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Original Research Article

Rhizophora apiculata extracts improved memory function through inhibition of acetylcholinesterase and oxidative stress in scopolamine-induced memory deficits in rats

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Abstract

Objective: This study aims to investigate the neuroprotective, memory enhancement effects and phytochemical profile of *Rhizophora apiculata*.

Materials and Methods: Ethanolic and aqueous extracts of *R. apiculata* leaves were prepared and screened for their antioxidant potential. *In vitro* studies were performed to assess the neuroprotective effects of *R. apiculata* extracts against scopolamine-induced neurotoxicity in SH-SY5Y cells. Further, *in vivo*, memory-enhancing effects of the extracts were evaluated in a scopolamine-induced amnesia model in rats by measuring brain acetylcholinesterase (AChE) levels, lipid peroxidation, and glutathione (GSH) activity. Furthermore, phytochemicals were identified through HR-LCMS analysis, and their binding interactions with the target protein AChE were investigated through *in silico* studies.

Results: Treatment with ethanolic (100 µg/ml) and aqueous extracts (100 µg/ml) significantly reduced oxidative stress up to 89.386±2.37% in DPPH assay and 84.167±5.80% ABTS assays, respectively. The extracts (100 µg/ml) notably increased the viability (97.49%) of SH-SY5Y neuroblastoma cells against scopolamine-induced neurotoxicity. *In vivo*, studies revealed that both extracts improved memory function in scopolamine-induced amnesia by inhibiting the AChE activity and enhancing brain GSH levels while reducing lipid peroxidation. HR-LCMS analysis identified 54 distinct phytochemicals, with several compounds showing promising binding affinities like olitorin (-11.5 kcal) and gambiriin A3 (-10.7 kcal) for AChE in *in-silico* studies.

Conclusion: Based on the findings of this study, *R. apiculata* leaves may be considered a promising source of neuroprotective compounds, with potential therapeutic applications for various neurological diseases.

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Introduction

The incidence of neurological disease is escalating regularly and most of them are accompanied by cognitive and memory impairment (Hemat Jouy et al. 2024). Acetylcholinesterase (AChE) enzyme degrades acetylcholine (ACh), a neurotransmitter involved in cognition and memory. Indeed, AChE enzyme activity is significantly elevated in neurodegenerative illnesses, resulting in decreased ACh levels, thereby affecting cognition and memory (Gutti et al. 2019). Therefore, it can be suggested that inhibition of AChE enzyme activities could be a promising avenue for combating the memory decline associated with various neurological diseases (Srikanth et al. 2025). Apart from AChE activity, oxidative stress is one of the leading pathological events that contribute to the degeneration of ACh neurons, thereby causing memory impairment (Singh et al. 2022). Elevation of lipid peroxidase and reduction in glutathione (GSH) levels have a direct relationship with amnesia in neurological disorders (Goboza et al. 2019). The current drug therapy that exists for the management of memory impairment has several limitations, including side effects and not being able to modify the disease pathology, therefore, they are recommended only for symptomatic relief (Yiannopoulou and Papageorgiou 2020). Hence, there is a dire need for effective and safe memory-enhancing agents to alleviate cognitive and memory impairment.

Indigenous coastal communities across the globe exploit the diverse pharmacologically active flora, particularly plants present within mangrove ecosystems, to address a multitude of health ailments, encompassing neurological, hepatic, and diabetic disorders (Sarvan Kumar et al. 2022). *Rhizophora apiculata* is found throughout the world's coastal subtropical and tropical regions and is reported to have various beneficial effects including antidepressant activity (Prabhu and Guruvayoorappan 2012). Additionally, the plant extracts of *R. apiculata* have been reported to exhibit antioxidant activity (Ramalingam and Rajaram 2018). Given the importance of its antioxidant and neuroprotective effects, this plant can be used to mitigate neurological complications, including memory impairment. Indeed, the phytochemical profiling of *R. apiculata* is not fully known. Besides, *R. apiculata* role in cognitive and memory enhancement activity is partially understood (Mande et al. 2023a). Therefore, there is a need for the assessment of the memory enhancement activity of *R. apiculata* and its mechanism of action in improving the memory function. Further, in this connection, the present study aims to investigate the memory enhancement activity and antioxidant activity of *R. apiculata* extracts against scopolamine-induced cognitive and memory impairment *in vitro* and *in vivo*. Additionally, we have investigated phytochemical profiling of the plant extracts using HR-LCMS and several phytochemicals were subsequently screened for AChE binding activity.

Overall, this study for the first time investigated memory enhancement activity, antioxidant activity, phytochemical profiling, and AChE modulating effects of *R. apiculata* using *in vitro, in vivo*, and *in-silico* studies.

Materials and Methods

All the chemicals and solvents were purchased from Thermo Fisher Scientific India Pvt. Ltd, SD Fine Chemicals, India, and Merck, India. Scopolamine was procured from Mylan Pharmaceuticals Inc., India and donepezil was obtained from Cipla Ltd, India.

Plant collection and authentication

R. apiculata was collected from the coastal mangrove forests of Andhra Pradesh, India, and authenticated by the taxonomist Dr. Raghu Ram, Acharya Nagarjuna University, with the voucher specimen number (RAR/7-

2(872)/2019/ANU/BOT). The leaves were separated, cleaned, and shade-dried. The dried leaves were powdered and preserved.

Preparation of the crude extract

The ethanolic (EERA) and aqueous (AERA) extracts of *R. apiculata* were obtained by successive extraction of leaf powder. The extract was filtered, concentrated, weighed, and refrigerated.

HR-LCMS analysis

EERA and AERA were subjected to HR-LCMS analysis using the ESI±VE_MSMS with the help of an instrument Hip sampler (model No. G 4226A) and Binary pump (model No. G4220B). The ion source used for the study was Dual AJS ESI. The source parameters included Gas Temp 250^{o}C, Gas Flow 13 l/min, Nebulizer 35 psig, Sheath Gas Temp 300^{o}C, and sheath Gas Flow 11 l/min. The draw speed and eject speed were maintained at 100 µl/min. The sample was injected with a needle at a speed of 5S µl. The column was maintained at a temperature of 40°C throughout the acquisition process.

Antioxidant activity

The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis,3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods (Islam et al. 2021). Stock solutions of DPPH and ABTS were prepared and both the extracts of the plant were subjected to serial dilutions at 2, 5, 10, 20, 50, and 100 µg/ml from a 1000 µg/ml stock solution. Ascorbic acid (10–100 mg) was used as a standard to plot the standard curve. The absorbances were measured .

