

Unlocking Neurodegeneration: Scaffold-Derived Blockers of MAO-B and AChE inspired by *Bryophyllum pinnatum*: A Structural Exploration

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Unlocking Neurodegeneration: Scaffold-Derived Blockers of MAO-B and AChE inspired by *Bryophyllum pinnatum*: A Structural Exploration

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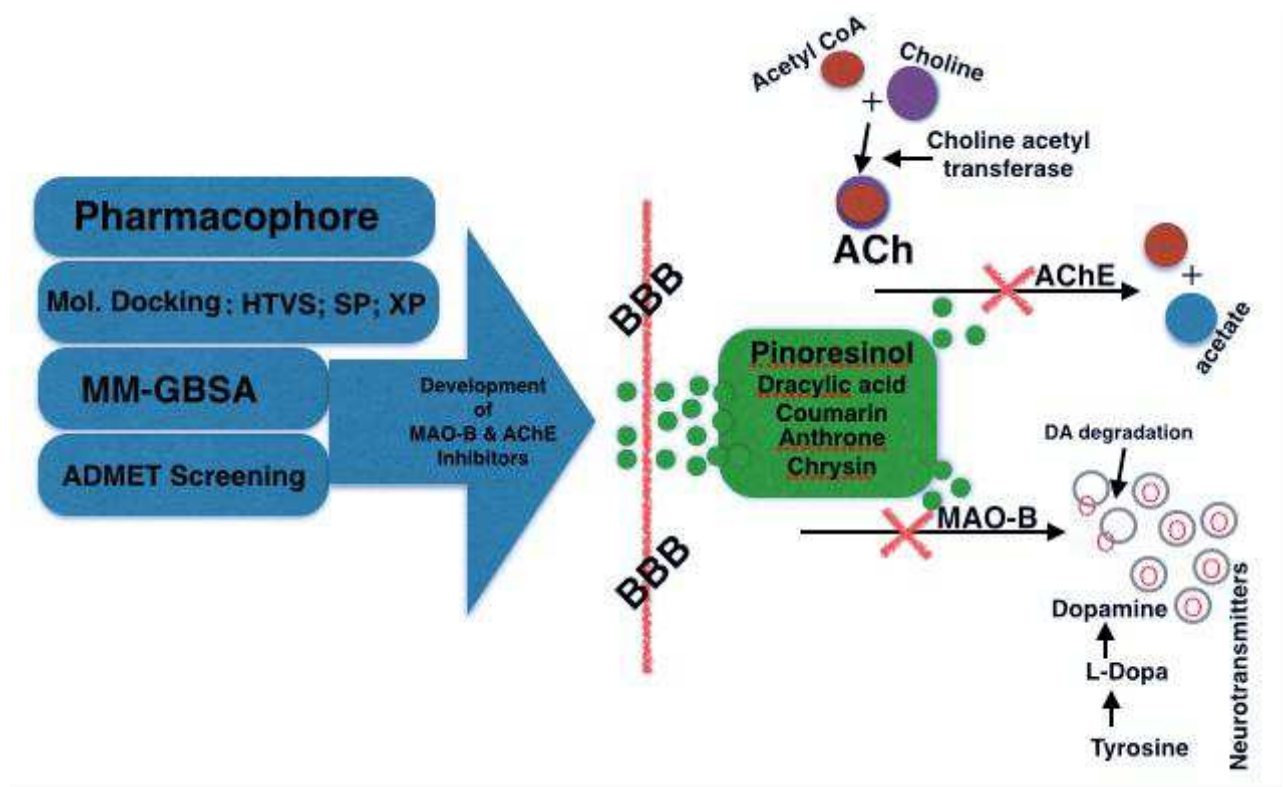
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Abstract

Alzheimer's disease (AD) and Parkinson's disease (PD) are progressive neurodegenerative disorders with overlapping symptoms. Reduced acetylcholine levels in AD are addressed using Acetylcholinesterase (AChE) inhibitors. Monoamine oxidase B (MAO-B) inhibitors prevent dopamine breakdown in PD. *Bryophyllum pinnatum*, a medicinal plant, that has been used as traditional treatments for various disorders because of its phytochemicals. This study explores its ligands as potential medications for AD and PD by inhibition of AChE and MAO-B. Computer-aided drug design was conducted using Schrödinger Suite software and Maestro 12.8. Ligands from *B. pinnatum* and standard drugs were docked to the active sites of MAO-B and AChE. ADMET screening and MM/GBSA analysis were performed. Pharmacophore modeling was used to identify compounds matching the reference ligands. After application of all screenings, 4 and 6 hit compounds were found for MAO B & AChE respectively based on good docking score and MM/GBSA as well as good ADMET properties. Pinorexinol was found to be the most potent of the hit compounds. These compounds could be used as neuroprotective agent in near future. Hence, this study provides evidence for consideration of valuable ligands in *Bryophyllum pinnatum* as

potential AChE and MAO-B inhibitors and further *in vivo* and *in vitro* investigation might prove their therapeutic potential.

Keywords: Alzheimer's disease; Parkinson's disease; *Bryophyllum pinnatum*; AChE and MAO-B inhibitors; Computer-aided drug design.



Significant Statement

This study delves into the potential of *Bryophyllum pinnatum*, a medicinal plant rich in phytochemicals, to address the overlapping symptoms of Alzheimer's disease (AD) and Parkinson's disease (PD). These neurodegenerative disorders have presented challenges in finding effective treatments. The research focuses on two key enzymes, Acetylcholinesterase (AChE) and Monoamine oxidase B (MAO-B), and aims to inhibit them as a means to alleviate the respective conditions.

By employing computer-aided drug design techniques, the study successfully identified a promising selection of compounds within *Bryophyllum pinnatum* that show potential in inhibiting AChE and MAO-B. Rigorous screenings and analyses, including docking simulations, ADMET assessments, and MM/GBSA analysis, have identified four promising compounds for MAO-B inhibition and six for AChE inhibition.

Among these, pinoresinol emerged as the most potent candidate, suggesting its significance as a neuroprotective agent for future exploration. This study not only sheds light on the therapeutic potential of *Bryophyllum pinnatum* but also paves the way for further *in vivo* and *in vitro* investigations. Ultimately, these findings offer a crucial foundation for considering *Bryophyllum*

pinnatum's valuable compounds as potential inhibitors for AChE and MAO-B, potentially leading to groundbreaking advancements in the treatment of AD and PD.

Introduction

Alzheimer's Disease (AD) and Parkinson's Disease (PD) are prevalent neurodegenerative disorders with increasing rate due to longer life span [1]. AD and PD are both progressive and incurable but differ in clinical presentation underlying pathology and affected brain regions. AD is characterized by progressive memory loss and cognitive decline, it is caused by impairment in the memory and cognition related regions [2,3]. PD is characterized by motor symptoms such as tremors, bradykinesia (slowness of movement), rigidity, and postural instability [4]. Both AD and PD have overlapping non-motor symptoms and increase in prevalence with age [5,6,7,8]. Current treatments focus on symptoms management rather than a cure.

Acetylcholinesterase (AChE) In Alzheimer's Disease

Acetylcholinesterase (AChE) is an enzyme that breaks down a neurotransmitter called acetylcholine in the brain [9]. In Alzheimer's disease, acetylcholine levels decrease leading to cognitive problems. Cholinesterase inhibitors blocks AChE, temporarily leading to increasing acetylcholine (ACh) and improving memory and cognitive function [10]. AChE's active site consists of two subsites: the anionic site and the esteratic subsite. The esteratic subsite contains the catalytic triad of amino acids involving in hydrolyzing acetylcholine [11,12]. Acetylcholinesterase inhibitors (AChEIs) are commonly prescribed to address reduced levels of acetylcholine in AD. They inhibit the enzyme AChE, increasing the level and duration of acetylcholine action. AChEIs bind to the peripheral anionic site and prevent the breakdown of acetylcholine. In some cases, such as organophosphate poisoning, this inhibition can be toxic due to the accumulation of acetylcholine in the synapses [13]. The cholinesterase inhibitor drugs which inhibit AChE activity are rivastigmine, galantamine and donepezil. They decrease the breakdown of acetylcholine, maintaining its level [14]. Rivastigmine inhibits AChE and its catalytic activities, while galantamine interacts with the anionic subsite and modulates nicotinic cholinergic receptors [15]. Donepezil binds to the catalytic site of the enzyme, controlling key symptoms of AD [16]. These drugs do not halt disease progression but improve memory and cognitive impairment. They are the main marketed drugs for Alzheimer's treatment.

Monoamine oxidase-B is an enzyme in the body that breaks down several chemicals in the brain, including dopamine [17]. Dopamine is a neurotransmitter responsible for coordinating movement and muscle control [18]. In PD, there is a loss of dopaminergic neurons in the substantia nigra, leading to reduced dopamine levels [19, 20, 21]. MAO-B inhibitors, such as selegiline and rasagiline, are used to address deficit. These inhibitors block MAO-B activity, preventing the breakdown of dopamine and increasing its availability in the brain [22, 23]. By inhibiting MAO-B, these medications help maintain higher dopamine levels, improving neurotransmission and alleviating PD symptoms.

