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Original Article

In vitro and *in silico* Acetylcholinesterase Inhibitory Activity of *Premna serratifolia* Linn.

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ABSTRACT

Background: *Premna serratifolia* Linn. (PS) was studied for phytochemical properties and antioxidant activity, as well as for its ability to inhibit the enzyme Acetylcholinesterase (AchE) and dock with PS molecules. **Materials and Methods:** An LC-MS/MS and GC-MS analysis of dried leaf extracts was performed for phytochemical identification, radical scavenging assays, and enzyme-based AchE inhibitory activity, as well as structurally-based molecular design of AchE inhibitors. **Results:** PS leaf extract was screened using phytochemical methods such as GC-MS and LC-MS/MS to identify its active constituents. DPPH radical (alcoholic 49.94 µg/mL and hydroalcoholic 49.89 µg/mL) was significantly inhibited by PS extracts at 20 and 40 µg/mL, as were their respective IC_{50} values. PS-alcoholic and PS-hydro alcoholic leaf extracts inhibited AchE by 49.45 µg/mL and 49.56 µg/mL, respectively. **Conclusion:** PS inhibits AchE and *in vitro* radicals scavenges, demonstrating its potential for reversing cholinergic deficits in Alzheimer's patients.

Keywords: *Premna serratifolia* Linn, Antioxidants, Acetylcholinesterase, Phytochemicals, Molecular docking, GC-MS/LC-MS analysis, Oxidative stress.

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INTRODUCTION

Worldwide, millions of people are affected by neurodegenerative diseases.[1] A neurodegenerative disease that causes behavioral changes, memory loss, and depression, AD causes behavioral changes, memory loss, and cognitive decline. There is currently no cure for AD.[2] Treatment consisting of acetylcholinesterase inhibitors such as tacrine, galantamine, donepezil, and rivastigmine is a comprehensive approach to managing acetylcholine-related disorders.[3] There have been over 200 promising drugs that have failed clinical trials in the last decade, suggesting the disease and its causes are complex. The current focus of Alzheimer's disease research is on symptomatic treatments and finding a cure.[4] A growing number of herbal remedies and medicinal plants are being used as complementary and alternative therapies for AD, and these plants are being used to develop new drugs.[5]

Drug discovery and structural molecular biology depend on molecular docking and computer-aided drug design. Ligand-protein docking predicts how a ligand will interact with a three-dimensional receptor.[6] Biovivo Discovery Studio was used to study the binding orientations and binding affinities of phenolic compounds. These studies were designed to determine how phenolic compounds interact with the active site of acetylcholinesterase.

Despite their long history of use, traditional herbal remedies have received little scientific research, despite their safety and efficacy. Traditional medicine recommends many plants to improve cognitive function and alleviate symptoms associated with Alzheimer's, including fatigue, memory loss, and depression.[7] Ayurvedic medicinal plants have been studied for their ability to inhibit acetylcholine esterase activity, a well-studied therapeutic target implicated in multiple neurological disorders.[8] Studies have shown that medicinal plants and their phytochemicals have the potential to treat Alzheimer's disease.[9] An extremely widespread Lamiaceae plant, *Premna serratifolia* Linn., is found in tropical and subtropical regions of the world.[10] Trees or shrubs with many branches and relatively short trunks with dark green, opposite leaves.[11] According to Singh *et al.*, 2021, PS methanolic root extract can inhibit hepatoblastoma cancer cell growth.[11] PS leaves extracts exhibit antioxidant activity, DNA protection, and metabolic enzyme activity (glucosidase, amylase, xanthine oxidase, protease, etc.,).[12] An ethanol extract of PS showed anti-arthritic effects when rats were treated with Freund's adjuvant for 21 days.[13] This extract acts as an antioxidant and scavenger

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of radicals, such as superoxide, nitric oxide, hydroxyl, DPPH, and ABTS. Additionally, the compound inhibits cytotoxicity in MCF7, HepG2, and A549 cells.[14] Many Ayurvedic drugs, such as dashmool, are prepared from the Ayurvedic medicinal plant.[15] There are many diseases that can be treated by PS leaves, including colic, rheumatism, and neuralgia.[16] The authentication of ayurvedic medicinal plants used in the preparation of herbal drugs remains a challenge. *Premna serratifolia* Linn. (PS) has been authenticated using molecular and pharmacognostic methods in our laboratory.[17] This study aimed to identify phytochemicals by LC-MC/MS and GC-MS, determine AchE inhibitory activity, and predict binding orientations and affinity predictions of phenolic compounds with AchE activity.

MATERIALS AND METHODS

Plant collection

Premna serratifolia Linn. (PS) leaves were collected from the Regional Ayurvedic Research Institute in Pune, Maharashtra, India. This plant specimen was authenticated by Dr. Arun M. Gurav, and was deposited with the RARI, Pune, Maharashtra, India, with accession number SVUBS 4536 (Figure 1).

Powder preparation

Using an electric blender, shade-dried leaves were ground into coarse powder, which was then stored in an airtight container.

Extract preparation

The coarsely ground air-dried leaves were transferred to a 250 mL conical flask, and 100 mL of solvent was added. For the first six hours, the mixture was shaken frequently, and then it was allowed to stand for 18 hr. After 18 hr, the solution was filtered with Whatman Filter Paper No. 1. Hydro-alcoholic and alcoholic filtered solutions were evaporated using a rotary vacuum evaporator. After that, alcoholic, and hydro-alcoholic solutions were lyophilized to create a concentrated powder. The yield of the extraction method was then calculated.

