Universidade do Minho
Escola de Ciências

João Pedro Sousa Barbosa

Evaluation of neuroprotective capacity of macroalgae extracts, in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* models

October 2022

Evaluation of neuroprotective capacity of macroalgae extracts, in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* models

João Barbosa

UMinho | 2022

Universidade do Minho
Escola de Ciências

João Pedro Sousa Barbosa

Evaluation of neuroprotective capacity of macroalgae extracts, in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* models

Master Thesis

Master's Degree in Molecular Biology, Biotechnology and Bioentrepreneurship in Plants

Work developed under supervision of

Professor Doctor Rui Pedro Soares Oliveira

Doctor Marta Daniela Araújo Costa

October 2022

Despacho RT - 31 /2019 - Anexo 3

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.

Atribuição-NãoComercial-SemDerivações
CC BY-NC-ND

https://creativecommons.org/licenses/by-nc-nd/4.0/

Agradrecimentos

Gostaria de agradecer em primeiro lugar ao Professor Rui Oliveira por ter aceitado o desafio e de me acolher durante o último ano no seu grupo de investigação, e pela sua dedicação e orientação. Uma palavra também aos meus colegas, elementos participantes neste grupo, onde certamente fiz amigos para a vida: Ana, Luara, Noversa, João Gonçalves, Mariana e Ricardo, pela partilha de bons momentos e discussão de desafios diários tornando os dias mais difíceis, significativamente mais fáceis, obrigado pessoal! P.s. se alguém vos aconselhar a sobremesa da casa desconfiem.

Um agradecimento especial à Professora Patrícia Maciel, por abrir as portas ao seu grande grupo (em elementos e sobretudo em qualidade), e sempre com um espírito critico muito construtivo dando um grande contributo no meu desenvolvimento pessoa e profissional. À doutora Marta Costa, pela paciência, orientação, dedicação e motivação ao longo destes últimos meses. Obrigado pela introdução ao mundo das *C. elegans*, esses seres fantásticos. Neste sentido uma palavra para o grupo das *C. elegans* que me acompanhou, pelo sentido partilha e entreajuda: Marta, Jorge, Daniela e Joana, grande abraço. Por último e não menos importante agradeço aos restantes membros do grupo: Andreia Castro, Sara Silva, Stéphanie Oliveira, Daniela Monteiro, Cármen Vieira, Sara Guerreiro Joana Correia, Liliana Costa, Daniela Garcia e Rita Fernandes.

Não poderia deixar de expressar o meu eterno agradecimento a todos os meus amigos, com que poderei sempre contar e discutir de futebol (principalmente os grandes: Gil Vicente F.C e S.L.B, sorry Raquel) como as grandes questões da sociedade, e também aquelas conversas que se prolooooongam: Abreu, Carol, Gil, Loureiro, Miguel, Raquel, Xana, Obi, grande abraço a todos, tornaram certamente esta etapa mais leve e incrível. Sempre presentes, nos bons e principalmente nos menos bons.

Por último, à minha família, principalmente aos meus pais e ao meu irmão Tiago, pelo apoio e esforço que fazem todos os dias para que todo este trabalho fosse realizado. Espero que não tenha sido em vão. Uma palavra para os meus avós, em especial ao Sr Severino que partiu na reta final deste trabalho. A vossa capacidade de trabalho e superação serão sempre uma inspiração.

A todos o meu profundo obrigado!!

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

Avaliação da capacidade neuroprotetora de extratos de macroalgas em modelos de *Saccharomyces cerevisiae* e *Caenorhabditis elegans*

Nos últimos anos, o consumo de algas tem vindo a aumentar, tanto na dieta quanto como nutracêutico,e as características antioxidantes destas plantas contribuem muito para isso. A desregulação de mecanismos antioxidantes está associada a muitas doenças, que também têm vindo a aumentar devido ao aumento da esperança média de vida. É o caso de doenças neurodegenerativas como Alzheimer ou Parkinson que ainda permanecem sem cura e que são tratadas com medicamentos capazes de atenuar ou retardar alguns sintomas da doença. No entanto, evidências de que extratos de algas podem ser uma fonte de novos compostos para o tratamento destas doenças tem aumentado a curiosidade sobre o seu potencial efeito terapêutico.

Neste estudo, foi explorada a relevância e o potencial terapêutico de um conjunto de extratos de macroalgas. Em primeiro lugar, os extratos de macroalgas foram selecionados pela sua atividade anti radicalar com base no ensaio DPPH. Quatro extratos de *Pelvetia caniculata* (PC), *Fucus spiralis* (FS) e *Fucus vesiculosus* (FV) e *Himanthalia elongata* (HE) foram os que apresentaram maior capacidade anti radicalar foram selecionados para estudos posteriores em *Saccharomyces cerevisiae* e *Caenorhabditis elegans*. PC, FS e FV foram capazes de aumentar a termotolerância de *S. cerevisiae* e diminuir a agregação de α-sinucleína em um modelo de *S. cerevisiae* da doença de Parkinson. Em *C. elegans*, o tratamento com o extrato de FV preveniu a degeneração de neurônios dopaminérgicos e glutamatérgicos em modelos neuronais de Parkinson e Alzheimer e mostrou um efeito promissor na melhora do fenótipo motor de um modelo FTDP-17 expressando uma *tau* mutante. Estudos adicionais com estirpes repórter de *C. elegans* deram algumas pistas sobre o mecanismo de ação deste extrato mostrando sua capacidade de induzir significativamente a resposta mitocondrial à malformação de proteínas. Este estudo mostrou efeitos promissores dos tratamentos com extratos de macroalgas em dois modelos biológicos, nomeadamente os seus impactos protetores em contextos de neurodegeneração. Estes resultados reforçam a importância da investigação por novos compostos bioativos para o desenvolvimento de novas formas de prevenção ou tratamento de doenças neurodegenerativas que permanecem sem cura.

Palavras-chave: *Caenorhabditis elegans*; doenças neurodegenerativas; *Fucus vesiculosus*; Macroalgas; *Saccharomyces cerevisiae*:;

Abstract

Evaluation of neuroprotective capacity of macroalgae extracts, in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* models

In recent years, the consumption of algae has been increasing, both in the diet and as a nutraceutical, and the antioxidant characteristics of these plants contribute greatly to this. Dysregulation of antioxidant mechanisms is associated with many diseases whose prevalence has also increased due to the higher life expectancy. This is the case of neurodegenerative diseases such as Alzheimer's or Parkinson's that still remain without a cure and that are treated with drugs capable of attenuating or delaying some symptoms of the disease. However, evidence that algae extracts could be a source of new compounds for the treatment of these diseases has encouraged research about their potential therapeutic effect.

In this study we explored the relevance and therapeutic potential of a set of macroalgae extracts: Firstly, the macroalgae extracts were screened for their antiradicalar activity based on the DPPH assay. Four extracts *Pelvetia caniculata* (PC), *Fucus spiralis* (FS) and *Fucus vesiculosus* (FV) and *Himanthalia elongata* (HE) with the highest antiradicalar measures were selected for further studies in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*. PC, FS, FV, were able to increase *S. cerevisiae* the thermotolerance and to decrease α-synuclein aggregation in a *S cerevisiae* model of Parkinson's disease. In *C. elegans*, the treatment with the FV extract prevented the degeneration of dopaminergic and glutamatergic neurons in Parkinson's and Alzheimer's neuronal models and showed a promising effect in the amelioration of the motor phenotype of a FTDP-17 model expressing a mutant *tau*. Additional studies with *C. elegans* transcriptional reporter strains gave some clues about the mechanism of action of this extract showing its ability to significantly induce the mitochondrial unfolded response. This study showed promising effects of macroalgae extracts treatments in two biological models, namely their protective impacts in contexts of neurodegeneration. These results reinforce the importance of the search for new bioactive compounds and of its research for the development of new ways to prevent or treat neurodegenerative diseases that remain without a cure.

Keywords: *Caenorhabditis elegans*; *Fucus vesiculosus*; Macroalgae; Neurodegenerative diseases; *Saccharomyces cerevisiae*.

Index

Abbreviation list

AD- Alzheimer's disease

CFU's- Colony forming units

DCFH-DA- Dichloro-dihydro-fluorescein diacetate

DPPH- 2,2-Diphenyl-1-picrylhydrazyl

ER- Endoplasmic reticulum

FS- *Fucus spiralis* extract

FTDP-17- Frontotemporal Dementia with Parkinsonism associated to chromosome 17

FV- *Fucus vesiculosus* extract

GRAS- Generally regarded as safe

GSH- Glutathione

HE- *Himanthalia elongata* extract

ND- Neurodegenerative disease

NGM- Nematode Growth Medium

OS- Oxidative stress

PC- *Pelvetia caniculata* extract

PD- Parkinson's disease

PUFA's- polyunsaturated fatty acids

ROS- Reactive oxygen species

SOD- Superoxide dismutase

UPR- unfolded protein response

UPR^{ER} - Endoplasmic reticulum unfolded protein response

UPR^{MT}- Mitochondrial unfolded protein response

Figure list

Table list

1. INTRODUCTION

1.1. Neurodegenerative diseases

As the average life expectancy increases, new health problems arise, neurodegenerative diseases (ND) and dementia being no exception to that. The World Health Organization, (2018) estimates that dementia affects 50 million people worldwide (approximately 5% of the world's older population). Despite the investment in investigation, the tendency of the disease evolution is increasing, expecting to affect 82 million people in 2030, and 152 million by 2050. Globally, this comorbidity is the seventh leading cause of death and the major cause of disability and dependency among older people, affecting families and societies (WHO, 2018). The increase of lifespan, combined with an unhealthy lifestyle, increases the probability of developing a ND, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Frontotemporal dementia (FTD) (Armstrong, 2020; Salahuddin et al., 2021). These diseases are chronic disabling disorders, characterized by the gradual and selective loss of neurons in the central or peripheral nervous system. However, current treatments have not been effective, only alleviating the symptoms or slowing the progress of the disease (Agnihotri & Aruoma, 2020; Armstrong, 2020). Although age is the most important risk factor,other relevant demographic factors, such as education, are related with the lack of cognitive activity. These two factors decrease the brain plasticity and the capacity to create new cerebral connections. Also, lifestyle, medical and environmental factors are related with an increased risk of developing neurological diseases (Figure 1).

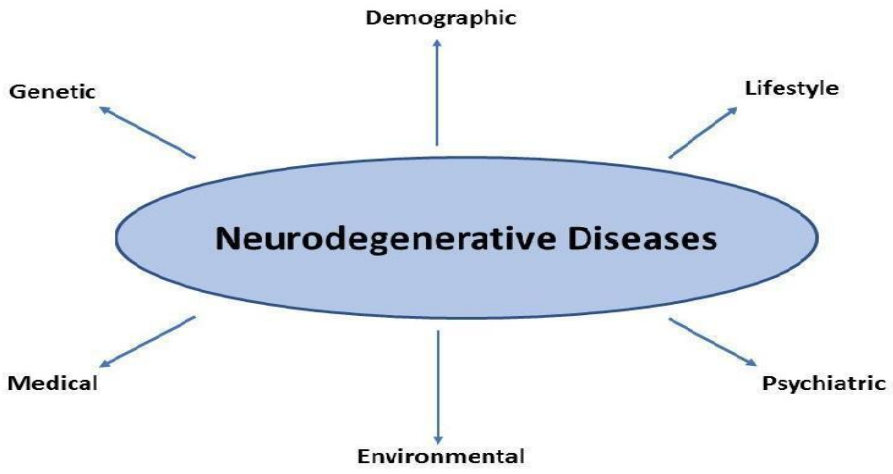


Figure 1 Risk factors associated with the development of neurodegenerative diseases. Adapted from Armstrong, 2020.

People that are exposed to some of these risk factors namely alcohol consumption, smoking, poor diet (lifestyle factors) and air pollution (environmental), obesity and diabetes (medical), for example, are more prone to develop ND in the future (Armstrong, 2020). Thus, minimizing these risk factors throughout life maximizing physical and cognitive exercise and having a balanced diet decreases the likelihood of developing ND.

1.1.1. Protein misfolding and neurodegenerative diseases

In healthy cells, misfolded proteins are either degraded or refolded correctly by chaperone proteins that are involved in protein folding and trafficking as well as intermediate stabilization. The ND involve the misfolding and aggregation of specific proteins into abnormal, toxic species (Table 1).

Table 1 Cerebral regions and proteins associated with different neurodegenerative diseases. Adapted from Ross & Poirier, (2004).

Disease	Regions most affected	Characteristic pathology	Disease proteins deposited
Parkinson's disease	Substantia nigra, cortex, locus coeruleus	Lewy bodies	α-Synuclein
Alzheimer's disease	Cortex, hippocampus, basal forebrain, brain stem	Neuritic plaques and neurofibrillary tangles	Aβ peptide and hyperphosphorylated *tau*
Huntington's disease	Striatum, cortex, other regions	Intranuclear inclusions and cytoplasmic aggregates	Huntingtin
Other polyglutamine diseases	Basal ganglia, brain stem cerebellum, and spinal cord	Intranuclear inclusions	Atrophin-1, Ataxin

Once formed, amyloid aggregates are very difficult to degrade. To cope with these stresses, there are several responses in the cell. Part of the network that synchronizes cellular adaptation to a variety of stressors is called the unfolded mitochondrial protein (UPR^{MT}) response. Thus, UPR^{MT} is the mechanism that can stabilize mitochondrial homeostasis and reduce the amount of misfolded protein in the organelle (Muñoz-Carvajal & Sanhueza, 2020). The UPR^{MT} is primarily coordinated by the transcription factor ATFS-

1, which promotes the expression of genes encoding chaperones, proteases, antioxidant enzymes or import components of mitochondrial proteins. Presumably these proteins preserve function, and recover the activity of damaged organelles. Mitochondrial function is also known to be disturbed in diseases attributed to aggregation-prone proteins, such as most neurodegenerative diseases. In mitochondrial dysfunction UPR^{MT} activation promotes development and extends organelle lifespan, suggesting that approaches to enhance UPR^{MT} activation may be useful therapeutics. (Melber & Haynes, 2018).

When the endoplasmic reticulum (ER) is under stress and the amount of proteins (folded and unfolded) exceeds the capacity of the ER machinery, the unfolded endoplasmic reticulum protein response (UPR^{ER}) is activated to recover proteostasis. UPR^{ER} is prompted in response to the accumulation of damaged proteins of the secretory pathway in the ER, which trigger the inhibition of protein synthesis, to reduce the protein load, and recruit chaperones to the ER lumen, to increase the refolding ability (Hetz, 2012).

With the passage of time, the cellular stress begins to be greater, either by cellular aging or by the presence of mutations. These factors cause some proteins to escape the quality control of the cell itself, starting to aggregate, varying between oligomers and amyloid plaques. In this way, cells have the ability to produce heat shock proteins (HSP's), which have the ability to sequester and signal defective proteins, sending these proteins to cellular compartments where they can be degraded, through the ubiquitin proteasome system or autophagy (Sweeney et al., 2017).

The HSP's are chaperones, and their function is to protect the proteome from denaturing environmental stressors, including heat and reactive oxygen species (ROS). Sequestration of misfolded aggregated proteins can, in many cases, play a beneficial role – preventing misfolded proteins from saturating chaperones and proteasomes, and thus making it possible for them to be destroyed via autophagy or preserving them for subsequent refolding and reuse by the cell. Cellular responses to proteotoxic stress, such as the heat shock response and the unfolded protein response (UPR), involve large-scale rebalancing of the proteostatic network through transcriptional regulation of two major chaperones (e.g., Hsp70, Hsp90) and non-chaperones (transcription factors, signaling proteins and Hsp70 receptors and cell cycle regulators) (Sweeney et al., 2017).

1.1.2. Neurodegenerative diseases and oxidative stress

Oxidative stress (OS) is induced by an imbalanced redox state, involving either excessive generation of ROS or dysfunction of the antioxidant system. ROS are defined as a group of reactive

molecules derived from oxygen, which are generally highly reactive because of their unpaired valence electrons. Cumulative OS may induce cellular damage, impairment of the DNA repair system, and mitochondrial dysfunction, all of which have been known as key factors in acceleration of aging process and the development of neurodegenerative disorders. The ROS can be classified as exogenous, like ultraviolet radiation, drugs, chemicals, and toxins, or endogenous, which can be mediated by mitochondrial metabolism or enzymes (xanthine oxidase or cytochrome P450 of ER (G. H. Kim et al., 2015).

Although ROS may not be the key factor for neurodegeneration, they are linked to the faster progression of these diseases. Allied to this, the neural cells are rich in polyunsaturated fatty acids (PUFA's), that are highly prone to peroxidation and the brain needs a high amount of oxygen (20% of the body oxygen consumption). In order to counter these insults, cells have the ability to activate some pathways, namely antioxidant enzymes such as superoxide dismutase (SOD) or Glutathione (GSH) for example. SOD is a very important enzyme that is capable of catalyze the breakdown of highly reactive, superoxide anion (O_2^-) to less reactive H_2O_2 and oxygen. SOD1 and SOD2 are intimately involved in the elimination of O_2^- in the cytosol and mitochondria, respectively. GSH, is synthesized from glutamate, cysteine, and glycine and exerts protective function of cell survival against OS and is able to react with ROS such as O_2^- and $^{\cdot}OH$, inhibiting DNA damage in cells and avoiding their death. Vitamin E is an antioxidant that can attenuate the effects of peroxide and protect against lipid peroxidation in cell membranes, and this is possible because this vitamin is lipid-soluble. On other side, Vitamin C is water-soluble, and it is involved in the removal of free radicals by electron transfer. Nonetheless, neuronal cells have a weak antioxidant response to counteract these factors. OS also contributes to aggregation by modulating the proteasome and autophagy capacity (G. H. Kim et al., 2015; Z. Liu et al., 2017).

Oxidative imbalance and consequent cellular dysregulation can be very important in the initiation and progression of AD. The excessive accumulation of ROS is intimately linked with accumulation of Aβ aggregates that induce mitochondrial dysfunction. Also, in PD it seems that the mitochondrial dysfunction is related with disease progression since, reduced activity in Complex I of the respiratory chain, may contribute to the generation of excessive ROS and, in turn, induce neuronal apoptosis (G. H. Kim et al., 2015).

Thus, under normal circumstances, autophagic and lysosomal clearance degrades protein in the cell, but in ND these pathways are blocked causing an accumulation of aggregated protein that could itself lead to more aggregation. While in healthy cells mitochondrial function and oxidative

balance are maintained, in ND the electron transport chain and mitochondria function are compromised which causes an increase in ROS that leads to OS (Fields et al., 2019).

1.1.3. Alzheimer's disease

When *tau* protein is associated with microtubules, it helps to maintain the structure of neurons, but when this protein is hyperphosphorylated, it becomes cytotoxic, causing microtubule destabilization and oligomerization, forming neurofibrillary tangles (NFTs). This phosphorylation is also related to the malfunction of mitochondria and the production of ROS (Mishan et al., 2019; Pohanka, 2018; Figure 2). AD has as main symptoms, loss of memory and cognitive ability, affecting the patient's behavior, and its development is related principally to the deposition of beta amyloids (Aβ) and accumulation of hyperphosphorylated *tau* protein (Rege et al., 2014). Just 1% of the cases of AD are related to family history, whereas 99% are sporadic and have a late onset (Manjula et al., 2021).

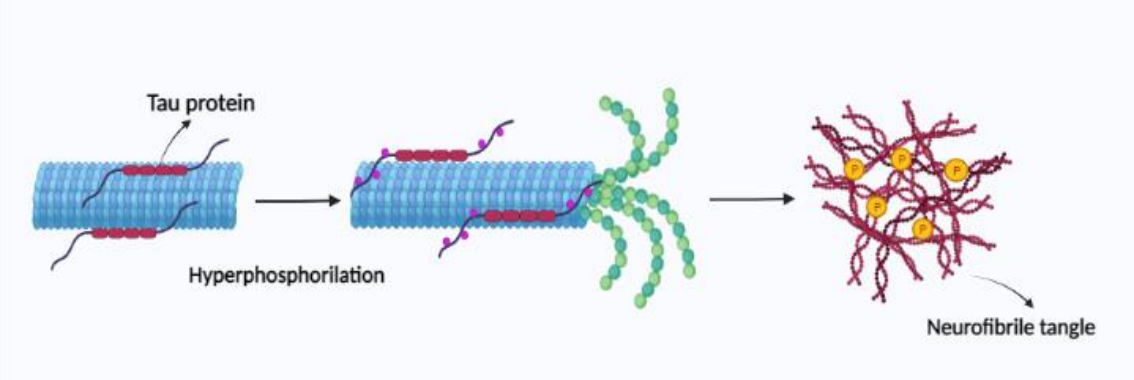


Figure 2 *Tau* protein is phosphorylated leading to neuronal microtubule destabilization, leading to aggregation of *tau* protein molecules, forming neurofibrillary tangles. Adapted from Mishan et al., 2019.

The amyloidogenic pathway leads to Aβ production. The formation of Aβ amyloids is related to the cleavage activity of β-secretase, followed by γ-secretase on APP, which results in the formation of Aβ protein fragments and Aβ plaques (Ahmad et al., 2016; Ahmed et al., 2017; Figure 3). The accumulation of Aβ aggregates, will release nitric oxide synthetase, increasing nitric oxide levels, causing OS, leading to cellular imbalance and consequently neurodegeneration. In the non-amyloidogenic pathway, α secretase acts on "amyloid precursor proteins" (APP) followed by γ-secretase forming a peptide-p3.