However, excessive inhibition of MAO-B can lead to adverse effects due to excessive dopamine in the brain [22, 24]. Excess dopamine in different brain region can result in psychosis, addiction [25], or movement disorders [4, 26]. Therefore, MAO-B inhibitors must be used in a targeted and controlled manner to prevent excess accumulation of dopamine in the brain, with careful monitoring to maximize therapeutic effects and minimize potential harm.

Bryophyllum pinnatum

Bryophyllum pinnatum (*B. pinnatum*), also known as the air plant or leaf of life, is a succulent plant with medicinal potentials [27]. It contains various phytochemicals such as flavonoids, alkaloids, tannins, saponins, sterols, and glycosides [28, 29, 30]. Notable phytochemicals reported in *B. pinnatum* include quercetin-3-o-rutinoside, kaempferol-3-o-rutinoside, and quercetin-3-o-glucoside [31]. Traditionally, it has been used topically in for wound healing and treating skin conditions [32, 33]. It has been employed to alleviate respiratory symptoms [34, 35, 36], reduce inflammation [37, 38], address gastrointestinal issues [39, 40], and exhibit antioxidant and potential anti-diabetic properties [41, 42, 43, 44].

Computer-Aided Drug Design

Computer Aided Drug Design (CADD) is a highly effective approach for expediting the drug discovery process and reducing time [45, 46]. It employs various methods, such as database screening and generation of novel scaffolds, to efficiently identify potential drug leads [47]. Molecular docking, a crucial method CADD was used in this study. It investigates how tiny compounds such as phytochemicals interact or behave in a target protein active region [48]. Among the techniques employed are ligand library development and preparation from an online

database, target retrieval from a PDB database, receptor grid generation, molecular docking, and ADME-Tox Screening.

Material and Methods

Target Preparation and Molecular Docking

Schrödinger Suite software (Maestro 12.8) was used to conduct computer-based drug screening. A total of 121 compounds that have been described with *B. Pinnatum* and the standard drugs were collected from an online database and docked to the active site of MAO-B and AChE to predict compounds with the best inhibitory potential to inhibit MAO-B and AChE action in the treatment of Parkinson's disease and Alzheimer's disease. The 3D structure of MAO-B in complex with rosiglitazone (PDB ID: 4A7A) and AChE in complex with Donepezil (PDB ID:7E3H) was downloaded from RCSB protein data bank (<https://www.rcsb.org>). The structure was imported and the protein preparation wizard tool in Maestro's Schrodinger Suite was used to prepare the protein. Bond orders were assigned, hydrogens were added, zero-order metal bonds were made, disulfide bonds were created, water molecules were removed, and het states were generated using Epik at pH 7.0 ± 2.0 during the protein production.

The protein was refined by optimizing the H-bond assignment and PROPKA pH 7.0 was used. Water molecules were removed beyond hets 3.0 Å then restrained minimization was done using force field OPLS4 (Optimized potentials for liquid simulation) to minimize the protein. The Ramachandran's Plot was then deduced and saved. The Glide tool of the maestro Schrodinger package was used to perform the molecular docking using the generated receptor grid file. The prepared ligands were docked following the standard docking protocols which includes High Throughput Virtual Screening (HTVS), Standard Precision (SP), setting the ligand sampling to rigid and Extra Precision (XP) setting the ligand sampling to rigid and flexible.

Receptor Grid Generation

The receptor grid depicts the area where the ligand and protein interact [49]. The Receptor Grid Generation tool was used to create the prepared protein grid on the binding site (Glide Grid) [50]. Selecting the co-crystallized ligand at the active site of 7E3H and 4A7A revealed the binding location. A cubic grid box including all the amino acid residues at the active site was automatically produced. The produced grid's three-dimensional coordinates X, Y, and Z were -43.57 Å, 37.99 Å & -30.21 Å and 50.73 Å, 157.29 Å & 29.10 Å for AChE and MAO-B respectively.

Ligand preparation

The selected 121 ligands used for this study were gotten from Pubchem (<https://pubchem.ncbi.nlm.nih.gov>). The compound(ligands) was downloaded in SDF format. The ligands were imported and prepared using Ligprep tool Maestro 12.8 using OPLS4 force field and neutralizing the ionization and generation of at most 1 stereoisomer per ligand.

ADMET screening

The pharmacokinetic profile, drug-likeness, and toxicity study of the ligands were determined using the SwissADME (<http://www.swissadme.ch>), Pro-Tox II online servers (https://tox-new.charite.de/protox_II), and ADMETSar was then used to carry out the ADMET properties prediction [51]. The ADMET properties of a compound shows the absorption, distribution, metabolism, excretion, and toxicity in and through the human body [52]. MW (Molecular weight), HBA (number of hydrogen bond acceptors), HBD (number of hydrogen bond donors), ILogP, Logkp and TPSA (Topological polar surface area) were calculated on the basis of Lipinski rule of five [53].

MM/GBSA

The Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) was used to determine the docked protein–ligand complex binding free energy [54]. To complete this project, we selected the protein-ligand complex and VGSB salvation model and OPLS4 force field was used.

Receptor Ligand Complex Pharmacophore Modelling

Pharmacophore was used to identify the compounds that match the pharmacophoric features of the reference ligands of the target receptor. The crystal structure of the protein AChE (PDB ID:7E3H) and MAO B (PDB ID:4A7A) in complex with the reference ligand (Donepezil for AChE and Rosiglitazone for MAO-B) was used. Pharmacophore hypothesis tool in Schrodinger maestro 12.8 was used to develop pharmacophore and identify the pharmacophoric features exhibited by the reference ligand, and it revealed the features; Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD), Aromatic ring and hydrophobic region. The selected compounds were subjected to pharmacophore feature matching screening using phase ligand screening module, (2/3 matches for 4a7a) and (4/5 matches for 7e3h). After the screening, all hit compound were subjected to molecular docking and their fitness score were also gotten.

Results

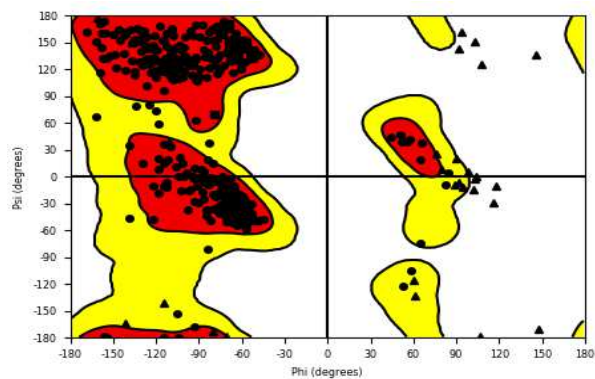


Fig. 1i: Ramachandran Plot of the crystal structure of Monoamine oxidase B MAO-B (4A7A)

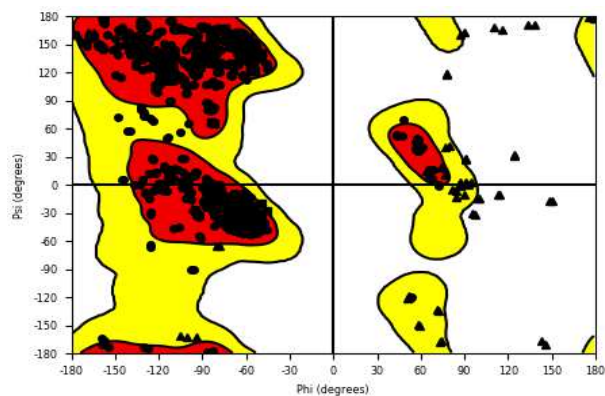


Fig. 1ii: Ramachandran Plot of the crystal structure of Acetylcholinesterase AChE (7E3H)

Compound name	Docking Score (Kcal/mol)	MM/GBSA (ΔG_{bind})
Quercetin	-11.582	-24.93
Taxifolin	-11.446	-40.38
Betaxanthin	-11.322	-48.79
Catechin	-10.557	-38.26
Pinoresinol	-10.281	-35.82
Myricetol	-10.221	-38.33
Chlorogenic Acid	-9.549	-36.92
Hesperetin	-9.508	-26.53
Ellagic acid	-9.415	-34.33

Chrysin	-9.214	-18.54
Artocarpone	-8.997	-25.30
Morin	-8.974	-32.76
Inositol	-8.773	-23.84
Anthrone	-8.748	-33.50
Peonidin	-8.692	-60.74
Kaempferol	-8.578	-36.30
Superoxide dismutase	-8.388	-23.87
Xylose	-8.179	-42.93
Fructose	-8.127	-41.59
Caffeic acid	-8.061	-37.77

TABLE 1i: Docking and MM/GBSA scores of the top 20 phytochemicals of *B. pinnatum* with the crystal structure of Monoamine oxidase-B (4A7A)

Compound name	Docking Score (Kcal/mol)	MM/GBSA (ΔG_{bind})
Myricitol	-13.609	-50.59
Taxifolin	-13.234	-56.25
Catechin	-12.827	-54.04
Kaempferitrin	-12.826	-40.4
Thiamine	-11.762	-63.00
Isorhamnetin	-11.550	-46.64
Betuletol	-11.435	-47.48
Artocarpone	-11.405	-33.13
Morin	-11.269	-22.74
Kaempferol	-11.257	-47.52
Hesperetin	-11.126	-32.32
Betaxanthin	-11.041	-20.89
Chlorogenic acid	-10.898	-29.61
Betacyanin	-10.853	-63.96

Pinoresinol	-10.767	-36.90
Ellagic acid	-10.726	-67.11
Methyl brevifolincarboxylate	-10.669	-57.13
Peonidin	-10.651	-78.44
Artoflavanone	-10.612	-36.27
Betalains	-10.542	-47.36

TABLE 1ii: Docking and MM/GBSA scores of the top 20 phytochemicals of *Bryophyllum pinnatum* with the crystal structure of Acetylcholinesterase (7E3H).