Qualitative phytochemical analysis

According to standard procedures, alcoholic and hydroalcoholic extracts were screened for phytochemical content.[18-20]

GC-MS analysis

The GC-MS analysis was conducted at the Sophisticated Instrumentation Facility (SIF), School of Advanced Sciences, Chemistry Division, Vellore Institute of Technology University, Vellore, Tamil Nadu, India. To compare the GC-MS spectra with those in databases of known components stored in the NIST (2008) library, the components were compared to those in the NIST (2008) database.[21]

LC-MS/MS analysis

Two grams of *Premna serratifolia* leaf powder was accurately weighed, dissolved in 10 mL of acidified methanol (formic acid), and vortexed for five minutes. After solubilization, the mixture was centrifuged at 4000 RPM for five minutes at 15°C. In HPLC vials, the upper layer was collected and filtered through nylon syringe filters, and then injected into the LC-MS/MS instrument. A triple quad combined with Alliance HPLC with an autosampler and PDA detector was used by Agilent Technologies to analyze polyphenolic compounds using LC-MS/MS-6460. With Multiple Reaction Modes (MRM), the MS/MS data from the validation standards were integrated into MassHunter Quantitative Analysis B.10.0 (Agilent Technologies).[22]

In vitro radical scavenging assay

DPPH assay

The 2,2-Diphenyl-1-picrylhydrazyl assay is the most commonly used antioxidant assay for extracts. This method is used to determine the antioxidant scavenging capacity of extracts. We used the previously described method of Gomez-Alonso *et al.*, 2003[23,24] with minor modifications to evaluate the radical scavenging activity of the extract. Fifty microliters of extract or standard antioxidant solution was added to 150 µL of DPPH solution (0.1 mM DPPH in methanol). The DPPH solution and extracts were then mixed properly and allowed to stand at room temperature for 30 min. The absorbance of the resultant mixture was measured at 517 nm using a spectrophotometer. Butylated Hydroxy Anisole (BHA) was used as a reference.

FRAP assay

Plant extracts and standards were determined using the Oyaizu (1986) method with minor modifications.[25] In a 96-well plate, about 30 µL of plant extract or positive control will be mixed with phosphate buffer (30 µL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (30 µL, 1%). The mixture was incubated at 50°C for 20 min. A portion (30 µL) of trichloroacetic acid (10% in PBS) was added to the mixture, and then 120 µL of distilled water and Ferric Chloride ($FeCl_3$) (25 µL, 0.1%) were added. The mixture was mixed well, and the reaction was allowed to incubate at room temperature for 30 min. At 700 nm, the absorbance was measured using a microplate reader. BHT was used as the reference material. An average of three observations was used for the graph, and all tests were performed in triplicate.

Anticholine esterase assay

The assay for Acetylcholinesterase (AChE) inhibition was standardized in 96-well microplates according to the methodology described previously (Mathew & Subramanian, 2014).[26,27] In the 96-well plates, 100 µL of 3 mM Ellman's reagent (DTNB), 20 µL of 0.26 U/mL AChE, 40 µL of 50 mM Tris pH 8.0 buffer, and 20 µL of drug/standard galanthamine at different concentrations

dissolved in buffer were added to the wells. After gentle mixing, the plate was incubated for 15 min at RT, and then the absorbance was measured at 412 nm in a microplate reader. These readings were used as blanks. The enzymatic reaction was initiated by the addition of 20 µL of 15 mM Acetylthiocholine Iodide (ATCI), and the hydrolysis of acetylthiocholine was monitored by reading the absorbance every 30 sec for 20 min. The positive control was galantamine hydrobromide. As a result, all reactions were performed in triplicate. The percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = (E\ S)/E \times 100$$

where E is the activity of the enzyme without extract/positive control and S is the activity of the enzyme with the extract. The IC_{50} values were calculated based on the % inhibition value of the positive controls or plant extracts.

Molecular docking

A molecular docking study was conducted on the compounds identified by LC-MS/MS. In this experiment, galantamine was used as a positive control. All identified compounds were retrieved from Pubchem for their Three-Dimensional (3D) structures (http://pubchem.ncbi.nlm.nih.gov). Discover Studio's small molecule tab provides the option to prepare ligands for downloaded compounds (Figure 2). The CHARMm force field was used to minimize compounds, generate conformations, and create separate isomers.[28] The 3D structure of the protein can be downloaded from a website (http://www.rcsb.org/pdb). Docking was carried out on the crystal structure of human acetylcholinesterase PDB ID 1B41 with a resolution of 2.76 (Figure 3). BIOVIA Discovery Studio (DS) 2022 was used to prepare the proteins. In addition to removing water molecules and bound ligands, hydrogen was added to the protein.[29] Additionally, CHARMm was used to minimize the protein's energy consumption. The binding pocket of the AChE protein was identified using Discovery Studio's "Define and edit binding site" protocol.[30]

Statistical analysis

Statistical analysis was performed using one way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using Statistical Package of Social Science (SPSS) version 10.0 for windows. The values are mean ±SD for three experiments in each group. p values ≤ 0.05 were considered as level of significance.

RESULTS

Qualitative test

Table 1 shows the phytochemical constituents of hydroalcoholic and alcoholic leaf extracts of *Premna serratifolia* Linn. The hydroalcoholic extract contained carbohydrates, reducing sugars, proteins, steroids, saponins, flavonoids, tannins, and phenols. The alcoholic extract contained reducing sugars, proteins, steroids, cardiac glycosides, flavonoids, and phenols.

Figure 1: Photography images of *Premna serratifolia* Linn.

GC-MS analysis

Figure 4 shows a phytochemical screening study of *Premna serratifolia* Linn. using GC-MS analysis. Based on the retention time, the gas chromatogram illustrates the relative concentrations of various compounds eluted. The heights of the peaks indicate the relative concentrations of the components in the ethanolic leaf extract. The structure and nature of compounds eluted at different times were determined by mass spectrometry. Mass spectra are fingerprints of compounds identified in the NIST library databases. A list of the compounds identified by the GC-MS analysis can be found in Table 2.

LC-MS/MS analysis

The leaves were examined by LC-MS/MS for identification and quantification of four phenolic compounds: gallic acid (3.16 µg/mL), chlorogenic acid (0.56 µg/mL), quercetin (1.25 µg/mL), and luteolin (0.30 µg/mL) mL). A chromatogram of the identified compounds is shown in Figure 5. Table 3 provides the quantity of each phenolic compound in each chromatogram.

In vitro radical scavenging assay

Figures 6 and 7 show the results of the DPPH and FRAP assays. The alcoholic and hydro-alcoholic extracts of PS inhibited DPPH activity in a dose-dependent manner at 49.94 µg/mL and 49.89 µg/mL, respectively, when compared with standard butylated hydroxy toluene (49.88 µg/mL). The results were found to be statistically significant ($p < 0.05$). In the DPPH radical assay and the FRAP assay at 100 µg/mL, PS had good scavenging activity. Further increases in PS concentrations did not significantly increase the scavenging effect at the optimum concentration of 100 µg/mL; perhaps this is due to saturation of the system with PS.