Experimental design

The experiments associated with this study have been divided into three sub-experiments. First, AERA and EERA extracts were tested for antioxidant potential. Second, the extracts were subjected to investigate the neuroprotective and memory enhancement activities. Third, both the extracts were subjected to phytochemical analysis using HR-LCMS and their phytochemicals were studied for binding properties against AChE using *in silico* studies.

***In vivo* studies**

Healthy adult male albino Wistar rats were used to evaluate the memory enhancement activity of AERA and EERA extracts against scopolamine-induced memory impairment. Briefly, animals were randomly divided into nine groups with six animals in each group. The first group served as control (healthy rats); the second group received scopolamine 1 mg/kg only by oral route, and the third, fourth, and fifth groups received scopolamine 1 mg/kg along with EERA in three test doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg, respectively (administered orally). The sixth, seventh, and eighth groups were given scopolamine 1 mg/kg along with AERA in three test doses 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively (administered orally). The ninth group was administered orally with donepezil (2.5 mg/kg) and scopolamine (1 mg/kg) (Ahidjo et al. 2021). The test doses of leaf extracts were selected based on their neuroprotective abilities (Mande et al. 2022). AERA and EERA extracts and scopolamine were given orally for seven days. After 30 min of the final dose of the extracts, the last dose of scopolamine was administered. Memory enhancement activity was studied through the passive avoidance test. After the experimentation, the animals were anesthetized by using thiopentone sodium (40 mg/kg, intra peritoneal route) and the brains were isolated to estimate the biochemical AchE enzyme activity, and oxidative stress markers were estimated. The study was performed after obtaining approval from the Institutional Animal Ethical Committee (IAEC), Bapatla College of Pharmacy, Bapatla, Andhra Pradesh with reference number IAEC/XIV/06/BCOP/2021.

Passive avoidance test

The present *in vivo* test was done with the help of an Evasion box that was internally divided into light and dark areas by a guillotine door. The dark area was equipped with an electric shock instrument fitted to the floor that was absent in the light compartment of the evasion box. During the acquisition trial, the rats were first placed in a dark room and given an electric shock (0.3A for 15 sec), then the rats were moved from a dark area to a bright area. After 24 hrs of acquisition trial, rats were placed in a dark chamber, without electric shock, and the latency(sec) to enter the light chamber was noted as a measure of cognition and memory function (Choi et al. 2021).

Estimation of lipid peroxidation

Lipid peroxidation (LPO) initiates when a cell injury leads to organ damage. The quantity of lipid peroxidase present in the brain homogenate of extract and standard-treated rats was measured following the standard procedure as per the given reference (Hossain et al. 2012).

Estimation of reduced GSH

The method employed to assess the GSH from brain tissue homogenate was taken from the following reference (Chandrashekhar et al. 2013).

Estimation of AchE

AChE activity was measured as reported earlier (Khokar et al. 2021).

Evaluation of neuroprotective activity by using SH-SY5Y cell lines

The neuroprotective effects of AERA and EERA against scopolamine-induced neurotoxicity in Human neuroblastoma cell lines (SH-SY5Y) were evaluated. SH-SY5Y cells were cultured in Dulbeccos modified Eagle medium (DMEM) with 10% Fetal bovine serum, centrifuged, and adjusted to $1x10^5$ cells/ml. A 100-µl cell suspension was added to each well of a 96-well plate and incubated for 24 hr. Cells were treated with AERA or EERA (25, 50, and 100 µg/ml) and scopolamine (20 µg/ml). After 24 hr, 20 µl of MTT (2 mg/ml) was added, and plates were incubated for 2 hr. Formazan was solubilized with 100 µl of Dimethyl sulfoxide and absorbance was measured at 540 nm.

***In silico* docking study**

The target protein AChE binding affinities of the phytocompounds in both extracts were assessed using *in-silico* docking. The protein bank (rcsb.com/pdb database) provided the crystal structure of protein AChE (1EVE). Discovery Studio Visualiser 2021 was used to draw the crystal ligand structure in pdbqt format. Plotting the Ramachandran plot revealed structural issues in the co-crystallized structure. The PyRx virtual screening program 0.8's Auto dock tool's 'macromolecule' option converted the pdb protein file to pdbqt. Vin Wizard selected protein pdbqt files and ligands for docking research. A grid box was formed around the cocrystal ligand amino acid interaction. Ligands dock with the protein's active site. Discovery Studio Visualizer 2021 visualizes interacting amino acids.

Statistical analysis

All the results are expressed as Mean±Standard Error of Mean (SEM). The statistical significance was determined by applying one-way ANOVA followed by Tukey's post hoc test. The entire data was analyzed by using GraphPad Prism version 8 (GraphPad Software Inc., San Diego, CA, USA). In all cases, a p-value less than 0.05 was considered significant.

Results

HR-LCMS/MS analysis

Several bioactive phytocompounds were detected in both extracts based on HR-LCMS analysis (Table 1). The EERA was comprised of isomaltulose, hypoglycine, 5'O-β-D-glucosyl pyridoxine, caffeic acid, veranisatin C, 2', 3'-dihydroxy

acetophenone, manghaslin, and quarcetagetin, etc. On the other hand, the AERA exhibited physalolactone, protaphin aglucone, vindoline, piperochromanoic acid, ranunculin, deoxycoformycin, etc.

Table 1. Phytochemicals detected in EERA and AERA as per HR-LCMS analysis.