Compound name	Docking Score (Kcal/mol)	MM/GBSA (Δ Gbind)
Chrysin	-9.214	-18.54
Pinoresinol	-10.281	-35.82
Anthrone	-8.748	-33.50
Ferulic acid	-7.939	-35.93
Selegiline	-7.416	-24.89
Rasagiline	-7.161	-21.48

TABLE 2i: Docking and MM/GBSA scores of the selected phytochemicals of *B. pinnatum* and the standard Monoamine oxidase-B inhibitors with the crystal structure of Monoamine oxidase-B (4A7A).

Compound name	Docking Score (Kcal/mol)	MM/GBSA
Draculyic acid	-7.490	-28.59
Octanoic acid	-6.406	-31.47
Niacin	-7.102	-26.11
Pinoresinol	-10.767	-36.90
Coumarin	-7.696	-40.03
Donepezil	-11.186	-54.30
Galantamine	-7.002	-35.25

Rivastigmine	-6.104	-29.57
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TABLE 2ii: Docking and *MM/GBSA* scores of the selected phytochemicals of *B. pinnatum* and the standard Acetylcholinesterase inhibitors with the crystal structure of Acetylcholinesterase (7E3H).

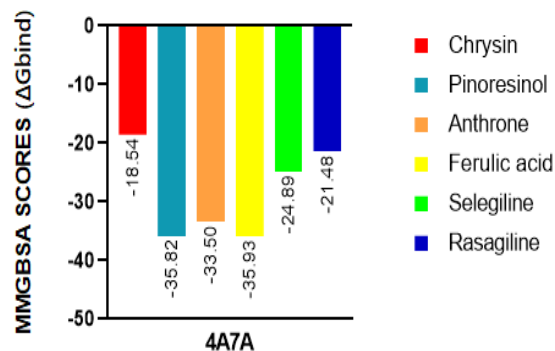


Fig. 2i: Graphical representation of the *MM/GBSA* scores of the selected phytochemicals of *B. pinnatum* and the standard Monoamine oxidase-B inhibitors with the crystal structure of Monoamine oxidase-B (4A7A).

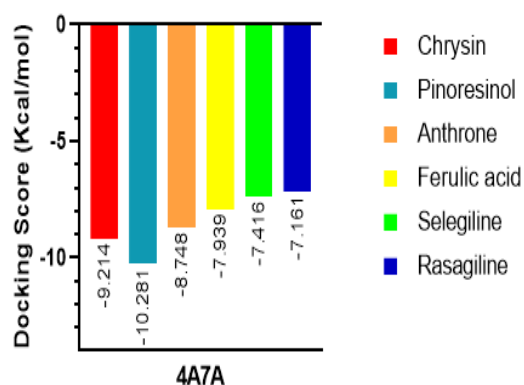


Fig. 2ii: Graphical representation of the docking scores of the selected phytochemicals of *B. pinnatum* and the standard Monoamine oxidase-B inhibitors with the crystal structure of Monoamine oxidase-B (4A7A).

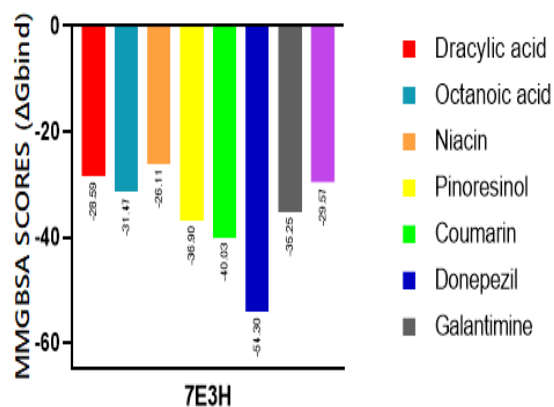


Fig 3i: Graphical representation of the *MM/GBSA* scores of the selected phytochemicals of *B. pinnatum* and the standard Acetylcholinesterase inhibitors with the crystal structure of Acetylcholinesterase (7E3H).

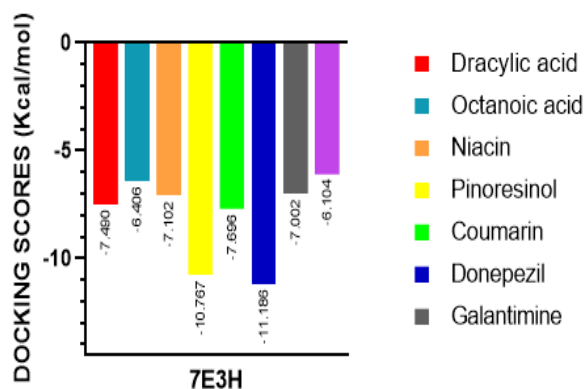


Fig 3ii: Graphical representation of the docking scores of the selected phytochemicals of *Bryophyllum pinnatum* and the standard Acetylcholinesterase inhibitors with the crystal structure of Acetylcholinesterase (7E3H).

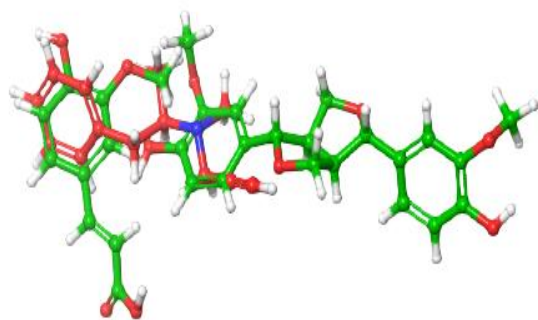


Fig. 4i. Superimposed ball-and-stick view of the native (red) and docked (green) pose of the reference ligand (Selegiline) in the active site of MAO-B.

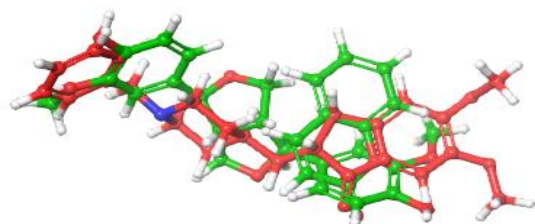


Fig. 4ii. Superimposed ball-and-stick view of the native (red) and docked (green) pose of the reference ligand (Donepezil) in the active site of AChE.

Compound Name (4A7A)	Fitness Score
Chrysin	0.407
Pinoresinol	1.848
Anthrone	0.412
Ferulic acid	0.423
Selegiline	0.421

TABLE 3i: Fitness scores of the phytochemicals of *B. pinnatum* and the standard MAO-B inhibitors with the crystal structure of Monoamine oxidase-B (4A7A).

Compound Name (7E3H)	Fitness Score
Octanoic acid	0.117
Pinoresinol	1.563
Coumarin	0.182
Donepezil	2.53
Galantamine	1.423
Rivastigmine	1.492

TABLE 3ii: Fitness scores of the phytochemicals of *B. pinnatum* and the standard AChE inhibitors with the crystal structure of Acetylcholinesterase (7E3H).