AchE inhibitory activity

Figure 8 shows acetylcholinesterase inhibition. There was significant inhibition of acetylcholinesterase at 5 different concentrations (20, 40, 60, 80, and 100 µg/mL) and the IC_{50} values were 49.45 µg/mL for the alcoholic extract and 49.56 µg/mL for the hydro-alcoholic extract of PS. The alcoholic and hydroalcoholic extracts of PS showed significant inhibition compared to galantamine hydrobromide. It was determined that the IC_{50} of standard galantamine was 46.73 µg/mL. The results were found to be statistically significant ($p < 0.05$).

Molecular docking

The hydrogen bonds formed by galanthamine are shown in Figure 9 (A). The hydrophobic interactions were shown at residues PHE A:338, PHE A:298, GLY A:120, GLY A:122, GLU A:202, SER A:203, ALA A:204, TYR A:446, HIS A:446, PRO A:446, TYRA:124, ASN A:87, ASP A:74, TRP A:86, TYR A:341, THR A:83, GLY A:82, TYR A:337, GLY A:448, TRP A:438, PRO A:446, TYR A:446, TYR A:449, and CDOCKER interaction energy -37.22 Kcal/mol. Compared with galanthamine, Phytol had the greatest CDOCKER interaction energy of -56.22 Kcal/mol. A

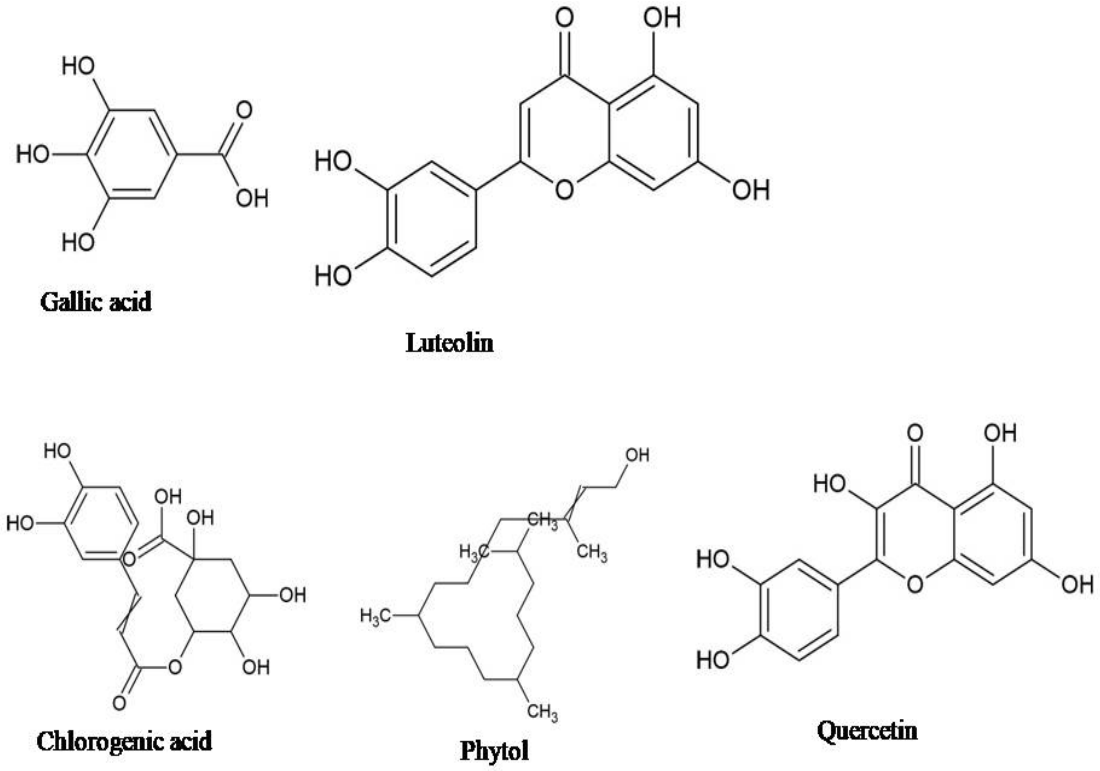

Figure 2: Chemical structure of compounds identified by LC-MS/MS and GC-MS analysis.

Figure 3: X-ray crystallography structure of Acetylcholine esterase (PDB ID of 1B41).

hydrogen bond was formed between phytol and GLU, as shown in Figure 9 (B). Additionally, several hydrophobic interactions were formed between GLY A:126, ALA A:127, GLY A:120, TYR A:119, GLY A:448, GLY A:122, TRP A:286, ARG A:296, VAL A:294, GLU A:202, GLY A:121, ALA A:204, ILE A:451, SER A:203, HIS A:447, TYR A:124, TYR A:449, TYR A:341, PHE A:297, PHE A:295, and PRO B:31. The CDOCKER interaction energy of luteolin is -37.22 (Table 4), and four hydrogen bonds are formed, as shown in Figure 10 (A). PRO A:446, TYR A:448, GLY A:448, ILE A:451, GLY A:120, SER A:203, TYR A:133, GLY A:121, GLY A:126, LEU A:130, SER A:125, PRO A:88, ASN A:87, GLN A:71, VAL A:73, TYR A:341, MET B:33, TYR A:337, TYR A:124, TRP A:86. Figure 10 (B) shows that quercetin formed two hydrogen bonds at TYR A:124 and ASN A:87 with a CDOCKER interaction energy of -30.23 Kcal/mol.

DISCUSSION

In ancient Ayurvedic medicine, herbal remedies were prescribed to enhance memory and cognition.[31] Plant-based therapies may improve memory and cognition in people with neurodegenerative diseases, including Ginkgo biloba, Panax ginseng, and Salvia officinalis.[32] Alzheimer's disease is the most common and detrimental condition affecting the quality of life and general health of the elderly. In Medhya Rasayana, herbs and/or herbo-mineral preparations delay the onset of dementia and enhance neurocognitive function.[33] Our study investigated plants antioxidant activity as well as potential inhibitors of cholinesterase.

Phytochemicals, which are compounds plants produce to protect themselves, are bioactive compounds derived from plants. Phytochemical-rich foods include whole grains, fruits, vegetables, nuts, and herbs.[34] Foods contain a variety of phytochemicals, such as carotenes, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides.[35] Additionally, phytochemicals have antimicrobial, antidiarrheal, antihelmintic, antiallergic, antispasmodic, and antiviral properties. Phytochemicals in plants regulate gene transcription, improve gap junction communication, boost immunity, and prevent lung and prostate cancer.[36] In the present study, qualitative tests, GC-MS/LC-MS analysis, and various *in vitro* assays were used to assess PS phytochemical content, antioxidant properties, and acetylcholinesterase inhibition. The antioxidant properties of PS were found to be concentrated dependent. A phytochemical screening of PS leaf extracts identified fifteen chemical constituents based on qualitative and GC-MS analysis. An LC-MS/MS triple quadrupole analysis of phenolic compounds in leaves revealed flavonoids as the most abundant phenolic compounds.