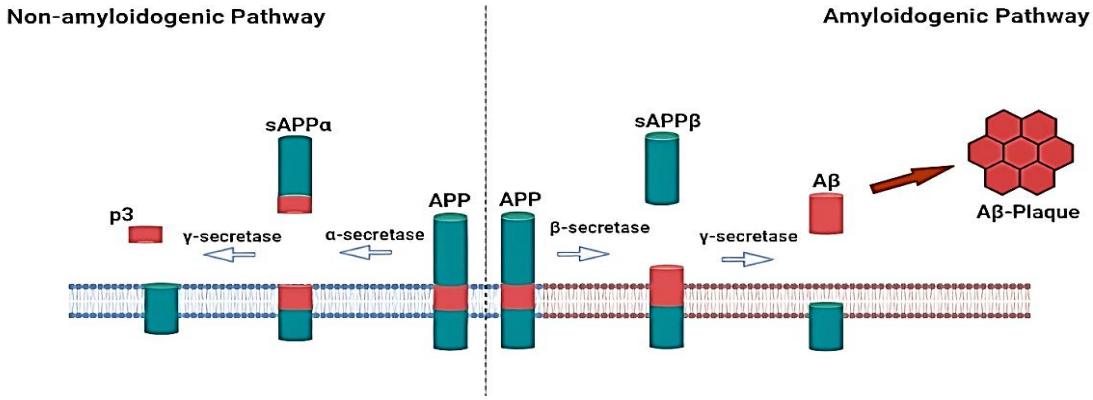


Figure 3 Role of α-secretase and β-secretase in the formation of Aβ plaque. Adapted from (Rahman et al., 2021).

In common to these two factors (Aβ amyloids and *tau* proteins) are one type of enzymes: sirtuins. These enzymes, especially SIRT-1, have an important role in the development of AD. SIRT-1 can direct the cleavage of APP to α-secretase, avoiding the formation of Aβ plaque. In a normal situation, the α-secretase pathway is the chosen one, forming p3 peptide (Ahmad et al., 2016). At the same time, the SIRT-1 enzyme acts through *tau* proteins reducing the amount of NFTs. This happens because, SIRT-1 deacetylates the *tau* proteins, forming spaces to permit the ubiquitination, and lately the protein degradation by proteasomes (Ahmed et al., 2017; Min et al., 2013). The role of p3 peptide remains unclear. Two contradictory situations have been hypothesized. Han et al., 2011, refer that p3 may play a neuroprotective role in the brain, contrarily to Kuhn et al., 2020, which showed p3-mediated aggregation and fibrils formation.

1.1.4. Frontotemporal dementia

Frontotemporal dementia (FTD) is associated mainly with behavioral impairment such as disinhibition, loss of initiative or apathy. Loss of interest in the environment, negligence of personal hygiene, verbal and physical aggressiveness are additional features of FTD. Patients with FTD often display asymmetrical atrophy of the frontal and temporal cortex. There is evidence that motor neuron disease and FTD coexist, and that the motor symptoms might precede the development of cognitive and behavioral changes (Wider & Wszolek, 2008). Nearly 30% of the cases are related to *tau* protein aggregation or malfunction related with mutations in MAPT gene.

This type of mutation originated other designation- Frontotemporal Dementia with Parkinsonism associated to chromosome 17 (FTDP-17). This is one of the familial forms of FTD with autosomal dominant inheritance, characterized by frontotemporal atrophy, neuronal loss, degeneration of substantia nigra and basal ganglia and abundant *tau* inclusions. Accumulation and consequent

oligomerization of *tau* protein, is capable of inducing ER stress, leading to cell death (van Swieten & Spillantini, 2007).

1.1.5. Parkinson's disease

The second most prevalent neurodegenerative condition next to AD is PD. This illness affects approximately 10 million people worldwide, and the number may double by 2030 (Zesiewicz, 2019). In general, symptoms can be divided into motor, neuropsychiatric and cognitive, and in particular, tremors, depression, and memory loss, respectively (Zesiewicz, 2019).

In some cases, the degeneration of dopaminergic neurons and a reduction of dopamine levels in the striatum and corresponding loss of dopamine transporters, cause most of the clinical symptoms of PD (Barbosa et al., 2014).Dopamine replacement therapy is usually used to treat these cases however, this treatment gives only symptomatic relief, without delaying or reducing the disease itself (Mehra et al., 2019). Linked with neurodegeneration, some studies indicated that α-synuclein protein is responsible for the development of neurodegenerative disorders, such PD, dementia with Lewy body, and Hallervorden–Spatz disease, also known as synucleinopathies (Fields et al., 2019; Stefanis, 2012).

Even though the precise physiological function of α-synuclein is unclear, it plays an essential role in synaptic vesicle trafficking and is involved in the regulatory mechanisms associated with synaptic homeostasis (Mehra et al., 2019). But, the accumulation of α-synuclein into prefibrillar forms, and then its assembly into higher molecular weight aggregates, induces cellular toxicity being the greatest contributor to pathogenesis in PD (Mehra et al., 2019). It is known that the accumulation of misfolded oligomers and larger aggregates of α-synuclein are the cause of these perturbations, but the mechanisms remain unknown (Burré et al., 2018; Uversky, 2018). There are also four dominant mutations related with early-onset (A30P, E46K, A53T, G51D) and H50Q that are related to late-onset. The major difference between them is the faster aggregation compared with the wild type protein (Mehra et al., 2019).

1.2. Therapeutic strategies

ND remain without a cure so far. The therapeutic drugs used in the clinics are able to attenuate or delay the symptoms of the disease but are not disease modifying. To face these limitation the search for new therapies is an urgent need.

To date, there are no effective therapies for the cognitive symptoms in FTD, which often involve executive function, memory, and language. For the motor difficulties associated with FTD, the prescription of riluzole is indicated as therapy (Tsai & Boxer, 2014).

In the case of AD patients, the symptoms are characterized by memory and cognitive loss and are often accompanied by disorientation, mood swings, and eventually delirium (Wang et al., 2022). Actually, the most used drugs for treatment of AD symptoms are neurotransmitters or cholinesterase inhibitors, for example, tacrine, donepezil, rivastigmine. These therapies only alleviate symptoms of dementia and cognitive decline by preserving acetylcholine levels in the synaptic gaps between neurons, preventing their degradation by acetylcholinesterase and butyrylcholiesterase (Chopade et al., 2022).

For PD, treatment is focused on drugs that have the capacity of stimulate the dopaminergic system, such as levodopa which is the drug most used. Levodopa is a precursor to dopamine, and it is administered with carbidopa which inhibitings dopa decarboxylase reduces extracerebral metabolism of levodopa before it reaches the brain (Chopade et al., 2022).

One hypothesis is reducing the production of protein, like Aβ amyloids or α-synuclein, reducing toxicity. Another alternative is enhancing the degradation of intracellular aggregates (Fields et al., 2019).

1.3. Macroalgae and neuroprotector compounds

Algae are a group of photosynthetic organisms with high variance of species. They can be divided into unicellular organisms (microalgae) or pluricellular known as macroalgae. These seaweeds can also be divided based on their pigmentation: red seaweeds (Rhodophyceae), green seaweeds (Chlorophyta) and brown seaweeds (Phaeophyceae). Seaweed cultivation is the most rapidly expanding sector in aquaculture production, accounting for more than 50 percent of total global marine production, equating to around 34.7 million tones. With seafood increasingly used for food (and animal feed), as well as in fertilizers, food supplements, and even alternatives to plastics, the industry has grown rapidly in the past 50 years, reaching a value of 14.7 billion dollars in 2019 (International Science Council, 2022 https://council.science/current/blog/further-action-on-biosecurity-is-needed-to-safeguard-the-rapidly-growing-global-seaweed-industry/; accessed in 13 October, 2022).

Macroalgae are widely spread in Asian diet, but this scenario is different in Europe or in the United States. According to the report of The United Nations Decade of Ocean Science for Sustainable Development 2021-2030 (ONU, 2021), to date, only 19% of the ocean floor is mapped and there are vast areas, as well as in the arctic and polar regions, over which practically nothing is known about the

distribution of species, ecosystems, or oceanic processes. However, the investigation in this area is growing, not just to identify new species, but also to identify and find new bioactive compounds. Seaweeds are generally rich in photosynthetic pigments that have great antioxidant capacity, and polysaccharides that have great therapeutic potential (Pereira & Valado, 2021). In this way macroalgae gain a particular interest because most of them are edible and have multifactorial activity, acting in several antioxidant pathways (Barbalace et al., 2019; Pereira & Valado, 2021). Several studies have explored the neuroprotective effect of macroalgae extracts but the majority is based in *in vitro* assays (Table 2).

Olasehinde et al, (2019), use antioxidant chemical assays, as DPPH or ABTS and biochemical assays as cholinesterase activity or β-secretase inhibition assay, and conclude that the seaweed extracts of *Gracilaria gracilis*, *Gelidium pristoides*, *Ulva lactuca*, and *Ecklonia maxima* have neuroprotector potential. Custódio et al, (2016) also performed several chemical assays, nonetheless this study has a cytotoxic assay in SH-SY5Y (human neuroblastoma cell line), where they evaluated the cell viability after a hydrogen peroxide insult with several extract concentrations of *Cystoseira tamariscifolia* and *Cystoseira nodicaulis* and they concluded that these extracts have cholinesterase inhibition antioxidant activity.

In order to find extracts with neuroprotective potential Liu et al., 2015 used as a model, *C. elegans*. The study was focused on the objective of protecting Parkinson's model strains against dopaminergic neurons loss and to decrease of α-synuclein accumulation, and in fact the *Chondrus crispus* extracts were able to protect the nematodes from neurodegeneration.

Table 2 Potential neuroprotection activities of some seaweeds

Color	Seaweed	Potential application	Reference
Red	*Gracilaria gracilis*	Nutraceutic, neuroprotection	Rodrigues et al., 2015 Olasehinde et al., 2019
	Gelidium pristoides	Neuroprotection	Olasehinde et al., 2019
	Gracilariopsis chorda	Neuroprotection	Mohibbullah et al., 2018
	Chondrus crispus	Neuroprotective Effects in a *C. elegans* Model of Parkinson's Disease	Liu et al., 2015
Green	*Codium tomentosum*	Nutraceutic. Antioxidant and therapeutic potential in PD	Rodrigues et al., 2015; Silva et al., 2020
	Ulva lactuca	Antioxidant and therapeutic potential neurodegenerative diseases	Olasehinde et al., 2019
Brown	*Sargassum muticum*	Nutraceutic, neuroprotection	Rodrigues et al., 2015; Silva et al., 2018
	Saccorhiza polyschides	Food or nutraceutic	Rodrigues et al., 2015
	Ecklonia maxima	Neuroprotection	Olasehinde et al., 2019
	Bifurcaria bifurcata	Neuroprotection	Silva et al., 2019
	Scytothamnus australis	Potential therapeutic effects in Alzheimer's disease	Wozniak et al., 2015
	Marginariella boryana		
	Splachnidium rugosum		
	Undaria pinnatifida		
	Ecklonia stolonifera	Cholinesterase inhibition	Yoon et al., 2008
	Sargassum sagamianum	Cholinesterase inhibition	Choi et al., 2007
	Cystoseira tamariscifolia	Cholinesterase inhibition. Antioxidant	Custódio et al., 2015
	Cystoseira nodicaulis		Custódio et al., 2015

1.3.1. *Himanthalia elongata*

Himanthalia elongata is a common brown seaweed that can be found in Norway, Britain, Ireland, northwest coast of France, northern Spain, and Portugal. Plants commonly live for 2-3 years and reproduce once before dying (Garcia-Perez et al., 2022; Figure 4). *H. elongata* is eaten as a food in Ireland and France. It is sold dried and pickled or may be eaten fresh in a salad. It has several properties that make it attractive as an ingredient in personal care and cosmetic products, it has an ability to help maintain skin moisture levels and can also be used as a thickening agent in cooking (Macoi, 2008).

Figure 4 *Himanthalia elongata* (A) and habitat distribution (B; Macoi. 2008).

1.3.2. Pelvetia caniculata

Pelvetia caniculata is found in Norway, Iceland, UK, Ireland, Atlantic coast of France, Spain, and Portugal (Garcia-Perez et al., 2022; Figure 5). It is very tolerant to desiccation and a wide range of exposure conditions (Macoi, 2008). It can survive several days without being covered by the tide and often only gets splashed by the sea's spray. In fact, it requires these relatively dry conditions - if the plant is submerged for more than 6 hours out of 12 it begins to decay. Its physiology is perfectly adapted to these harsh conditions (variations in temperature, light, current, freshwater rain, and salinity). Chemical and biological defense mechanisms have arisen in *P. canicuata* allowing the seaweed to adapt to light, desiccation, or the rhythm of the tides. Historically harvested for use as animal fodder and a source of food for people, nowadays it is sold in dried form. In the chemical composition was found that arabinose, fucoidan and alginate are present (Macoi, 2008)

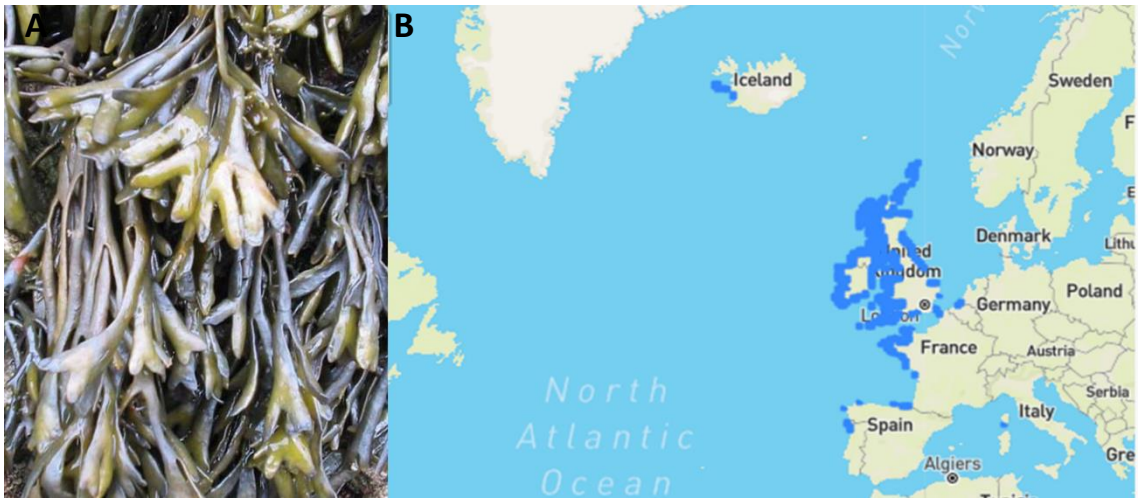


Figure 5 Pelvetia caniculata (A) and habitat distribution (B; Macoi, 2008).

1.3.3. Fucus spiralis

Fucus spiralis is found in Iceland, Norway, Denmark, Netherlands, UK, Ireland, atlantic coast of France, Spain, Morocco, Azores, east coast of America from New Jersey to Nova Scotia and isolated reports in the northern Pacific (Garcia-Perez et al., 2022; Figure 6). The presence or absence of suitable substrata is one of the most important factors determining the distribution of this seaweed. It spends up to 90 percent of the time out of the water and can tolerate a high level of desiccation, being able to survive with 70 to 80 percent water loss. This seaweed, is regularly exposed to sun radiation and consequently OS, having adapted to these inhospitable conditions, producing compounds with antioxidant capacity such as phloroglucinol derivatives or fucoidans. This macroalga also has anticoagulant properties and contain, alginate, copper, iron, manganese. It has been used for treatment of obesity, gout and goiter (Macoi, 2008; Paiva et al., 2018).

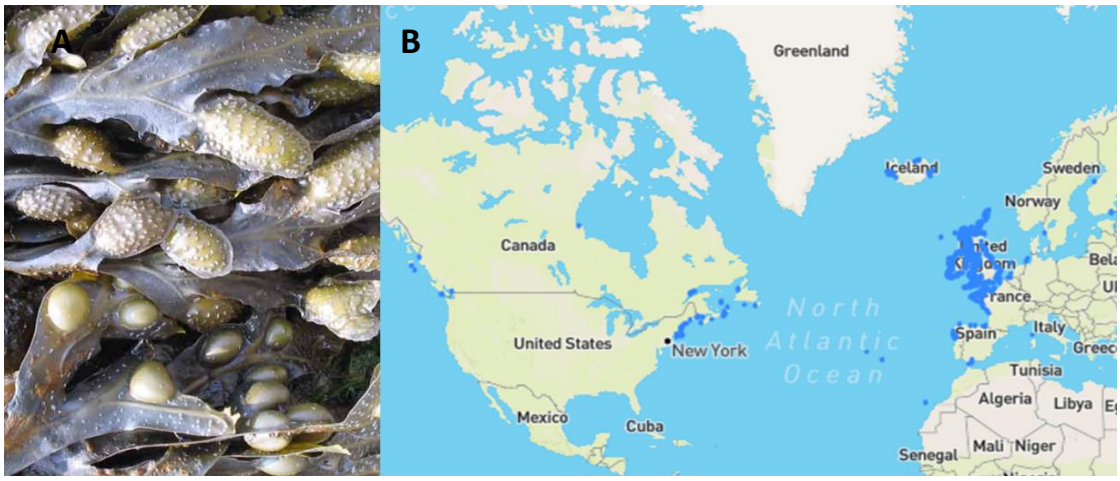


Figure 6 *Fucus spiralis* (A) and habitat distribution (B; Macoi 2008).

1.3.4. Fucus vesiculosus

Fucus vesiculosus can be found on the coasts of the North Sea, the western Baltic Sea, and the Atlantic and Pacific Oceans. It occurs around the coastline of Greenland, Britain, Ireland, Norway, the Atlantic coast of France, Spain and Morocco, and the Atlantic coasts of Canada and the United States from Hudson Bay to North Carolina (Garcia-Perez et al., 2022; Figure 7). It is commonly used as a food in Japan, and it can be stored dried. Chemicals constituents of *F. vesiculosus* include algin, mannitol, β-carotene, zeaxanthin, bromine, retinol, ascorbic acid (vitamin C), squalene. *F. vesiculosus* in herbal medicine is as a source of iodine, an essential nutrient for the thyroid gland; it can be used in the treatment of underactive thyroid glands (hypothyroidism). Its ability as a thyroid stimulant may also help counter obesity by increasing metabolic rate (Macoi, 2008; Balina et al., 2016; Obluchinskaya et al., 2022).

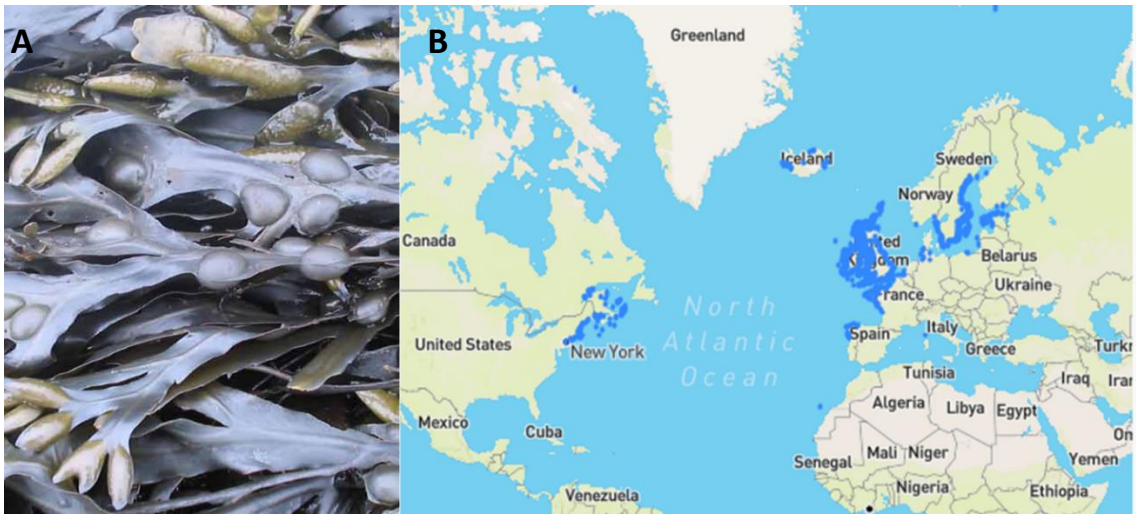


Figure 7 *Fucus vesiculosus* (A) and habitat distribution (B; Macoi, 2008).

1.4. Biological models

Cell cultures most of the time do not offer the necessary complexity and, for some studies, it is not appropriate to use mammals, such as rodents, as the time and number of animals required are too large, raising ethical issues. In this sense, *S. cerevisiae* and *C. elegans*, can be complementary models, for new neuroprotector compounds research (Tardiff et al., 2013).