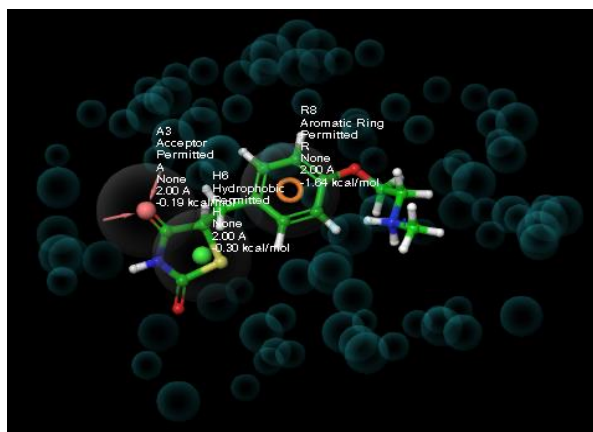


Fig 5i. The pharmacophore features of the reference ligand and the crystal structure of Monoamine oxidase B (4A7A)

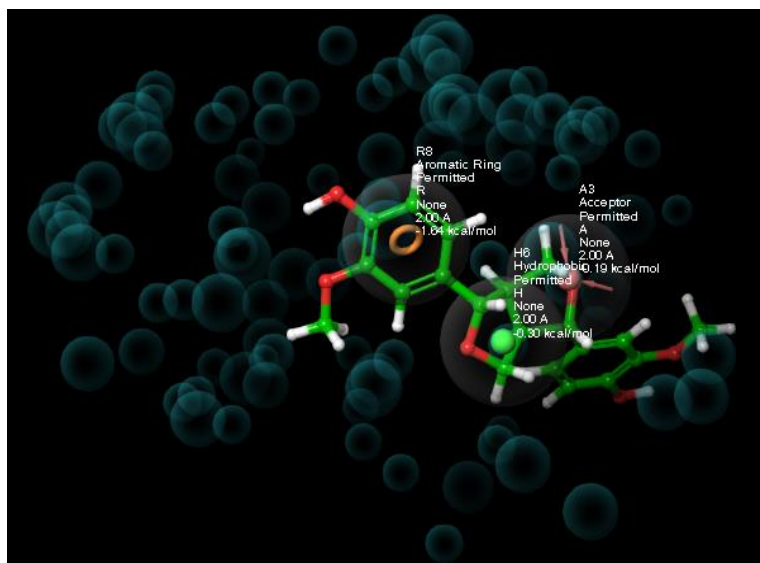


Fig. 5ii: The pharmacophore features of *B. pinnatum*'s ligand: pinoresinol and the crystal structure of Monoamine oxidase-B (4A7A)

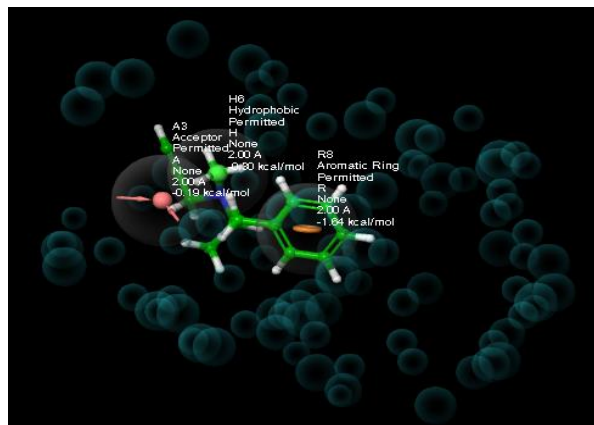


Fig. 5iii: The pharmacophore features of a standard drug, selegiline and the crystal structure of Monoamine oxidase-B (4A7A).

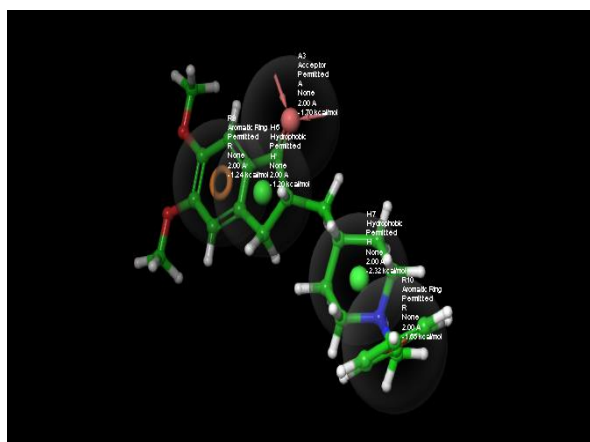


Fig 6i: The pharmacophore features of the reference ligand and the crystal structure of Acetylcholinesterase (AChE).

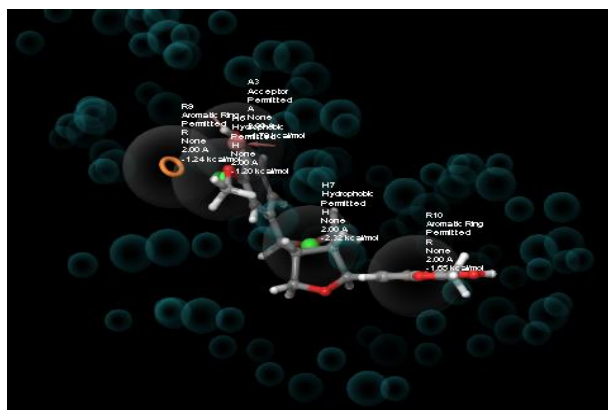


Fig. 6ii: The pharmacophore features of *B. pinnatum*'s ligand: pinoresinol and the crystal structure of Acetylcholinesterase (7E3H).

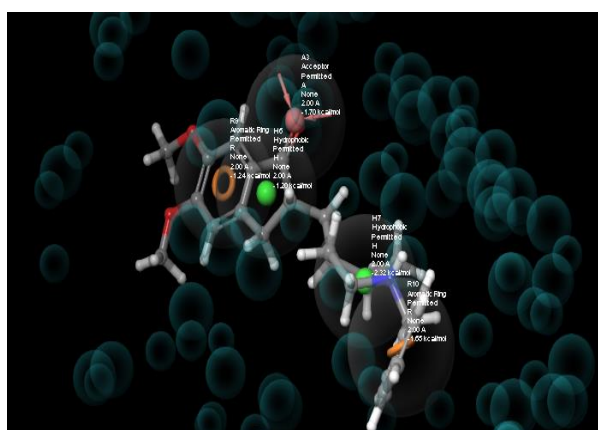
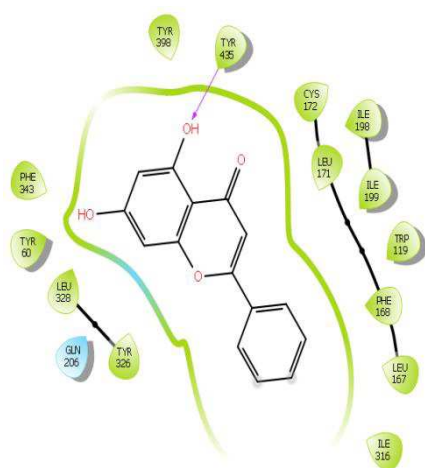
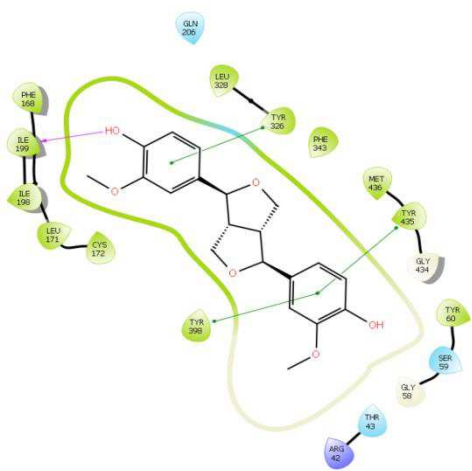


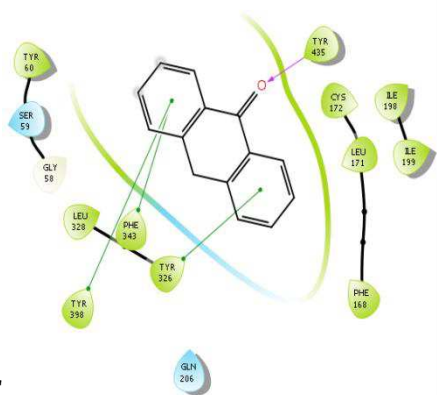
Fig. 6iii: The pharmacophore features of the standard drug (donepezil) and the crystal structure of Acetylcholinesterase (7E3H).



A



B



C

D



Fig. 7i: 2D-Molecular interactions of amino-acid residues of the crystal structure of MAO-B (4A7A) with the photochemical constituents of *B. pinnatum*: **A** Chrysin **B** Pinoresinol **C** Anthrone **D** Ferulic acid.