S.no	Name of the compound	Rt (Mins)	Mass (g/mol)	Chemical Formula	Category of the compound	Pharmacological use	Dbd (ppm)
1	Caffeic acid	3.391	180.04	$C_9H_8O_4$	Phenolic compound	Anti-oxidant (Khan et al. 2016)	0.44
2	Veranisatin C	4.57	372.10	$C_{16}H_{20}O_{10}$	Terpenoid	Neurotropic (Nakamura et al. 1996)	1.23
3	2',3'-Dihydroxy acetophenone	4.58	152.04	$C_8H_8O_3$	Flavonoids	Anti-oxidant (Siddiqa et al. 2022)	1.23
4	Manghaslin	5.95	756.21	$C_{33}H_{40}O_{20}$	Flavonoid	Anti-AChE (Olennikov et al. 2017)	0.7
5	Quercetagetin	6.001	318.03	$C_{15}H_{10}O_8$	Flavonoid	Antioxidant (Parejo et al. 2005)	1.51
6	Rutin	6.49	610.15	$C_{27}H_{30}O_{16}$	Flavonoid	Neuroprotective (Barbara et al. 2019), Anti-Alzheimer (Solomon 2016)	0.66
7	3'-(2"-Galloyl glucosyl)- phloro acetophenone	6.64	182.10	$C_{21}H_{22}O_{13}$	Flavonoid	Anticancer (Cho et al. 2012)	1.26
8	Safflomin A	7.03	612.1685	$C_{27}H_{32}O_{16}$	Glycoside	Anti-inflammatory (Wang et al. 2011)	
9	Phytosphingosine	14.22	317.2925	$C_{18}H_{39}NO_3$	Steroid	Anti-proliferative agent (Sun et al. 2023)	1.64
10	Capsoside A	14.88	694.3768	$C_{33}H_{58}O_{15}$	Polyphenols	Anti-oxidant (Guevara et al. 2021)	1.11
11	Tosyllysine Chloromethyl Ketone	1.268	332.0967	$C_{14}H_{21}CN_{2\,O3}S$	Terpenoid	Anti-cancer (Sárközy et al. 2020)	1.55
12	Rhodojaponin I	4.311	452.2375	$C_{24}H_{36}O_8$	Terpenoid	Neuropathic pain inhibitor (Chen et al. 2023)	7.69
13	Sesamolinol	4.534	372.1167	$C_{20}H_{20}O_7$	Phenolic compound	Anti-Alzheimer (Katayama et al. 2016)	11.3
14	Harpagoside	5.688	494.1788	$C_{24}H_{30}O_{11}$	Glycoside	Anti-amnesic (Chen et al. 2018)	0.03
15	Gambiriin A3	5.877	580.1613	$C_{30}H_{28}O_{12}$	Glycoside	None	5.94
16	Physalolactone	6.842	538.2364	$C_{28}H_{39}ClO_8$	Glycoside	Anti-cancer (Rao et al. 2016)	5.61
17	Volemolide	10.1	346.2472	$C_{22}H_{34}O_3$	Terpenoid	Anti-cancer (Zhang et al. 2020)	10.31
18	Olitorin	10.415	696.3288	$C_{35}H_{52}O_{14}$	Glycoside	None	9.91
19	Borrelidin	19.13	489.3121	$C_{28}H_{43}NO_6$	Flavonoid	Anti-Alzheimer (Shin et al. 2021)	6.28
20	Vindoline	9.15	456.2257	$C_{25}H_{32}N_2O_6$	Alkaloid	Anti-oxidant, anti-inflammatory (Goboza et al. 2019)	0.73
21	Dioscorine	5.016	221.1416	$C_{13}H_{19}NO_2$	Alkaloid	Anti-oxidant (Hou et al. 2001)	0.06

R. apiculata inhibited oxidative stress

Figure 1 presents the free radical scavenging activity of the extracts. The EERA shows DPPH free radical scavenging activity in a dose-dependent manner (Figure 1A). Further, the EERA (100 µg/ml) exhibited about 89.386±2.37% of DPPH free radical scavenging activity, which is equal to standard ascorbic acid. Similarly, the AERA showed dose-dependent DPPH free radical scavenging activity and AERA (100 µg/ml) showed the highest % of inhibition of DPPH free radical (84.167±5.80%) (Figure 1B). These findings imply that the EERA has a high free radical scavenging activity compared to the AERA. The results of the ABTS assay indicated that EERA has 99.649±0.22% free radical scavenging activity, which is close to the percentage inhibition of free radicals of standard compound ascorbic acid 99.05±0.62 % (Figure 1C).

Extracts of *R. apiculata* improved the memory

The results of the effect on memory enhancement study of EERA and AERA are shown in Figure 2. A significant decline in the transfer latency into the dark compartment from a light compartment in control animals than scopolamine-treated rats ($p<0.0001$) was observed. The group that received Scop+ AERA (400 mg/kg) and Scop + EERA (600 mg/kg) showed a significant ($p<0.001$) increase in the transfer latency to enter the dark compartment when compared with the scopolamine (1 mg/kg)-treated group.