1.4.1. *Saccharomyces cerevisiae*

This simple organism has been mostly used in the food industry, but in the last decades, new approaches have been investigated. The yeast *S. cerevisiae* or budding/baker's yeast is the most studied unicellular eukaryotic organism, its genome was fully sequenced in 1996 and it was the first one to be

published from an eukaryote (Goffeau et al., 1996). With this information it was possible to make genetic manipulations also due to a stable haploid phase in the life cycle and genetic amenability of this model. *S. cerevisiae* shares with humans numerous key aspects in functional pathways, including cell cycle, metabolism, programmed cell death among others (Mohammadi et al., 2015; Tenreiro & Outeiro, 2010). The budding yeast is also cheap to maintain in the laboratory, fast growing, easy to manipulate, and a GRAS (generally regarded as safe) investigation model. In summary, this is an important experimental model that, in complement with other biological models, can be an interesting tool to investigate new compounds that may have protective activity for human diseases (Mohammadi et al., 2015).

1.4.2. *S. cerevisiae* as a model of neurodegenerative diseases

The basic mechanisms and pathways underlying neurodegenerative diseases, such as mitochondrial dysfunction, transcriptional dysregulation or proteasomal deficiency, are highly conserved between humans and yeast. In this way it is possible to investigate the fundamental molecular events involved in these pathological processes in *S. cerevisiae*. However, two different strategies can be used. If the gene of interest has a yeast homolog, it is possible to study its function directly. If, on the other hand, the gene underlying the disease is absent in yeast, it can be modeled through heterologous expression of the human gene in yeast cells (Tenreiro & Outeiro, 2010).

Genetic transformation of yeast is relatively simple, and in this way it's possible to use *S. cerevisiae* as a model of ND and test the therapeutic potential of diverse compounds. One example is the study of Tardiff et al. 2013, which demonstrates that N-aryl benzimidazole protects *S. cerevisiae* mutants from wild type α-synuclein protein toxicity. More recently Popova et al, (2021), identified two new peptides that inhibited of α-synuclein toxicity and aggregation, using a yeast model expressing α-synuclein under the GAL1 promoter. It was also found that simvastatin for example, had the capacity to reduce the amount of Aβ, in a yeast strain that was transformed with Aβ fused with GFP (Dhakal et al., 2019).

1.4.3. *Caenorhabditis elegans*

C.elegans is a free-living nematode. The life cycle after egg hatching, includes four larval stages, L1, L2, L3, L4, followed by the adult stage (Figure 8). This process is relatively short, taking about three days until the nematode reaches adulthood. The overall lifespan is around two or three weeks. There are two *C. elegans* sexes: hermaphrodite and males (Apfeld & Alper, 2018). When under stress or unfavorable conditions, such as starvation, L2 animals can enter the Dauer Stage. In order to increase their lifespan, these animals limit their metabolic activity, exhibit adaptations in their behavior and structure, and

increase resistance to multiple stresses. When food is available again, the worms exit the Dauer larval stage and proceed to the adult stage.

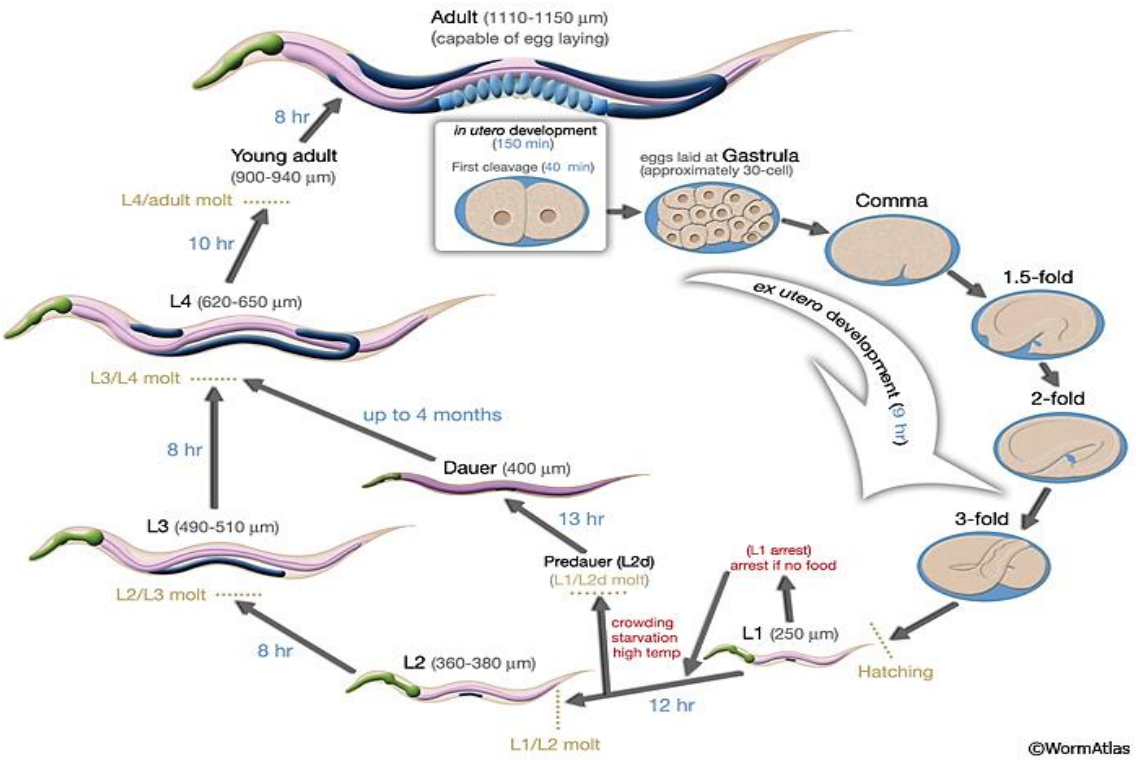


Figure 8 Life cycle of *Caenorhabditis elegans* at 22 °C. An animal reachs the adult stage takes in 3-4 days at 20°C and goes through three stages: embryogenesis; larval development and adult stage. When under stress or unfavorable conditions, such as starvation, animals can enter the Dauer Stage, becoming resistant to environmental stresses and being able to last a few more months until conditions improve. Figure adapted from Wormatlas (*https://www.wormatlas.org/*)

Despite its small size, *C. elegans* has digestive, nervous, muscular, and reproductive systems. It is anatomically transparent and the neurons are easily visualized by expressing fluorescent proteins in live animals, allowing the study of neuronal properties throughout development and in real time experiences (Caldwell et al., 2020). In the wild, *C. elegans* feeds on bacteria present in the soil, however, in the laboratory, it is maintained in Petri dishes, seeded mostly with *Escherichia coli*. The manipulation of these nematodes is simple, with a small platinum wire, *C. elegans* can be moved from one plate to another. To store for larger periods, these nematodes can be frozen and kept at -80 °C and liquid nitrogen for decades (Apfeld & Alper, 2018).

1.4.4. *C. elegans* as neurodegenerative disease model

Despite the differences between *C. elegans* and humans, this model has been used to investigate ND. This happens because humans share many conserved molecular pathways and cellular mechanisms with *C. elegans*. So, this conservation provides great potential to mimic human diseases. Though the

mammalian nervous system consists of billions of neurons, an adult *C. elegans* has 302 neurons over its body, increasing the accuracy of neuronal analysis.

Furthermore, their neuronal circuit has also been fully mapped since 1980, facilitating the understanding of neuronal signaling. Another advantage is the ease which it is possible to make genetic transformations. Thus, the formation of transgenic strains is possible, presenting phenotypes in key aspects of the disease (Caldwell et al., 2020). Considering these factors, this model is of particular interest in studies of neurodegenerative diseases, because they share forms or mechanisms of disease development, such as the expression of mutant proteins and their accumulation/aggregation. Thus, the possibility of replicating complex human diseases, and at the same time the simplicity of the model, having a known genome, easy-to-evaluate phenotypes and genetic tools, make *C. elegans* a robust model for the screening of new drugs (O'Reilly et al., 2014). One example of investigation is the use of *C. elegans* to test some compounds or compounds mixtures (like plant extracts), in model strains of PD or AD Chalorak et al. (2018), demonstrated that *Holonthuria scabra* extract could significantly reduce the concentration of α-synuclein in the muscle cells. In this study, they used a strain capable of producing α-synuclein protein, responsible for the physiopathology of PD. In addition, Dostal et al. (2010), using a model of β-amyloid peptide, found that coffee extracts could reduce *C. elegans* toxicity induced by the protein aggregation. In addition to neuroprotection studies, this model was also used to study neurodegeneration. A bacterial metabolite was found to induce glutathione-tractable proteostatic damage, leading to neurodegeneration of glutamatergic neurons (Martinez et al., 2015).

2. THE AIM

This study aims to investigate the neuroprotective effects of macroalgae and also to valorize as the ocean resources as a source of new molecules to be used for medical purposes. For this, several macroalgae were tested for their antiradical activity. The most promising seaweed extracts were further studied in two distict biological systems:*Saccharomyces cerevisiae* and *Caenorhabditis elegans* aiming to select macroalgae with potential neuroprotective activity in FTD-17, AD and PD models contexts. This study also explored to understand which targets and pathways are activated by the most effective seaweed extract, unraveling its mechanism of action. Thus, it was possible to complement two topics that are currently in vogue: exploration of marine resources and neurodegenerative diseases.

3. MATERIALS AND METHODS

3.1. Plant material and extract preparation

All vegetable materials were collected manually on the Galician coast by Algamar company and ceded by the University of Vigo in the form of extract. The algae tested were: *Undaria pinnafitida Himanthalia elongata, Pelvetia canaliculata, Fucus vesiculosus, Fucus spiralis, Mastocarpus stellatus, Gigartina pistillata, Saccharina latisima, Chondrus crispus, Codium tomentosum, Bifurcaria bifurcata, Laminaria ochroleuca, Sargassum muticum, Ulva rigida, Ascophyllum nodosum*. They were washed abundantly with tap water to remove salt, sand, and other debris. Afterwards, samples were stored in plastic zip bags at −80 °C and lyophilized (LyoAlfa10/15, Telstar, Thermo Fisher Scientific), pulverized into a fine powder with a blender, and stored at −20 °C until extraction. All samples were subjected to heat-assisted extraction from 15 g of algae with 100 mL of 60% (v/v) methanol for 3 h at 45 °C and protected from light. The extracts were later concentrated in a rotary evaporator at 40 °C to obtain the dry extracts and 50 mg/mL stock solutions were made in pure DMSO.

3.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

In order to do the first screening of algae extracts, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was made. This assay was performed in 96 well plates, as described previously (B. A. Silva et al., 2005). In this method, the absorbance decreases as a result of a color change as the radical is scavenged.

The antiradical activity of each extract was evaluated in a large range of sample concentrations:1 mg/mL to 0.125 mg/Ml. In each well, 10 µl extract (100% DMSO) and 140 µl DPPH (stock solution, 400 µM, ethanol 100%, Sigma) were added. For each concentration, three independent replicates were performed and the absorbance was monitored after 60 min at 515 nm with a microplate reader (SpectraMax Plus 384, USA). The blank of the samples and the negative control consisted of 10 µl of the respective solutions at each concentration and 140 µl of 100% ethanol and 140µl DPPH, respectively. The DPPH solution was prepared and stored at 4 °C in the dark between analyses. From the absorbance readings, the percentage of antioxidant activity (% AA) corresponding to the amount of DPPH reduced by the samples was determined using the following formula: % AA = ((Abs C - (Abs t60 - Abs B)) / Abs C)) * 100, where Abs t60: absorbance of the samples for each concentration in time 60 min; Abs B: absorbance of the blank of the samples for each concentration at time 60; Abs C: absorbance of negative

control. The efficient concentration (EC) was obtained from the percent reduction of the DPPH curve of each extract necessary for a 50% discoloration.

3.3. Strains, media and growth conditions

The yeast and the mutant strains used in this study are listed in Table 3 and were gently offered by Dr Paula Ludovico and Dr Belém Marques from ICVS/UMinho (Table 3). All yeast cultures were maintained in YPDA medium plates (1 % w/v yeast extract BD Bacto™, 2 % w/v BD Bacto™peptone, 2 % w/v dextrose and 2 % w/v Labchem agar agar) with 48 h incubation at 30 °C and finally stored at 4 °C. Pre-inocula of W303-1A and derived mutant strains were prepared using an isolated colony from the stock culture and subsequently suspended in YPD medium and incubated overnight at 30 °C and 200 rpm. Growth was monitored by spectrophotometry at OD_{600}. After cell culture proliferation, the transformed strains were washed two times in sterilized 1X PBS at 25 °C and 4300*g* speed. The cell culture was resuspended in SCG medium containing 0.67 (w/v) (Sigma Life Science Y0626) with 2% (w/v) galactose (SGC) and supplemented with the nutritional requirements: 300 mg/L leucine (Sigma, L8000), 100 mg/L adenine (Sigma, A5665) and 50 mg/L histidine (Alfa Aesar, A10413). All media and solutions used are presented in: Supplementary Table 1.

Table 3 Genotype of yeast strains used in the study

Yeast strain		Genotype	Origin
Description	Name		
Wild type	W303-1A	*Mat a, ade2-1, ura3-1, leu2- 3112, trp1-1, his3-1115 can1-100*	-
Transformed from W303-1A	Vector control (CV)	W303-1A [pRS306]	Paula Ludovico and Belém Marques
	SNCA WT	W303-1A [pRS306 GALSNCAWT::GFP]	
	SNCA A53T	W303-1A [pRS306 GALSNCAA53T::GFP]	

3.4. SNCA strains microscopy

The strains SNCA WT, SNCA A53T and CV were incubated overnight in YPD medium with dextrose 2% (w/v) and the medium refreshed to SCG as said before. The cells were distributed for the control (DMSO) and the extracts PC, FS, FV and HE (1 mg/mL). All the strains were maintained at 30 °C and 200 rpm during 6 h.

Each sample was photographed in fluorescence microscope Leica DM5000B at 20x magnification and at least 200 cells per condition were counted for quantification of aggregation. The same settings of exposure, gain and intensity were used between control and the correspondent condition.

Antiaggregant activity was evaluated by the percentage of cells without inclusions for SNCA WT and SNCA A53T strains treated with extracts in comparison with corresponding controls. This percentage was then calculated dividing the number of cells without inclusions (tx) by the total cells (tc): % Cell without inclusions= (tx/tc)*100. Cells presenting at least 1 fluorescent spot in the cytoplasm were considered as containing aggregates.

At the same time viability was evaluated by CFU's at 0, 2, 4 and 6 h. Aliquots of 100 µl were taken to perform serial dilutions up to 10^{-5} and 40 µl were pipetted 3 times onto YPDA plates. After 48 h of incubation at 30 °C, the number of colonies was counted. This percentage of viability in CFUs was calculated for each extract and timepoint tested, as percentage of the mean value of colonies at different times, taking time 0 h as 100% viability.

3.5. Heat stress assay in *S. cerevisiae*

The parental yeast strain W303-1A was incubated overnight in YPD medium under the conditions described above. The pre-inocula was diluted and the OD_{600} was adjusted to 0.4 with the following treatments being added: DMSO at the same volume as the extracts (control) or 1 mg/mL of each extract. Viability was evaluated by CFU's at 0, 2, 4 and 6 h. Aliquots of 100 µl were taken to perform serial dilutions up to 10^{-4} and 40 µl were pipetted 3 times onto YPDA plates. Before shifting cultures to 42 °C, the first timepoint was performed. The number of colonies was counted and the percentage of viability in CFUs was calculated for each extract and timepoint tested, as percentage of the mean value of colonies at different times, taking time 0 h as 100% viability.

3.6. Strains and general maintenance of *C. elegans*

All strains were cultured and maintained on Nematode Growth Medium (NGM) plates, seeded with *Escherichia coli* OP50 strain at 20 °C. The strains used in the study are presented in supplementary Table 2 and all the media and buffers used in the study are listed in supplementary Table 3.

3.7. *E. coli* OP50 growth and inactivation

The OP50 used as nematode food was prepared in 200 mL of Luria Bertani (LB) medium, overnight at 37°C and 200 rpm. When the growth was stopped, the culture was maintained at 4 °C and was then is ready to be used. The seed of plates consists in pipetting 0.75 mL of bacterial suspension in the center of the NGM plate (90 mm) and was leaving them to dry during 3-4 days.

For the treatment of nematodes in liquid and solid medium the *E. coli* OP50 bacteria was inactivated. LB was inoculated with OP50 and the growth occurred overnight (16 h, approximately) in the same conditions mentioned above. The culture was centrifuged at 4000 g/4 °C, for 30 min. The pellet was maintained, and the bacteria was inactivated by 3 cycles of freeze/thaw in liquid nitrogen and water bath at 37 °C. Pellets were kept, in liquid nitrogen and stored at -80 °C.

3.8. Animal synchronization

The method used to synchronize the growth/development of a *C. elegans* population was base on the use of an alkaline hypochlorite solution (bleaching solution: 2.6% NaClO and 0.5M NaOH) was used. Firstly, the NGM plates were washed with M9 and the animals were collected to a 15 mL conical tube and left to deposit. The M9 was discarded and a bleaching solution was added for 5 minutes with vigorous shaking until the animals were lysed and release eggs. The tubes were centrifuged for 1 minute and 2000 *g* using a Thermo Electron Heraeus Multifuge 3L-R (Thermo Electron Corporation, Waltham, Massachusetts, USA). The supernatant was discarded, and the animals were washed two times with M9 at the same conditions referred above. To the assays that used eggs, the next step was to resuspend the resultant eggs in S-medium. For the assays that used L1 stage animals, the eggs were resuspended in M9 and allowed to hatch overnight.

3.9. Food clearance assay for toxicity evaluation

A food clearance assay was used to evaluate the toxicity of the various extracts in a range of concentrations. N2 Bristol strain eggs obtained by bleach treatment were resuspend in S-medium to a density of 20-25 eggs per 15 µL of volume. In a 96 well plate, egg suspension was added to 20 µL of inactivated *E. coli* OP50 with OD_{595} 0,7 (food source) and 25 µL of the extract, with a final concentration between 1 mg/mL and 0,125 mg/mL in 1% DMSO. The negative control was 5% DMSO because it causes toxicity for *C. elegans* represents and 1% DMSO was used as positive control (non-toxic).

The plates were incubated for 7 days and involved in wet paper and aluminum foil to protect the animals and the extracts from drought and light. The absorbance was measured daily at 595 nm using a Tecan Infinite M200 Pro plate reader (Männedorf, Switzerland). In this way, the consumption of the food source was evaluated along time, being an indirect measure for animals viability, reproductivity and survival when in the presence of extracts. Four independent trials were performed.

3.10. WMicroTracker motility assessment

The WMicroTracker from Nanometrix is a device that measures the locomotor activity of worm populations in liquid media in microplates. The reading method is based on infrared beams that cross each well of the plate. When the infrared beams are interrupted by the animals in movement, these interruptions are scored as animal activity. This device has the advantage of being able to read all the wells at the same time.

After synchronization, the eggs were allowed to hatch overnight and in the next day the animals were diluted to a final concentration of 40/50 L1 nematodes per 35 µL. The animals were added to 55 µL of *E. coli* suspension with $O.D_{595}$ 0,9 and 10 µL of extract. For each condition 4 replicates were performed. The plate was incubated during 4 days at 20 °C and was involved in humidified paper and aluminum foil to protect from drought and light. At the day 4, the plates were read for 60/180 min in the WMicroTracker device depending of the objective of the assay (Figure 9).

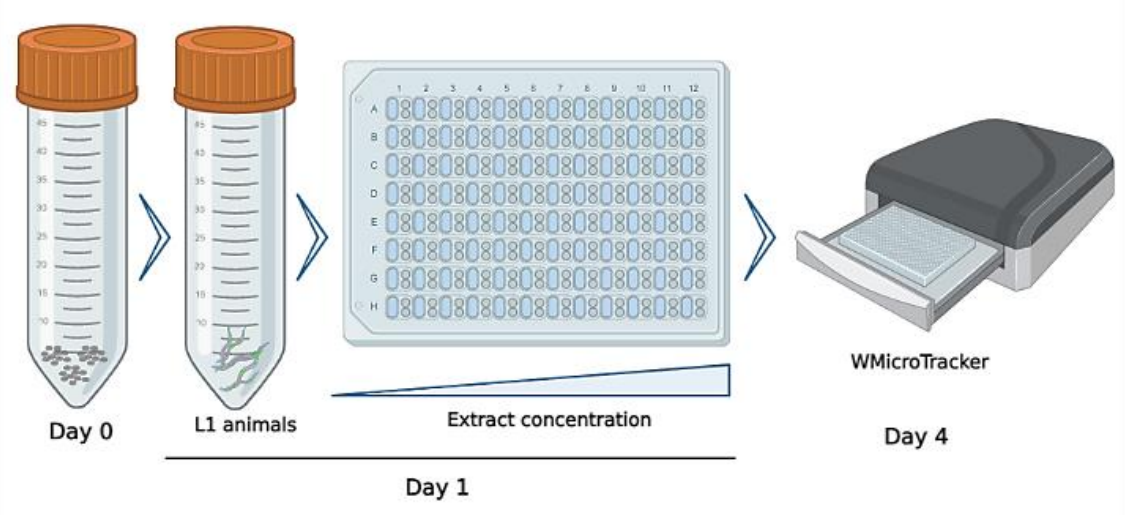


Figure 9 Experimental WMicroTracker design during the 4 day period, in liquid medium.