As shown by our *in vitro* results, 100 µg/mL PS showed optimal inhibition of cholinesterase activity. The results of the DPPH and FRAP assays of PS can be attributed to the presence of phytol, squalene, β-sitosterol and γ-sitosterol which donate hydrogen

Table 1: Phytocomponents identified in the ethanolic leaf extract of *Premna serratifolia* Linn. by GC-MS analysis.

Sl. No.	RT (Min)	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	17.53	Phytol	$C_{20}H_{40}O$	296	7.65
2	17.84	Octadecyne	$C_{18}H_{34}$	250	3.19
3	18.07	1-Octadecyne	$C_{18}H_{34}$	250	9.26
4	19.13	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}O_9N$	343	1.91
5	19.84	Palmitic acid	$C_{16}H_{32}O_2$	256	15.94
6	20.36	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	5.71
7	21.21	3-Decyn-2-ol	$C_{10}H_{18}O$	154	5.62
8	21.39	Z,Z-10,12-Hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	280	12.94
9	21.84	2-Pentadecyn-1-ol	$C_{15}H_{28}O$	224	6.72
10	22.33	19,19-Dimethyl-eicosa-8,11-dienoic acid	$C_{22}H_{40}O_2$	336	11.91
11	23.51	Beta-Sitosterol	$C_{33}H_{58}OSi$	498	6.55
12	24.79	Squalene	$C_{30}H_50$	410	8.33
13	25.78	Silane, [[(3. beta.)-gorgost-5-en-3-yl]oxy]trimethyl-	$C_{33}H_{58}OSi$	498	1.74
14	26.91	Pseduosarsasapogenin-5,20-dien	$C_{27}H_{42}O_3$	414	1.12
15	27.40	(2s,3s)-(-)-3-Propyloxiranemethanol	$C_6H_{12}O_2$	116	1.33

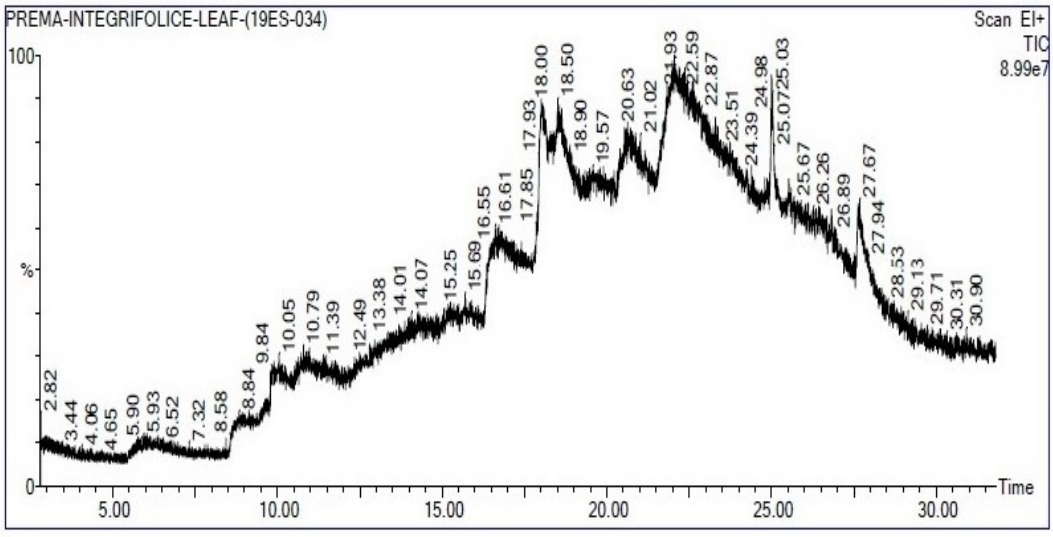

Figure 4: GC-MS analysis of leaf extract of *Premna serratifolia* Linn.

Table 2: **Qualitative estimation of *Premna serratifolia* Linn. leaves.**

Sl. No.	Phyto constituent tested	Test performed	*Premna serratifolia* Linn. leaves	
			Hydroalcoholic extract	Alcoholic extract
1	Carbohydrates	Molish's test	+	-
2	Reducing sugar	Fehling's test	+	+
		Benedict's test	+	+
3	Pentose sugar	Phloroglucinol Reagent test	-	-
4	Hexose sugar	Tollen's Phloroglucinol Test	-	-
		Cobalt chloride test	++	++
5	Protein	Biuret test	-	-
6	Amino acid	Ninhydrin Test	-	-
		Test for cysteine	-	-
7	Steroids	Libermann-Burchard Test	-	-
		Salkowaski reaction	-	-
8	Glycoside	General test	-	-
9	Cardiac glycosides	Legal test	-	+
		Keller-Killiani Test	-	+
10	Anthroquinone glycoside	Borntrager's test	-	-
		Modified Borntrager's test	-	-
11	Saponins	Foam test	+	-
		Lead acetate solution test	+	-
12	Coumarin glycoside	Aromatic odour test	-	-
		Fluorescence test	-	-
13	Flavonoids	Shinoda test	+	+
		Lead acetate test	+	-
14	Alkaloids	Dragendorff's test	-	-
		Mayer's test	-	-
		Wagner's test	-	-
15	Tannins	Lead acetate solution test	+	-
		Gelatin solution test	+	-
16	Phenol	Neutral Fecl3 test	+	-
		Indophenol reaction	+	+
17	Starch	Iodine test	-	-

Table 3: **Phytocomponents identified by LC-MS/MS analysis.**

Sl. No.	Name of the compound	RT (Min)	Molecular weight	Molecular formula	Concentration (µg/mL)
1	Gallic acid	3.02	170.12	$C_7H_6O_5$	3.16
2	Chlorogenic acid	5.14	354.31	$C_{16}H_{18}O_9$	0.56
3	Quercetin	7.72	302.23	$C_{15}H_{10}O_7$	1.25
4	Luteolin	7.65	286.24	$C_{15}H_{10}O_6$	0.30