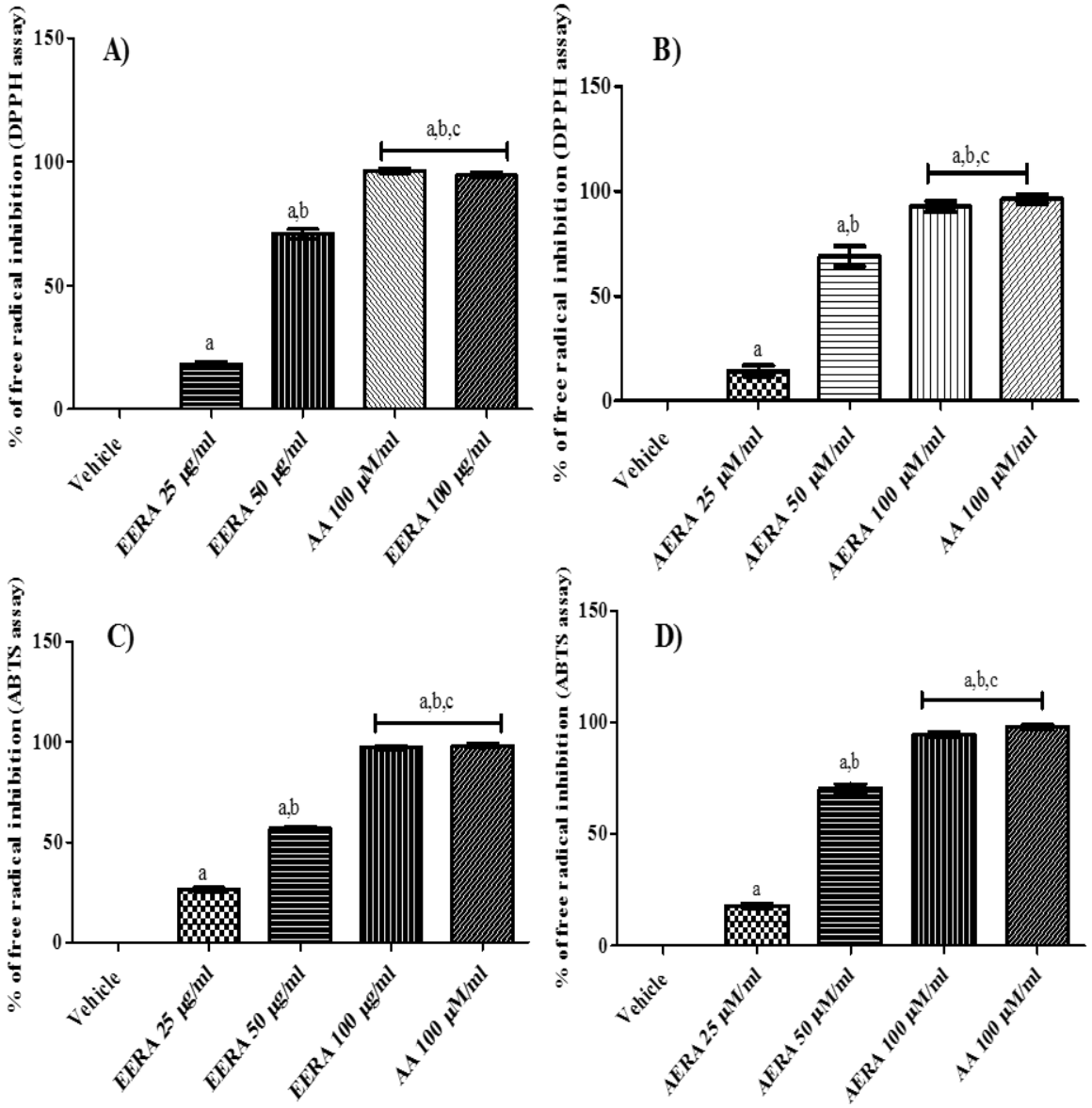

Figure 1. Antioxidant potentials of EERA and AERA. 1A and 1B represent the free radical scavenging inhibition activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, respectively for ethyl alcohol extract of *R. apiculata* (EERA), and aqueous extract of *R. apiculata* (AERA); 1C, and 1D represent the free radical scavenging inhibition activity by 2,2′-azino-bis,3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay for EERA and AERA, respectively. AA: Ascorbic acid. $^{a}p<0.05$, $^{b}p<0.05$, and $^{c}p<0.05$ vs vehicle. All values are expressed as Mean±SEM (n = 3).

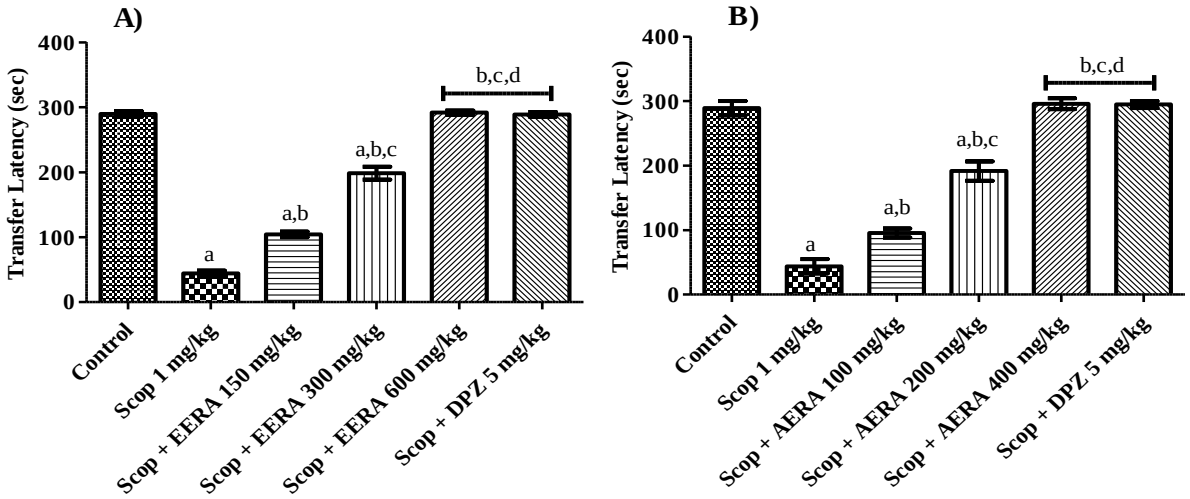

Figure 2. Effect of EERA and AERA on memory enhancement. 2A and 2B present the transfer latency into the dark compartment by ethyl alcohol extract of *R. apiculata* (EERA), and aqueous extract of *R. apiculata* (AERA), respectively. $^{a}p < 0.05$. vs. Normal, $^{b}p < 0.05$, vs. scopolamine (scop) 20 mg/kg, $^{c}p<0.05$, vs. scop + EERA 300 mg/kg and $^{d}p<0.05$ vs scop + EERA 300 mg/kg. All values are expressed as Mean ± SEM (n = 6).

Extracts of *R. apiculata* enhanced the GSH and reduced the lipid peroxidation

Figure 3 illustrates the levels of reduced glutathione and lipid peroxidase. Reduced glutathione levels were significantly lower in the Scop + EERA (150 mg/kg) group ($p<0.0001$) compared to the negative control (scopolamine 1 mg/kg). Groups receiving Scop + EERA (600 mg/kg) and Scop + Donepezil showed a significant ($p<0.001$) decrease in reduced glutathione compared to the Scop + EERA (300 mg/kg) group. Scop + AERA (100 mg/kg) showed lower glutathione than the negative control, with Scop + AERA (200 mg/kg) and (400 mg/kg) further reducing glutathione levels.

Regarding lipid peroxidase, the Scop + EERA (150 mg/kg) group exhibited a notable reduction ($p<0.05$) compared to the negative control, with Scop + EERA (300 mg/kg) and Scop + EERA (600 mg/kg) showing additional reductions ($p<0.05$) relative to lower doses, aligning with the antioxidant potential shown by Scop + Donepezil.

Extracts of *R. apiculata* inhibited AChE activity

The levels of AChE following treatment with extracts of *R. apiculata* are depicted in Figure 4. The group that received Scop+ EERA (150 mg/kg) showed a significant ($p<0.05$) decrease in levels of reduced AChE when compared with the control. The group that received Scop + EERA (600 mg/kg) and the standard group (Scop+ Donepezil) have shown significant ($p<0.05$) decreases in the levels of AchE when compared with the group that received Scop + EERA (300 mg/kg).

Extracts of *R. apiculata* protected the neurons from scopolamine-induced neurotoxicity

Figure 5 presents the neuroprotective effects of AERA and EERA extracts against scopolamine-induced neurotoxicity in SH-SY5Y cells. We have observed a significant decrease in neuronal cell viability in scopolamine-treated groups than control group cells ($p<0.0001$). Treatment with AERA and EERA significantly enhanced neuronal cell viability. Treatment with EERA at concentrations of 25, 50, and 100 µg/ml significantly improved neuronal cell viability by 31.60%, 70.07%, and 97.49%, respectively ($p<0.001$). Similarly, AERA treatment at 25, 50, and 100 µg/ml resulted in cell viability improvements of 35.60%, 67.48%, and 92.26% ($p<0.001$), respectively. These findings indicate that both extracts proved to be neuroprotective without significant differences.