3.11. Evaluation of glutamatergic and dopaminergic neurons degeneration

The strains UA198, DA1240, UA44 and BZ555 were grown synchronized in NGM agar plates until day 7, when neuronal loss was evaluated: loss of glutamatergic neurons in U1198 and DA1240 and loss of dopaminergic neurons in UA44 and BZ555. After day 4 after hatching, adults were isolated daily from the plates to avoid the presence of the progeny. The extract and the respective vehicle were incorporated in inactivated OP50 at 0.5 mg/mL

To examine the neurons, hermaphrodites were immobilized using 5 mM levamisole on glass cover slips and inverted onto 3% agarose pads on microscope slides and the evaluation was performed using the IX81 Olympus Widefield Inverted Microscope (objective 40X). The presence/absence of five glutamatergic neurons located on the animal tail was evaluated in UA198 and DA1240 strains while the presence/absence of six pairs of dopaminergic neurons was scored in UA44 and BZ555. Representative photos were taken using Olympus LPS Confocal FV1000 microscope at 640x magnification. Images were acquired every 0.5 μm along the Z-axis using the 515 nm excitation laser. The confocal microscope pinhole was adjusted to 1 Airy-unit optical slice.

3.12. Gene expression analysis using *C. elegans* fluorescence reporter strains

3.12.1. Antioxidant response pathways

The strains LD1171 and CL2166 (supplementary Table 3), expressing GFP under the regulation of the *gcs-1* and *gst-4* promoters, respectively, were grown in liquid media and treated with 0.5 mg/mL of FV extract as described previously. At day 4 after hatching, worms were picked to slides with a 3% agarose pad, anesthetized with levamisole (5 mM) and oriented using an eyelash. Excess levamisole was removed and animals were covered with a cover slide and sealed using 3% agarose. In each assay, 10 worms were analyzed per condition. As positive control, animals grown in vehicle (1% DMSO) were incubated in 5-Hydroxy-1,4 naphthoquinone (juglone) (Sigma-Aldrich, St. Louis, MO) at 150 μM for 1h and allowed to recover at 20 °C for 3h.

Brightfield and Fluorescence (GFP filter) images were obtained using IX81 Olympus Widefield Inverted Microscope 100x magnification. Fluorescence exposure time was adjusted to control conditions and applied equally for treated animals in each experiment. The fluorescence intensity was calculated by the following equation: Integrated Density – (Area of animal x Mean Fluorescence of Background readings). All parameters were quantified using Image J software.

3.12.2. Heat-shock response pathways

The SJ4100 and SJ4005 strain (supplementary table 3), expressing GFP under the regulation of the *hsp-6* and *hsp-4* promoters respectively, were used to assess the ability of FV treatment to activate the mitochondrial or endoplasmic reticulum UPRs, respectively. The nematodes were synchronized and grown in liquid media as described and treated with 0.5 mg/mL of FV or vehicle (DMSO 1%). At day 4, adult animals were mounted onto slides with 3% agarose pads, anesthetized with levamisole (5 mM), oriented using an eyelash and covered with a cover slide.

Twenty-four hours prior to use, 1 µL of antimycin A was added to wells containing animals treated with vehicle only, to a final concentration of 20 µM. Antimycin A is an oxidative phosphorylation inhibitor, able to cause mitochondrial stress and activating the UPR^{MT} and was therefore used as a positive control for SJ4100 strain. For SJ4005 strain, sixteen hours (approximately) prior to the microscope analysis, 1 µL of tunicamycin to a final concentration of 5 µg/mL was used as positive control. Tunicamycin is a chemical that promotes protein misfolding by inhibiting protein glycosylation.

Brightfield and Fluorescence (GFP filter) images were obtained using IX81 Olympus Widefield Inverted Microscope 100x magnification. Fluorescence exposure time was adjusted to control conditions and applied equally for treated animals in each experiment. The fluorescence intensity was calculated by the following equation: Integrated Density – (Area of animal x Mean Fluorescence of Background readings). All parameters were quantified using Image J software.

3.13. Statistical analysis

Continuous variables were tested for normal distribution and homogeneity of variances. One-way ANOVA and Dunnett multiple comparison tests were used for comparison of the percentage of cells with aggregates in *S. cerevisiae* and comparison of motility values between conditions. For reporter strains of *C. elegans*, when data normality was not guaranteed, Kruskal-Wallis non-parametrical test was applied. The comparison of the number of neurons in Alzheimer's and Parkinson's strains was evaluated using a chi-square test, and at least 15 animals were analyzed for each trial. For toxicity evaluation of the extracts in *C. elegans,* a non-linear regression, sigmoidal, 4PL, X is log(concentration) analysis was performed for all extracts. Results were expressed as mean ± standard error (SEM) from three independent assays. This significance is indicated in the figures with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). The software Graphpad Prism v9.0 was used to elaborate the graphs and the statistical analysis.

4. RESULTS AND DISCUSSION

4.1. Extracts from PC, HE, FV and FS with radical scavenging against DPPH

The DPPH assay was performed as a pre-screening before the assays in biological models because OS is related with the development of several ND. In this way, through a colorimetric analysis it is possible to determine if a compound has antiradical activity and with potential for neuroprotection.

The extracts with more activity were PC, HE, FV and FS (Figure 10). These were the chosen ones to proceed with the biological assays with EC_{50} below the 500 μg/mL, with emphasis to PC with EC_{50} below 150 μg/mL. Gallic acid is a polyphenol compound that can be found in fruits, vegetables, and herbal medicines and was used as positive control, because of its high antiradical capacity (Wang et al., 2022). The results for the remaining macroalgae extracts are presented in the Supplementary Table 4.

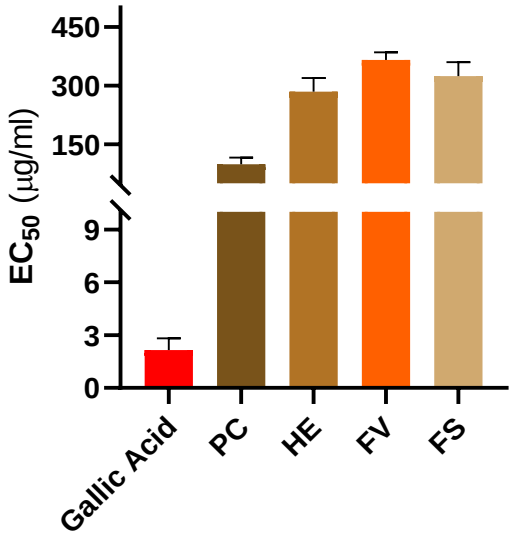


Figure 10 The DPPH anti-radical activity represented as EC_{50} of macroalgae extracts with higher antioxidant potential and a positive control- gallic acid, after 60 min of DPPH reaction. Gallic acid is a potent antioxidant. Results are presented as Mean ± SEM of three independent replicates.

Sabeena Farvin & Jacobsen, (2013) performed a study comparing the phenolic compounds and the antioxidant capacity of 16 algae extracts. They also compared, ethanolic and water extractions, which the water extracts more activity overall. For these two algae the antiradical activity was quite superior compared to our results. In the case of FV in both, aqueous and ethanolic extracts, the authors present EC_{50} values below 10 μg/mL, while our extract presents an EC_{50} of approximately 370 μg/mL. For FS the EC_{50} was around 74 μg/mL, while our extract presented 325 μg/mL. This can be explained by the type of solvent that in our case is 60% methanol (v/v), the harvest zone, or the moment of the year. In this

case the harvest zone was in Denmark between April and September and in our case the seaweeds were collected in the Galician coast. All these factors can induce different secondary metabolism, which will lead to different chemical composition, and consequently different bioactivities. However, we must keep in mind that the antioxidant and antiradical response is only one of them. Other assays could be important to complement this first screening such as FRAP, ORAC, ABTS or TEAC assays.

In summary PC, HE, FV and FS have a substantial antiradical activity compared with the remaining extracts, therefore they were selected to proceed for biological assays.

4.2. PC, HE, FV and FS Extracts do not affect growth of *S. cerevisiae* cultures

To understand the behavior of strains (CV, SNCA WT and SNCA A53T) growth of cultures in the absence and presence of the extracts was measured. As Sampaio-Marques et al., 2012 demonstrated, CV does not have toxicity associated with the transformation. This strain functions as a control for the transformation.

Since growth of cultures treated with the extracts is similar to the control, we concluded that the extracts did not present toxicity (Figure 11 A). With the genetic manipulation of *S. cerevisiae,* it is possible to induce the production of exogenous α-synuclein by the GAL1 promoter. Unlike CV strain, SNCA A53T and SNCA WT strains growth is extremely slow, suggesting that the expression of the human protein is toxic to cells (Figure 11 B and 11 C). Although only one replication was made, the results suggest that the extracts at the concentration of 1 mg/mL showed no toxicity.

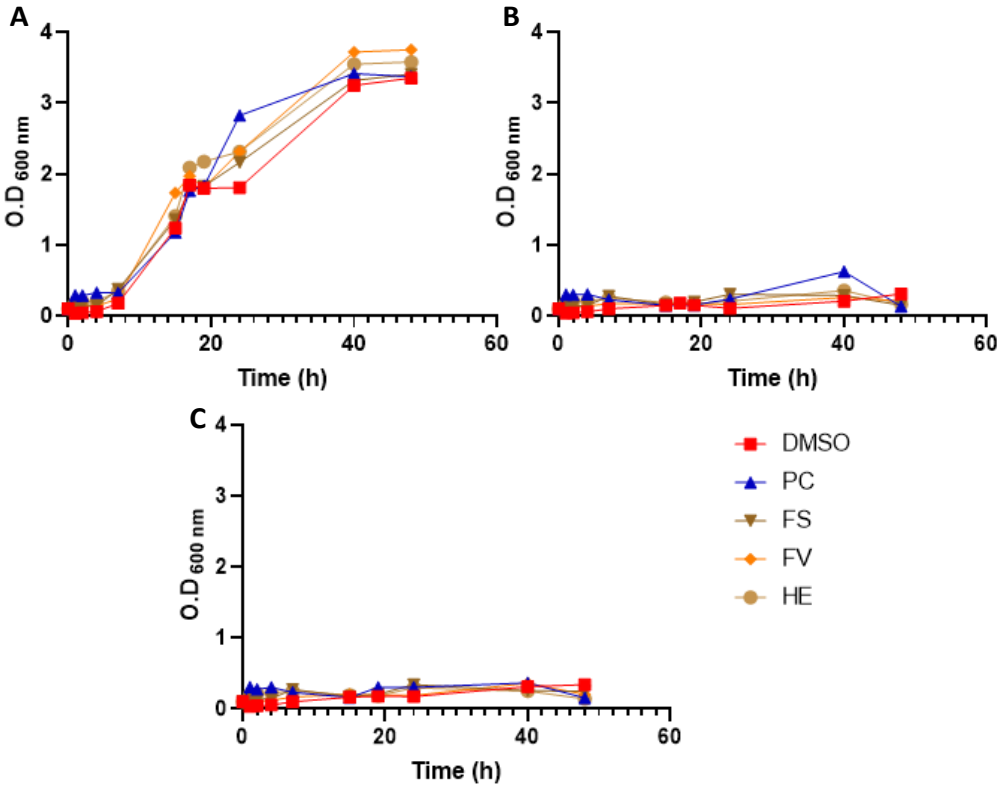


Figure 11 Extracts treatment do not affect the viability of *S. cerevisiae*. Growth curve of CV (**A**), SNCA A53T (**B**) and SNCA WT (**C**) in SCG with galactose as unique carbon source, during 48 h, at 30 °C, 200 rpm treated with, DMSO (solvent of extracts, *used as control)*, and 4 different extracts (1 mg/mL), *Pelvetia caniculata* (PC), *Fucus spiralis* (FS), *Fucus vesiculosus* (FV) or *Himanthalia elongata* (HE) n=1.

4.3. PC, FV and FS treatment increases the number of cells without α-synuclein inclusions

For the assessment of antiaggregant activity, genetically modified yeast strains, expressing the human α-synuclein protein fused with GFP were used. α-synuclein inclusions are visible as bright green spots in the cells under the fluorescence microscope. By putting cells with galactose as a unique carbon source, the galactose inducible promoter triggers expression of the protein α-Synuclein fused with GFP. Thus, this system allows us to understand if the extracts have the capacity to avoid α-synuclein aggregation.

After 6 h induction photos were taken of SNCA A53T strain, of the control culture (Figure 12A), in culture with PC (Figure 12B), with FV (Figure 12C), with FS (Figure 12D) and with HE (Figure 12E) and then the percentage of cells without inclusions calculated (Popova et al., 2021). Significant differences were found for PC, FV and FS extract in SNCA A53T strain, and the percentage of cells without inclusions is nearly the double in comparison with DMSO control. The treatment with HE extract did not show significant differences (Figure 12F). In this way, it can be said that the presence of 1 mg/mL FS, FV or PC extract can reduce significantly the number of aggregates in SNCA A53T strain. Nonetheless, the

presence of extracts did not protect the strain against the toxicity induced by αSynA53T-GFP protein after 6 h of treatment (Figure 12G).

When the same experiment was done with strain SNCA WT, expressing αSynWT-GFP, similar results were obtained in the control treatment compared with the control of SNCA A53T (Figure 13A). Significant differences were found only for the FS extract (Figure 13D). The treatment with PC (Figure 13B), FV (Figure 13C) and HE (Figure 13E) extract did not show significant differences in terms of cells without inclusions (Figure 13F). Curiously, PC extract, did not significantly reduce the number of cells with aggregates but protected against αSynWT-GFP toxicity (Figure 13G). Thus, it seems that the aggregation *per se* is not responsible for the toxicity, but other mechanisms might be present.

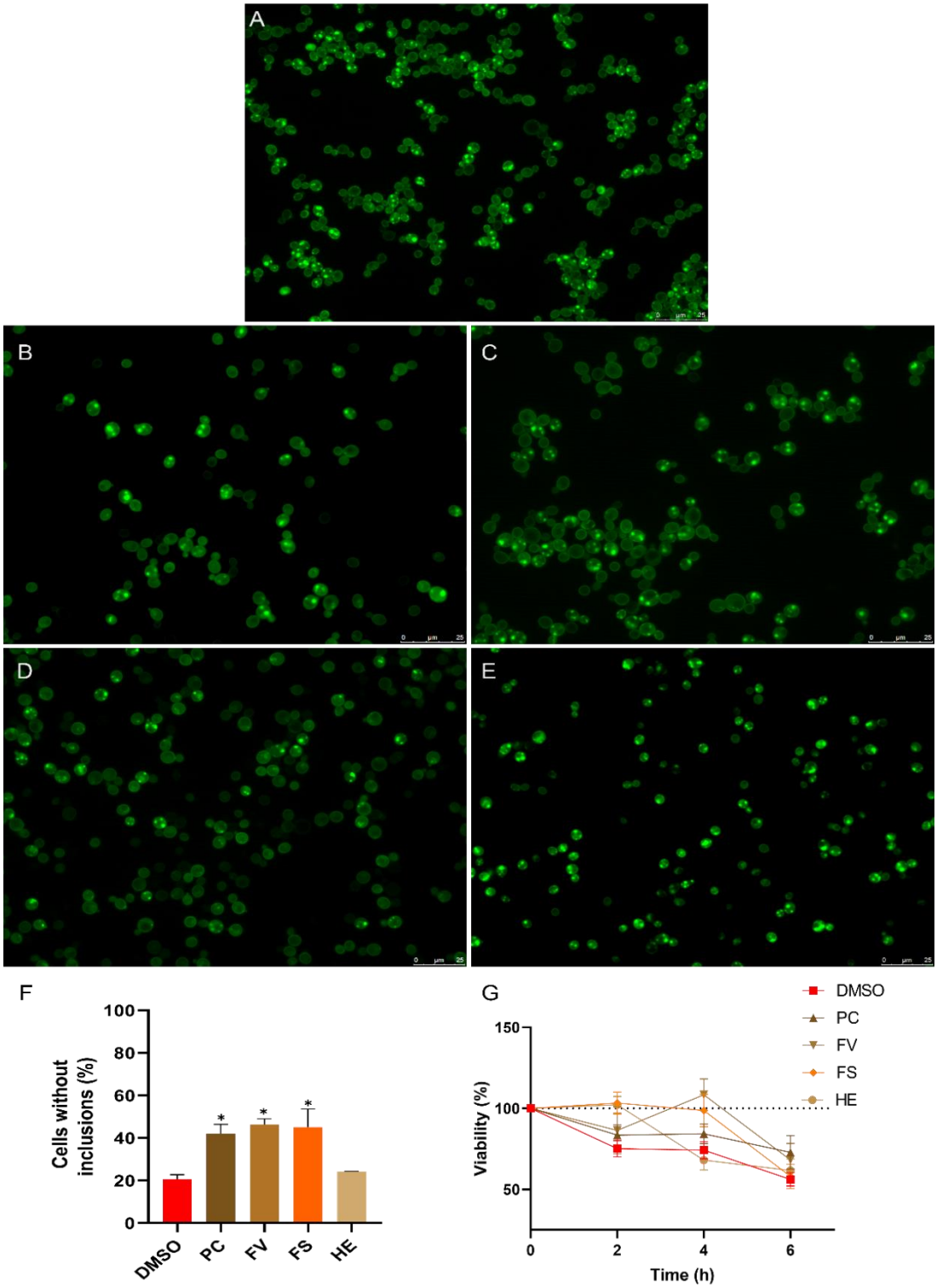


Figure 12 PC, FV and FS treatment reduces αSynA53T-GFP aggregation in *Saccharomyces cerevisiae* SNCA A53T strain. Cells were incubated in SCG medium to induce αSynA53T-GFP expression in the presence of DMSO control (**A**), *Pelvetia caniculata* extract-PC (**B**), *Fucus vesiculosus* extract-FV (**C**), *Fucus spiralis* extract-FS (**D**) or *Himanthalia elongata* extract-HE (**E**), all at 1 mg/mL, and after 6 h they were observed for the presence of fluorescent green spots. Cells without intracellular fluorescent spots were counted and the percentage was calculated and plotted (**F**). Fluorescent microphotographs at 200x magnification. Scale bar represents 25 µm. Results are present as Mean ± SEM of four independent replicates and statistical analysis was performed by one-way ANOVA, *p<0.05. Viability was assessed over the time by CFU's, taking time 0 min as 100% viability (**G**). Results are present as Mean ± SEM of four independent replicates and statistical analysis was performed by Two-way ANOVA.

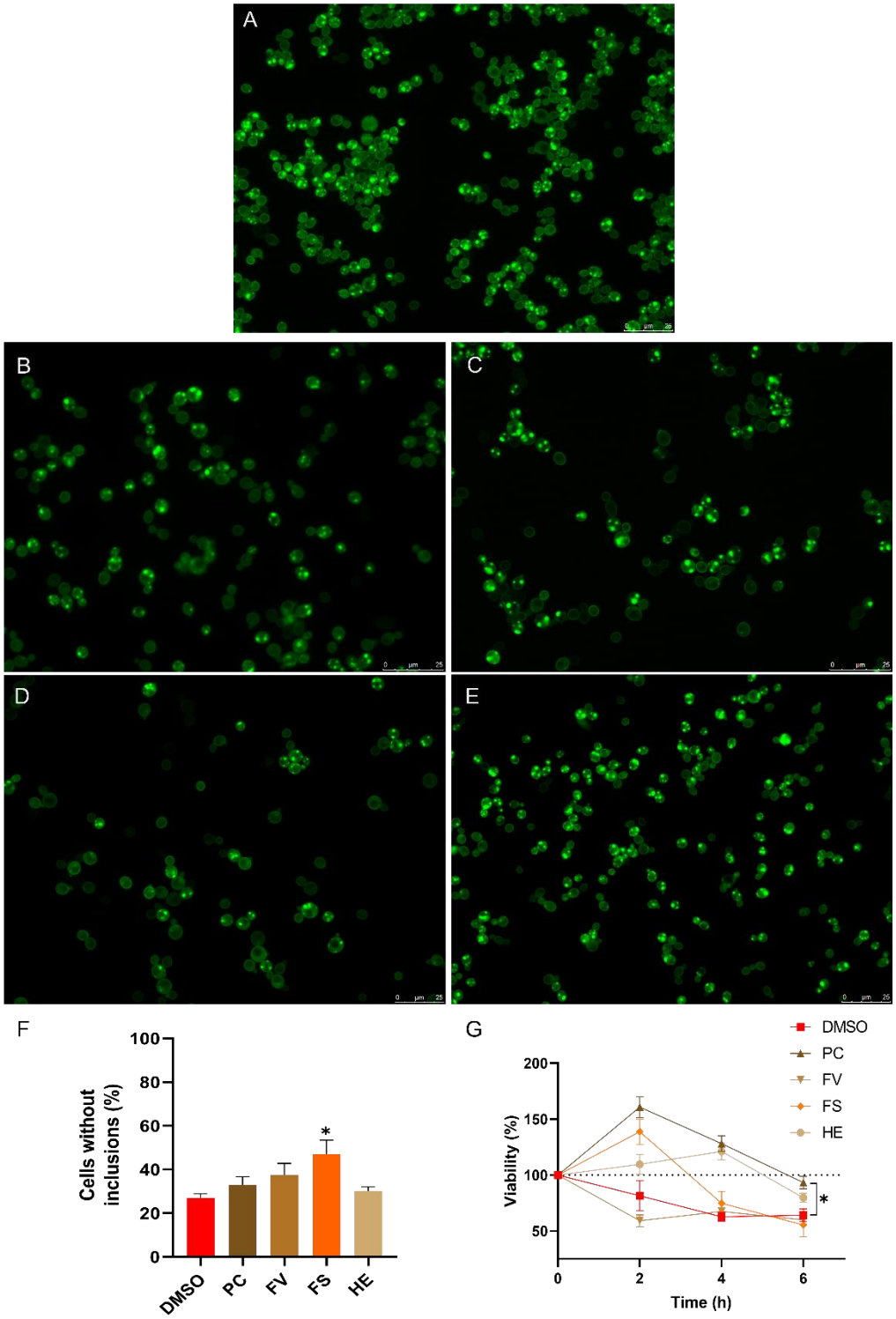


Figure 13 FS treatment reduce αSynWT-GFP aggregation in *Saccharomyces cerevisiae* SNCA WT strain. Cells were incubated in SCG medium to induce αSynWT-GFP expression in the presence of DMSO control (**A**), *Pelvetia caniculata* extract-PC (**B**), *Fucus vesiculosus* extract-FV (**C**), *Fucus spiralis* extract-FS (**D**) or *Himanthalia elongata* extract-HE (**E**), all at 1 mg/mL, and after 6 h they were observed for the presence of fluorescent green spots. Cells without intracellular fluorescent spots were counted and the percentage was calculated and plotted (**F**). Fluorescent microphotographs at 200x magnification. Scale bar represents 25 µm. Results are present as Mean ± SEM of four independent replicates and statistical analysis was performed by one-way ANOVA, *p<0.05. Viability was assessed over the time by CFU's, taking time 0 min as 100% viability (**G**). Results are present as Mean ± SEM of four independent replicates and statistical analysis was performed by Two-way ANOVA, *p<0.05.