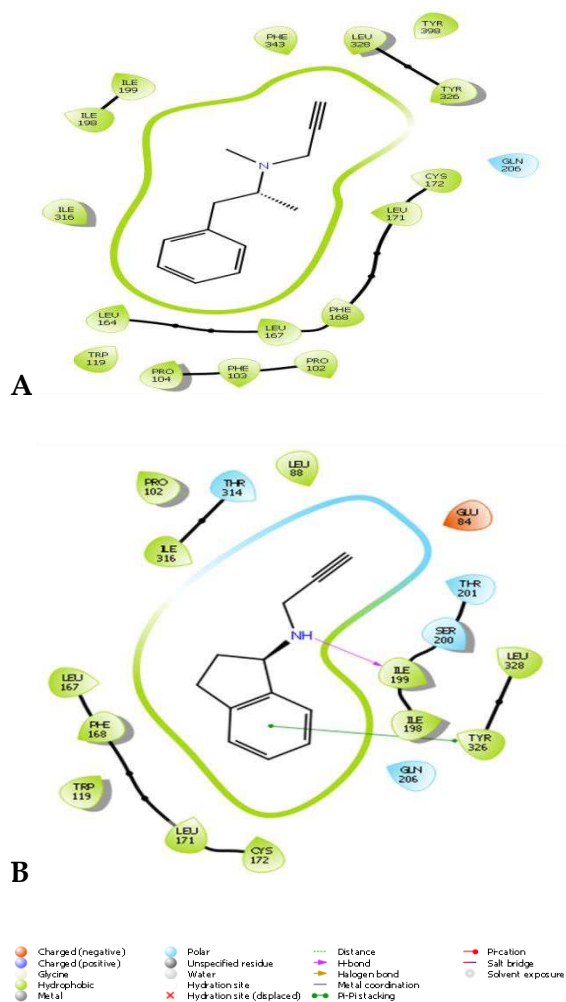
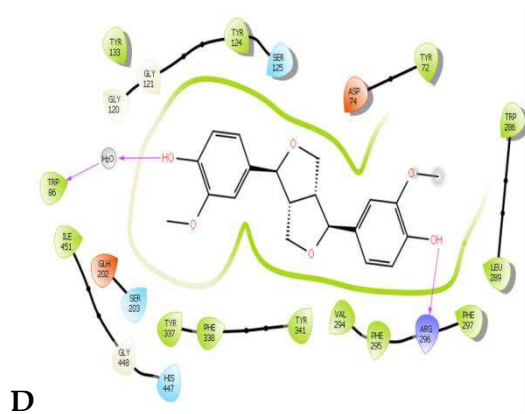
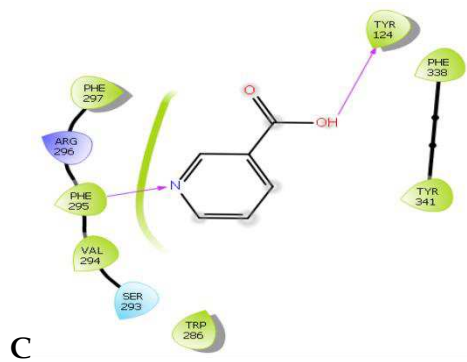
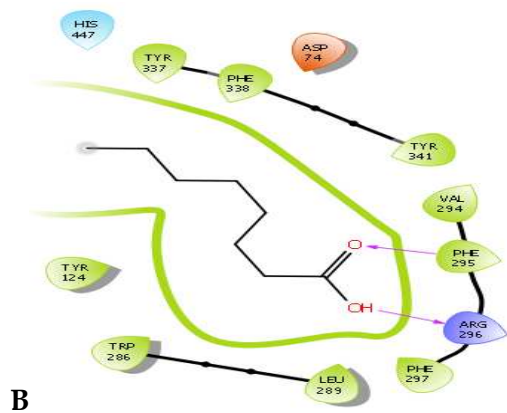
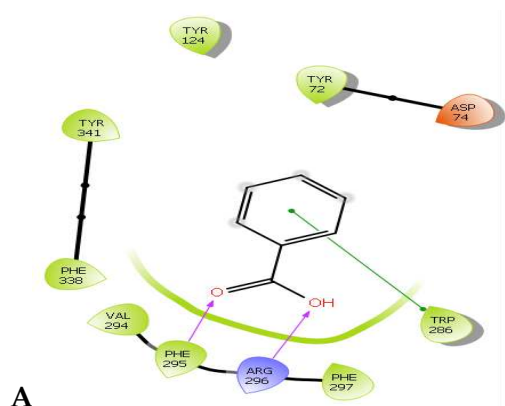


Fig. 7ii: 2D-Molecular interactions of amino-acid residues of the crystal structure of MAO-B (4A7A) with MAO-B inhibitors: **A** Selegiline **B** Rasagiline.



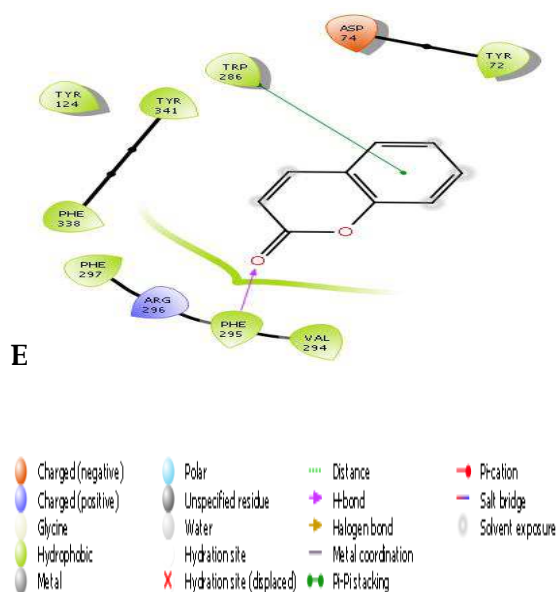
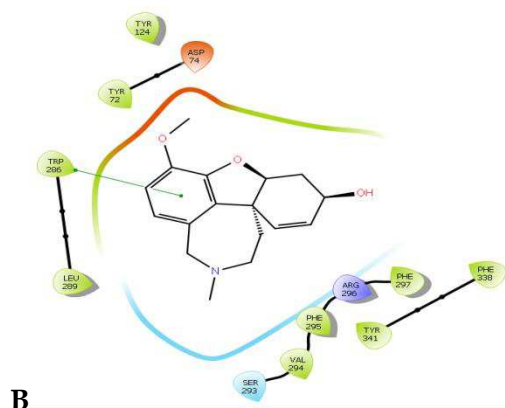
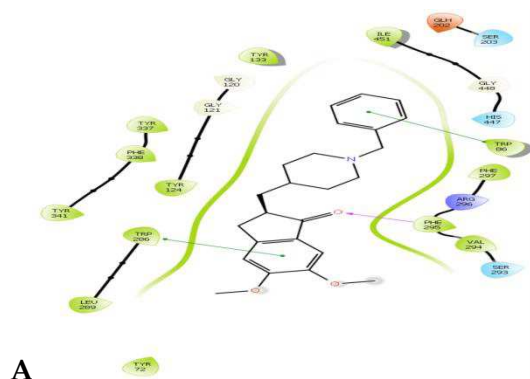


Fig. 8i: 2D-Molecular interactions of amino-acid residues of the crystal structure of AChE (7E3H) with the photochemical constituents of *B. pinnatum*: **A** Draculyic acid **B** Octanoic acid **C** Niacin **D** Pinoresinol **E** Coumarin.



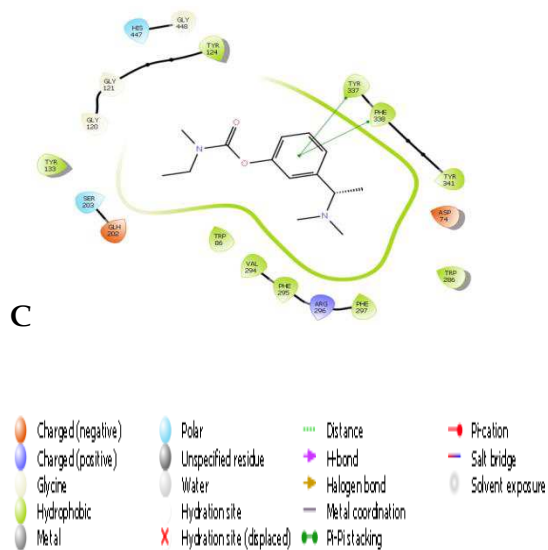
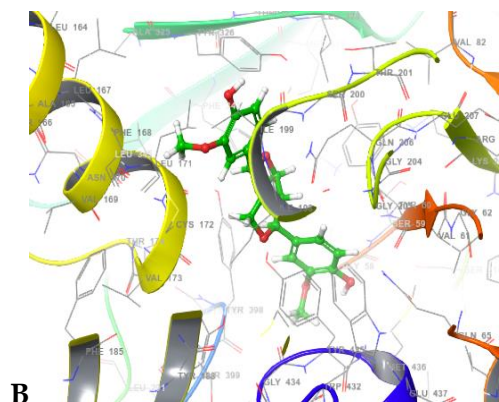
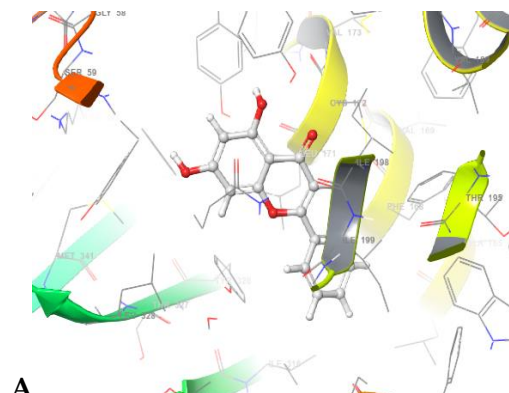


Fig. 8ii: 2D-Molecular interactions of amino-acid residues of the crystal structure of AChE (7E3H) with AChE inhibitors: **A** Donepezil **B** Galantamine **C** Rivastigmine.



