Electronic Journal of Biotechnology 72 (2024) 12–19

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

Research article

Acetylcholinesterase inhibition exerted by the extract of *Daldinia eschscholtzii*, a marine fungus associated with the coral *Siderastrea siderea*: GC-MS analysis and molecular docking of identified compounds ☆

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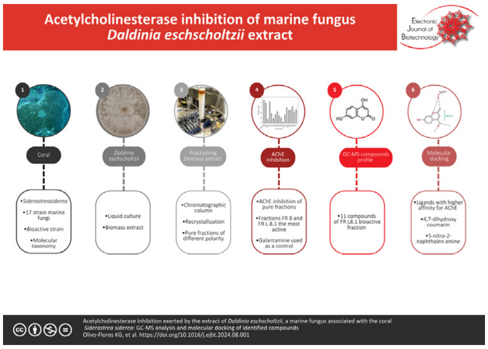
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 March 2024
Accepted 13 August 2024
Available online 6 September 2024

Keywords:
4,7-dihydroxycoumarin
Ligand-enzyme interactions
Acetylcholinesterase
AChE Inhibition
Bioprospecting
Coral-derived fungus
Daldinia eschscholtzii
GC-MS analysis
Marine fungus
Molecular docking

ABSTRACT

Background: Alzheimer's disease is a neurodegenerative disease that has no cure. The drugs used to treat Alzheimer's disease are based on the inhibition of the enzyme acetylcholinesterase (AChE). In this sense, marine fungal metabolites represent an alternative source for the discovery of drugs based on AChE inhibition (IAChE). The present research carried out a bioprospecting study of marine fungi with acetylcholinesterase inhibition potential.

Results: A total of 17 marine fungi were isolated from three stony corals from the Veracruz Reef System. The fungal genera identified were *Geotrichum*, *Curvularia*, *Penicillium*, *Aspergillus,* and *Daldinia*. The IAChE was evaluated from the broth and biomass extracts of each fungal strain. As a result, *Daldinia eschscholtzii* was one of the three fungi with the greatest IAChE effect (32.7%). Subsequently, a bio-directed chromatographic purification of the methanolic extract of the biomass of *D. eschscholtzii* was carried out. The FRL8.1 fraction was the most active with an IAChE of 41.0%, a value close to Galantamine positive control (44.0%). Furthermore, compound analysis was carried out by GC-MS. Finally, it was determined that the metabolites responsible for the inhibitory effect are probably 4,7-dihydroxycoumarin and 5-nitro-2-naphthalenamine, which was demonstrated by their interactions with the AChE enzyme receptor

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☆ Audio abstract available in Supplementary material.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso

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https://doi.org/10.1016/j.ejbt.2024.08.001

Siderastrea siderea

through molecular docking studies.
Conclusions: Coral-associated marine fungi produce secondary metabolites that inhibit acetylcholinesterase. This is the first report of *D. eschscholtzii* isolated from the stony coral *Siderastrea siderea*. Finally, we consider that more studies are needed to demonstrate the IAChE potential of marine fungi associated with corals or other marine organisms.

How to cite: Olivo-Flores KG, Couttolenc A, Suárez-Medellín J, et al. Acetylcholinesterase inhibition exerted by the extract of *Daldinia eschscholtzii*, a marine fungus associated with the coral *Siderastrea siderea*: GC–MS analysis and molecular docking of identified compounds. Electron J Biotechnol 2024;72. https://doi.org/10.1016/j.ejbt.2024.08.001.