In a previous study the same percentage of α-synuclein aggregates in the control treatment was found (Popova et al., 2021). Liang et al, (2008) has found a group of genes whose deletion of any of them enhanced the toxicity of α-synuclein as judged by growth defects compared with wild-type yeast cells expressing α-synuclein, suggesting a protective effect of these genes. One of the genes, *HSP82* encodes for a ubiquitous chaperone Hsp90 that, in yeast, binds to transcription factors, kinases, and other chaperones to regulate various signaling pathways. Their hypothesis was that Hsp90 might bind to α-synuclein, and this complexation could prevent the formation of toxic oligomers. Zondler et al. (2014) found a 15% reduction in the number of yeast cells with inclusions when the Hsps (*HSP31*, *HSP32*, *HSP33*, *HSP34*) were co-expressed. In this study it was also shown that the protein levels of α-synuclein did not change in the presence of the Hsps, suggesting that the effect on toxicity was not simply because of decreased levels of α-synuclein in the cell, but because of the capacity to reduce the high molecular weight α-synuclein species (oligomers).

Taking into consideration the effect of Hsps, the antiaggregating activity of the macroalgae extracts may be due to activating this mechanism of protein protection in the first hours, preventing the high toxicity induced by α-synuclein and reducing the number of cells with aggregates. But it seems that six hours of induction of GAL1 promoter and accumulation of α-synuclein can be too aggressive for yeast cells. The observation of an anti-aggregative effect of the extracts in the yeast model may be an indication of neuroprotective activity that needs to be confirmed in further experiments and also in more phylogenetically evolved models.

Paiva et al., 2018 when analyzing *Fucus spiralis* chemically, showed that it has a low percentage of lipids, compared to terrestrial plants. But in spite of this, it has higher levels of PUFAs. They also reported the presence of flavonoids and phenolics. Pigment composition analysis of extracts from brown seaweeds, showed that xantophylls especially fucoxanthin and carotenes such as β-carotene, are the most predominant compounds (Garcia-Perez et al., 2022). Various activities of these compounds (fucoxanthin especially) are known, such as antioxidant, antidiabetic, anticancer and neuroprotective, for example (Abdul et al., 2016; Barbalace et al., 2019).

Fucoxanthin acts on multiple targets, such as β-secretase avoiding protein aggregation in AD or OS (Yang et al., 2021). It was reported that have capacity to inhibits β-secretase and Aβ assembling in *in vitro* assays and can minimize cognitive impairments in mice(Jung et al., 2016; Xiang et al., 2017). Furthermore, fucoxanthin at 3 μM effectively reduced the levels of ROS induced by protein Aβ in cell lines (Xiang et al., 2017). This approach could in the future be adapted to other neurodegenerative diseases

that share the fact that there is protein dysregulation and aggregation, such as AD, HD or Machado-Joseph disease.

4.4. PC, FV and FS extracts protect *S. cerevisiae* cells from heat stress

As stated above, one explanation for the anti-aggregation activity of the extracts is by induction of Hsps. If this is the mechanism, then the extracts might protect cells from heat denaturation of proteins. So, we decided to perform a viability assay exposing cells to heat stress and assess viability in the presence and absence of the extracts. For 6 hours the cell cultures were incubated at 42 °C in the presence of each extract or DMSO, as control and viability was assessed over time. As expected, in the control culture viability decreased throughout the experiment until nearly 60% after 6h (Figure 14). In the culture with HE, viability was similar to the control, however in cultures with FS, FV or PC, viability was significantly higher than the control. These results suggest that FS, FV and PC can induce Hsps, and thus protect and maintain the proteostatic balance.

.

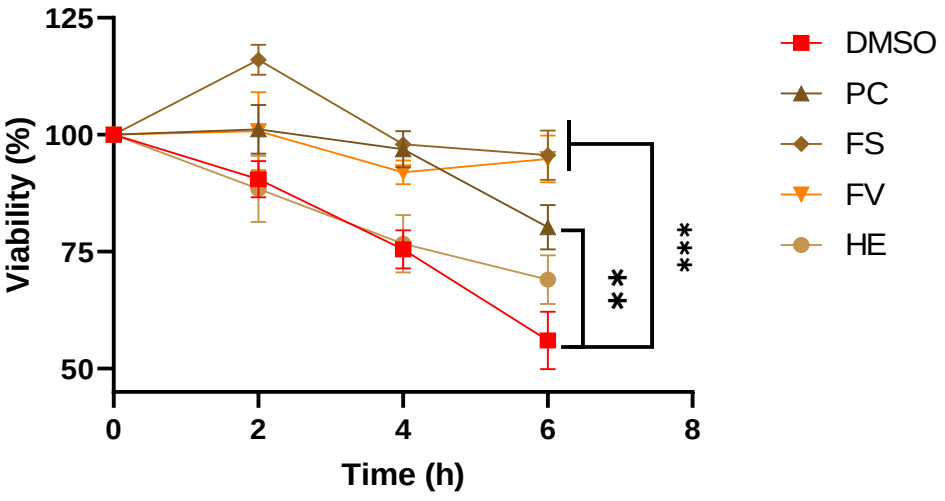


Figure 14 PC, FV and FS extracts protect *Saccharomyces cerevisiae* from heat stress. Viability curve of parental strain W303-1A at 42 °C, supplemented with, DMSO, or the extracts (1 mg/mL), *Pelvetia caniculata* (PC), *Fucus spiralis* (FS), *Fucus vesiculosus* (FV) and *Himanthalia elongata* (HE). Results are presented as Mean ± SEM of four independent replicates and statistical analysis was performed by Two-way ANOVA, $^{**}p < 0.01$, $^{***}p < 0.001$.

At 42 °C the proteostasis is unbalanced and the amount of misfolding proteins is higher. The cellular response to this type of stress is upregulation of chaperones that target the misfolded proteins for degradation or activate transcription factors capable of inducing synthesis of protective proteins. Bayliak et al, (2014) showed that plant extract at low concentrations can protect *S. cerevisiae* from heat and OS and extend the lifespan. They suggest that the extract is capable of activating transcription factors,

such as Yap1 and Msn2/Msn4, inducing synthesis of protective proteins. Other studies demonstrate that celastrol, can activate heat shock responses after upshift the temperature to 42 °C. They conclude that when in contact with celastrol, Yap1 accumulates in the nucleus and consequently promotes the expression of various genes involved in xenobiotics detoxification like *PDR5, ATR1* or *TRX2* (Trott et al., 2008). These genes are responsible for encoding proteins linked to antioxidant capacity or to promote DNA repair, for example. The hypothesis is that the action mechanism of the extracts, passes through the ability of assisting in some way the recruitment and the proper functioning of chaperones. The yeast *SSQ1* gene is an ortholog of mammalian *HSP70* (and *HSP6* in *C. elegans*) and is essential for viability in *Saccharomyces cerevisiae*. In the absence of this gene the yeast is more sensitive to heat stress (Nwaka et al., 1996; Verghese et al., 2012). Thus, we can conclude that the FV extract may be acting in this pathway, increasing resistance to high temperatures protecting cells from thermal stress-possibly by activating chaperones which in turn help with misfolded protein degradation and in proteostatic balance. These results can corroborate or supplement the antiaggregant activity of extracts. Other studies could be used, such as thermorecovery where you would heat-shock the cells and evaluate the recovery over time. Also the quantification of expression of genes involved in the response, such as *SSQ1*, could be assessed.

4.5. Higher concentrations of PC, FV and FS exhibit toxicity in *C. elegans*

In order to supplement and corroborate these data, the next step was testing these 4 extracts in a model with more complexity. The chosen model was *C. elegans* because it is a pluricellular organism and it has a neuronal system that has been completely studied.

The food clearance assay based on the study by Voisine et al. (2007) was used to evaluate a range of extracts concentrations regarding their ability to cause toxicity on *C. elegans* wild-type strain. In this assay, the animals are grown in the presence of food (bacteria) and the optical density of bacteria is measured daily. The consumption of the bacteria by the animals is an indirect measure of their survival, viability and reproductivity, meaning that in a non-toxic scenario (such as the chronic treatment with DMSO 1%), the consumption of bacteria increases over time, which is particularly evident after day 3 when animals progeny hatches. In a toxic situation, such as the 5% DMSO treatment, the animals are unable to grow/survive and therefore there is no food consumption and no alteration in optical density. When animals were chronically treated with seaweed extracts, we concluded that the 1 mg/mL of FV, PC, and FS were toxic, the case of PC being the most evident, because at day four the eggs had still not

hatched. In the case of treatment, the concentration of 0.75 mg/mL also showed some toxicity, noticeably delaying the development of the animals.

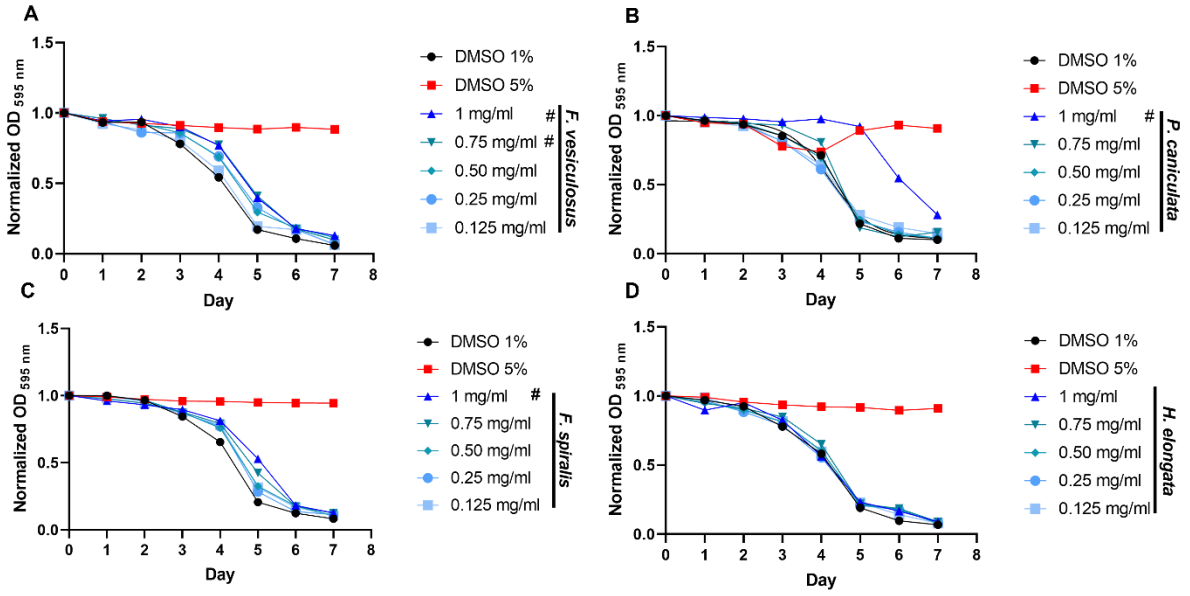


Figure 15 Higher concentrations of FV, PC, and FS were toxic to *Caenorhabditis elegans*. Evaluation of the toxic effects of *Fucus vesiculosus* (**A**); *Pelvetia caniculata* (**B**); *Fucus spiralis* (**C**); *Himanthalia elongata* (**D**), extracts, using a food clearance assay. The OD_{595} of *E. coli* was evaluated daily for each concentration and normalized for day 0 values. Statistical analysis revealed significant differences for FV, PC, and FS at 1 mg/mL and for FV at 0.75 mg/mL (marked concentrations with (#)) in comparison with DMSO 1% (drug vehicle, non-toxic). A representative assay was selected for representation of each extract toxicity. Non-linear regression, sigmoidal, 4PL, X is log(concentration), profile likelihood, $p \leq 0{,}05$.

Moreover, the effect of the chronic treatment of WT animals with the different extracts (at the non-toxic concentrations 0.5 and 0.25 mg/mL) was evaluated using the locomotion of adult animals as readout. For this, animals were treated with the extracts from the egg to day 4 after hatching. At this time, the locomotor activity was quantified in Wmicrotracker during 60 min. At these concentrations, FS (at both concentrations) and PC (at 0.5 mg/mL) treatments significantly reduced the motor activity of animals (Figure 16).

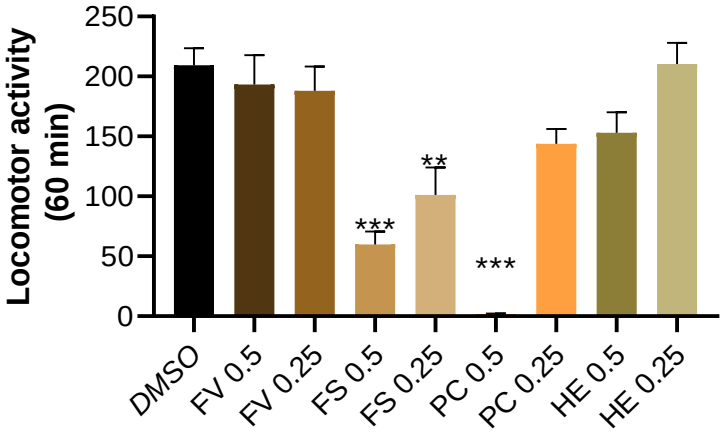


Figure 16 FS and PC extract treatments significantly reduced the motor activity of adult animals. *Pelvetia caniculata* (PC), *Fucus spiralis* (FS), *Fucus vesiculosus* (FV) and *Himanthalia elongata* (HE), locomotor activity of adult N2 animals chronically treated with macroalgae extract at 0.5 and 0.25 mg/mL. Results are present as Mean ± SEM of locomotor activity estimated in Wmicrotracker in one representative assay. Statistical analysis was performed by One-way ANOVA, ** $p<0.01$; *** $p<0.001$.

These results showed that although not affecting the development, survival and reproductivity of *C.elegans*, these concentrations of FS and PC extracts, somehow interfered with the motor activity of adult animals. Additional measures of wellbeing could be evaluated in this model to validate or complement these results. The characterization of the chemical composition of the extracts would also help to understand if this toxicity can be associated with specific compounds present in the extract.

When used between 0.125 and 0.5 mg/mL, the FV extract did not show any sign of toxicity in *C. elegans*. For this reason and taking in consideration the beneficial impact of this extract demonstrated previously in yeast, it was chosen for further studies, in *C. elegans*.

4.6. Protection of glutamatergic neurons by FV extract

A *C. elegans* model of AD was treated with FV extract (0.50 mg/mL) for 7 days. The presence of five glutamatergic neurons located at the tail of the animals was evaluated by fluorescence microscopy. Comparing the percentage of animals with five neurons between the strain control (Figure 17 A) and the strain that has coexpression of Aβ protein and GFP (Figure 17 B) it is possible to conclude that the expression of Aβ protein, significantly reduces the number of animals with 5 normal glutamatergic neurons. However, when treated with the extract the percentage of animals with the total number of neurons increases by about 15 % (Figure 17 C-D).

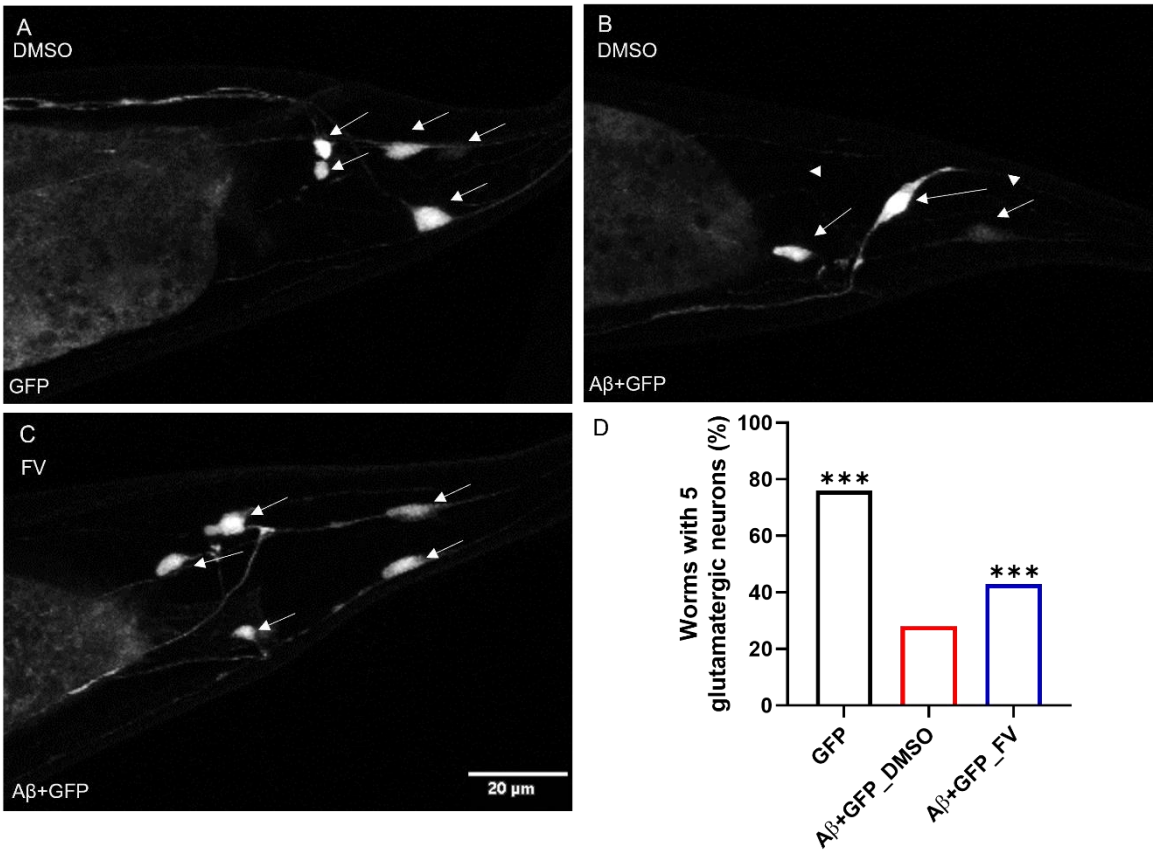


Figure 17 Overexpression of Aβ induces neurodegeneration but is reduced by FV extract. The proportion of animals with intact glutaminergic neurons for each condition was determined by counting the number of animals with WT neurons, in animals expressing GFP (**A**) and the animals that express Aβ+GFP, treated with DMSO (**B**) and FV (**C**). There is no representation of error bars because of the statistical test used, which is a comparison of categorical data (having or not having all WT glutamatergic neurons) (**D**). Statistical analysis was performed with Pearson's chi-square test, ***$p < 0.001$. A total of 53-71 animals were assayed per group across three independent experiments. Arrows represent the glutamatergic neurons, and the arrowhead indicates where the neuron should be (neurodegeneration). Scale bar represents 20 µm.

The information concerning the effects of extracts of this alga in biological models is quite scarce, so we also searched for evidence of neuroprotection in the brown seaweed family.

Previously it was shown that fucoidan isolated from FV influence cholinergic neuronal death induced by $A\beta_{1-42}$, in rats. In this case the hypothesis is that fucoidan pretreatment could block the activation of caspase-9 and caspase-3. These two caspases are directly involved in the apoptosis (Jhamandas et al., 2005). Also, phlorotannins from *Ecklonia* sp. possessed neuroprotection due to protective effects against OS and prevented the $A\beta_{1-42}$ neurotoxicity, in PC12 cells (Shrestha et al., 2022). In another study they demonstrated that *Ishige foliacea* (a brown macroalgae) extract had positive effects in viability of cells against H_2O_2 and Aβ in the human neuroblastoma SH-SY5Y cells and also improved induced memory deficit in rats (Kim et al., 2020). Mixtures of compounds were also tested, in this case, the authors have

mixed phlorotannin and fucoidan extracted from *Ecklonia cava* and they concluded that the mixture have a preventive effect on Aβ-induced cognitive, learning and memory impairment in mice (Han et al., 2021).

