RESEARCH PAPER

Phytochemical Profiling and Neuroprotective Potential of *Zephyranthes rosea* Leaf Extract Against Aluminium-Induced Neurotoxicity in SH-SY5Y Cells

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ABSTRACT

Zephyranthes rosea, member of the family Amaryllidaceae, contains a rich source of several neuroactive alkaloids, including Galantamine, with established relevance in Alzheimer's disease management. While the bulb constituents have been investigated previously, the biological potential of the leaves remain largely unexplored.

The present study aimed to characterize the phytochemical composition of ethanolic extract of *Z. rosea* leaves (ZRLE) by GC–MS and to evaluate its antioxidant and acetylcholinesterase inhibitory activities, along with its cytotoxicity and neuroprotective efficacy against aluminium-induced neurotoxicity in SH-SY5Y cells.

GC–MS identified 33 phytochemicals in ZRLE, including galantamine-related alkaloids. The extract demonstrated notable antioxidant activity as well as moderate AChE inhibition. Cytotoxicity studies showed an LC50 of 85.38 µg/mL in SH-SY5Y cells. Aluminium chloride markedly reduced cell viability, while extract pre-treatment significantly attenuated neurotoxicity and restored cell viability with observable morphological protection. These findings suggest that ZRLE exerts significant neuroprotective activity.

Keywords: *Zephyranthes rosea*; GC–MS; anti-oxidant; neuroprotection; aluminium chloride; SH-SY5Y cells; MTT assay

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INTRODUCTION

Neurodegenerative diseases pose a major health and economic burden, with costs of dementia estimated to rise to USD 2 trillion by 2030, emphasising the need for efficient neuroprotective agents[1]. Current treatment strategies primarily provide symptomatic relief with little efficacy in halting disease progression. It is the need of the hour to investigate novel therapies targeting multiple pathological mechanisms-including amyloid aggregation, tau pathology, neuroinflammation, synaptic dysfunction and oxidative stress.[2,3]

Natural phytochemicals, including polysaccharides, flavonoids, such as kaempferol and hesperetin, and plant-derived compounds such as astragaloside IV, are promising due to their anti-inflammatory, antioxidant, and anti-apoptotic effects[1,4–6]. Emerging therapeutic approaches such as extracellular vesicles, cell-penetrating peptides and microRNA-based strategies, are also being explored to address the challenges of blood-brain barrier permeability and limited bioavailability[7]. Despite promising preclinical data, it is challenging to translate these approaches into clinical success, highlighting the necessity for precision medicine strategies supported by robust biomarkers to monitor therapeutic effects[2,8].

The SH-SY5Y human neuroblastoma cell line is a commonly used *in vitro* neuronal model for studying neurodegenerative mechanisms[9]. Aluminium exposure induces AD-like pathology in SH-SY5Y cells by promoting oxidative stress, mitochondrial dysfunction, apoptosis, and dysregulation of amyloid precursor protein processing[10–12]. Aluminium chloride ($AlCl_3$) has therefore been extensively used to model metal-induced neurotoxicity. Several natural compounds have exhibited protective effects against aluminium-induced neuronal damage by modulating oxidative stress and various cell survival pathways[13–15].

The genus *Zephyranthes* (family Amaryllidaceae) is renowned for its wide range of biologically active alkaloids with notable therapeutic relevance, particularly in neurodegenerative disorders[16,17]. Amaryllidaceae alkaloids such as galantamine are clinically approved acetylcholinesterase inhibitors used in the treatment of mild to moderate AD[18–20]. Several *Zephyranthes* species have been reported to contain galantamine, haemanthamine, and related alkaloids exhibiting cholinesterase inhibitory and neuroprotective

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activities[17,21–23]. Although previous studies have predominantly focused on bulbs, there is not much information available regarding the phytochemical composition and neuroprotective potential of *Zephyranthes* leaves.

Therefore, the present study represents the first comprehensive profile on the phytochemical constituents of ethanolic leaf extract of *Z. rosea* (ZRLE) using GC–MS and to assess its *in vitro* cytotoxicity and neuroprotective potential against $AlCl_3$-induced neurotoxicity in SH-SY5Y cells using the MTT assay.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaves of *Z. rosea* were gathered from Kanchiyar, Idukki District, Kerala in April 2023. Dr. Toji Thomas, Professor, Department of Botany, St. Thomas college, Pala, Kerala authenticated the fresh specimen and the prepared herbarium. A voucher herbarium specimen under the voucher number STCP2102 has been deposited at the herbarium of St. Thomas College, Pala, Kerala.