1. Introduction

Marine fungi are widely distributed and can be found in marine plants such as mangroves, algae, and grasses. They also establish associations with some invertebrates such as corals and sponges, and vertebrates such as fish and can even survive on driftwood and sediments [1]. Marine fungi can produce structurally different metabolites compared to their analogs in the terrestrial environment, this is because marine fungi are exposed to extreme conditions such as high salt concentrations, exposure to UV rays, little access to substrate for their growth, and hydrostatic pressure [2]. Anthraquinones and their derivatives have been reported isolated from the marine environment such as mangroves, coral, sponges, algae, sediments and deep waters. In this sense, until 2021, 296 anthraquinones and derivatives had been registered, with biological properties such as enzyme inhibition, among others [3]. It is important to highlight that from 2020 to 2022, 184 new metabolites derived from 46 marine fungi were reported [4], indicating the great chemical-biological potential that they present. The main groups of compounds reported are terpenes, peptides, alkaloids, and halogenated compounds [5,6,7,8]. On the other hand, the main biological activities evaluated are antioxidant, antiproliferative, anti-inflammatory, antimicrobial, and enzyme inhibition. An example of the latter is the inhibition of acetylcholinesterase (AchE), which is associated with neurodegenerative conditions such as Alzheimer's disease (AD). In this sense, marine fungal metabolites represent an important source for the development of drugs based on the inhibition of AChE (IAChE). For example, from a *Cladosporium cladosporioides* strain isolated from mangrove leaves, the compounds ladamide, cinnamic acid, *p*-coumaric acid, stigmasterol-3-O-β-D-glucoside and uracil were identified. Cinnamic acid showed the highest IAChE activity with an IC_{50} value of 0.057 ± 0.003 μM [9]. Another compound with potential use for the treatment of AD is lumichrome (IAChE, IC_{50}: 12.24 ± 0.12 μM) produced by an *Acremonium persicinum* strain associated with the sponge *Mycale* sp. [10]. Likewise, from an endophytic fungus *Acrostalagmus luteoalbus* TK-43, three pairs of enantiomers were isolated, with acrozin A (+) being the one that presented the greatest inhibition of AchE (IC_{50} = 2.3 μM), also, the alkaloid penicillamine produced by *Penicillium commune* 366606, a fungus isolated from seawater in China, exhibited an anti-AChE activity (32.4% inhibition) [11]. Recently, beauvericin isolated from a mangrove endophytic fungus, *Aspergillus terreus* (no. GX7-3B), showed anti-AChE activity with an IC_{50} value of 3.09 M and scopularide I peptide isolated from a soft coral-associated fungus, *A. sclerotiorum* SCSIO41031, inhibited AChE activity with an IC_{50} = 15.6 M, furthermore, through molecular docking study showed that the alkyl chain of scopularide I formed a hydrophobic interaction with the active site residues Trp84, Asp72, Try70 and Trp279 [12]. Due to the fact that currently, the only FDA-approved medications for AD are based on IAChE Galantamine, Donepezil, and Rivastigmine which helps to relieve symptoms and improve or stabilize cognitive functions [13]. The present study aimed to carry out bioprospecting of fungi isolated from stony corals of the Veracruz Reef System as a source of AChE inhibitory metabolites.

2. Materials and methods

2.1. Biological material and culture

The study area included the reefs: Verde (19°15′29″ N, 96°07′ 19″ W) at 5 m depth and Gallega (19°13′19″ N, 96°07′36″ W) at 3 m depth. These reefs belong to the northern reef complex of the Parque Nacional Sistema Arrecifal Veracruzano (PNSAV). The coral species obtained from the sampling were *Millepora alcicornis* Linnaeus, *Porites astreoides* Lamarck and *Siderastrea siderea* Blainville. The coral samples were placed in triplicate in Petri dishes containing modified Potato Dextrose Agar (PDA) (50% seawater and 0.2 g/L chloramphenicol) and incubated at 25 ± 2°C for an interval of 7–14 d. Pure strains were identified by their reproductive structures using taxonomic keys [11,13].

2.2. Liquid fermentation and extraction

For each fungal isolation, Potato Dextrose Broth (PDB) with 50% seawater was used as the culture medium. The fermentation was maintained for 14 d in the absence of light at 25 ± 2°C at 150 rpm. After this period, they were maintained for another 14 d in static conditions. Subsequently, the biomass and culture broth were separated by vacuum filtration and were frozen and lyophilized. The dehydrated material was extracted with a 1:1 mixture of $CHCl_3$:CH_3OH and kept in an ultrasound bath for 2 intervals of 25 min. Finally, the biomass and culture broth extracts were evaporated in a rotary evaporator and stored at −4°C until their use in the IAChE bioassay.

2.3. In vitro AChE inhibition

The biomass and culture broth extracts of the isolated marine fungi were tested for their ability to inhibit the AChE enzyme following the procedure of Ellman et al. [14] and Alves et al. [10] with some modifications, where 10 μL of the extract, fraction or control, 20 μL of electric eel AChE and 150 μL of dithiobenzoate (DTNB) at 6 mM were mixed. Phosphate buffer (0.01 M, pH = 7.4) was used as a solvent for the mixture. The reaction starts by adding 20 μL of 15 mM acetylcholine iodide (AChI). The absorbance was measured at 414 nm after 10 min of reaction, and 3.5 mM galantamine was used as a positive control. The result was expressed as a percentage (%) of AChE inhibition and was calculated as follows in [**Equation 1**].

$$IAChE(\%) = \left[1 - \frac{A_{test}}{A_{blank}}\right] \times 100 \qquad (1)$$

2.4. Molecular identification of bioactive strain

Molecular identification of the bioactive strain was carried out using nuclear rDNA-ITS sequence data. Genomic DNA was isolated based on the methodology proposed by Franceschy et al. [15]. Primers ITS1 and ITS4 were used to amplify the nuclear rDNA-ITS regions by direct PCR. Sequences were compared to the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/). Blast search software was used to confirm species identity. The phylogenetic tree was built using the RaxmlGUI v. 2.0.10 according to Stadler et al. [16]. The most likely molecular-phylogenetic tree was reconstructed using the GTR + G + I model, and the parameters of the free model were estimated by the software. The bootstrap supports were estimated by 500 replicates.