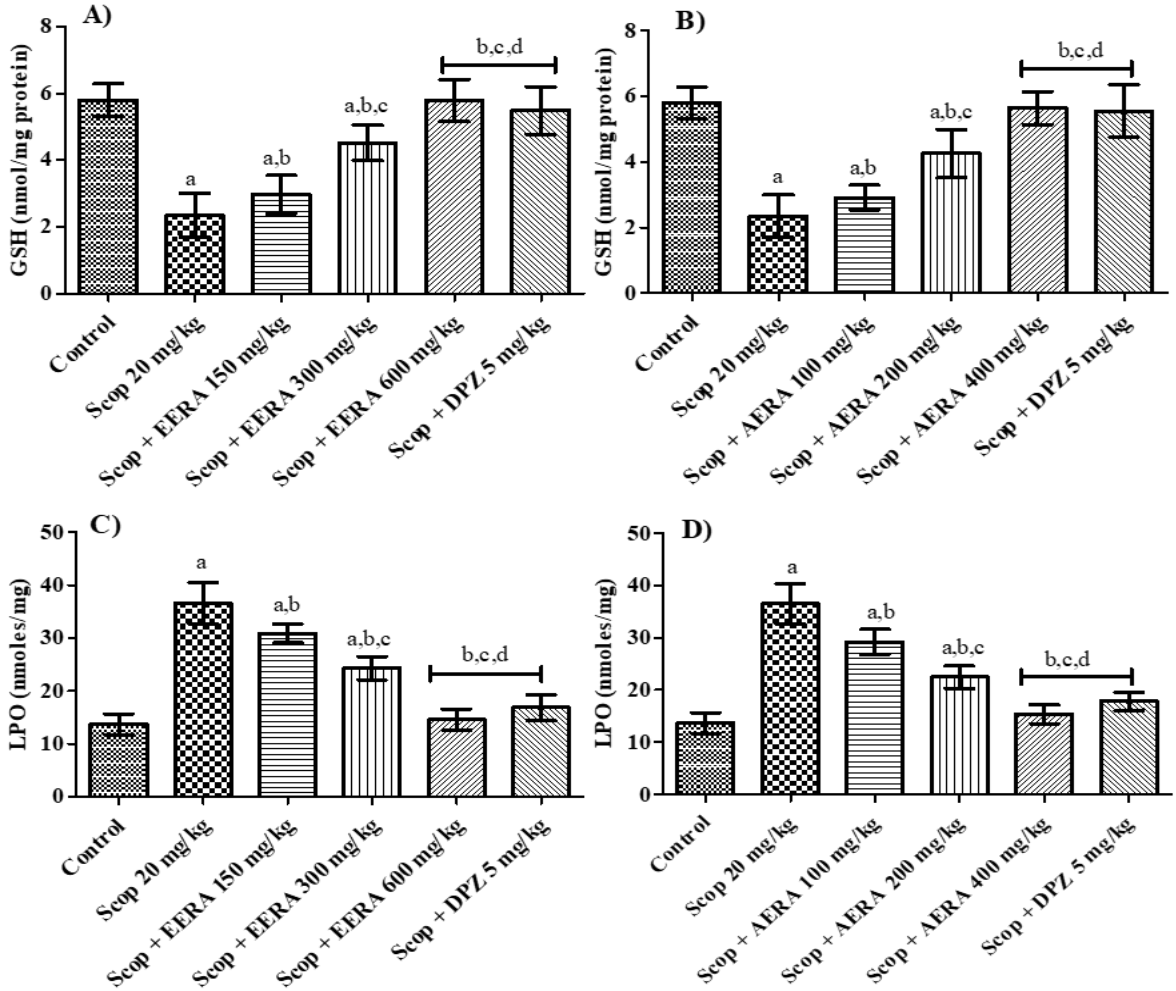

Figure 3. Effect of EERA and AERA on Biochemical parameters. 3A and 3B present the level of reduced glutathione (GSH) (nmol/mg of protein), respectively for ethyl alcohol extract of *R. apiculata* (EERA) and aqueous extract of *R. apiculata* AERA. Figure 3C, and 3D present the level of lipid peroxidation (LPO) (nmol/mg of protein), respectively for EERA, and AERA. $^{a}p < 0.05$ vs control, $^{b}p < 0.05$ vs scopolamine (scop) 20mg/kg, $^{c}p<0.05$ vs scop + EERA 150 mg/kg/AERA 100mg/kg, and $^{d}p<0.05$ vs scop + EERA 150 mg/kg/AERA 100mg/kg for the respective treatment groups. All values are expressed as Mean ± SEM (n = 6).

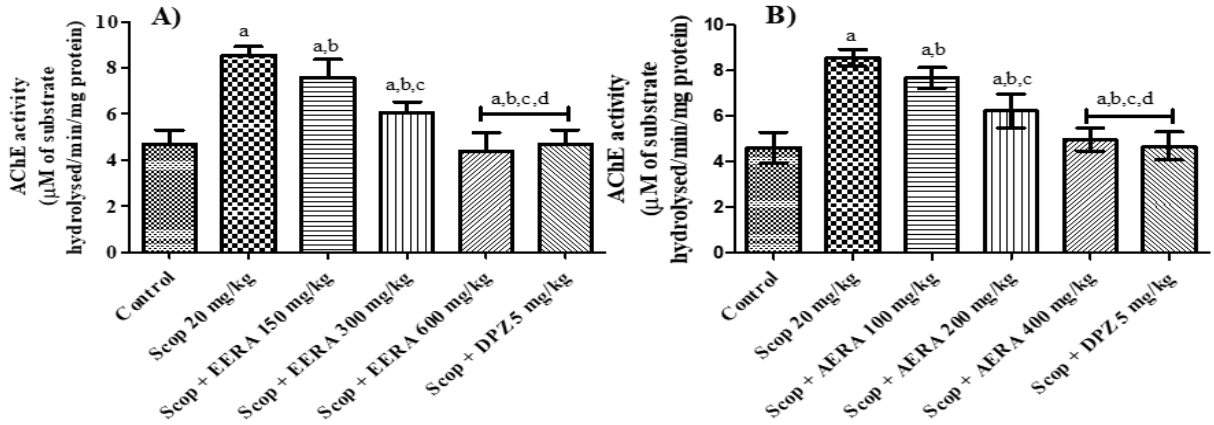

Figure 4. Effect of EERA and AERA on the level of AChE. Effect of ethyl alcohol extract of *R. apiculata* (EERA) and aqueous extract of *R. apiculata* (AERA) on the level of acetylcholinesterase (AChE). Figure 4A and 4B present the level of AChE (µM of the substrate), respectively for EERA and AERA. $^{a}p<0.05$ vs control, $^{b}p<0.05$ vs scopolamine (scop) 20 mg/kg, $^{c}p<0.05$ vs scop + EERA 150 mg/kg/AERA 100mg/kg and $^{d}p<0.05$ vs scop + EERA 150 mg/kg/AERA 100mg/kg for the respective treatment groups. All values are expressed as Mean ± SEM (n = 6).