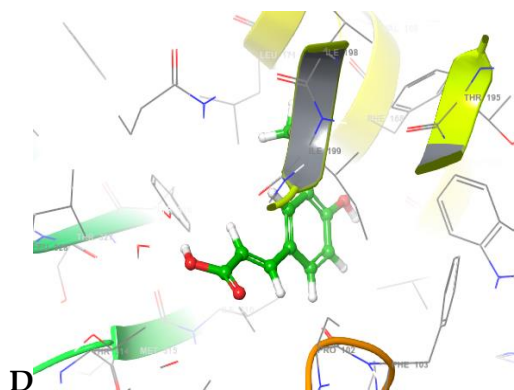
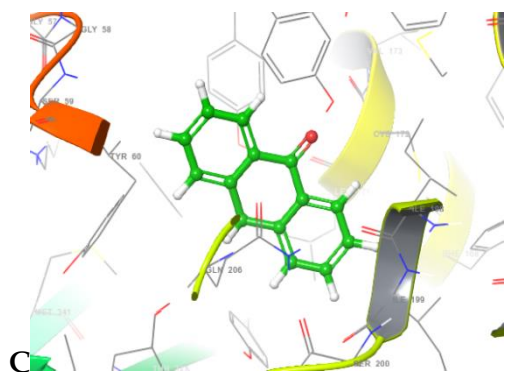
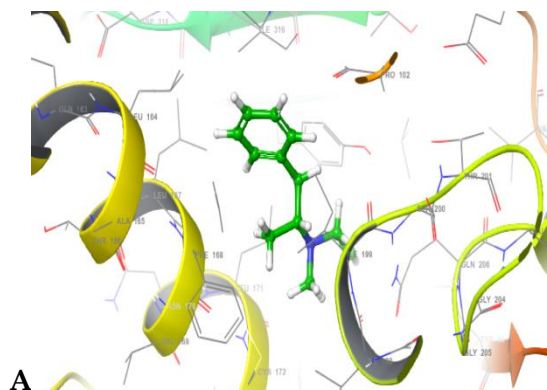


Fig. 9i: 3D-Molecular interactions of amino-acid residues of the crystal structure of Monoamine oxidase-B (4A7A) with the ligands of *B. pinnatum*: **A** Chrysin **B** Pinoresinol **C** Anthrone **D** Ferulic acid.



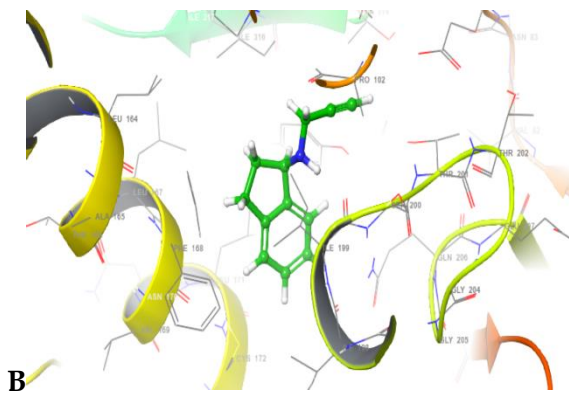
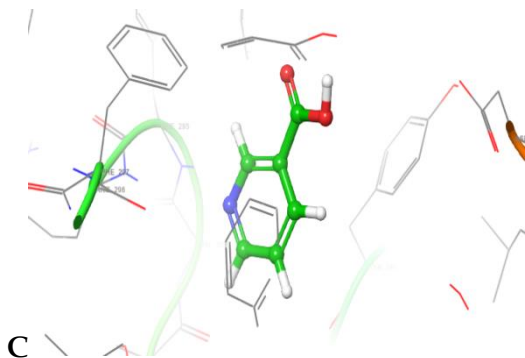
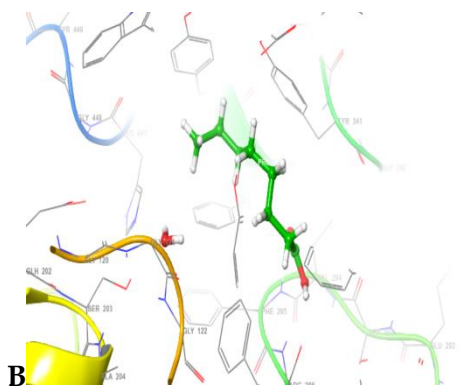


Fig. 9ii: 3D-Molecular interactions of amino-acid residues of the crystal structure of MAO-B (4A7A) with MAO-B inhibitors: **A** Selegiline, **B** Rasagiline.



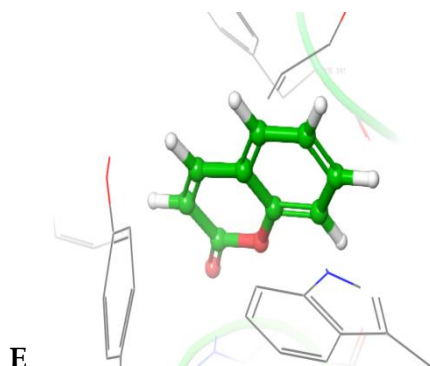
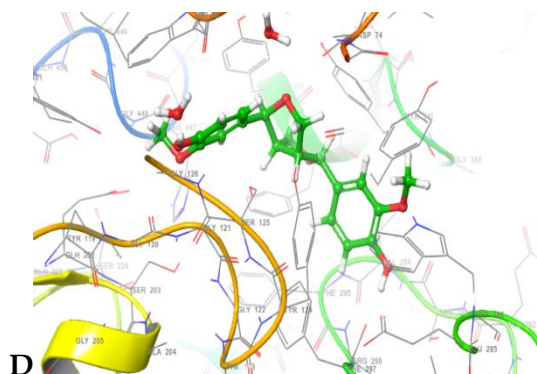
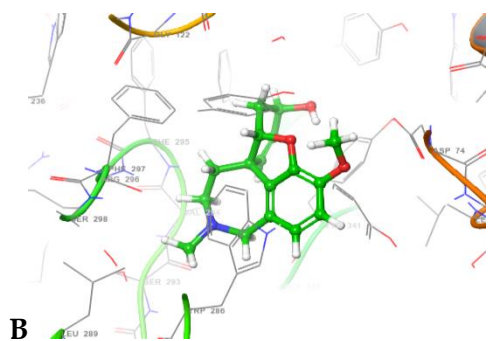
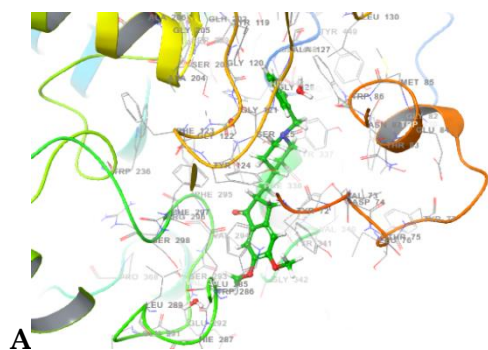


Fig. 10i: 3D-Molecular interactions of amino-acid residues of the crystal structure of AChE (7E3H) with the ligands of *B. pinnatum*: **A** Draclyic acid **B** Octanoic acid **C** Niacin **D** Pinorexinol **E** Coumarin.



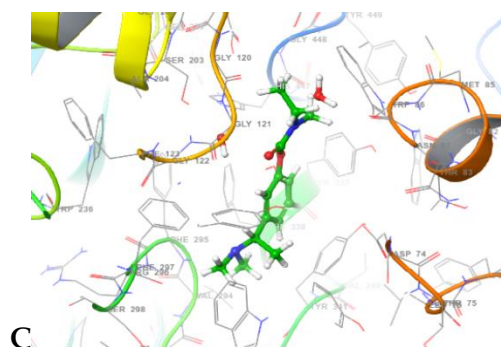


Fig. 10ii: 3D-Molecular interactions of amino-acid residues of the crystal structure of Acetylcholinesterase (7E3H) with AChE inhibitors: **A** Donepezil, **B** Galantimine, **C** Rivastigmine.

Compound Name	Docking Score (kcal/mol)	H-BOND	Hydrophobic interacting amino acids	Other Interactions
Chrysin	-9.214	TYR 435	TYR 398, TYR 435, CYS 172, LEU 171, PHE 168, LEU 167, ILE 316, ILE 198, ILE 199, TRP 119, PHE 343, TYR 60, LEU 328, TYR 326	None
Pinoresinol	-10.281	ILE 199	TYR 398, CYS 172, LEU 171, ILE 198, ILE 199, PHE 168, LEU 328, TYR 326, PHE 343, MET 436, TYR 435, TYR 60	Pi-Pi Stack: TYR 435, TYR 398, TYR 326
Anthrone	-8.748	TYR 435	TYR 60, LEU 328, PHE 343, TYR 326, TYR 398, PHE 168, LEU 171, CYS 172, TYR 435, ILE 198, ILE 199	Pi-Pi Stack: PHE 343, TYR 326, TYR 398
Ferulic acid	-7.939	THR 201, TYR 326	LEU 328, TYR 326, PHE 99, PRO 102, PHE 103, PRO 104, ILE 316, LEU 88, ILE 199, CYS 172, LEU 171, PHE 168, LEU 167, LEU 164, TRP 119	None
Selegiline	-7.416	None	TRP 199, ILE 316, ILE 199, ILE 198, CYS 172, LEU 171, PHE 168, LEU 167, LEU 164, PRO 104, PHE 103, PRO 102, TYR 326, LEU 328, PHE 343	None
Rasagiline	-7.161	ILE 199	LEU 88, ILE 316, PRO 102, LEU 167, PHE 168, TRP 119, LEU 171, CYS 172, TYR 326, ILE 198, ILE 199, LEU 328	Pi-Pi Stack: TYR 326

TABLE 4i: Hydrogen Bonds, Hydrophobic interactions, and other contacts of the selected ligands of *B. pinnatum* and the standard MAO-B inhibitors with the crystal structure of MAO-B (4A7A).