2.5. Fractionation of Daldinia eschscholtzii biomass extract

The most bioactive strain was inoculated in 40 L of PDB with 50% seawater using 500 mL Erlenmeyer flasks. The biomass was separated from the culture broth, following the methodology previously described. In this way, the lyophilized biomass (160 g) was extracted with methanol (E-1) and re-extracted with chloroform (E-2). From the chromatographic purification of E-2 using mixtures of n-hexane:ethyl acetate (Hx:EtOAc) as mobile phase, fractions of different polarity were eluted [17], and IAChE potential was evaluated.

2.6. Analysis of compounds GC-MS

An Agilent Technologies gas chromatograph (model 6890 N, Net Work GC system) was used for the analysis of chemical components from the most active fraction obtained from the *D. eschscholtzii* extract E-2. The gas chromatograph has a DB-5, 5%-phenyl-methylpolysiloxane column (Agilent Technologies) of 60 m length, 0.25 mm internal diameter and 0.25 μm film thickness. The column temperature started at 50°C and was maintained for 5 min; then, it was raised at 20°C/min to 250°C and remained for 10 min. Then, it was increased to 280°C and maintained for 20 min at a flow of 1 mL/min of ultrapure Helium. The injector temperature was 250°C. Split injection mode with a ratio of 10:1. The identification of each peak was carried out by mass spectrometry using an Agilent Technologies mass spectrometer (model 5975 inert XL). Mass spectra were obtained by electron impact ionization at 70 eV. For the identification of the compound profile, the mass spectra obtained for each compound were compared with a database (HP Chemstation-NIST 05 Mass Spectral search program, version 2.0).

2.7. Molecular docking

AChE crystallographic structure was downloaded from Protein Data Bank (PDB ID:5HFA). Solvent and co-crystalized molecules were eliminated from the PDB file, and a single monomer (chain A) was chosen. The protein structure was prepared with the dock prep routine of UCSF Chimera software [18]. The structure of ligands identified from the in vitro assay was downloaded from the PubChem database, and their geometry was optimized with the mmff94 force field in Avogrado2 (version 1.97.0) software [19]. Molecular docking analysis was carried out with AutoDock VINA [20]. Configuration files were generated with Autodock Tools 1.5.6. The enzyme active site was identified from their catalytic relevant residues: Ser 203, Glut 334, and His 447 [21], with Grid Box dimensions of 15.75 Å for each side. Ligand-enzyme interactions were analyzed with the PoseView online tool [22].

2.8. Statistical analysis

Each assay was carried out in triplicate, and the results are expressed as the mean ± standard deviation (SD). For the *in silico* molecular docking results, a Shapiro-Wilk test was run ($p < 0.01$), and since the data violated the assumption of normality, a Kruskal Wallis test followed by a Dunn's multiple comparison test as post-hoc was used. The statistical analysis was performed with the RStudio program version 1.2.5033 [23].

3. Results and discussion

3.1. Fungal isolation

From the three stony corals collected in the Veracruz Reef System, 17 strains of fungi were isolated. Seven fungi were isolated from *Millepora alcicornis*, four fungi from *Porites astreoides* and six fungi from *S. siderea*. The morphological identification of the isolates was determined in 9 fungi that developed reproductive structures (Table 1). The genera identified by taxonomic keys were *Aspergillus* for isolates C14-H2, C16-H3 and C16-H6, *Penicillium* for C13-H5 and C16-H5, *Geotrichum* strain C13-H2, *Curvularia* strain C14-H3 [24] and *Daldinia* Ces. & De Not for C16-H2 and C16-H4 [16] (Fig. 1).

3.2. AChE inhibition of fungal extracts

The results of the IAChE assay for the biomass and culture broth extracts of the 17 isolated strains are shown in Fig. 2. Galantamine used as a control inhibited 41.0% of the AChE. Also, it was observed that culture broth extracts were more active compared to biomass extracts. The fungal extracts with the highest percentage of IAChE were from *Penicillium* sp. (C16-H5 and C16-H1 with values of 43.0 and 34.0%, respectively), *Daldinia* sp. (C16-H2 with 32.7%) and *Aspergillus* sp. (C16-H3 with 31.6% and C13-H7 with 29.4%).

The bioactive potential of marine fungi to inhibit AChE has been previously reported for genera such as *Penicillium* [25] and *Aspergillus* [26]. Furthermore, the potential of *Daldinia* sp. to inhibit AChE has already been reported. Benzopyran derivatives from the methanolic extract of *D. eschscholtzii* obtained percentages of 0.99–7.3% at a concentration of 50 μM, which were compared with Tacrine (64.88% at 0.333 μM) [27]. Subsequently, Wang et al. [28] reported the compound Daldinsin that obtained an AChE inhibition percentage of 38.8% at 50 μM.

Table 1
List of fungi isolated by coral and internal identification key.

