckmaxol, a Phlorotannin Extracted from *Ecklonia maxima*, Produces Anti-β-amyloid Oligomer Neuroprotective Effects Possibly via Directly Acting on Glycogen Synthase Kinase 3β. *ACS Chemical Neuroscience*, *9*(6), 1349–1356. https://doi.org/10.1021/acschemneuro.7b00527

Werner-Washburne, M., Stone, D. E., & Craig, E. A. (1987). Complex interactions among members of an essential subfamily of hsp70 genes in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, *7*(7), 2568–2577. https://doi.org/10.1128/mcb.7.7.2568-2577.1987

Williams, D. R., Watt, H. C., & Lees, A. J. (2006). Predictors of falls and fractures in bradykinetic rigid syndromes: a retrospective study. *Journal of Neurology, Neurosurgery, and Psychiatry*, *77*(4), 468–473. https://doi.org/10.1136/jnnp.2005.074070

Xiang, S. Y., Liu, F. F., & Lin, J. J. (2017). Fucoxanthin Inhibits beta-Amyloid Assembly and Attenuates beta-Amyloid Oligomer-Induced Cognitive Impairments. *J. Agric. Food Chem.*, *65*, 4092.

Yang, L.-B., Lindholm, K., Yan, R., Citron, M., Xia, W., Yang, X.-L., Beach, T., Sue, L., Wong, P., Price, D., Li, R., & Shen, Y. (2003). Elevated β-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nature Medicine*, *9*(1), 3–4. https://doi.org/10.1038/nm0103-3

Yao, T. P. (2010). The role of ubiquitin in autophagy-dependent protein aggregate processing. *Genes and Cancer*. https://doi.org/10.1177/1947601910383277

Yeger-Lotem, E., Riva, L., Su, L. J., Gitler, A. D., Cashikar, A. G., King, O. D., Auluck, P. K., Geddie, M. L., Valastyan, J. S., Karger, D. R., Lindquist, S., & Fraenkel, E. (2009). Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. *Nature Genetics*, *41*(3), 316–323. https://doi.org/10.1038/ng.337

Yoon, N. Y., Chung, H. Y., Kim, H. R., & Choi, J. E. (2008). Acetyl- and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. *Fisheries Science*, *74*(1), 200. https://doi.org/10.1111/j.1444-2906.2007.01511.x

Yu, J., Lin, J. J., & Yu, R. (2017). Fucoxanthin prevents H2O2-induced neuronal apoptosis via concurrently activating the PI3-K/Akt cascade and inhibiting the ERK pathway. *Food Nutr. Res.*, *61*, 1304678.

Yuan, Y., & Macquarrie, D. (2015). Microwave assisted extraction of sulfated polysaccharides

(fucoidan) from *Ascophyllum nodosum* and its antioxidant activity. *Carbohydrate Polymers*, *129*, 101–107.

Zhang, L., Wang, H., Fan, Y., Gao, Y., Li, X., Hu, Z., Ding, K., Wang, Y., & Wang, X. (2017). Fucoxanthin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE and Nrf2-autophagy pathways. *Scientific Reports*, *7*(1), 46763. https://doi.org/10.1038/srep46763