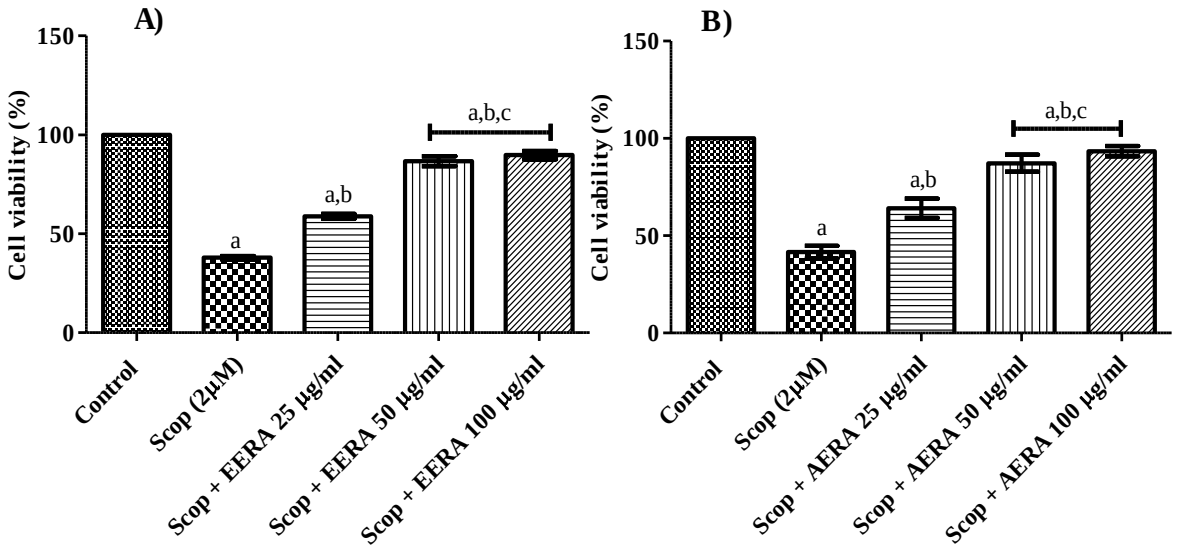

Figure 5. Effect of EERA and AERA on cell viability of SH-SY5Y cell lines. Effect of ethyl alcohol extract of *R. apiculata* (EERA) and aqueous extract of *R. apiculata* (AERA) on cell viability of human neuroblastoma cell lines (SH-SY5Y cell lines. Figs. 5A and 5B present the cell viability of SH-SY5Y cell lines, respectively for EERA, and AERA. $^{a}p < 0.05$ vs control, $^{b}p < 0.05$ vs scopolamine (scop) 2 μM, and $^{c}p<0.05$ vs scop 2 μM + EERA 25 μg/ml AERA 25 μg/ml for the respective treatment groups. All values are expressed as Mean ± SEM (n = 3).

In silico docking study

The binding energies of the few ligand molecules and their interactions are summarized in Tables 2 and 3. Donepezil was used as a reference standard. The results of *in silico* studies confirmed that the compounds ent-17-acetoxy-16β-kauran-19-al (-10.3 kcal/mole), gambiriin A3 (-10.7 kcal/mole), methyl 4,6-di-*O*-galloyl-βD-glucopyranoside (-10.0 kcal/mole), Olitorin (-11.5 kcal/mole), and volemolide (-10.2 kcal/mole) had significant binding affinities with the target protein (Figures 6 and 7).

Table 2. Binding energies of phytochemicals that were detected in EERA and AERA in an HR-LCMS study against the target protein AChE**.**

S. No.	Compound name	Binding energy (kcal/mol) against 1EVE
1	1-Hexanol arabinosyl glucoside	-8.9
2	2-Aminoacridone	-9.1
3	3-Methyl butyl 2-furan butanoate	-7
4	4(2-Furyl)pyridine	-6.5
5	Allosamidine	-8.3
6	Armillatin	-9.2
7	Bis-γ-glutamylcysteinylbis-β-alanine	-8.8
8	Carvyl propionate	-8
9	Desacetylvindoline	-7.1
10	D-Glucose, cyclic 1,2-ethanediyl mercaptal	-6
11	ent-17-Acetoxy-16b-kauran-19-al	-10.3
12	Gambiriin A3	-10.7
13	Glycine, *N*-(*N*-glycyl-L-leucyl)-	-7.2
14	Haemocorin	-8.9
15	Isomaltulose	-8
16	L-iso leucyl-L-proline	-7.2
17	L-Oleandrosyl-oleandolide	-7.4
18	Lyoniresinol	-8.1
19	Methyl 4,6-di-O-galloyl-βD-glucopyranoside	-10.0
20	Methyl glycocholate, 3TMS derivative	-6.1
21	*N*-*N*-glycyl leucyl glycine	-6.9
22	Olitorin	-11.5
23	Volemolide	-10.2

Table 3. Binding interactions of the selected phytochemicals against AChE (PDB ID: 1EVE).

S. No.	Compound Name	Binding Energy (Kcal/mol)	Interaction		Interacting amino acid	Bond length (Å)
			Type	No. of Bonds		
1	Olitorin	-11.5	H-bond	4	Trp84, Ser286, Phe288, Arg289	1.87, 2.29, 2.36, 2.39,
			Donor-Donor	1	Phe288	1.80
2	Gambiriin A3	-10.7	H-bond	4	Asp72, Arg289, Tyr334, His440	2.04, 2.10, 2.23, 2.99
			Pi-Pi T Shaped	3	Trp279, Phe330	4.78, 5.61, 5.72
			Pi-Pi Stacked	1	Tyr334	2.23
3	ent-17-Acetoxy-16b-kauran-19-al	-10.3	H-bond	2	Asp72, Tyr121	2.06, 2.60
			C-H –bond	1	Asp72	3.12
			Pi-Sigma	2	Phe334	3.58, 3.91
			Pi-Alkyl	3	Phe331, Tyr334, His440	4.56, 4.87, 5.43
4	Donepezil	-11.3	C-H-bond	1	Ser286	3.51
			Pi-Sigma	1	Trp279	3.67
			Pi-Pi Stacked	3	Trp84, His440	3.95, 4.16, 5.87
			Pi-Alkyl	4	Tyr70, Phe330, Phe331, Tyr334	4.01, 4.63, 4.87, 5.44

Note: Binding interactions of compounds with amino acids of target protein.