Reef	Coral	Strain key	Genus identificated
Verde	*Millepora alcicornis*	C13-H1	NP-ER
		C13-H2	*Geotrichum* sp.
		C13-H3	NP-ER
		C13-H4	NP-ER
		C13-H5	*Penicillium* sp.
		C13-H6	NP-ER
		C13-H7	NP-ER
	Porites astreoides	C14-H1	NP-ER
		C14-H2	*Aspergillus* sp.
		C14-H3	*Curvularia* sp.
		C14-H4	NP-ER
Gallega	*Siderastrea siderea*	C16-H1	NP-ER
		C16-H2	*Daldinia* sp.
		C16-H3	*Aspergillus* sp.
		C16-H4	*Daldinia* sp.
		C16-H5	*Penicillium* sp
		C16-H6	*Aspergillus* sp.

NP-ER: did not present reproduction structures.

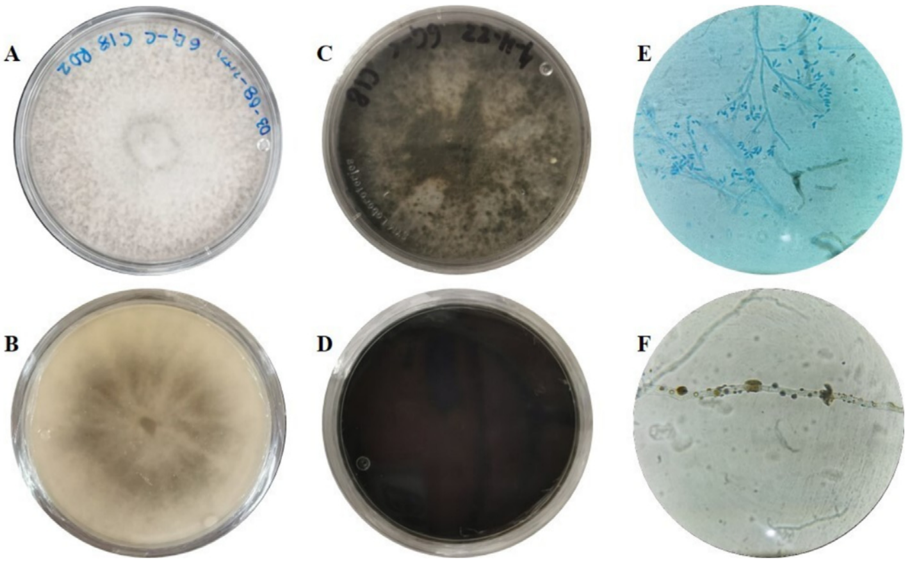

Fig. 1. *Daldinia eschscholtzii*. A-B) Young strain, white color. C-D) Mature strain, smoky grey color with some shades of olive green. E) Conidiophore is branched with cylindrical, smooth and hyaline conidiogenous cells. F) Thick-walled hyphae with blackish exudate. Magnification at 100x using lactophenol blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

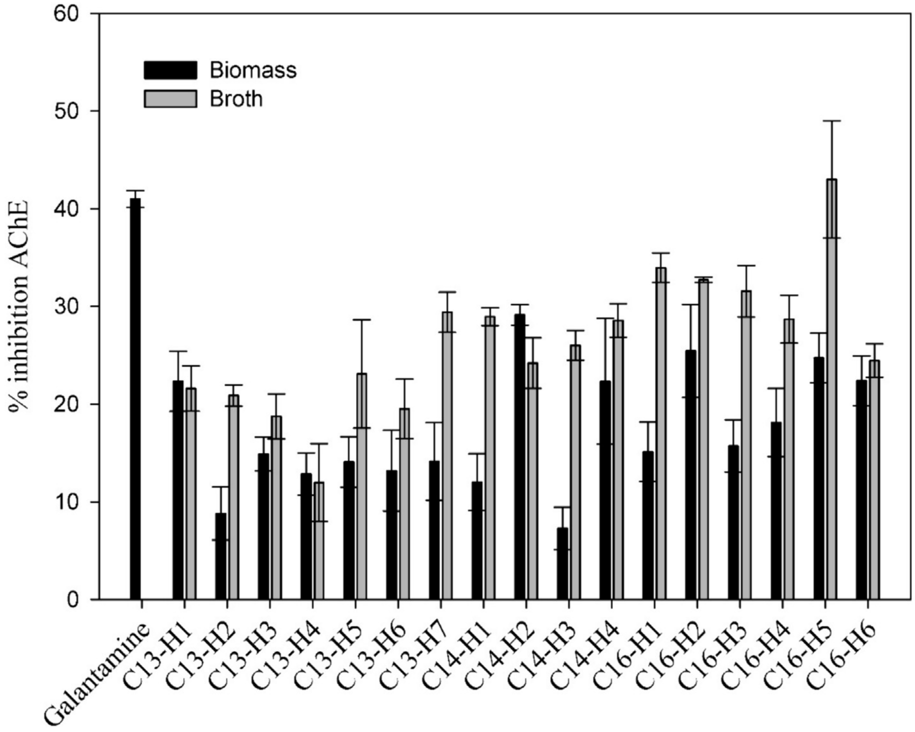

Fig. 2. Percentage of inhibition of broth and biomass extracts of marine fungi. Results are expressed as the mean ± standard deviation (SD) with n = 3.