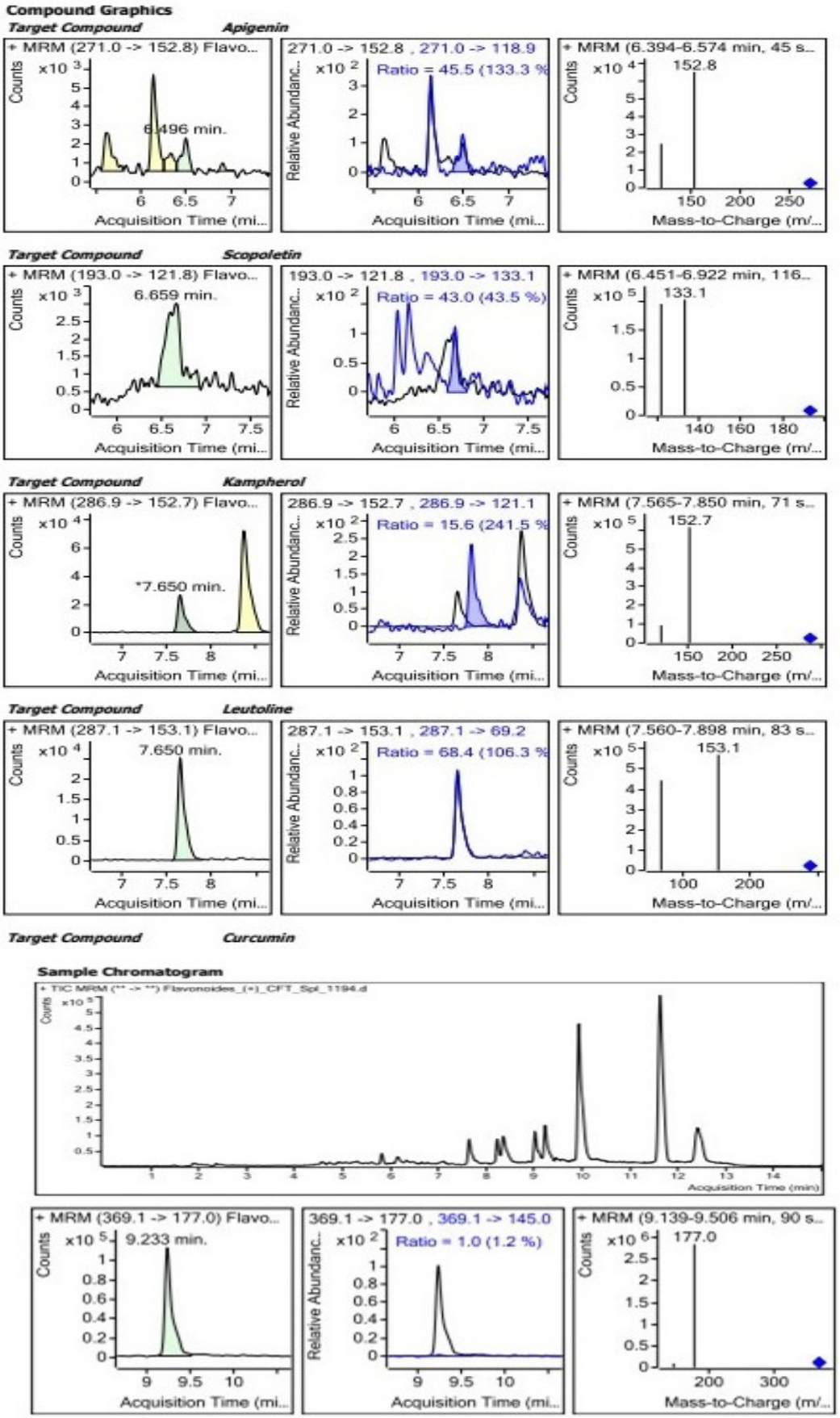

Figure 5: LC-MS/MS analysis of leaf extract of *Premna serratifolia* Linn.

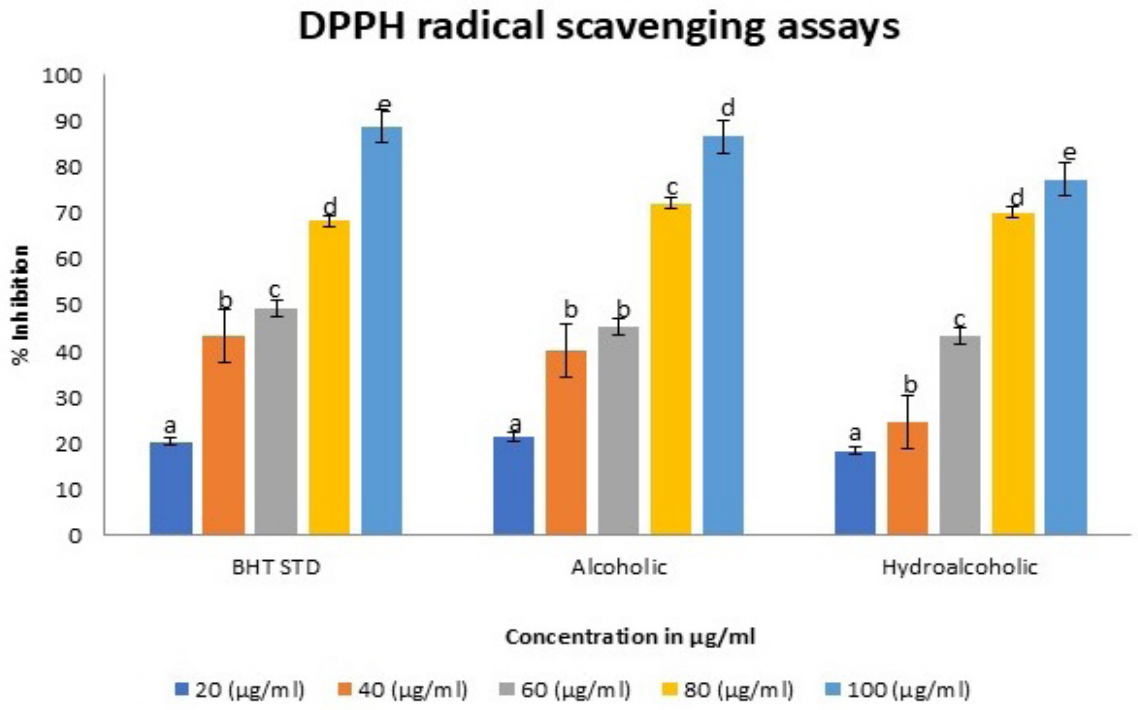

Figure 6: DPPH radical scavenging assays of alcoholic and hydroalcoholic extract of *Premna serratifolia* Linn. Values are mean ± SD from three experiments in each group. Values not sharing a common superscript significantly at $p \leq 0.05$.