Compound Name	Docking Score	H-BOND	Hydrophobic interacting amino acids	Other Interactions
Draculyic acid	-7.490	PHE 295, ARG 296	TYR 124, TYR 72, TYR 341, TRP 286, PHE 297, PHE 295, VAL 294, PHE 338	Pi-Pi Stack: TRP 286
Octanoic acid	-6.406	ARG 296, PHE 295	TYR 337, PHE 338, TYR 341, VAL 294, PHE 295, PHE 297, LEU 289, TRP 286, TYR 124	None
Niacin	-7.102	PHE 295, TYR 124	TYR 341, PHE 338, TYR 124, PHE 297, PHE 295, VAL 294, TRP 286	None
Pinoresinol	-10.767	TRP 86, ARG 296	TYR 72, TYR 124, TYR 133, TRP 86, ILE 451, TYR 337, PHE 338, TYR 341, VAL 294, PHE 295, PHE 297, LEU 289, TRP 286	None
Coumarin	-7.696	PHE 295	TYR 72, TRP 286, TYR 341, TYR 124, PHE 338, PHE 297, PHE, 295, VAL 294	Pi-Pi Stack: TRP 286
Donepezil	-11.186	PHE 295	TYR 72, LEU 289, TRP 286, TYR 341, TYR 124, PHE 338, TYR 337, TYR 133, ILE 451, TRP 86, PHE 297, PHE 295, VAL 294	Pi-Pi Stack: TRP 286, TRP 86
Galantamine	-7.002	None	TYR 124, TYR 72, TRP 286, LEU 289, PHE 338, TYR 341, PHE 297, PHE 295, VAL 294	Pi-Pi Stack: TRP 286
Rivastigmine	-6.104	None	TYR 124, TYR 337, PHE 338, TYR 341, TRP 286, PHE 297, PHE 295, VAL 294, TRP 86, TYR 124	Pi-Pi Stack: TYR 337, PHE 338

TABLE 4ii: Hydrogen Bonds, Hydrophobic interactions, and other contacts of the selected ligands of *B. pinnatum* and the standard AChE inhibitors with the crystal structure of AChE (7E3H).

COMPOUNDS	MW	HBA	HBD	TPSA	ILOGP	LOGKP	ROV
Chrysin	254.24	4	2	70.67	2.27	-5.35	0
Pinoresinol	358.40	6	2	77.38	2.67	-6.87	0
Anthrone	194.23	1	0	17.07	2.21	-4.89	0
Ferulic acid	194.18	4	2	66.76	1.62	-6.41	0

Selegiline	187.28	1	0	3.24	2.80	-5.38	0
Rasagiline	171.24	1	1	12.03	2.51	-6.05	0

TABLE 5i: In-silico drug-likeness predictions of the selected *B. pinnatum* ligands and the standard MAO-B inhibitors.

COMPOUN DS	MW	HBA	HBD	TPSA	ILOGP	LOGKP	ROV
Dracrylic acid	122.12	2	1	37.30	1.11	-5.72	0
Octanoic acid	144.21	2	1	37.30	1.95	-5.01	0
Niacin	123.11	3	1	50.19	0.86	-6.80	0
Pinoresinol	358.40	6	2	77.38	2.67	-6.87	0
Coumarin	146.14	2	0	30.21	1.75	-6.20	0
Donepezil	379.50	4	0	38.77	3.92	-5.58	0
Galantamine	287.35	4	1	41.93	2.66	-6.75	0
Rivastigmine	250.34	3	0	32.78	3.20	-6.20	0

TABLE 5ii: In-silico drug-likeness predictions of the selected *B. pinnatum* ligands and the standard AChE inhibitors. (7E3H).



Positive/Active/Inhibitor
 Negative/Inactive/Non-inhibitor

Fig. 11i: A heat map illustration of the ADMET parameters of selected *B. pinnatum* phytochemicals and the standard MAO-B inhibitors (Selegiline and Rasagiline).

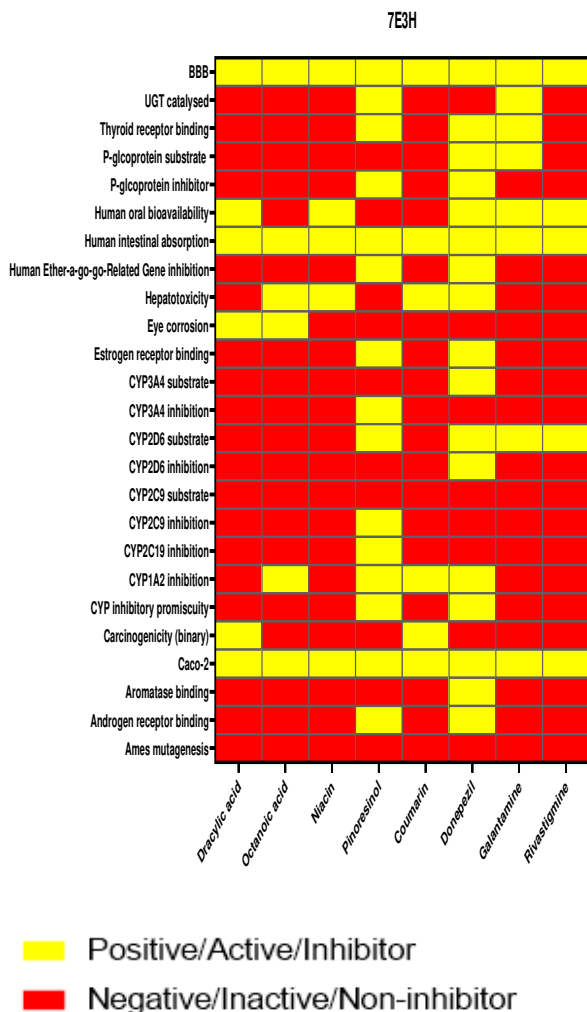


Fig. 11ii: A heat map illustration of the ADMET parameters of selected *B. pinnatum* phytochemicals and the standard AChE inhibitors (Donepezil, Galantamine and Rivastigmine).

Discussion

Neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease (PD) result in the gradual deterioration of specific groups of neurons [55]. Current therapeutic strategies focus on symptom management rather than disease modification. AD is characterized by progressive memory disorder, while PD primarily manifests motor symptoms due to the loss of dopaminergic neurons [56]. Pharmacological interventions such as acetylcholinesterase inhibitors (AChEI) for AD and MAO-B inhibitors for PD aim to alleviate symptoms by balancing neurotransmitters [57, 58]. *B. pinnatum* shows potential as a source of natural inhibitors against AChE and MAO-B, with promising compounds like Pinoresinol and ferulic acid. These findings suggest the plant's neuroprotective properties and its potential for developing novel therapeutics for AD and PD. In

this research, we assessed via *in-silico* study the ligands of *B. pinnatum* with the highest overall potential to be a medication based on its inhibitory ability, blood brain barrier permeability, and toxicity but not limited to against both MAO-B and AChE target proteins for PD and AD. This was thoroughly explored and compared with the standard drugs for MAO-B and AChE inhibition respectively.

The Ramachandran plot provides a statistical distribution of Phi and Psi dihedral angles for an unidentified amino acid (X) within an Ala-X-Ala tripeptide [52, 59]. It helps predict the secondary structure of a protein by analyzing the Psi and Phi values. Figure (1i) and (1ii) show the Ramachandran plots of the crystal structures of MAO-B (4A7A) and AChE (7E3H), respectively, which aid in understanding the secondary structure of these proteins.

Table 1(i) and (ii) present the docking scores and MM/GBSA values of the top scoring 20 ligands of *B. pinnatum* screened against MAO-B and AChE, respectively. Table 2(i) and (ii) include the selected ligands and standard drugs, that can penetrate the Blood-Brain Barrier (BBB) for neurologically related diseases. Chrysin, pinorexinol, anthrone, and ferulic acid exhibit strong binding energy with MAO-B, while draculyic acid, octanoic acid, niacin, pinorexinol, and coumarin show strong binding energy with AChE. These ligands outperformed synthetic inhibitors in terms of docking scores and binding free energy as demonstrated in table 2(i) and (ii).