3.3. Molecular identification

The nucleotide sequence of C16-H2 was registered in GenBank, obtaining accession number **PP035219**. According to the cladogram (Fig. 3), C16-H2 was grouped in the clade of the species *D. eschscholtzii*. In the alignment, representative sequences of the *Daldinia* genus were compared, generating 17 groups for *Daldinia* species, five sequences correspond to *D. eschscholtzii* isolated from marine habitat, and four sequences were obtained from the BLAST search. The *Hypoxylon fragiforme* sequence (GenBank accession No. **MH860609**) was used as an outgroup.

Daldinia genus belongs to the order Xylariales, so it is widely distributed in the terrestrial habitat; however, it has also been isolated from marine organisms. For example, the strain *Daldinia* sp. isolated from the sponge *Dragmacidon reticulatum* [29] or *D. eschscholtzii* associated with the sponge *Xestospongia* sp. [30]. It has also been isolated from the algae *Gracilaria* sp. [31] and the gorgonian *Annella* sp. [32]. In other studies, *D. eschscholtzii* has been isolated from mangroves, for example, from branches of *Scaevola sericea* [33], from a leaf of *Bruguiera gymnorrhiza* [34], from the root of *Pluchea indica* Less [35] or *Bruguiera sexangula* var. Rhynchopetala [36]. Although *Daldinia* sp. has already been identified

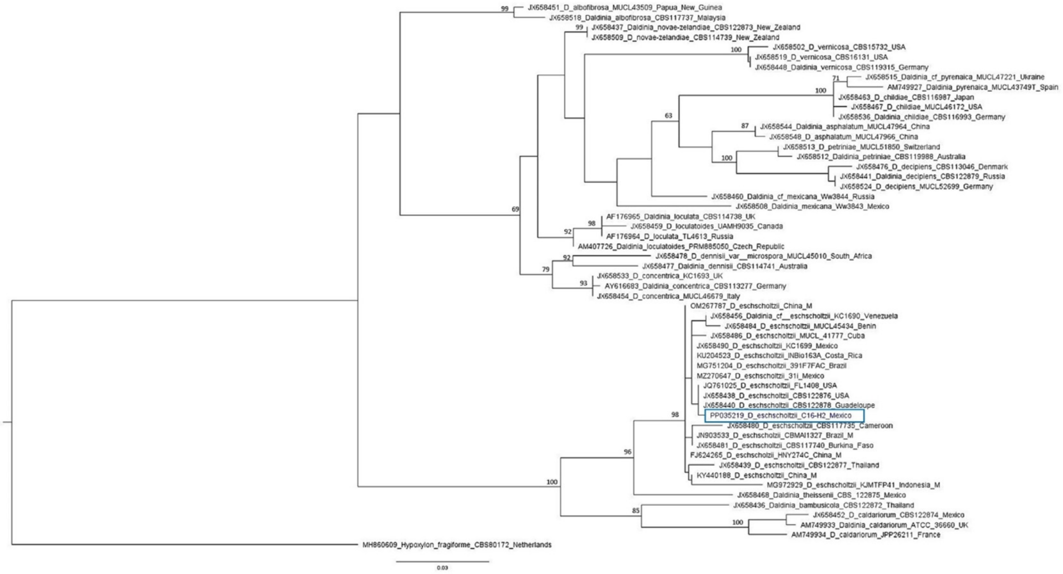

Fig. 3. Cladogram of the genus *Daldinia*. The ITS1 and ITS4 sequence of C16-H2 was used. Sequences were compared to the GenBank database and aligned using the Clustal algorithm. The most likely molecular-phylogenetic tree was reconstructed using the GTR + G + I model and the software estimated the parameters of the free model. The bootstrap supports were estimated by 500 replicates.

in the marine habitat, this is the first report of the genus *Daldinia* associated with stony corals, particularly the coral *S. siderea*.

3.4. AChE inhibition of bio-guided fractionation of D. eschscholtzii extract

Chromatographic fractionation was performed using an ascending polarity gradient using Hx:EtOAc mixtures as mobile phase. Thus, 17 fractions were obtained and their IAChE potential was evaluated. The results of the IAChE assay for the fractions of *D. eschscholtzii* are shown in Fig. 4. Galantamine obtained a percentage of AChE inhibition of 44.0%. The most active fraction compared to the positive control is FR L8.1 (41.0%). This fraction was obtained from a recrystallization process of FR 8 (38.0%). Both fractions eluted at a polarity of 90:10 (Hx:EtOAc).