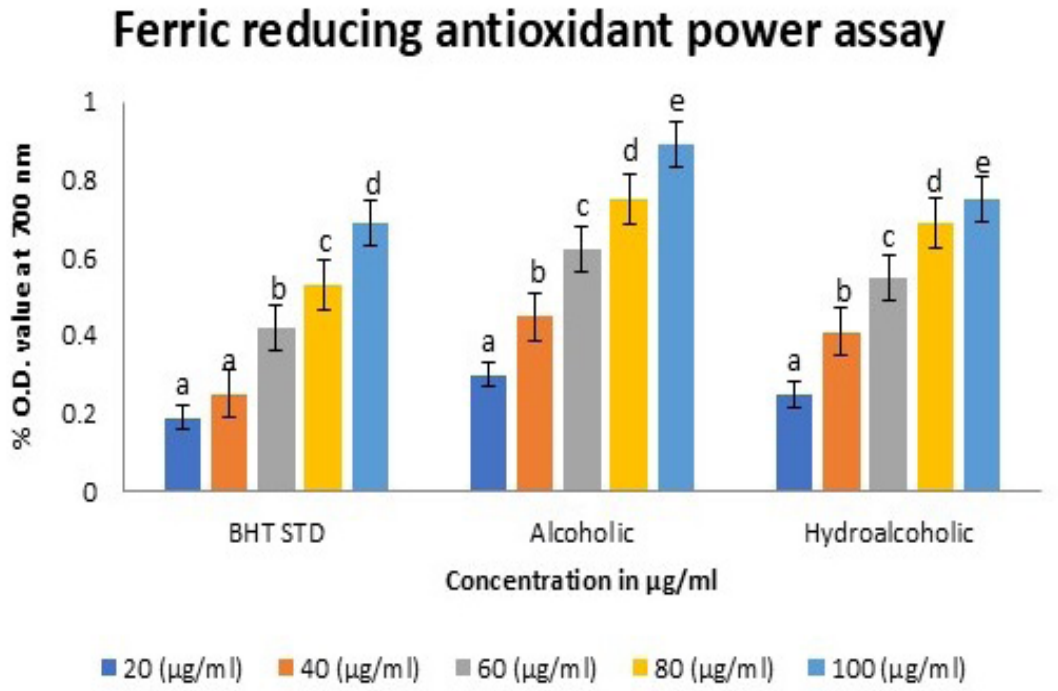

Figure 7: Ferric reducing antioxidant power of alcoholic and hydroalcoholic extract of *Premna serratifolia* Linn. Values are mean ± SD from three experiments in each group. Values not sharing a common superscript significantly at $p \leq 0.05$.

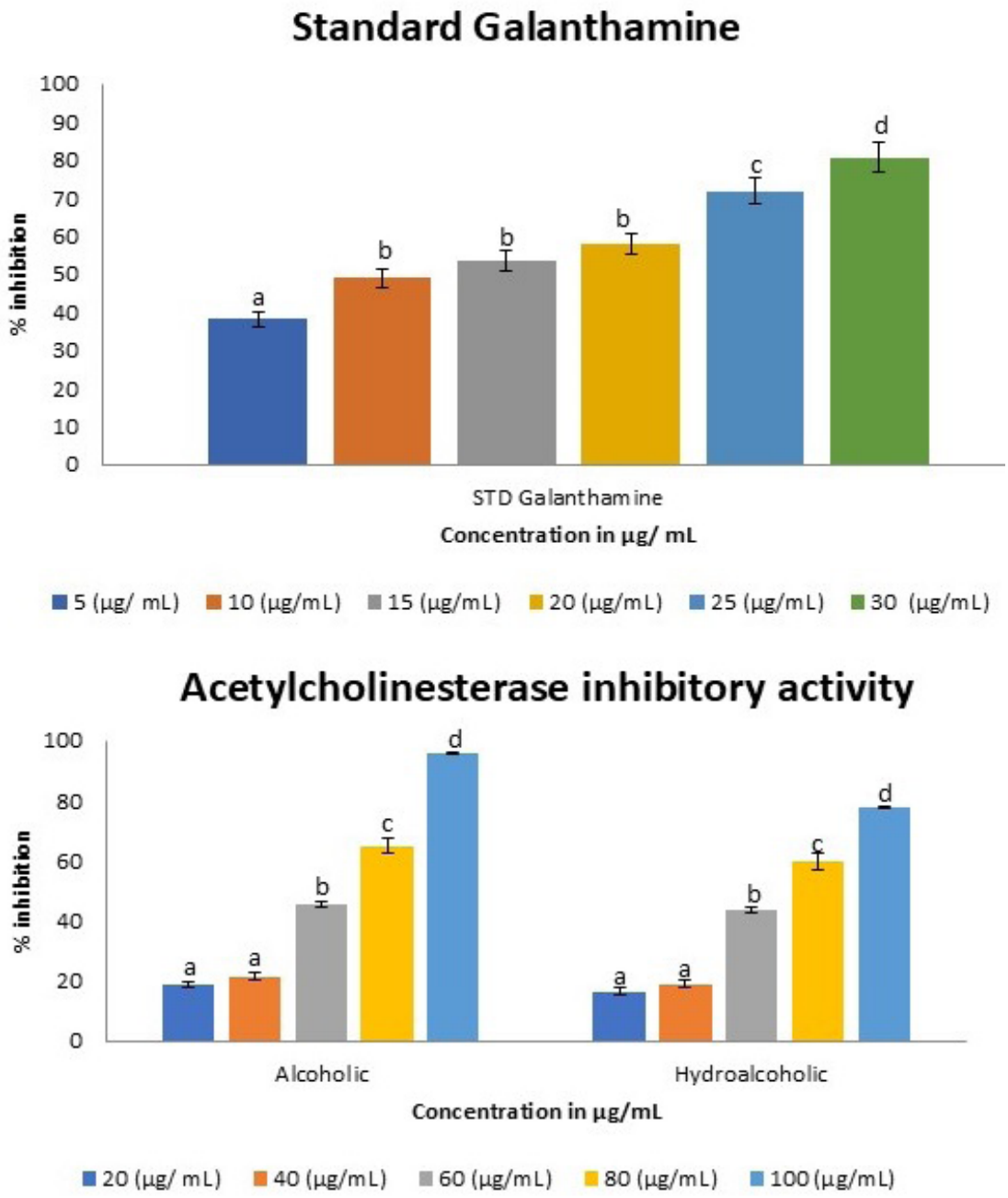

Figure 8: Acetylcholine esterase inhibitory activity of standard galantamine and alcoholic and hydroalcoholic extract of *Premna serratifolia* Linn. Values are mean ± SD from three experiments in each group. Values not sharing a common superscript significantly at $p \leq 0.05$.

and an electron to hydroxyl radicals, stabilizing them and giving rise to relatively stable radicals. Thus, the free hydroxyl group on the aromatic ring is responsible for the antioxidant properties.