4.7. Protection of dopaminergic neurons by FV extract

The same approach was made to evaluate the neuroprotector activity of FV extract in a model of PD. A *C. elegans* model of PD was treated with FV extract (0.50 mg/mL) for 7 days. The presence of six dopaminergic neurons located at the head (CEPs and ADEs) and another two located in the posterior half of the body of the animals (PDEs) was evaluated by fluorescence microscopy (representative localization in Supplementary Figure 1). Comparing the percentage of animals with all neurons of the strain control (Figure 18 A) and the strain that has coexpression of α-synuclein protein and GFP (Figure 18 B) it is possible to conclude that expression of α-synuclein protein, significantly reduces the number of animals with all glutamatergic neurons (Figure 18 D). However, when treated with the extract (Figure 18 C) the percentage of animals with the normal number of neurons increases by about 23 % (Figure 18 D).

In a previous study, Liu et al., 2015 found that a methanolic extract of *Chondrus crispus* (red macroalga) protects *C. elegans* from the 6-hydroxydopamine-mediated dopaminergic neuronal degeneration. The chemical analysis of *C. crispus* shows that floridoside and isethionic acid are the major components of the comprising 7.5% and 9.1%, respectively. They also found taurine and unsaturated fatty acids. They found that the treatment with the extract was able to reduce the amount of α-synuclein. And they further conclude that the decreased α-synuclein accumulation by *C. crispus* extract supplementation was associated with enhanced tolerance of OS, but not heat stress.

Analyzing the chemical composition of *F. vesiculosus*, collected in the Galician coast, it was possible to conclude that, it is very rich in PUFAs, amino acids and minerals (Lorenzo et al., 2017). In this regard the administration of omega-3 PUFA in PD rats showed potent antioxidant, neuroprotective, and anti-inflammatory effects (Mori et al., 2018). This is another clue as to what kind of compounds may be triggering a cellular response.

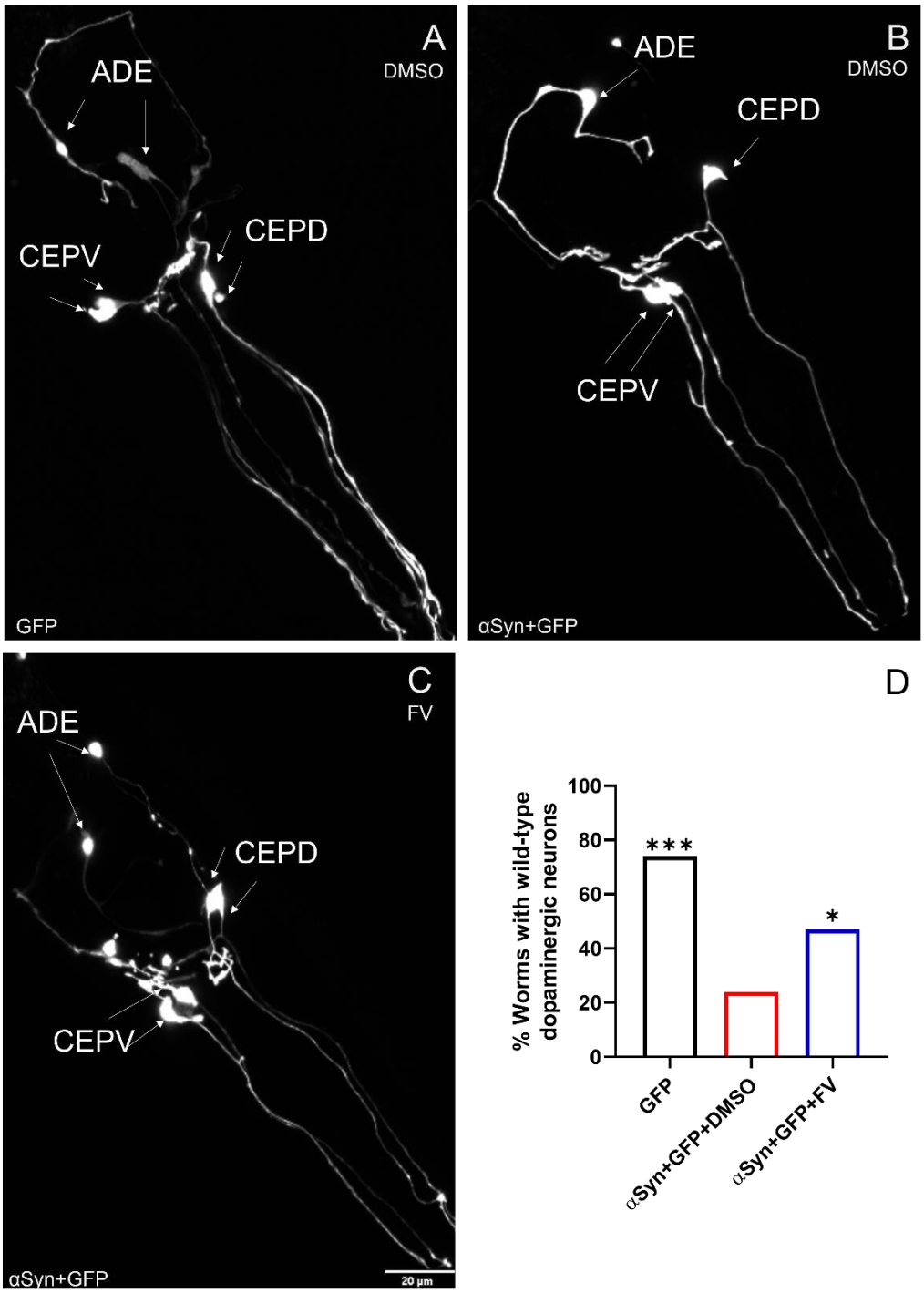


Figure 18 Overexpression of α-synuclein induces neurodegeneration but is reduced by the treatment with FV extract. The proportion of animals with intact dopaminergic neurons for each condition was determined by counting the number of animals with six dopaminergic neurons present in the head (CEPs and ADEs), and two PDEs (present in posterior half of the body-not shown) in animals expressing GFP (**A**) and the animals that express α-syn+GFP, treated with DMSO (**B**) and FV (**C**). There is no representation of error bars because of the statistical test used, which is a comparison of categorical data (having or not having all WT dopaminergic neurons) (**D**). Statistical analysis was performed with Pearson's chi-square test, ***$p<0.001$, $p<0.033$. A total of 34-44 animals were assayed per group across three independent experiments. Scale bar represents 20 µm.

4.8. FV extract treatment show a promising motor function of the FTD-17 model

The impact of a chronic treatment with distinct concentrations of FV extract was studied in a *C. elegans* model of FTDP-17 (CK10 strain). These animals express a mutant *tau* protein (V337M) in the entire nervous system, presenting motor dysfunction. The effect of the extract treatment towards the motor phenotype of these animals was evaluated using the WMicrotracker. At day 4 after hatching, *C. elegans* WT (N2) animals, that do not display any impairment in locomotor activity, presented activity counts, in the Wmicrotracker, of around 1600-2000 after 150 min. On the other hand, mutant *tau*-expressing animals (CK10 model) presented the expected significant motor defects, scoring around 600-800 counts after 150 min. The chronic treatment with algae extracts did not cause statistically significant differences in the motor dysfunction of the CK10 animals, however they improved the motor activity of animals by 22 % (Figure 19). Based on previous studies, in this model despite not having significant differences, we can conclude that this improvement can have biological relevance (dashed line on graph) (Fernandes, 2021).

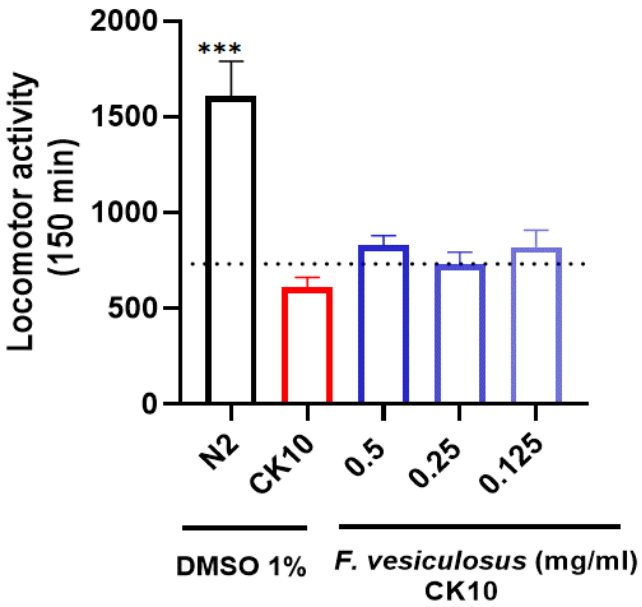


Figure 19 FV extract does not improve locomotor activity of the FTD-17. Motility assays using the WMicrotracker, were performed using N2 (WT) treated just with DMSO 1% and CK10 (FTDP-17 model) strains treated with 1% DMSO (drug vehicle) in comparison with CK10 treated with FV extract (0.5-0.125 mg/mL). Results are present as Mean ± SEM of locomotor activity estimated in Wmicrotracker in three independent replicates. Statistical analysis was performed with One-way ANOVA, *** $p<0.001$. Dashed line indicates 20 % of improvement, compared to strain CK10 treated with DMSO 1%.

It is important to refer that other motor behavioral tests could be used to and to complement Wmicrotracker results. While the WMicroTracker measured the movement of the animal in liquid medium,

there are motility assays that evaluate the movement of the animal as they crawl in solid NGM medium. Using other ways of motor function evaluation or measuring the impact of treatment towards other disease-related phenotypes could help determine with more certainty the impact of FV extract treatment in this model.

4.9. Transcriptional and translational activation of cellular responses with FV treatment

To understand what pathways are behind the neuroprotective activity of FV extract, *C. elegans* transcriptional reporter strains of diverse cellular stress responses were used. In these strains, fluorescent proteins, such as GFP, are expressed under the regulation of specific gene promoters, enabling to assess the levels of transcriptional induction of those genes. It is particularly informative when these genes are markers of diverse cellular adaptative responses, such as oxidative or UPRs.

4.9.1. Antioxidant response

In this way were analyzed the expression levels of two genes, which measure the induction of transcription of genes encoding detoxification enzymes, namely the expression of γ-glutamyl cysteine synthetase-1 (GCS-1) and glutathione transferase-4 (GST-4). Glutathione S-transferases (GSTs) are cellular detoxification enzymes. This family of multifunctional enzymes has the ability to respond to exogenously and endogenously derived toxic compounds. The gst-4 and gcs-1 gene are a target of the SKN-1 transcription factor. In the case of gst-4 gene, encodes for the enzyme glutathione-S-transferase 4, involved in the metabolism of glutathione. In the case of gcs-1 gene encodes: γ-glutamyl cysteine synthetase-1 (Pohl et al., 2019).

The impact of FV treatment on the modulation of endogenous antioxidant pathways in *C.elegans* was evaluated using- transcriptional reporter strains for γ-glutamyl cysteine synthetase-1 (gcs-1) and glutathione-s-transferase-4 (gst-4). These animals were chronically treated with FV and acutely treated with juglone, a ROS inducer that was used as positive control in this experiment.

The fluorescence conditions (exposure time and gain) used for the positive control and for the animals treated with the extract were defined in the untreated condition (Figure 20 A;B). We observed as expected an activation of the response mediated by the juglone (Figure 20 C;D), but FV treatment was not able to induce significantly the expression of *gst-4* (Figure 20 E;F).

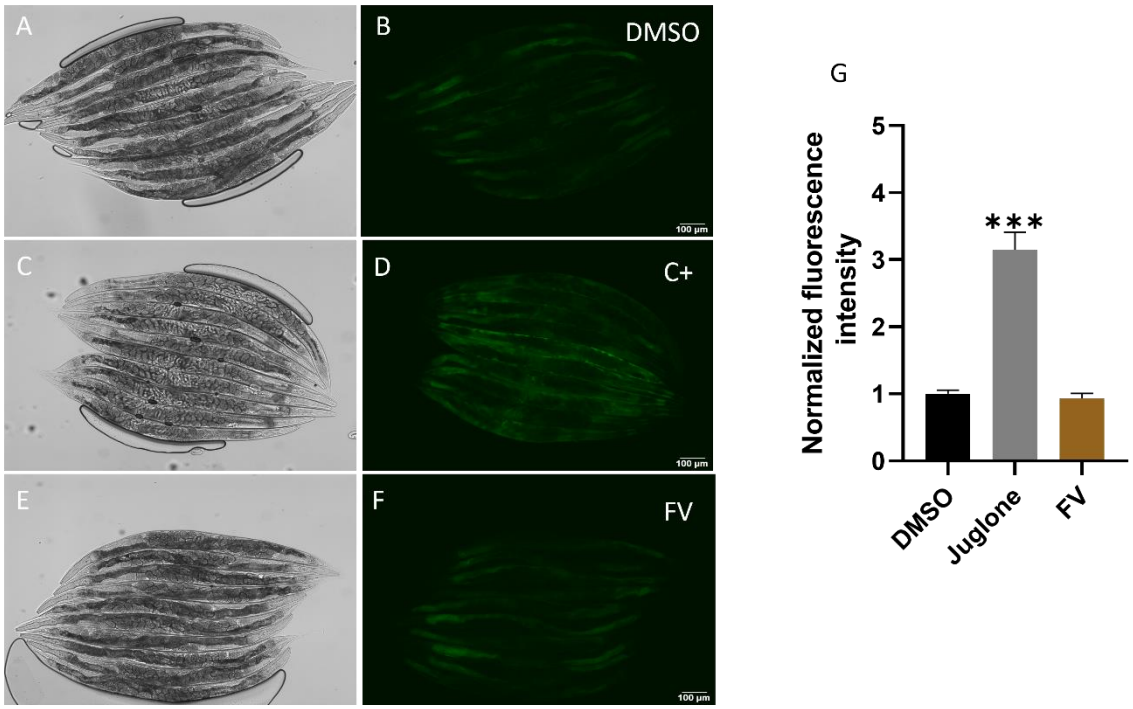


Figure 20 Impact of FV treatment in the CL2166 reporter strain (*gst-4*:GFP). Representative microphotographs of a strain expressing gst-4 promoter (A-F). Brightfield and GFP fluorescence images were acquired in animals (**A-B**) treated with vehicle (DMSO 1%) for 4 days, (**C-D**) grown in vehicle, subjected to juglone for 1 hour and allowed to recover, (**E-F**) treated with FV 0.5 mg/m. Normalized representation of GFP fluorescence intensity (**G**). Juglone used as positive control. Results are present as Mean ± SEM of normalized fluorescence intensity of three independent replicates). Statistical analysis was performed with One-way ANOVA; *** p<0.001; n=30.

Pohl et al., 2019, prove that the neuroprotection in PD and Machado-Joseph disease models was also associated with the activation of the *gst-4* gene. However, they don't exclude that additional pathways are involved such as SOD-3, other GSTs and other pathways.

The activation of the *gcs-1* gene, which encodes for the γ-glutamyl cysteine synthetase enzyme, involved in glutathione synthesis, was also analyzed. This gene is also a target of the SKN-1 transcription factor. The fluorescence conditions (exposure time and gain) used for the positive control and for the animals treated with the extract were defined in the untreated condition (Figure 21 A;B). In the case of gcs-1 reporter strain we observed as expected an activation of the response mediated by the juglone (Figure 21 C;D), but FV treatment was not able to induce significantly the expression of *gcs-1*, however a weak activation was noticed (Figure 21 E;F).

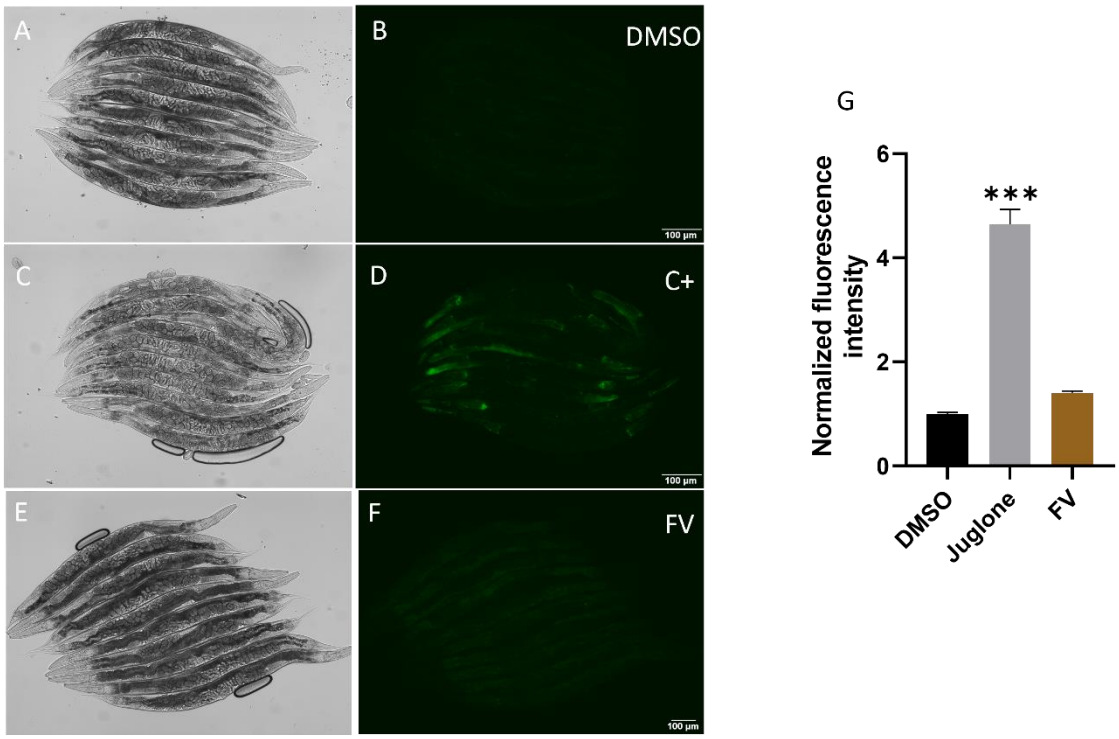


Figure 21 Impact of FV treatment in the LD1171 reporter strain (*gcs-1*::GFP transcription. Representative microphotographs of a strain expressing GFP under the regulation of gcs-1 promoter (**A-F**). Brightfield and GFP fluorescence images were acquired in animals (A and B) treated with vehicle (DMSO 1%) for 4 days, (**C-D**) grown in vehicle, subjected to juglone for 1 hour and allowed to recover, (**E-F**) treated with FV 0.5 mg/mL over 4 days. Normalized representation of GFP fluorescence intensity (**G**). Juglone used as positive control. Results are present as Mean ± SEM of normalized fluorescence intensity of three independent replicates. Statistical analysis was performed with Kruskal-Wallis test; *** p<0.001; n=30.

Interestingly even though the extract exerts an antiradical ability, it does not seem to activate significantly these two antioxidant pathways in *C. elegans*. Perhaps, the activation of these reporter strains could be also measured after causing an oxidative insult (with juglone for instance). Other antioxidant enzymes could be used to understand further the extract antioxidant capacity (such as the superoxide dismutases, catalase, thioredoxins, glutaredoxins or peroxiredoxins). The precise amount of these enzymes could be evaluated by qPCR. Liu et al. (2015) showed that in wild type worms, the oxidative response gene, *sod-3*, showed a 15-fold up-regulation in the *Chondrus crispus* methanolic extract treated group, compared to the control, using a qPCR assay. In *C. elegans* the total amount of intracellular ROS can be quantified after reaction with specific dyes, such as 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA). This dye is particularly interesting, because it is sensitive to H_2O_2, hydroxyl radicals (•HO) and Peroxyl radicals (ROO•;Ayuda-Durán et al., 2020). Perhaps using these additional methodologies, the antioxidant capacity of this extract could be more studied in this biological model.

4.9.2. Unfolded protein response

The potential activation of the UPR^{ER} by FV treatment was also investigated using the strain SJ4005. These animals express GFP under the regulation of the *hsp-4* gene promoter, that encodes for an ERF stress-responsive chaperone.

The fluorescence conditions (exposure time and gain) used for the positive control and for the animals treated with the extract were defined in the untreated condition (Figure 22 A;B). We observed as expected an activation of the response mediated by the tunicamycin (Figure 22 C;D), but FV treatment was not able to induce the expression of *hsp-4* (Figure 22 E;F;G).