3.5. Analysis of compounds by GC-MS of the active fraction of D. eschscholtzii

The GC-MS profile of the FR L8.1 fraction showed 11 compounds, the two most abundant were 5-(4-methoxyphenyl)-pyrazole and 4,7-dihydroxy-2-H-1-benzopyran-2-one, also known

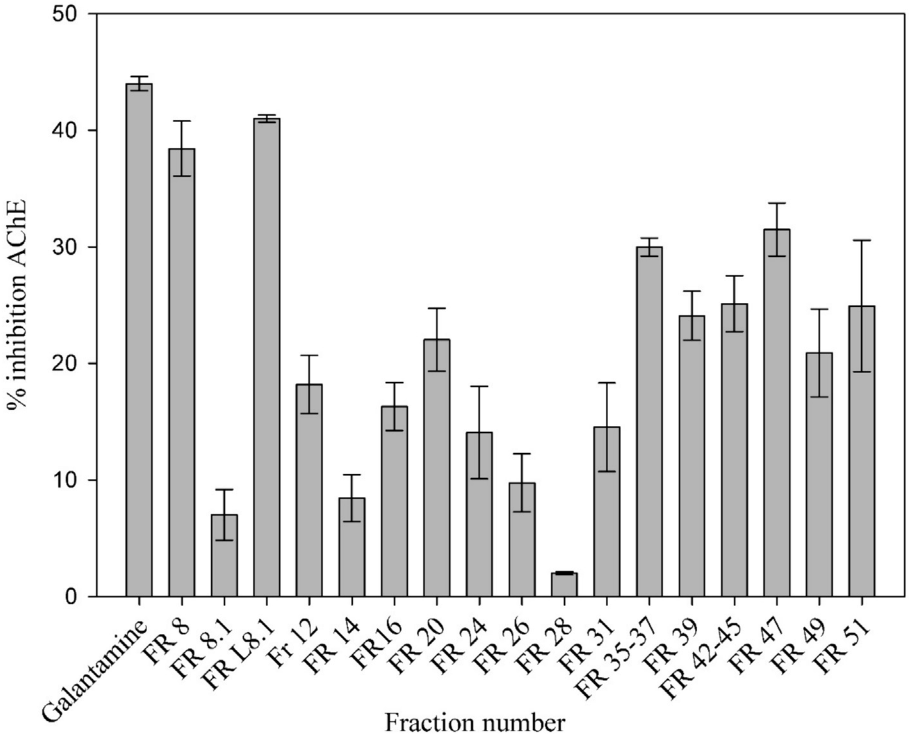

Fig. 4. AChE inhibition percentages from chromatographic fractionation of *D. eschscholtzii* biomass extract. Results are expressed as the mean ± standard deviation (SD) with n = 3.

as 4,7-dihydroxycoumarin (Table 2). These results coincide with Pandey and Banerjee [37], they identified 4,7-dihydroxycoumarin from the dichloromethane extract of *Daldinia bambusicola*. On the other hand, the methyl esters of hexadecanoic acid, 9,12-octadecadienoic acid and 9-octadecenoic acid were identified from the ethyl acetate extract of *D. eschscholtzii* [38]. Also, pyrazoles have been reported such as 5-methoxy-1,3-dimethyl-1-H-pyrazole produced by *Daldinia* cf. *childiae* [39]. In addition, the saturated fatty acids C14:0 tetradecanoic acid (myristic acid, 0.5%), C16:0 hexadecanoic acid (palmitic acid, 24.0%), C18:0 octadecanoic acid (stearic acid, 10%) and unsaturated fatty acids C16:1 9-hexadecenoic acid (palmitoleic acid, 1.5%), C18:1 *cis*-9-octadecadienoic acid (oleic acid, 25%) and C18:2 *cis*-9,12-octadecadienoic acid (linoleic acid, 39%) were detected. These results coincide with previous reports of *Daldinia* sp. [40] characterized palmitic acid, oleic acid and the ethyl ester of hexadecanoic acid from the methanolic extract of *D. concentrica*. On the other hand, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, margaric, behenic and lignoceric acids have been detected in *D. eschscholtzii* [41].

To our best knowledge, there are no previous reports of the isolation of 5-nitro-2-naphthylamine from marine fungi. However, other naturally occurring naphthalenes have been isolated from several fungi belonging to the order Xylariales, including daldinol and 8-methoxy-1-napthol from the genus *Daldinia* [42,43], demonstrating this genus has the enzymatic machinery to synthesize this group of metabolites.