After carefully cleaning the collected leaves in running water, they were shade-dried to a constant weight and were coarsely powdered and extracted using a Soxhlet apparatus with 95% ethanol. The solvent was removed under reduced pressure and the obtained concentrate was stored at -4° C until further use[24,25].

Gas chromatography–mass spectrometry (GC–MS) analysis

Phytochemical analysis using GC–MS was performed with the help of Shimadzu Nexis GC- 2030 with an autosampler AOC-30/20i to characterize the chemical constituents of ZRLE. Separation was achieved using a capillary column with helium as the carrier gas under programmed oven temperature conditions. Samples were injected in splitless mode. Mass spectra and data were recorded at an electron impact ionization of 70eV and were compared to entries in the National Institute of Standards and Technology (NIST) library to identify the compounds, and high-confidence matches were only considered and expressed in relative percentage peak area [26].

In-vitro antioxidant assays

DPPH radical scavenging assay

The *in-vitro* free radical scavenging ability of ZRLE was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. 24 mg of DPPH was dissolved in 100 mL of methanol to create a stock solution, which was then kept at 20 °C in the dark. Further dilution of the stock solution with methanol produced a working solution of 0.267 mM (0.004%) DPPH, with an absorbance of around 0.98 ± 0.02 at 517 nm.

An aliquot of 1.5 mL of the DPPH working solution was added to 50 µL of ZRLE at various neuroprotective concentrations (1.5–25 µg/mL). After thorough mixing, the solution was incubated at room temperature for 30 minutes in the dark and absorbance was measured at 517 nm using a spectrophotometer. A control sample without extract was prepared under identical conditions and ascorbic acid was used as the positive control.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A_0\text{-}A_1}{A_0} \times 100$$

where, A_0 and A_1 represents the absorbance of control and the extract or standard, respectively. All experiments were performed in triplicate and IC_{50} was determined using non-linear regression analysis using a constrained four-parameter logistic model in GraphPad Prism [27].

Nitric oxide scavenging assay

Nitric oxide (NO) scavenging ability of ZRLE were evaluated using sodium nitroprusside (SNP) and the Griess reagent. At physiological pH, SNP releases NO in aqueous solution, and the resultant nitrite ions formed was measured using Griess reagent.

After mixing different neuroprotective concentrations of the ZRLE (1.5, 3.1, 6.25, 12.5, and 25 µg/mL) with 10mM SNP in phosphate-buffered saline (pH 7.4), the mixture was incubated for 3 hours at 25 °C. The resultant mixture was mixed with an equal volume of freshly prepared Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, and 3% phosphoric acid) and absorbance was measured at 546 nm using a spectrophotometer. Ascorbic acid was employed as the positive control.

The percentage inhibition of nitric oxide production was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A_0\text{-}A_1}{A_0} \times 100$$

where:

A_0 and A_1 represents the absorbance of the control and extract or standard, respectively. After performing the experiments in triplicate, IC_{50} was determined using non-linear regression analysis using a constrained four-parameter logistic model in GraphPad Prism 11.0.0 (84)[28].

In vitro Acetylcholine esterase inhibition assay

Acetylcholinesterase (AChE) inhibitory activity of ZRLE was evaluated by Ellmann's colorimetric method with slight modifications. The assay was performed in a 96-well microplate with each well containing 140 µL of 0.1 M phosphate buffer (pH 8.0), 10 µL of ZRLE at different concentrations (1.5, 3.1, 6.25, 12.5, and 25 µg/mL), and 10 µL of AChE enzyme solution (0.1 U/mL). After incubating at 25°C for 10 minutes in the dark, 10 µL of 10 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and 10 µL of 14 mM acetylthiocholine iodide was added as the substrate to initiate the enzymatic reaction. The plate was gently shaken for 1

minute and the reaction was terminated by adding 20 µL of 5% sodium dodecyl sulfate (SDS).
Control wells contained the same components as above, except that the extract was replaced with 10 µL of 70% ethanol. The formation of the yellow 5-thio-2-nitrobenzoate anion was measured at 412 nm using a microplate reader and all experiments were performed in triplicate, and results were expressed as mean ± SD. Galantamine was used as standard. The percentage inhibition of AChE activity was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract or standard. IC_{50} was determined using non-linear regression analysis using a constrained four-parameter logistic model in GraphPad Prism.