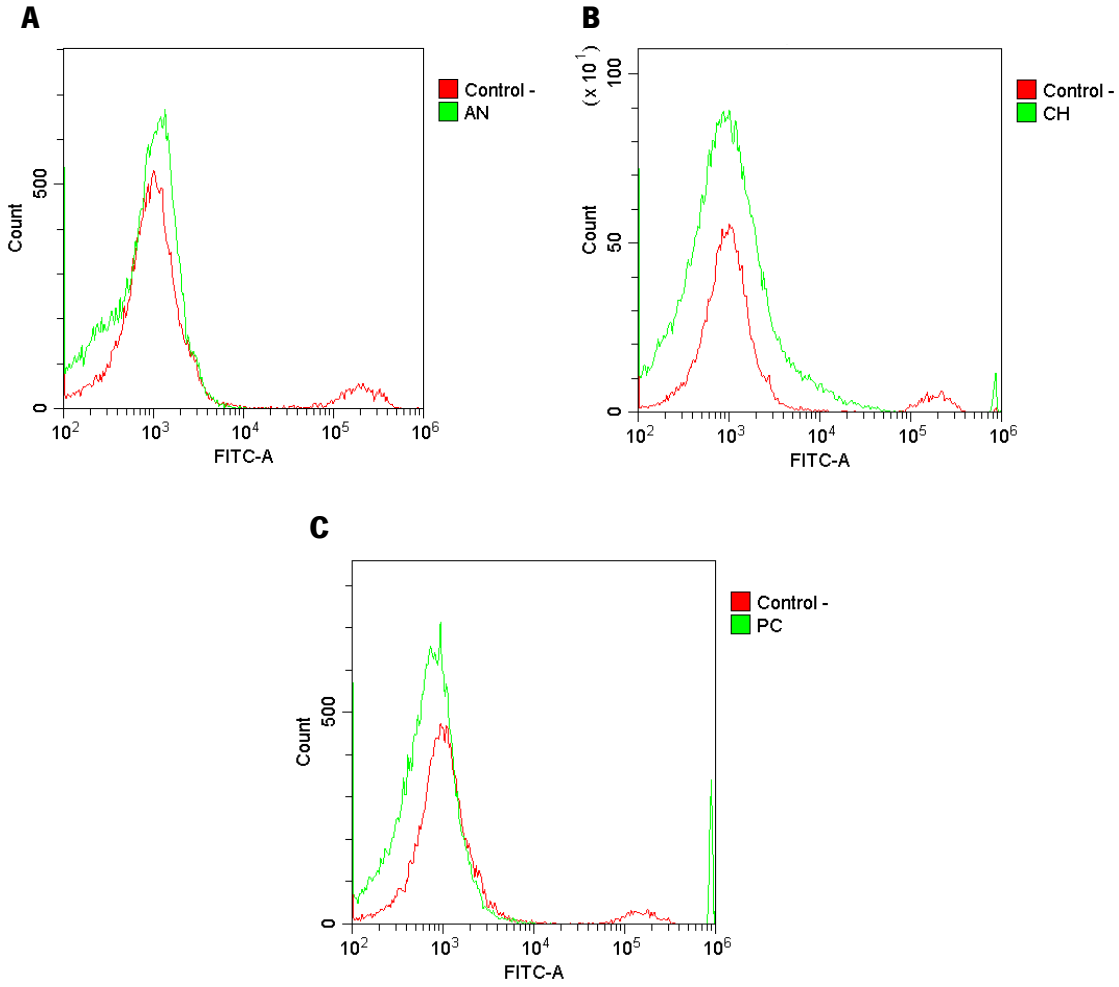
Zheng, H., & Koo, E. H. (2011). Biology and pathophysiology of the amyloid precursor protein. *Molecular Neurodegeneration*, *6*(1), 27. https://doi.org/10.1186/1750-1326-6-27

SUPPLEMENTARY MATERIAL

Supplementary Table 1. List of media and buffer for *Saccharomyces cerevisiae* used in this work.

Media/Buffer	Composition
YPD	1% Yeast extract, 2% dextrose, 2% peptone
YPDA	1% Yeast extract, 2% dextrose, 2% peptone, 2% agar
SCG (supplemented with aminoacids) for SNCA strain	0.67% YNB without aminoacids, 2% dextrose, 2% agar, 80 mg/L adenine, 80 mg/L histidine and 80 mg/L leucine
SCG (supplemented with aminoacids) for HTT strain	0.67% YNB without aminoacids, 2% dextrose, 1,4 g/L drop-out, 80 mg/L histidine, 80 mg/L tryptophan and 80 mg/L uracil
SCG (supplemented with aminoacids) for SSA2 and HSP104 strain	0.67% YNB without aminoacids, 2% dextrose, 1,4 g/L drop-out, 80 mg/mL adenine 80 mg/L histidine, 80 mg/mL leucine, 80 mg/L tryptophan and 80 mg/L uracil
SGCD for HTT strain	0.67% YNB without aminoacids, 2% dextrose, 2% agar, 1,4 g/L drop-out, 80 mg/L histidine, 80 mg/L tryptophan and 80 mg/L uracil
SGCD for SSA2 and HSP104 strain	0.67% YNB without aminoacids, 2% dextrose, 2% agar, 1,4 g/L drop-out, 80 mg/mL adenine 80 mg/L histidine, 80 mg/mL leucine, 80 mg/L tryptophan and 80 mg/L uracil
PBS	NaCl: 137 mM; KCl: 2.7 mM; Na_2HPO_4: 10 mM; KH2PO4: 1.8 mM

Anexo 1. *Ascophyllum nodosum* (AN; A), *Cystoseira humilis* (CH; B) and *Pterocladiella Capillacea* (PC; C) extract did not promote significant effect on intracellular oxidation in *Saccharomyces cerevisiae* strain BY4741. Negative control was prepared with cells treated only with DMSO (C-, red line; A, B and C) and extract control was prepared with cells treated only with 1000 μg/mL of AN (green line; A), CH (green line; B) or PC (green line; C). Results are representative of at least three independent experiments.



Anexo 2. *Ascophyllum nodosum* (AN; A), *Cystoseira humilis* (CH; B) and *Pterocladiella Capillacea* (PC; C) extracts did not affect cell viability in *Saccharomyces cerevisiae* strain BY4741. In cells from exponentially growing cultures treated with 1000 µg/mL of each extract and 5 mM H_2O_2 incubated for 20 min at 30 °C, 200 rpm, labelled with 2',7'-dichlorofluorescein diacetate (50 µM; H2DCFDA) and propidium iodide (3 µg/ml) in the dark, at 30 °C for 1h or 10 min, respectively and the fluorescence was measured by flow cytometry. Percentage of negative events for PI in AN treatment was 99,32% (A); in CH treatment was 99,91 % and in the PC treatment was 96,21%. Results are representative of at least three independent experiments.