3.6. Molecular docking

Molecular docking analysis of the previously identified compounds in complex with AChE enzyme active site, using galantamine as the positive control, was carried out to determine which metabolite from fraction FR L8.1 would be responsible for the inhibitory effect found. Table 3 summarizes free binding energy (FBE) calculated for each ligand. Since 4,7-dihydroxycoumarin and 5-nitro-2-naphthylamine chemical structures in Fig. 5 are the ligands with the most affinity to AChE, we explore their interactions with the enzyme active site. Both metabolites form hydrogen bonds with the amino acids belonging to the catalytic triad Ser203 and His447 and also interact with the anionic subsite residues Glu202, Trp86 and Tyr337, which are responsible for binding the quaternary trimethylammonium tail group of acetylcholine. Finally, both 4,7-dihydroxycoumarin and 5-nitro-2-naphthylamine show non-polar interactions with Gly121 from the oxyanion hole, the essential catalytic functional unit of AChE [44]. Such ligand-enzyme interactions are consistent with the AChE inhibitory activity of fraction FR L8.1. Fig. 6 summarizes the ligand-enzyme interactions found for several tridimensional conformations of 4,7-dihydroxycoumarin and 5-nitro-2-naphthylamine obtained by molecular docking analysis.

Table 2
GC-MS compound profile of FR L8.1 fraction of *D. eschscholtzii* biomass extract.

Compound	% Abundance
1,4-benzodioxan-6-amine	0.34
4,7-dihydroxycoumarin	17.62
5-nitro-2-naphthylamine	3.53
5-(4-methoxyphenyl)-pyrazole	48.96
9-hexadecanoic acid, methyl ester	0.45
hexadecanoic acid, methyl ester	7.44
isopropyl palmitate	0.46
9-octadecenoic acid, methyl ester	16.77
octadecenoic acid, methyl ester	2.70
9,12-octadecadienoic acid, ethyl ester	0.27
isopropyl linoleate	1.46

Table 3
Free binding energy (FBE) calculated for the best ligands coupled to the AChE enzyme active site. Data are expressed as mean ± standard deviation (kcal/mol).

Ligand	FBE (kcal/mol)
1,4-benzodioxan-6-amine	−6.12 ± 0.2
4,7-dihydroxycoumarin	−7.07 ± 0.4
5-nitro-2-naphthylamine	−7.23 ± 0.3
5-(4-methoxyphenyl)-pyrazole	−5.87 ± 0.5
hexadecanoic acid, methyl ester	−5.61 ± 0.2
isopropyl palmitate	−5.63 ± 0.2
9-octadecenoic acid, methyl ester	−5.33 ± 0.5
octadecenoic acid, methyl ester	−5.57 ± 0.3
9,12-octadecadienoic acid, ethyl ester	−5.20 ± 0.5
isopropyl linoleate	−5.01 ± 1.1
Galantamine (Positive control)	−4.33 ± 1.1

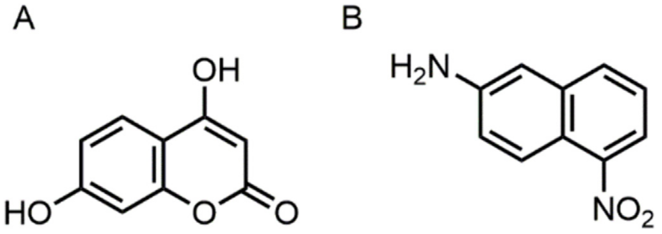

Fig. 5. Ligands with higher affinity for AChE present in the FR L8.1 fraction of the *D. eschscholtzii* biomass extract. **[A]** 4,7-dihydroxycoumarin and **[B]** 5-nitro-2-naphthylamine.

Although both metabolites show similar affinity with the AChE enzyme, the relative abundance of 4,7-dihydroxycoumarin (17.62%) suggests its role as responsible for the bioactivity exerted by fraction FR L8.1. It is worth noting that previous reports show that coumarins and their derivatives are efficient AChE inhibitors, while the coumarin ring is essential for optimum activity [45,46]. Selective influence for the substituent type and position with free binding energy was also previously identified [47]. Particularly, fungal coumarins represent an innovative source for metabolites with potential AChE inhibitory activity since several studies suggest that fungi are able to synthesize both simple and complex coumarins [48].

On the other hand, 5-(4-methoxyphenyl)-pyrazole, being the most abundant metabolite present (48.96%), might also play a role in the bioactivity exerted by fraction FR L8.1. Even though its BFE (−5.87 ± 0.5 kcal/mol) is significantly higher than the values estimated for 4,7-dihydroxycoumarin and 5-nitro-2-naphthylamine, it is nevertheless lower than the calculated values for the positive control Galantamine (−4.33 ± 1.1 kcal/mol). Interestingly, 5-(4-methoxyphenyl)-pyrazole, like 4,7-dihydroxycoumarin and 5-nitro-2-naphthylamine, also interacts with the aminoacid His447 from the AChE active site, which might partially explain the observed effect, especially since the reports of pyrazole derivatives capable of acetylcholinesterase inhibition are not uncommon in the literature [49,50]. However, fraction FR L8.1 is a complex mixture of several compounds with an acceptable calculated affinity for AChE, so the interactions between metabolites (including a possible synergistic effect) cannot be ruled out. Therefore, further research is needed to determine the relative role of each individual metabolite in the observed bioactivity.