AChE (hAChE) consists of three major sites: a catalytic triad, an active site (A-site); a Peripheral Anionic Site (PAS); and a long narrow hydrophobic gorge. The catalytic triad consists of Ser203 (a catalytically reactive residue), His447, and Glu334, all of which are positioned in the A-site. The PAS contains several aromatic residues, Tyr72, Asp74, Trp86, Tyr124, Trp286, and Tyr341.[37] Galantamine forms a hydrogen bond with His 447 and hydrophobic interactions with Ser203, which indicates that its interacts with the active site and most of the residues with the PAS site.

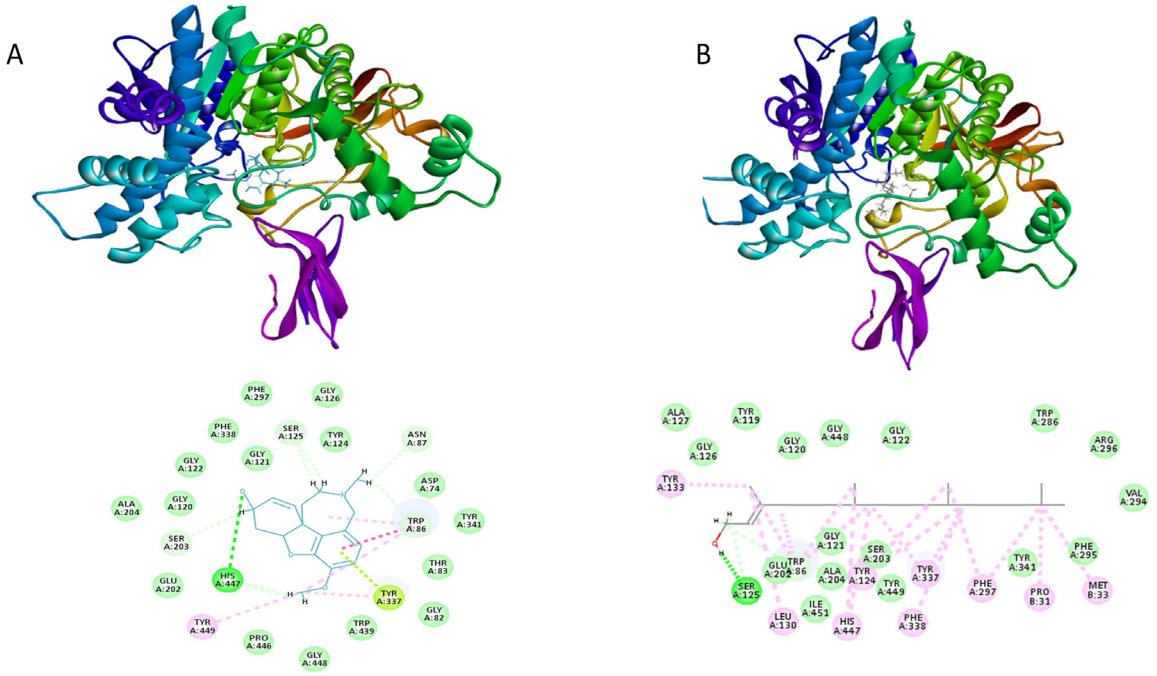

Figure 9: Three-dimensional and 2-dimensional interaction representation of (A) Galantamine and (B) Phytol with Acetylcholinesterase.