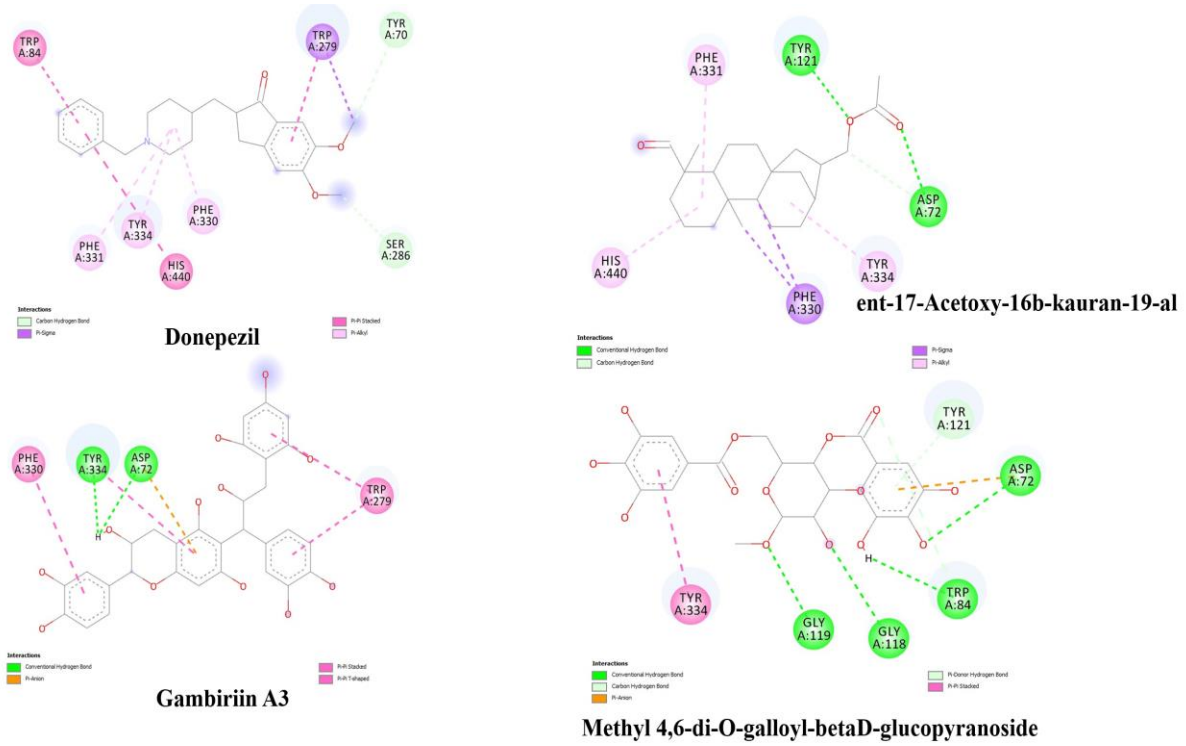

Figure 6. 2D interactions of phytochemicals against 1EVE and acetylcholinesterase (AChE) enzyme.

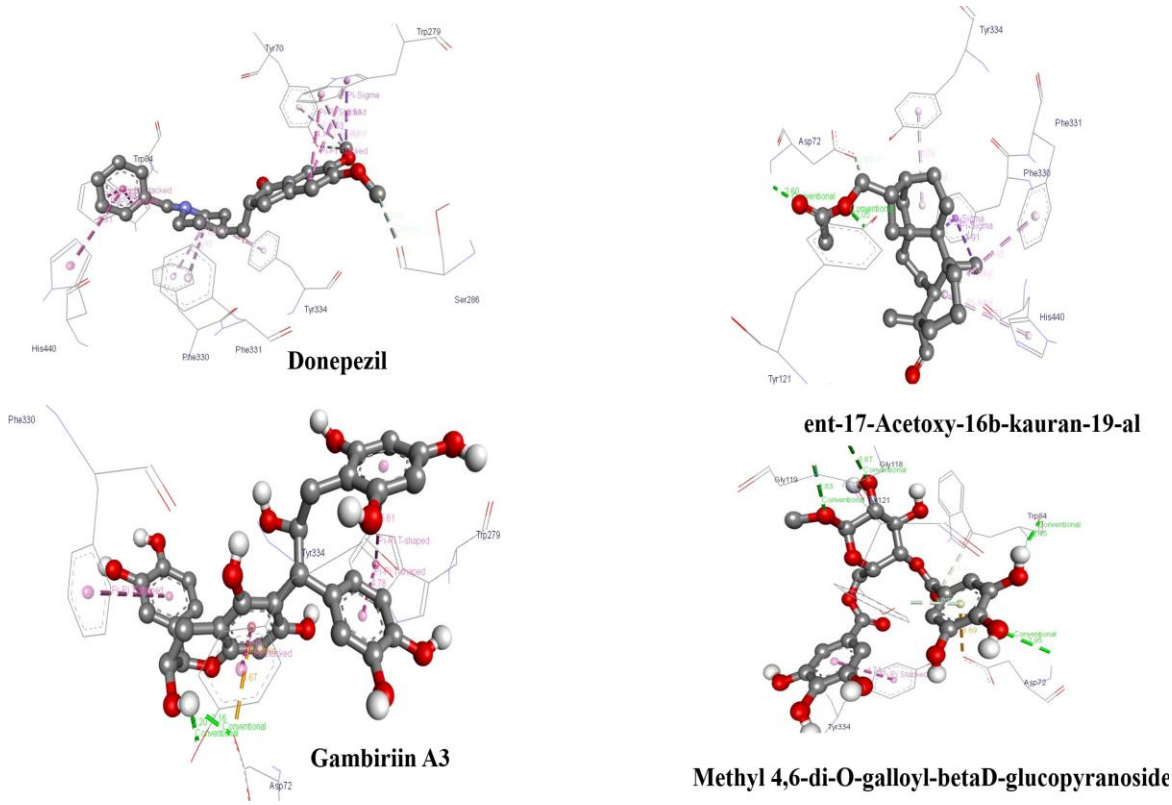

Figure 7. 3D interactions of phytochemicals against 1EVE acetylcholinesterase (AChE) enzyme.

Discussion

Cognitive and memory impairments are common in many neurological diseases like Alzheimer's disease (AD), Parkinson's disease (PD), stroke, etc. Therefore, improving cognition and memory function in affected individuals is highly desirable (Kakarla et al. 2024).

In the present study, we have investigated the cognition and memory enhancement activity of *R. apiculata* against scopolamine-induced cognition and memory impairment in rats. Our study shows significant improvement in cognition and memory activities in rats treated with *R. apiculata* plant extracts. Further, our mechanistic studies reported that both extracts, AERA and EERA inhibited the AChE activity and oxidative stress that resulted in improved cognition and memory function.