4. Conclusions

Marine fungi are an alternative in the search for bioactive secondary metabolites. In this study, the potential of marine fungi

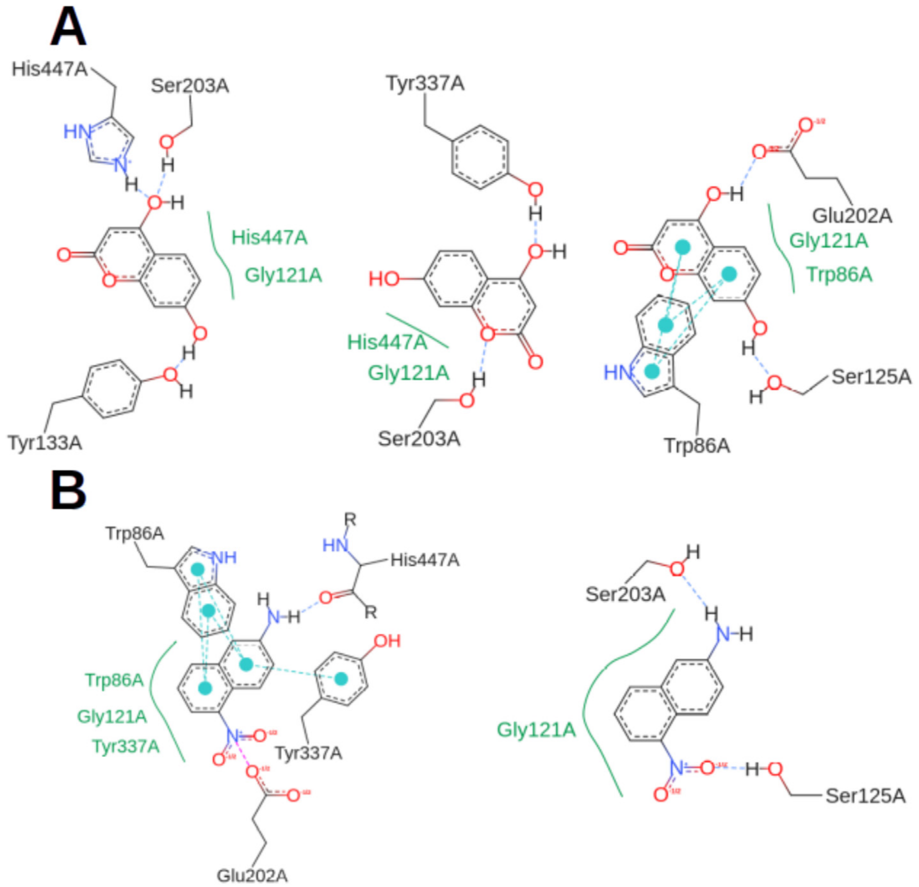

Fig. 6. Interactions map for the ligand-enzyme complexes with the best affinity: 4,7-dihydroxycoumarin (A) and 5-nitro-2-naphthylamine (B).

associated with Veracruz Reef System corals to inhibit AChE is reported. According to bioprospecting, *D. eschscholtzii* produces 4,7-dihydroxycoumarin and 5-nitro-2-naphthalenamine, which were detected in the fraction with the highest IAChE potential and their interaction with the AChE enzyme receptor, was demonstrated. This is the first report of *D. eschscholtzii* isolated from stony coral *S. siderea*. Therefore, these results support the importance of the conservation of coral reefs as a source of microorganisms with pharmaceutical potential. Furthermore, we consider that more studies are needed to demonstrate the IAChE potential of marine fungi associated with corals or other marine organisms.

CRediT authorship contribution statement

Karla G. Olivo-Flores: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Alan Couttolenc:** Writing – original draft, Supervision, Methodology, Data curation. **Jorge Suárez-Medellín:** Writing – original draft, Methodology, Formal analysis, Data curation. **Ángel Trigos:** Writing – review & editing, Investigation, Funding acquisition, Data curation. **César Espinoza:** Writing – review & editing, Project administration, Investigation, Formal analysis, Conceptualization.

Financial support

This work was supported by the Consejo Nacional de Humanidades, Ciencias y Tecnología (CONAHCYT) grant 784029. SIREI-UV (31506202246) project; the Universidad Veracruzana (UV-CA-354).

Conflict of interest

None.

Acknowledgements

JSM thanks the National Supercomputing Center from the Potosino Institute of Scientific and Technological Research for the computational resources used in this work. We thank Dr. José Luis Sánchez Castro, M.C. Alejandro Muñoz Aldape and M.C. Victor Hugo Rojas Ramírez for support in collecting the specimens.

Supplementary material

https://doi.org/10.1016/j.ejbt.2024.08.001.

Data availability

Data will be made available on request.

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