The selected ligands, particularly pinorexinol, show promising potential as inhibitors for MAO-B and AChE. It can penetrate the Blood-Brain Barrier and have superior docking scores compared to the standard drugs. Furtherly, the compound demonstrates a high binding affinity for MAO-B and the second best for AChE, suggesting its potential as a novel and effective treatment for these neurological diseases. In the context of Molecular Mechanics/Generalized Born Surface Area (MM/GBSA), a more negative value for the binding affinity indicates a stronger binding between a ligand and a target [60, 61]. The ligands exhibit stronger binding affinity, as indicated by more negative MM/GBSA scores, compared to the standard drugs. Figures (2i), (2ii), (3i), and (3ii) provide graphical representations of the docking scores and MM/GBSA values for the ligands and standard inhibitors.

Another computational parameter evaluated was pharmacophores, it is an essential molecular feature responsible for a compound's biological activity and interaction with target receptors or enzymes [62, 63]. The reference ligands of MAO-B and AChE crystal structures have specific pharmacophore features such as aromatic rings, hydrophobic regions, and hydrogen bond acceptors as shown in Fig 5i and 6i respectively. Pinorexinol, a phytochemical constituent of *B.*

pinnatum, shares common pharmacophore features with the reference ligands of both MAO-B and AChE indicating its potential for binding and activity similar to these reference ligands (Fig 5ii, 5iii, 6ii and 6iii).

The Ball and stick representation of the superimposed *B. pinnatum* ligands and the MAO-B and AChE inhibitors further showed the structural similarities and functional groups as confirmed by the pharmacophore screening enabling their binding affinity (Fig. 4i and 4ii respectively).

The fitness score of a drug measures its effectiveness and suitability for treating a specific condition [64]. In the case of selected phytochemical constituents from *B. pinnatum*, Table 3i indicates that these ligands have higher fitness scores compared to standard MAO-B inhibitors. Ligands like ferulic acid and pinoreosinol even have higher fitness scores than the standard drugs selegiline and rasagiline. Similarly, Table 3ii shows that the selected ligands have high fitness scores, particularly pinoreosinol, compared to standard AChE inhibitors. These factors suggest the potential of these phytochemicals as excellent blockers of MAO-B and AChE.

The 2D-molecular interactions depicted in Figure 7 and 8 and the 3D-molecular interactions depicted in Figure 9 and 10 show that the selected ligands and standard drugs exhibit some common hydrogen bonds, pi-pi stacking, and hydrophobic interactions with the amino acid residues of MAO-B (4A7A) and AChE (7E3H) crystal structures. These interactions are essential for ligand-protein binding, complex stability, and specificity. The selected ligands, chosen for their BBB-crossing ability [65], demonstrate strong hydrophobic interactions with the target proteins, as shown in Table 4(i) and 4(ii). For example, ferulic acid exhibits more hydrophobic interactions with MAO-B (ID: 4A7A) than the standard drug rasagiline, while pinoreosinol has more hydrophobic interactions with AChE (ID: 7E3H) than the standard drugs galantamine and rivastigmine. These findings suggest that the selected ligands could serve as potent antagonists of MAO-B and AChE in brain disorders, potentially surpassing the efficacy of standard drugs. Pi-pi stacking and hydrophobic interactions play crucial roles in protein folding, thermal stability, and ligand binding [66].

Table 5(i) and (ii) present the *in-silico* druglikeness predictions of *B. pinnatum* phyto-constituents and standard inhibitors with the target proteins. These predictions are based on the Lipinski Rule of Five (ROV), BBB, and toxicity study which evaluates the drug-like properties of a compound. ROV states that a compound should not exceed 500 Daltons in molecular weight (MW) [67], have no more than 10 hydrogen bond acceptors (HBA) and 5 hydrogen bond donors (HBD) [68], and have a LogP (partition coefficient) value not less than five [69]. Adhering to these rules is crucial

for a compound to be considered a drug [59, 70, 71]. The selected compounds, including those from *B. pinnatum* and standard inhibitors, comply with all the Lipinski ROV criteria, indicating their potential as orally active drugs. The absorption, distribution, metabolism, excretion, and toxicity properties of the selected *B. pinnatum* phytochemicals and standard inhibitors were assessed using admetSAR. Fig 11i displays a heat map of ADMET parameters, where yellow rectangles indicate positive or active compounds, and red rectangles indicate negative or inactive compounds.

The heat map reveals that all the compounds, including those from *B. pinnatum*, can cross the BBB. Additionally, chrysin, pinoreesinol, and ferulic acid exhibit high UGT catalysis, facilitating efficient metabolism and elimination of these compounds. Furthermore, chrysin, pinoreesinol, and anthrone show positive results for thyroid receptor binding, which is important for modulating neuroinflammation and oxidative stress in neurodegenerative disorders.

The study indicates that the selected compounds, including pinoreesinol, are non-substrates for P-glycoprotein, allowing for greater bioavailability and efficacy. Pinoreesinol also acts as an inhibitor of P-glycoprotein, enhancing the effectiveness and bioavailability of co-administered drugs. Anthrone and ferulic acid exhibit high oral bioavailability, indicating a significant fraction reaching the systemic circulation after oral administration. The compounds do not inhibit the human ether-go-go-related gene and can be absorbed by the human intestine. Chrysin and pinoreesinol are not hepatotoxic and do not cause damage to the liver.

Drug eye corrosion is the ability of a drug to cause damage to the eye and the surrounding tissues [72]. Chrysin, pinoreesinol and anthrone have no eye damaging properties. Cytochrome P450 is an enzyme that is involved in the metabolism of drugs and the most important isoforms of cytochrome P450 are CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 [73]. Thus, inhibition of cytochrome P450 isoforms can affect the metabolism of drugs and increase the risk of toxicity. Furthermore, all the compounds display no carcinogenicity or mutagenic effects based on the ADMET test data.

The heat map analysis (Fig 11ii) demonstrates that all selected compounds, including pinoreesinol, can cross the BBB. Pinoreesinol exhibits high catalysis by uridine glucuronyl transferase, aiding in the efficient metabolism and elimination of the compound. It also shows positive binding to thyroid receptors, which play a crucial role in neuroinflammation and oxidative stress modulation. Pinoreesinol acts as an inhibitor of P-glycoprotein, enhancing the effectiveness and bioavailability of co-administered drugs by impeding their transport out of cells. Dracylic acid and niacin have high oral bioavailability and are non-inhibitors of the human ether-go-go-related gene. All tested compounds can be absorbed by the human intestine. Dracylic acid and pinoreesinol are non-

hepatotoxic, posing no harm to the liver. Niacin, pinorexinol, and coumarin do not cause eye corrosion. The compounds do not exhibit inhibition of crucial cytochrome P450 isoforms, reducing the risk of drug metabolism interference. Octanoic acid, niacin, and pinorexinol do not show carcinogenicity effects. These compounds tested exhibit no mutagenic effects.

Overall, the test AChE inhibitors displayed significant inhibitory activity against AChE, suggesting its potential as a candidate for enhancing cholinergic neurotransmission and addressing cognitive impairments associated with AD. The test MAO-B inhibitors on the other hand, demonstrated significant inhibitory activity against MAO-B, pronouncing their potential in maintaining dopamine levels and alleviating motor symptoms in PD. The identification of phytochemicals in *Bryophyllum pinnatum* with inhibitory potential against AChE and MAO-B highlights its pharmacological relevance and validates its traditional medicinal uses. The binding free energies of these compounds to AChE and MAO-B were found to have a good correlation with other experimental inhibitory activities and the standard drugs.

Conclusion

This study explored *B. pinnatum* phyto-ligands as a source of natural inhibitors against AChE and MAO-B which are enzymes implicated in the pathophysiology of AD and PD respectively. The phytochemical composition of *B. pinnatum* was structurally analyzed, and the inhibitory effects of its compounds on AChE and MAO-B were evaluated. The phytochemical analysis revealed the presence of various bio-active compounds, including flavonoids, alkaloids, phenolics, and terpenoids in *B. pinnatum*. These compounds possess diverse pharmacological activities and have shown potential in the management of neurodegenerative diseases. In this study, Pinorexinol, octanoic acid, dracyleic acid, naicin and coumarin; ferulic acid, chrysin, anthrone and pinorexinol showed to be excellent blockers of AChE and MAO-B, respectively for the treatment of neurological disorders (AD and PD). The findings of this study contribute to the growing body of evidence supporting the use of natural products as sources of therapeutic agents for neurodegenerative disorders. Pinorexinol is strategically the best blocker of both enzymes in this study so it holds promise as lead molecules for further optimization and development of both therapeutics and treatment for AD and PD. However, *in-silico* study is pivotal in drug development, it is also important to verify these findings using *in-vivo* and *in-vitro* methods on the plant's ligand.

List of abbreviations

AChE: acetacholinesterase

MAO-B: Mono-amine oxidase B

AD: alzheimers' disease

PD: parkinson's disease

CADD: Computer Aided Drug Design

ADMET: adsorbtion distribution metabolism excretion and toxicity

PDB: Protein Data Bank

BBB: blood brain barrier

ROV: rule of thumb or Lipinski's rule of five

HBA: hydrogen bond acceptor

HBD: hydrogen bond donor

MM/GBSA: molecular mechanics/generalized born surface area

OPLS4: optimized potentials for liquid simulation-4

Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Availability of data and material

All data are available on the internet database (NCBI) or will be made available on request.

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