ACh is a neurotransmitter that plays a vital role in cognition and memory function. Physiologically, the ACh levels are regulated by AChE. This enzyme cleaves acetylcholine into acetyl and choline groups, which are reused to synthesize and maintain adequate ACh levels. However, under pathological conditions, AChE activity is exaggerated, leading to a decline in ACh levels, thereby impairing the ACh-mediated functions like cognition and memory (Alla et al. 2024). In this study, we have ascertained the cognition and memory dysfunction in scopolamine-treated rats using a passive avoidance test, indicating a decline in cognition and memory in rats. Further, we observed a significant increase in cognition and memory in rats with treatment of AERA and EERA leaf extracts, indicating that both extracts demonstrated a notable improvement in cognition and memory. Furthermore, we have observed a significant decline in the AChE activities with AERA and EERA extracts. These findings allow us to conclude that the improved cognition and memory function in rats with the intervention of both the

extract of *R. apiculata* could be due to the inhibition of AChE activities.

In earlier studies, we have reported the memory-enhancing effects *R. apiculata* extracts in a model of depression (Mande, Malothu, Areti, et al., 2023). Remarkably, in the current study, *R. apiculata* extracts demonstrate enhancements in cognition and memory, aligning with earlier research indicating that these extracts improve memory performance.

The emergence of cognitive and memory deterioration in individuals with dementia is not an abrupt occurrence. These symptoms arise as a result of ongoing neuronal damage. According to existing reports, neuronal damage occurs because of elevated oxidative stress, thereby causing various neurological diseases that are associated with cognition and memory dysfunction (Janloo et al. 2024; Rahimi et al. 2023). Therefore, prevention or inhibition of oxidative stress is considered a promising neuroprotective strategy. Our *in vitro* studies show that both extracts effectively alleviated the free radicals generated from DPPH and ABTS assays. Correspondingly, both extracts at various concentrations show an elevation of GSH enzyme activity and a decline in lipid peroxidation (LPO) in rats exposed to scopolamine. These findings confirm that *R. apiculata* extracts act as antioxidants that could lead to improved neuronal function. GSH is vital for detoxifying reactive oxygen species (ROS) and reactive nitrogen species (RNS), maintaining cellular redox balance, and protecting against oxidative stress, which is particularly important in various neurological diseases (Mande et al. 2023b). We have observed a significant improvement in brain GSH levels with the treatment of *R. apiculata* extracts against scopolamine-treated rats. To ascertain whether *R. apiculata* extracts possess the capacity to alter neuronal cell fate, we incubated various concentrations of both extracts with scopolamine-treated SH-S5Y5 cells, which exhibited considerable improvement in cell viability. These findings corroborate the hypothesis that the antioxidant properties may serve as a contributing factor to enhanced neuronal cell viability.

Natural products are the foundation for numerous pharmacologically active phytochemicals, which offer neurological benefits, including anti-inflammatory, antioxidant, antidepressant, cognition-enhancing, and AChE-inhibitory properties (Beheshtimanesh and Rajaei 2023). *In vitro*, *in vivo*, and *in-silico* studies suggested significant neuroprotection and cognition and memory-enhancing effects for AERA and EERA extracts. However, as of now, no studies have reported the phytochemical composition of *R. apiculata*. Therefore, we have subjected both extracts for HR-LCMS analysis to identify the bioactive secondary metabolites (Table 1).

Further, we have subjected the 30 phytochemicals for screening AchE binding properties using docking studies that reported strong binding affinities for AChE, with olitorin showing binding energy comparable to the reference standard drug donepezil (DPZ). Among the phytochemicals of EERA, vernasatin C, manghaslin, rutin, borrelidin, sesaminol, and harpagoside have been reported for their neuroprotective potential. Among the phytochemicals of AERA, vindoline, dioscorine have been reported to have neuroprotective potential and while protoaphin aglucone and 2-phenylethyl beta-D glucopyranoside have been reported to have antioxidant and anti-inflammatory activities. Therefore, we have assumed that the neuroprotective effects of AERA and EERA extracts could be due to the abundance of various phytochemicals that are responsible for neuroprotective effects. However, future studies are required to address their memory-enhancing effects.

Donepezil is commonly used to improve memory in patients. In this study, we have assessed the neuroprotective and memory-enhancing potential of both extracts with donepezil (Gutti et al. 2019; Gutti et al. 2023). Our results observed that

both the extracts exhibited equipotent neuroprotection and memory improvement in scopolamine-treated rats indicating that both extracts are potent in combating dementia. Both *in vitro* and *in vivo* findings demonstrate that AERA and EERA offer substantial neuroprotection against scopolamine-induced neurotoxicity in both cell lines and rat models. These findings indicate that *R. apiculata* holds promise as an antioxidant and neuroprotective agent, although further studies are necessary to evaluate its active phytochemicals.

Indeed, the present study has some limitations i) The present study did not evaluate the mechanism of action of antioxidant activities; hence, an in-depth investigation is required to understand the antioxidant mechanism of both extracts ii) the present study investigated the memory enhancement activity of *R. apiculata* in chemically induced dementia model, however, the memory enhancement property of this plant has to be further investigated in a disease model like AD, PD, and stroke, etc. iii) several potential phytochemicals has been identified through HR-LCMS studies, their neuroprotective and memory enhancement activities to be investigated in future studies.

The present study concludes that *R. apiculata* extracts (AERA and EERA) significantly improve cognition and memory in scopolamine-induced dementia models. These effects are mediated through AChE inhibition and antioxidant properties, reducing oxidative stress and enhancing neuronal survival. *In vitro, in vivo,* and *in-silico* studies support the neuroprotective potential of both extracts, comparable to donepezil. HR-LCMS analysis identified bioactive compounds with strong AChE binding affinities. While promising, further studies are needed to explore the antioxidant mechanisms, test efficacy in disease models, and validate the neuroprotective effects of individual phytochemicals for potential therapeutic applications in neurodegenerative diseases as possible sources for the isolation of chemical molecules with better neuroprotective activity.

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Conflicts of interest

There are no pertinent financial or non-financial interests that the authors need to disclose.

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Ethical Considerations

The study involved the rodents as animal models were approved by the Institutional Animal Ethical Committee (IAEC), Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India and experiments were conducted as per CCSEA guidelines, India.

Code of Ethics

IAEC/XIV/06/BCOP/2021.

Authors' Contributions

Annie M. has devoted a significant amount of time and energy to conducting the study and evaluating the findings. Narender M oversaw the research and provided insightful advice for conducting experiments, analyzing results, and writing the report. K Ramakrishna and Chakravarthi Guntupalli reviewed and edited the manuscript. S K Konidala assisted in *in silico* docking studies.

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