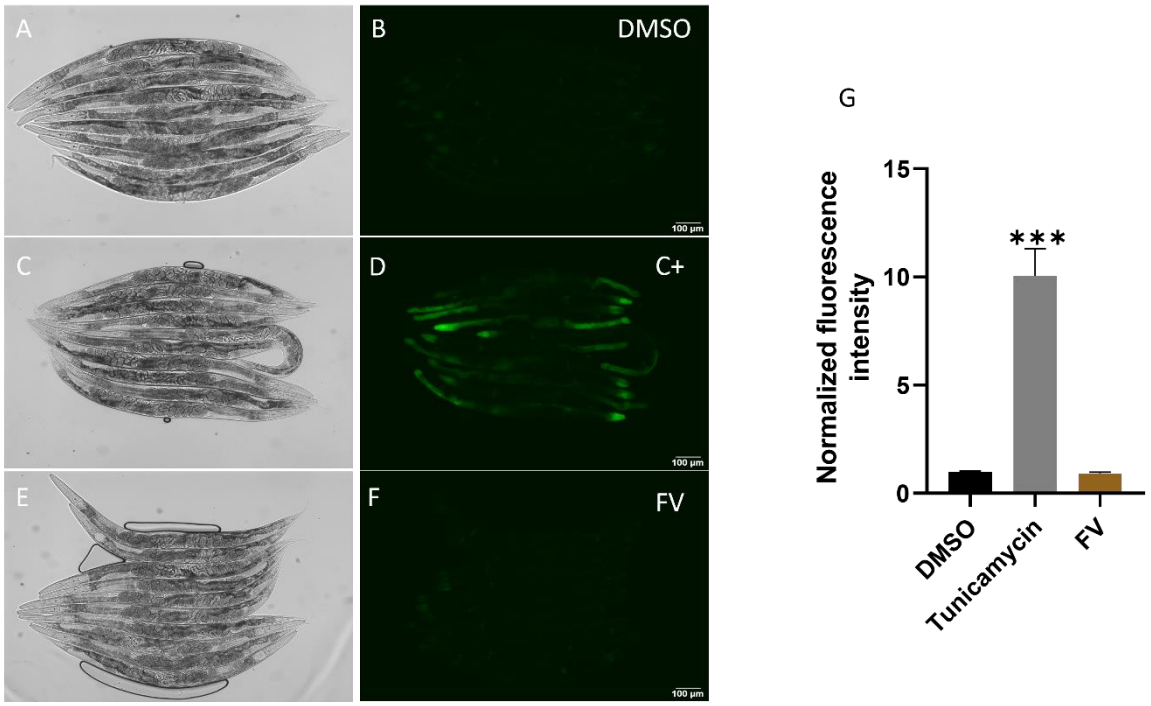


Figure 22 Impact of FV treatment in the SJ4005 reporter strain (*hsp-4*::GFP) transcription. Representative microphotographs of a strain expressing GFP under the regulation of hsp-4 promoter (**A-F**). Brightfield and GFP fluorescence images were acquired in animals (**A-B**) treated with vehicle (DMSO 1%) for 4 days, (**C-D**) grown in vehicle, subjected to tunicamycin for 16 hours (**E-F**) treated with FV 0.5 mg/mL over 4 days. Normalized representation of GFP fluorescence intensity (**G**). Tunicamycin was used as positive control. Results are present as Mean ± SEM of normalized fluorescence intensity of three independent replicates. Statistical analysis was performed with One-way ANOVA; ***p<0.001; n=30.

The potential activation of the mitochondrial stress response UPR^{MT} by FV was also investigated and to evaluate the activation of this pathway, we use the SJ4100 strain, expressing GFP under the regulation of the *hsp-6* gene promoter, that encodes for a mitochondrial stress-responsive chaperone (Figure 23 G). The fluorescence conditions (exposure time and gain) used for the positive control and for the animals treated with the extract were defined in the untreated condition (Figure 23 A;B). We observed as expected an activation of the response mediated by the antimycin A (Figure 23 C;D), and FV treatment

was able to induce the expression of *hsp-6* increasing the fluorescence by nearly 20 % when compared with the control condition (Figure 23 E;F).

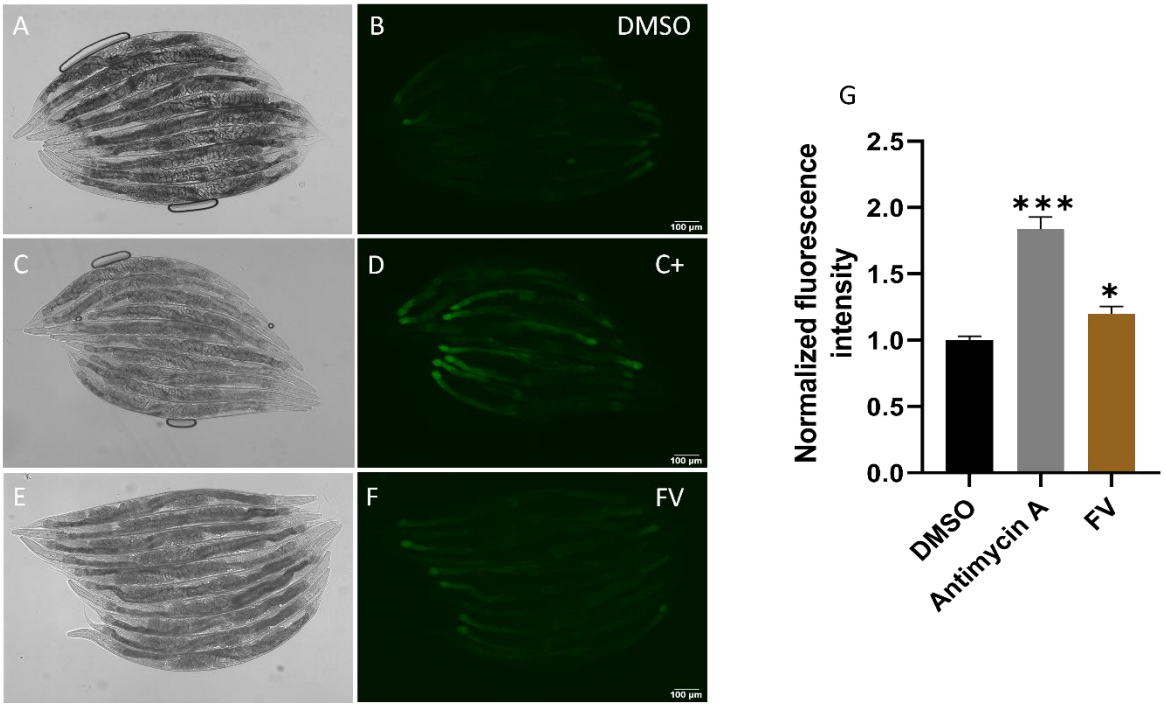


Figure 23 Impact of FV treatment in the SJ4100 reporter strain (*hsp-6*::GFP) transcription. Representative microphotographs of a strain expressing GFP under the regulation of hsp-6 promoter (**A-F**). Brightfield and GFP fluorescence images were acquired in animals (A and B) treated with vehicle (DMSO 1%) for 4 days, (**C-D**) grown in vehicle, subjected to antimycin A for 24 hours (**E-F**) treated with FV 0.5 mg/mL over 4 days. Normalized representation of GFP fluorescence intensity (**G**). Antimycin A was used as positive control. Results are present as Mean ± SEM of normalized fluorescence intensity of three independent replicates. Statistical analysis was performed with Kruskal-Wallis test, * $p<0.05$; *** $p<0.001$; n=30.

Accordingly with data, it is possible to conclude that the extract can induce significantly the expression of hsp-6, a marker of the UPR^{MT}. However, to have more evidence that this is the pathway induced by the extract, other reporter strains for the same mitochondrial response such as hsp-60 or atsf-1, could be used.When treated with FV extract, the cells acquired significant resistance to heat stress, possibly by recruiting chaperones, that contribute to maintain the cellular proteostasis. In fact, using *C. elegans* reporters the activation of a chaperone was demonstrated. It would be very interesting to understand how other heat stress- responsive strains, such as reporters for hsp-70 or hsp-90, respond to the extract. Moreover, thermotolerance and thermorecovery tests could also performed in *C. elegans*.

Autophagy would also be another pathway of interest since it is linked to the destruction of proteins in the cell. In this case an induction of this pathway could have a neuroprotective response, by the degradation of proteins such as Aβ or α-synuclein. Long et al., 2022 have shown, that ferulic acid can activate the autophagy in *C. elegans* by the activation of *lgg-1*, *vps-34* and *unc-51* genes (quantified

mRNA expression by qRT-PCR), reducing the amount of α-synuclein and the preservation of dopaminergic neurons.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

This study showed, for the first time in two biological systems, the potential of macroalgae treatments in the context of neurodegenerative diseases. A set of extracts with confirmed antiradical activity, increased the resistance of yeast cells to heat and showed anti-aggregation properties when used to treat a yeast model of PD. Additional studies in *C. elegans*, particularly focused on the impact of FV extract, prevented the neurodegeneration of dopaminergic and glutamatergic neurons in neuronal models of PD and AD, respectively and showed promising effects in the improvement of the motor function of a FTDP-17 *C. elegans* model. In these animals, it was also demonstrated the ability of the FV chronic treatment to significantly activate the UPR^{MT}, inducing the expression of its marker chaperone hsp-6. This result suggested that FV extract is able to modulate a specific branch of the proteostasis network, the cellular responses that monitor protein quality since their synthesis through their degradation, preventing protein misfolding and aggregation.

Since a proteostasis imbalance is associated with several ND, FV extract treatment can be effective for distinct diseases, as it is demonstrated in this study. The induction of chaperones can protect against the effect of misfolded proteins, preventing their aggregation and consequently reducing the associated toxicity. This can be corroborated with data found in *S. cerevisiae* relating anti-aggregation activity and thermotolerance, and lately in *C. elegans* model, having neuroprotection in distinct disease models. The majority of compounds present in the brown macroalgae group are fucoxanthin, PUFAS, and phlorotannins (Garcia-Perez et al., 2022). Without chemical analysis of this extract, we cannot conclude what molecule (or several molecules) are directly involved in neuroprotection. Therefore, the detailed characterization of the chemical composition of the seaweed extracts, particularly of FV, will be obtained in the future. It can be hypothesized, that fucoxanthin may be a key molecule behind the neuroprotection of these extracts. Studies with this molecule in a purified form should be performed in these and other superior models. Once confirmed its bioactivity, fucoxanthin could be used to assay different forms of administration like microencapsulation to increase bioavailability. Other alternatives could be to use this molecule in food supplements.

This study also raises awareness about the sustainable exploitation and consumption of algae. The use of algae for human consumption would provide health and nutritional benefits since they are rich in

fibers, minerals, proteins, PUFAs and poor in saturated lipids. Moreover, as demonstrated in this study, they can also be potential therapies for distinct neurodegenerative diseases.

This study demonstrated that ocean resources can be explored as a source of new bioactive agents that can be effective for the treatment of several neurodegenerative diseases that still remain without any disease-modifying therapy.

6. BIBLIOGRAPHY

Abdul, Q. A., Choi, R. J., Jung, H. A., & Choi, J. S. (2016). Health benefit of fucosterol from marine algae: A review. In *Journal of the Science of Food and Agriculture* (Vol. 96, Issue 6, pp. 1856–1866). John Wiley and Sons Ltd. https://doi.org/10.1002/jsfa.7489

Agnihotri, A., & Aruoma, O. I. (2020). Alzheimer's Disease and Parkinson's Disease: A Nutritional Toxicology Perspective of the Impact of Oxidative Stress, Mitochondrial Dysfunction, Nutrigenomics and Environmental Chemicals. *Journal of the American College of Nutrition*, *39*(1), 16–27. https://doi.org/10.1080/07315724.2019.1683379

Ahmad, S., Akhtar, S., Jamal, Q., Rizvi, S., Kamal, M., Khan, M., & Siddiqui, Mohd. (2016). Multiple Targets for the Management of Alzheimer's Disease. *CNS & Neurological Disorders - Drug Targets*, *15*(10), 1279–1289. https://doi.org/10.2174/1871527315666161003165855

Ahmed, T., Javed, S., Javed, S., Tariq, A., Šamec, D., Tejada, S., Nabavi, S. F., Braidy, N., & Nabavi, S. M. (2017). Resveratrol and Alzheimer's Disease: Mechanistic Insights. *Molecular Neurobiology*, *54*(4), 2622–2635. https://doi.org/10.1007/s12035-016-9839-9

Apfeld, J., & Alper, S. (2018). What can we learn about human disease from the nematode *C. elegans*? In *Methods in Molecular Biology* (Vol. 1706, pp. 53–75). Humana Press Inc. https://doi.org/10.1007/978-1-4939-7471-9_4

Armstrong, R. (2020). What causes neurodegenerative disease? *Folia Neuropathologica*, *58*(2), 93–112. https://doi.org/10.5114/FN.2020.96707

Ayuda-Durán, B., González-Manzano, S., González-Paramás, A. M., & Santos-Buelga, C. (2020). *Caernohabditis elegans* as a Model Organism to Evaluate the Antioxidant Effects of Phytochemicals. *Molecules*, *25*(14). https://doi.org/10.3390/molecules25143194

Balina, K., Romagnoli, F., & Blumberga, D. (2016). Chemical Composition and Potential Use of *Fucus vesiculosus* from Gulf of Riga. *Energy Procedia*, *95*, 43–49. https://doi.org/10.1016/j.egypro.2016.09.010

Barbalace, M. C., Malaguti, M., Giusti, L., Lucacchini, A., Hrelia, S., & Angeloni, C. (2019). Anti-inflammatory activities of marine algae in neurodegenerative diseases. In *International Journal of Molecular Sciences* (Vol. 20, Issue 12). MDPI AG. https://doi.org/10.3390/ijms20123061

Barbosa, M., Valentão, P., & Andrade, P. B. (2014). Bioactive compounds from macroalgae in the new millennium: Implications for neurodegenerative diseases. In *Marine Drugs* (Vol. 12, Issue 9, pp. 4934–4972). MDPI AG. https://doi.org/10.3390/md12094934

Bayliak, M. M., Burdyliuk, N. I., Izers'ka, L. I., & Lushchak, V. I. (2014). Concentration-dependent effects of *Rhodiola rosea* on longterm survival and stress resistance of yeast *Saccharomyces cerevisiae*: The involvement of Yap 1 and Msn2/4 regulatory proteins. *Dose-Response*, *12*(1), 93–109. https://doi.org/10.2203/dose-response.13-013.Bayliak

Burré, J., Sharma, M., & Südhof, T. C. (2018). Cell biology and pathophysiology of α-synuclein. *Cold Spring Harbor Perspectives in Medicine*, *8*(3), 1–28. https://doi.org/10.1101/cshperspect.a024091

Caldwell, K. A., Willicott, C. W., & Caldwell, G. A. (2020). Modeling neurodegeneration in *Caenorhabditis elegans*. *DMM Disease Models and Mechanisms*, *13*(10). https://doi.org/10.1242/dmm.046110

Chalorak, P., Jattujan, P., Nobsathian, S., Poomtong, T., Sobhon, P., & Meemon, K. (2018). Holothuria scabra extracts exhibit anti-Parkinson potential in *C. elegans*: A model for anti-Parkinson testing. *Nutritional Neuroscience*, *21*(6), 427–438. https://doi.org/10.1080/1028415X.2017.1299437

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 423 Anticholinesterase Activity of Plastoquinones from *Sargassum sagamianum*: Lead Compounds for Alzheimer's Disease Therapy. *Phytother. Res*, *21*, 423–426. https://doi.org/10.1002/ptr

Chopade, P., Chopade, N., Zhao, Z., Mitragotri, S., Liao, R., & Chandran Suja, V. (2022). Alzheimer's and Parkinson's disease therapies in the clinic. In *Bioengineering and Translational Medicine*. John Wiley and Sons Inc. https://doi.org/10.1002/btm2.10367

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

Dhakal, S., Subhan, M., Fraser, J. M., Gardiner, K., & Macreadie, I. (2019). Simvastatin Efficiently Reduces Levels of Alzheimer's Amyloid Beta in Yeast. *International Journal of Molecular Sciences*, *20*(14). https://doi.org/10.3390/ijms20143531

Dostal, V., Roberts, C. M., & Link, C. D. (2010). Genetic mechanisms of coffee extract protection in a *Caenorhabditis elegans* model of β-amyloid peptide toxicity. *Genetics*, *186*(3), 857–866. https://doi.org/10.1534/genetics.110.120436.

Fernandes, E (2021). Study of the neuroprotective activity of medicinal plants in *Caenorhabditis elegans* models of Neurodegenerative Diseases.

Fields, C. R., Bengoa-Vergniory, N., & Wade-Martins, R. (2019). Targeting Alpha-Synuclein as a Therapy for Parkinson's Disease. In *Frontiers in Molecular Neuroscience* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fnmol.2019.00299

Garcia-Perez, P., Lourenço-Lopes, C., Silva, A., Pereira, A. G., Fraga-Corral, M., Zhao, C., Xiao, J., Simal-Gandara, J., & Prieto, M. A. (2022). Pigment Composition of Nine Brown Algae from the Iberian Northwestern Coastline: Influence of the Extraction Solvent. *Marine Drugs*, *20*(2). https://doi.org/10.3390/md20020113

Goffeau, A., Barrell, G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., Murakami, Y., Philippsen, P., Tettelin, H., & Oliver, S. G. (1996). Life with 6000 genes. *Science*, *274*(5287), 546–567. https://doi.org/10.1126/science.274.5287.546

Han, H. J., Park, S. K., Kang, J. Y., Kim, J. M., Yoo, S. K., Kim, D. O., Kim, G. H., & Heo, H. J. (2021). Mixture of phlorotannin and fucoidan from *Ecklonia cava* prevents the aβ-induced cognitive decline with mitochondrial and cholinergic activation. *Marine Drugs*, *19*(8). https://doi.org/10.3390/md19080434

Han, W., Ji, T., Mei, B., & Su, J. (2011). Peptide p3 may play a neuroprotective role in the brain. *Medical Hypotheses*, *76*(4), 543–546. https://doi.org/10.1016/j.mehy.2010.12.013

Hetz, C. (2012). The unfolded protein response: Controlling cell fate decisions under ER stress and beyond. In *Nature Reviews Molecular Cell Biology* (Vol. 13, Issue 2, pp. 89–102). https://doi.org/10.1038/nrm3270

Higuchi-Sanabria, R., Frankino, P. A., Paul, J. W., Tronnes, S. U., & Dillin, A. (2018). A Futile Battle? Protein Quality Control and the Stress of Aging. In *Developmental Cell* (Vol. 44, Issue 2, pp. 139–163). Cell Press. https://doi.org/10.1016/j.devcel.2017.12.020

Jhamandas, J. H., Wie, M. B., Harris, K., MacTavish, D., & Kar, S. (2005). Fucoidan inhibits cellular and neurotoxic effects of β-amyloid (Aβ) in rat cholinergic basal forebrain neurons. *European Journal of Neuroscience*, *21*(10), 2649–2659. https://doi.org/10.1111/j.1460-9568.2005.04111.x

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/10.1016/j.fct.2016.01.014

Kim, G. H., Kim, J. E., Rhie, S. J., & Yoon, S. (2015). The Role of Oxidative Stress in Neurodegenerative Diseases. In *Experimental Neurobiology* (Vol. 24, Issue 4, pp. 325–340). Korean Society for Neurodegenerative Disease. https://doi.org/10.5607/en.2015.24.4.325

Kim, T. E., Son, H. J., Lim, D. W., Yoon, M., Lee, J., Kim, Y. T., Han, D., Lee, C., & Um, M. Y. (2020). Memory-enhancing effects of Ishige foliacea extract: In vitro and in vivo study. *Journal of Food Biochemistry*, *44*(4). https://doi.org/10.1111/jfbc.13162

Kuhn, A. J., Abrams, B. S., Knowlton, S., & Raskatov, J. A. (2020). Alzheimer's Disease "non-amyloidogenic" p3 Peptide Revisited: A Case for Amyloid-α. *ACS Chemical Neuroscience*, *11*(11), 1539–1544. https://doi.org/10.1021/acschemneuro.0c00160

Labbadia, J., & Morimoto, R. I. (2015). Repression of the Heat Shock Response Is a Programmed Event at the Onset of Reproduction. *Molecular Cell*, *59*(4), 639–650. https://doi.org/10.1016/j.molcel.2015.06.027

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 α-synuclein toxicity identified using yeast. *Human Molecular Genetics*, *17*(23), 3784–3795. https://doi.org/10.1093/hmg/ddn276

Liu, F., Wang, H., Zhu, X., Jiang, N., Pan, F., Song, C., Yu, C., Yu, C., Qin, Y., Hui, J., Li, S., Xiao, Y., & Liu, Y. (2022). Sanguinarine promotes healthspan and innate immunity through a conserved mechanism of ROS-mediated PMK-1/SKN-1 activation. *IScience*, *25*(3). https://doi.org/10.1016/j.isci.2022.103874

Liu, J., Banskota, A. H., Critchley, A. T., Hafting, J., & Prithiviraj, B. (2015). Neuroprotective effects of the cultivated *Chondrus crispus* in a *C. elegans* model of Parkinson's disease. *Marine Drugs*, *13*(4), 2250–2266. https://doi.org/10.3390/md13042250

Liu, Z., Zhou, T., Ziegler, A. C., Dimitrion, P., & Zuo, L. (2017). Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. In *Oxidative Medicine and Cellular Longevity* (Vol. 2017). Hindawi Limited. https://doi.org/10.1155/2017/2525967

Long, T., Wu, Q., Wei, J., Tang, Y., He, Y. N., He, C. L., Chen, X., Yu, L., Yu, C. L., Law, B. Y. K., Wu, J. M., Qin, D. L., Wu, A. G., & Zhou, X. G. (2022). Ferulic Acid Exerts Neuroprotective Effects via Autophagy

Induction in *C. elegans* and Cellular Models of Parkinson's Disease. *Oxidative Medicine and Cellular Longevity*, *2022*. https://doi.org/10.1155/2022/3723567

Lorenzo, J. M., Agregán, R., Munekata, P. E. S., Franco, D., Carballo, J., Şahin, S., Lacomba, R., & Barba, F. J. (2017). Proximate composition and nutritional value of three macroalgae: *Ascophyllum nodosum*, *Fucus vesiculosus* and *Bifurcaria bifurcata*. *Marine Drugs*, *15*(11). https://doi.org/10.3390/md15110360

Macoi,2008. Portugues seaweeds website, Universidade de Coimbra. http://macoi.ci.uc.pt/default.php . Accessed 1 October of 2022.