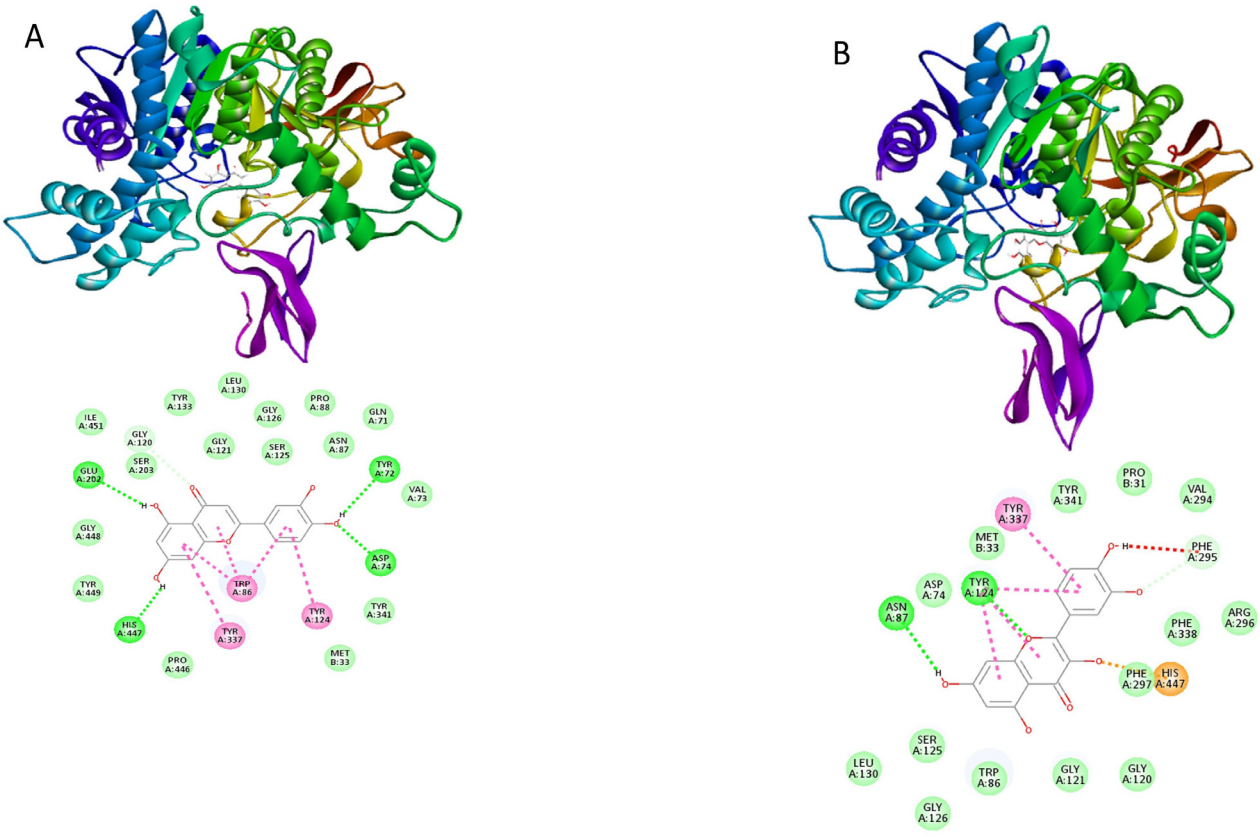

Figure 10: Three-dimensional and 2-dimensional interaction representation of (A) Luteolin and (B) quercetin with Acetylcholinesterase.

Table 4: Results of the docking of compounds of *Premna serratifolia* Linn. on the crystal structure of acetylcholinesterase

Ligand	CDOCKER interaction energy (-Kcal/mol)	Hydrogen bond interactions	Hydrophobic interactions
Luteol	33.304	TYR A:72, ASP A:74, GLU A:202, HIS A:447	PRO A:446, TYR A:449, GLY A:448, ILE A:451, GLY A:120, SER A:203, TYR A:133, GLY A:121, GLY A:126, LEU A:130, SER A:125, PRO A:88, ASN A:87, GLN A:71, VAL A:73, TYR A:341, MET B:33, TYR A:337, TYR A:124, TRP A:86
Phytol	56.22	SER A:125, GLU A:202	GLY A:126, ALA A:127, GLY A:120, TYR A:119, GLY A:448, GLY A:122, TRP A:286, ARG A:296, VAL A:294, GLU A:202,GLY A:121, ALA A:204, LEU A:130, ILE A:451, SER A:203, HIS A:447, TYR A:124, TYR A:449, TYR A:337, PHE A:338, TYR A:341, PHE A:297, PHE A:295, MET B:33, PRO B:31
Quercetin	30.23	TYR A:124, ASN A:87	MET B:33, PRO B:31, ASP A:74 TYR A:337, TYR A:341, VAL A:294, ARG A:296, PHE A:338, HIS A:447, GLY A:120, GLY A:121, TRP A:86, GLY A:126, LEU A:130, PHE A:297, PHE A:338
Eugenol	28.36	SER A:203	GLY A:120, ALA A:204, GLY A:121, TYR A:337, GLY A:122, TYR A:337, PHE A:338, PHE A:297, GLY A:448, SER A:125, ILE A:451, TYR A:133, ASN A:87, ASP A:74, TYR A:124, TYR A:341
Linalool	29.93	-	ASN A:87, ASP A:74,TYR A:124, TYR A:341, THR A:83, GLU A:202, GLY A:122, PHE A:338, GLY A:120, GLU A:202, GLY A:448, ILE A:451, TYR A:449, HIS A:447, SER A:125, TRP A:86, GLY A:121
Trans-caryophyllene	25.63	-	ALA A:204, LEU A:130, ILE A:451, SER A:203, GLY A:122, GLY A:121, GLY A:448, GLU A:202, PHE A:297, TYR A:341, MET B:33
Spathulenol	30.23	GLY A:122, GLY A:121	GLY A:448, TRP A:86, TYR A:337, ILEA:451, HIS A:447, GLU A:202, PHE A:338, GLY A:120, SER A:203, ALA A:204, PHE A:297, GLY A:122, GLY A:121, ASP A:74, THR A:83,TYR A:341, SER A:125, TYR A:124
Galanthamine	37.22	HIS A:447, SER A:125, ASN A:87, TRP A:86	PHE A:338, PHE A:297, GLY A:120, GLY A:122, GLU A:202, SER A:203, ALA A:204, TYR A:449, HIS A:447, PRO A:446, TYRA:124, ASN A:87, ASP A:74, TRP A:86, TYR A:341, THR A:83, GLY A:82, TYR A:337, GLY A:448, TRP A:439, PRO A:446, TYR A:449

The best-docked molecule, phytol, interacts with all active sites, the PAS, and the remaining hydrophobic regions of AChE, suggesting that it has a similar effect as galantamine. There were no significant interactions between the remaining phytochemicals and AChE residues, which indicates that these phenolics have an inhibitory effect on AChE residues, as demonstrated by these results.

The presence of phenolic compounds in the PS alcoholic extract may inhibit acetylcholinesterase. *In vitro* studies further validated their traditional use in treating CNS disorders by demonstrating AChE inhibition and antioxidant properties.[38] It is possible that those that did not inhibit AChE act on a different molecular target.

CONCLUSION

According to experimental observations, PS is highly antioxidant in terms of scavenging free radicals produced *in vitro*. Additionally, it inhibited AchE activity in both alcoholic and hydroalcoholic extracts in a dose-dependent manner.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

hACHE: Homodimerization of recombinant monomeric human acetylcholinesterase; **AChE:** Acetylcholinesterase; **PS:** *Premna serratifolia* Linn.; **GC-MS:** Gas chromatography–mass spectrometry; **LC-MS/MS:** Liquid chromatography with tandem mass spectrometry; **FRAP:** Ferric Reducing Antioxidant Power Assay; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **AD:** Alzheimer disease; **DNA:** Deoxyribonucleic acid; **ABTS:** 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; **MCF-7:** Michigan Cancer Foundation-7; **HepG2:** Hepatoblastoma cell line; **A549:** Adenocarcinomic human alveolar basal epithelial cells; **BHT:** Butylated hydroxytoluene; **BHA:** Butylated hydroxyanisole; **API:** Ayurvedic Pharmacopoeia of India; **SIF:** Sophisticated Instrumentation Facility; **NIST:** National Institute of Standards and Technology; **HPLC:** High-performance liquid chromatography; **PDA:** Photodiode Array Detector; **QTOF:** Quadrupole Time-of-Flight; **DTNB:** 5,5′-dithiobis-(2-nitrobenzoic acid).

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