Manjula, R., Anuja, K., & Alcain, F. J. (2021). SIRT1 and SIRT2 Activity Control in Neurodegenerative Diseases. *Frontiers in Pharmacology*, *11*(January), 1–26. https://doi.org/10.3389/fphar.2020.585821

Martinez, B. A., Kim, H., Ray, A., Caldwell, G. A., & Caldwell, K. A. (2015). A bacterial metabolite induces glutathione-tractable proteostatic damage, proteasomal disturbances, and PINK1-dependent autophagy in *C. elegans*. *Cell Death and Disease*, *6*(10). https://doi.org/10.1038/cddis.2015.270

Mehra, S., Sahay, S., & Maji, S. K. (2019). α-Synuclein misfolding and aggregation: Implications in Parkinson's disease pathogenesis. In *Biochimica et Biophysica Acta - Proteins and Proteomics* (Vol. 1867, Issue 10, pp. 890–908). Elsevier B.V. https://doi.org/10.1016/j.bbapap.2019.03.001

Melber, A., & Haynes, C. M. (2018). UPR mt regulation and output: A stress response mediated by mitochondrial-nuclear communication. In *Cell Research* (Vol. 28, Issue 3, pp. 281–295). Nature Publishing Group. https://doi.org/10.1038/cr.2018.16

Min, S. W., Sohn, P. D., Cho, S. H., Swanson, R. A., & Gan, L. (2013). Sirtuins in neurodegenerative diseases: An update on potential mechanisms. *Frontiers in Aging Neuroscience*, *5*(SEP). https://doi.org/10.3389/fnagi.2013.00053

Mishan, M. A., Kanavi, M. R., Shahpasand, K., & Ahmadieh, H. (2019). Pathogenic tau protein species: Promising therapeutic targets for ocular neurodegenerative diseases. *Journal of Ophthalmic and Vision Research*, *14*(4), 491–505. https://doi.org/10.18502/jovr.v14i4.5459

Mohammadi, S., Saberidokht, B., Subramaniam, S., & Grama, A. (2015). Scope and limitations of yeast as a model organism for studying human tissue-specific pathways. *BMC Systems Biology*, *9*(1). https://doi.org/10.1186/s12918-015-0253-0

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

Mori, M. A., Delattre, A. M., Carabelli, B., Pudell, C., Bortolanza, M., Staziaki, P. V., Visentainer, J. V., Montanher, P. F., del Bel, E. A., & Ferraz, A. C. (2018). Neuroprotective effect of omega-3 polyunsaturated fatty acids in the 6-OHDA model of Parkinson's disease is mediated by a reduction of inducible nitric oxide synthase. *Nutritional Neuroscience*, *21*(5), 341–351. https://doi.org/10.1080/1028415X.2017.1290928

Muñoz-Carvajal, F., & Sanhueza, M. (2020). The Mitochondrial Unfolded Protein Response: A Hinge Between Healthy and Pathological Aging. In *Frontiers in Aging Neuroscience* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fnagi.2020.581849

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/10.1016/S0014-5793(96)01336-1

Obluchinskaya, E. D., Pozharitskaya, O. N., Zakharov, D. v., Flisyuk, E. v., Terninko, I. I., Generalova, Y. E., Smekhova, I. E., & Shikov, A. N. (2022). The Biochemical Composition and Antioxidant Properties of *Fucus vesiculosus* from the Arctic Region. *Marine Drugs*, *20*(3). https://doi.org/10.3390/md20030193

Olasehinde, T. A., Olaniran, A. O., & Okoh, A. I. (2019). Aqueous–ethanol extracts of some South African seaweeds inhibit beta-amyloid aggregation, cholinesterases, and beta-secretase activities in vitro. *Journal of Food Biochemistry*, *43*(7). https://doi.org/10.1111/jfbc.12870

ONU. (2021). *United Nations Decade of Ocean Science for Sustainable Development Implementation Plan*. http://www.unesco.org/open-access/terms-use-ccbysa-en

O'Reilly, L. P., Luke, C. J., Perlmutter, D. H., Silverman, G. A., & Pak, S. C. (2014). C. elegans in high-throughput drug discovery. In *Advanced Drug Delivery Reviews* (Vols. 69–70, pp. 247–253). Elsevier. https://doi.org/10.1016/j.addr.2013.12.001

Paiva, L., Lima, E., Neto, A. I., & Baptista, J. (2018). Seasonal variability of the biochemical composition and antioxidant properties of *Fucus spiralis* at two Azorean Islands. *Marine Drugs*, *16*(8). https://doi.org/10.3390/md16080248

Pereira, L., & Valado, A. (2021). The Seaweed Diet in Prevention and Treatment of the Neurodegenerative Diseases. In *Marine drugs* (Vol. 19, Issue 3). NLM (Medline). https://doi.org/10.3390/md19030128

Pohanka. (2018). Oxidative stress in Alzheimer disease as a target for therapy. *Bratislava Medical Journal*, *119*(9), 535–543. https://doi.org/10.4149/BLL_2018_097

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*. https://doi.org/10.3389/fnmol.2021.659926

Rahman, M. A., Rahman, M. S., Rahman, M. H., Rasheduzzaman, M., Mamun-Or-rashid, A. N. M., Uddin, M. J., Rahman, M. R., Hwang, H., Pang, M. G., & Rhim, H. (2021). Modulatory effects of autophagy on app processing as a potential treatment target for Alzheimer's disease. In *Biomedicines* (Vol. 9, Issue 1, pp. 1–20). MDPI AG. https://doi.org/10.3390/biomedicines9010005

Rege, S. D., Geetha, T., Griffin, G. D., Broderick, T. L., & Babu, J. R. (2014). Neuroprotective effects of resveratrol in Alzheimer disease pathology. *Frontiers in Aging Neuroscience*, *6*(AUG), 1–27. https://doi.org/10.3389/fnagi.2014.00218

Rodrigues, D., Freitas, A. C., Pereira, L., Rocha-Santos, T. A. P., Vasconcelos, M. W., Roriz, M., Rodríguez-Alcalá, L. M., Gomes, A. M. P., & Duarte, A. C. (2015). Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal. *Food Chemistry*, *183*, 197–207. https://doi.org/10.1016/j.foodchem.2015.03.057

Ross, C. A., & Poirier, M. A. (2004). Protein aggregation and neurodegenerative disease. *Nature Medicine*, *10*(7), S10. https://doi.org/10.1038/nm1066

Sabeena Farvin, K. H., & Jacobsen, C. (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chemistry*, *138*(2–3), 1670–1681. https://doi.org/10.1016/j.foodchem.2012.10.078

Salahuddin, P., Fatima, M. T., Uversky, V. N., Khan, R. H., Islam, Z., & Furkan, M. (2021). The role of amyloids in Alzheimer's and Parkinson's diseases. In *International Journal of Biological Macromolecules* (Vol. 190, pp. 44–55). Elsevier B.V. https://doi.org/10.1016/j.ijbiomac.2021.08.197

Shrestha, S., Choi, J. S., Zhang, W., & Smid, S. D. (2022). Neuroprotective activity of macroalgal fucofuroeckols against amyloid β peptide-induced cell death and oxidative stress. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.15753

Silva, B. A., Ferreres, F., Malva, J. O., & Dias, A. C. P. (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chemistry*, *90*(1–2), 157–167. https://doi.org/10.1016/j.foodchem.2004.03.049

Silva, J., Alves, C., Freitas, R., Martins, A., Pinteus, S., Ribeiro, J., Gaspar, H., Alfonso, A., & Pedrosa, R. (2019). Antioxidant and neuroprotective potential of the brown seaweed bifurcaria bifurcata in an in vitro Parkinson's disease model. *Marine Drugs*, *17*(2). https://doi.org/10.3390/md17020085

Silva, J., Alves, C., Pinteus, S., Mendes, S., & Pedrosa, R. (2018). Neuroprotective effects of seaweeds against 6-hydroxidopamine-induced cell death on an in vitro human neuroblastoma model. *BMC Complementary and Alternative Medicine*, *18*(1). https://doi.org/10.1186/s12906-018-2103-2

Silva, J., Martins, A., Alves, C., Pinteus, S., Gaspar, H., Alfonso, A., & Pedrosa, R. (2020). Natural Approaches for Neurological Disorders-The Neuroprotective Potential of *Codium tomentosum*. *Molecules (Basel, Switzerland)*, *25*(22). https://doi.org/10.3390/molecules25225478

Stefanis, L. (2012). α-Synuclein in Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(2). https://doi.org/10.1101/cshperspect.a009399

Sweeney, P., Park, H., Baumann, M., Dunlop, J., Frydman, J., Kopito, R., McCampbell, A., Leblanc, G., Venkateswaran, A., Nurmi, A., & Hodgson, R. (2017). Protein misfolding in neurodegenerative diseases: Implications and strategies. *Translational Neurodegeneration*, *6*(1). https://doi.org/10.1186/s40035-017-0077-5

Tardiff, D. F., Jui, N. T., Khurana, V., Tambe, M. A., Thompson, M. L., Chung, C. Y., Kamadurai, H. B., Kim, H. T., Lancaster, A. K., Caldwell, K. A., Caldwell, G. A., Rochet, J. C., Buchwald, S. L., & Lindquist, S. (2013). Yeast reveal a "druggable" Rsp5/Nedd4 network that ameliorates α-Synuclein toxicity in neurons. *Science*, *342*(6161), 979–983. https://doi.org/10.1126/science.1245321

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

Trott, A., West, J. D., Klaic´,Klaic´, L., Westerheide, S. D., Silverman, R. B., Morimoto, R. I., & Morano, K. A. (2008). Activation of Heat Shock and Antioxidant Responses by the Natural Product Celastrol: Transcriptional Signatures of a Thiol-targeted Molecule. *Molecular Biology of the Cell*, *19*, 1104–1112. https://doi.org/10.1091/mbc.E07-10

Tsai, R. M., & Boxer, A. L. (2014). Treatment of frontotemporal dementia. In *Current Treatment Options in Neurology* (Vol. 16, Issue 11, pp. 1–14). Current Science Inc. https://doi.org/10.1007/s11940-014-0319-0

Uversky, V. N. (2018). Intrinsic Disorder, Protein–Protein Interactions, and Disease. In *Advances in Protein Chemistry and Structural Biology* (Vol. 110, pp. 85–121). Academic Press Inc. https://doi.org/10.1016/bs.apcsb.2017.06.005

van Swieten, J., & Spillantini, M. G. (2007). Hereditary frontotemporal dementia caused by Tau gene mutations. *Brain Pathology*, *17*(1), 63–73. https://doi.org/10.1111/j.1750-3639.2007.00052.x

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*, *76*(2), 115–158. https://doi.org/10.1128/mmbr.05018-11

Voisine, C., Varma, H., Walker, N., Bates, E. A., Stockwell, B. R., & Hart, A. C. (2007). Identification of Potential Therapeutic Drugs for Huntington's Disease using *Caenorhabditis elegans*. *PLoS ONE*, *2*(6). https://doi.org/10.1371/journal.pone.0000504

Wang, Y., Chen, R., Yang, Z., Wen, Q., Cao, X., Zhao, N., & Yan, J. (2022). Protective Effects of Polysaccharides in Neurodegenerative Diseases. In *Frontiers in Aging Neuroscience* (Vol. 14). Frontiers Media S.A. https://doi.org/10.3389/fnagi.2022.917629

WHO. (2018). *The Global Dementia Observatory Reference Guide World Health Organization*. 74. http://apps.who.int/bookorders.

Wider, C., & Wszolek, Z. K. (2008). Etiology and pathophysiology of frontotemporal dementia, Parkinson disease and Alzheimer disease: Lessons from genetic studies. *Neurodegenerative Diseases*, *5*(3–4), 122–125. https://doi.org/10.1159/000113680

Wozniak, M., Bell, T., Dénes, Á., Falshaw, R., & Itzhaki, R. (2015). Anti-HSV1 activity of brown algal polysaccharides and possible relevance to the treatment of Alzheimer's disease. *International Journal of Biological Macromolecules*, *74*, 530–540. https://doi.org/10.1016/j.ijbiomac.2015.01.003

Xiang, S., Liu, F., Lin, J., Chen, H., Huang, C., Chen, L., Zhou, Y., Ye, L., Zhang, K., Jin, J., Zheng, J., Wang, C., He, S., Wang, Q., Cui, W., & Zhang, J. (2017). Fucoxanthin inhibits β-amyloid assembly and attenuates β-amyloid oligomer-induced cognitive impairments. In *J. Agric. Food Chem., Just Accepted Manuscript • Publication Date*. http://pubs.acs.org

Yang, M., Xuan, Z., Wang, Q., Yan, S., Zhou, D., Naman, C. B., Zhang, J., He, S., Yan, X., & Cui, W. (2021). Fucoxanthin has potential for therapeutic efficacy in neurodegenerative disorders by acting on multiple targets. In *Nutritional Neuroscience*. Taylor and Francis Ltd. https://doi.org/10.1080/1028415X.2021.1926140

Yoon, N. Y., Chung, H. Y., Kim, H. R., & Choi, J. S. (2008). Acetyl- and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. *Fisheries Science*, *74*(1), 200–207. https://doi.org/10.1111/j.1444-2906.2007.01511.x

Zesiewicz, T. A. (2019). *Parkinson Disease*. http://journals.lww.com/continuum

Zondler, L., Miller-Fleming, L., Repici, M., Gonçalves, S., Tenreiro, S., Rosado-Ramos, R., Betzer, C., Straatman, K. R., Jensen, P. H., Giorgini, F., & Outeiro, T. F. (2014). DJ-1 interactions with a-synuclein attenuate aggregation and cellular toxicity in models of Parkinson's disease. *Cell Death and Disease*, *5*(7). https://doi.org/10.1038/cddis.2014.307

SUPPLEMENTARY MATERIAL

Supplemetary Table 1 List of media and buffer for *S. 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
SGC (supplemented with aminoacids)	0.67% SC media, 2% dextrose, 2% agar, 50 mg/L histidine, 300 mg/L leucine and 100 mg/L adenine
SGCD	0.67% SC media, 2% dextrose, 2% agar, 50 mg/L histidine, 300 mg/L leucine and 100 mg/L adenine, 2% agar
PBS	NaCl: 137 mM; KCl: 2.7 mM; Na_2HPO_4: 10 mM; KH2PO4: 1.8 mM

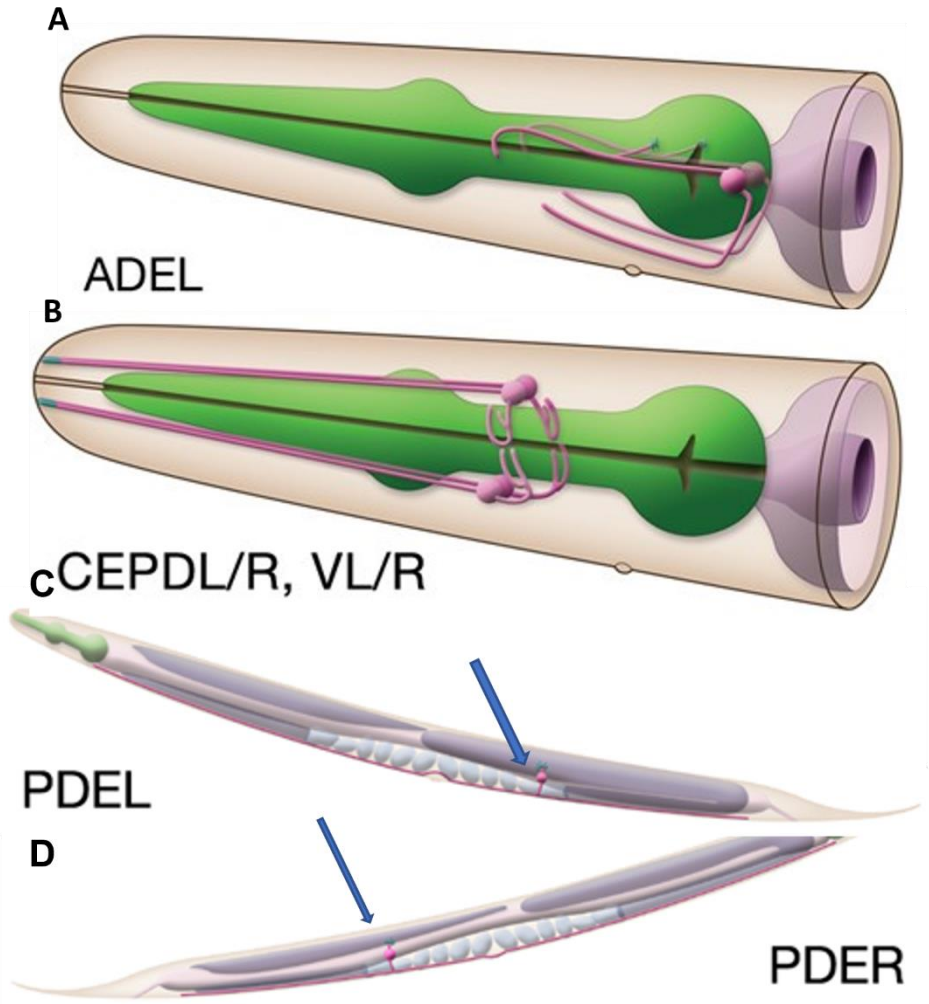
Supplemetary Table 2 Model strains used in this study (Wormbase.com).

Strains	Genotype
N2	Wild type
CK10	*bkIs10* [Paex-3 :: h4 R1NTauV337M; Pmyo-2 :: gfp]
UA198	*baIn34* [Peat-4::Aβ, Pmyo-2::mCherry]; adIs1240[Peat-4::GFP]
DA1240	*adIs1240* [Peat-4::GFP + lin-15(+)]
UA44	*baIn11* [Pdat-1 ::a-syn, Pdat-1 ::GFP
BZ555	*egIs1* [dat-1p:: GFP)
SJ4005	*zcIs4* [hsp-4::GFP] V
SJ4100	*zcIs13* [hsp-6p::GFP]
CL2166	*dvIs19* [(pAF15)gst-4p::GFP::NLS] III
LD1171	*ldIs3* [gcs-1p::GFP + rol-6(su1006)]

Supplementary Table 3 List of media and buffer for *C. elegans* used in this work

Media	Composition
NGM (Nematode growth medium)	17 g/L agar, 2.5 g/L bacto peptone, 3 g/L NaCl, 1 mM $CaCl_2$, 25 mM Phosphate Buffer, 1 mM MgSO4, and 5 mg/mL cholesterol
M9 Buffer	3 g/L KH_2PO_4, 6 g/L Na_2HPO_4, 5 g/L NaCl, 1 mM $MgSO_4$
S-Basal	NaCl 5.85 g/L, K_2HPO_4 1 g/L, KH_2PO_4 6 g/L, cholesterol (5 mg/mL in ethanol 100%) 1 mL dH_2O
Trace Metal	1.86 g/L disodium EDTA, 0.69 g/L $FeSO_4$ • 7 H2O, 0.2 g/L $MnCl_2$ • 4 H_2O, 0.29 g/L $ZnSO_4$ • 7 H_2O, 0.025 g/L $CuSO_4$ • 5 H_2O
Potassium Citrate	20 g/L citric acid $C_6H_8O_7$ • H_2O, 293.5 g/L tri-Potassium citrate $C_6H_5K_3O_7$ • H_2O
S-Medium	S-Basal, 10mL/L trace metal, 10mL/L potassium citrate
S-Medium Complete	S-Medium, x100 Penicillin/Streptomycin, x100 Nystatin
Freezing Solution	NaCl 5.8 g/L, KH_2PO_4 1M (pH=6) 50 mL, glycerol 85% 240 mL, dH2O 710 mL

Supplementary Figure 1 Schematic representation of 6 glutamatergic neurons in the head zone of *Caenorhabditis elegans*: anterior deirids (ADE left and right Supplementary Figure 1**A**) and neurons of cephalic sensilla (CEPs ventral left/right and dorsal left/right; Supplementary Figure 1**B**). The Posterior deirid neurons (PDEs neurons) were also evaluated (Supplementary Figure 1**C-D**).



Supplementary Table 4 DPPH antiradical activity of all macroalgae extracts. Gallic acid (GA) used as positive control. Three replicates were performed just for extracts with EC_{50} lower than 500 μg/mL.

Macroalgae extract	EC_{50} (μg/mL)
Pelvetia canicultа (PC)	66,2
Himanthalia elongata (HE)	221,0
Fucus spiralis (FS)	319,9
Fucus vesiculosus (FV)	385,7
Ascophylum nodosum (AN)	680,5
Bifurcaria bifurcata (BB)	832,6
Laminaria ochroleuca (LO)	953,3
Sargassum muticum (SM)	1175,6
Gigartina pistillata (GP)	No activity
Saccharina latíssima (SL)	No activity
Codium tomentosum (CT)	No activity
Undaria pinnatifida (UP)	No activity
Ulva rigida (UR)	No activity
Codium chrispus (CC)	No activity
Mastocarpus stellatus (MS)	No activity
Gallic acid (GA)	2,1±0,95