Cell culture

SH-SY5Y cell line procured from the national cell repository was cultured in Dulbecco's Modified Eagle Medium (DMEM), with foetal bovine serum, L-glutamine, buffering agents, and antibiotics added. The cultured cell lines were kept in a humidified incubator with 5% CO_2 at 37 °C. Two days old confluent monolayer of cells were suspended in 10% growth media after being trypsinized[29].

***In vitro* cytotoxicity assessment by MTT assay**

The cytotoxicity of ZRLE was evaluated by employing the MTT assay. In 96- well plates, a 100µl cell suspension (5×10^3 cells/well) was reseeded and were allowed to adhere under standard culture conditions. Cells were exposed to increasing concentrations of the ZRLE (6.25–100 µg/ml) followed by incubation at 37 °C in a humidified incubator with 5% CO_2. Additionally, untreated cells served as control. After 24 hours, entire plate was examined for any discernible changes in cell morphology using an inverted phase microscope.
Following ZRLE treatment, MTT reagent was added and the mixture was incubated for 4 hours to enable formazan crystal formation. After solubilising the formazan crystals, absorbance was measured at 540 nm. [30,31]. LC_{50} was determined using non-linear regression analysis using GraphPad Prism.

Neuroprotection assay

Aluminium chloride ($AlCl_3$) was used to induce neurotoxicity in SH-SY5Y cells. A stock solution of $AlCl_3$ was prepared in sterile distilled water, and a working concentration of 800 µM was freshly prepared in culture medium prior to use[32]. SH-SY5Y cells were seeded at a density of 5×10^3 cells/well and allowed to attach for 24 h. Based on MTT cytotoxicity assay, non-toxic concentrations of ZRLE were selected for further neuroprotection studies. Cells were pre-treated with ZRLE (1.5–25 µg/ml) for 24 h, and were exposed to $AlCl_3$ (800 µM) for 1 h to induce neurotoxicity[33].

Treatment groups included untreated control, $AlCl_3$ alone, and ZRLE pre-treated cells followed by $AlCl_3$ exposure. Morphological changes and cell viability were determined using MTT assay as described above. Data were expressed as mean ± standard deviation (SD). Statistical comparisons using one-way ANOVA followed by Dunnett's post-hoc test were performed, with $p < 0.05$ considered statistically significant[30].

RESULTS AND DISCUSSION

Phytochemical profiling of ZRLE by GC-MS

Phytochemical profiling of the ZRLE by GC-MS showed a complex phytochemical profile composed of 34 different compounds with a retention time (RT) range of 7.833-34.843 min, representing different chemical classes such as pyranones, furan derivatives, phenolics, fatty acids and alkaloids. The total ion chromatogram (TIC) showed obvious peaks, and the five highest area compounds were roughly 54.81% of the total peak area. Notably, 4H-pyran-4-one, 2,3-dihydroxy-6-methyl- (RT 7.833 min, 19.51%) and 4H-pyran-4-one, 3,5-dihydroxy-2-methyl- (RT 8.407 min, 2.33%), as pyranone derivatives, were the dominant and together represented 21.84%. 5-Hydroxymethylfurfural (RT 9.057 min, 15.97%) is a known product of the Maillard reaction[32].
Notably, Amaryllidaceae alkaloids, including galantamine and related derivatives, were detected along with several antioxidant and neuroactive constituents. The relative abundance of the identified compounds is presented as percentage peak area in **Table 1**, while the GC–MS chromatogram is shown in **Figure 1**. The presence of these compounds supports the potential neuropharmacological relevance of the leaf extract.

Table 1: GC–MS phytochemical composition of *Z. rosea* ethanolic leaf extract

Sl. No	RT (min)	Name of the Compound	Mol. Formula	Mol. Weight	Peak Area (%)
1.	7.833	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	19.51
2.	8.407	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$	142	2.33
3.	8.649	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	102	0.72
4.	8.844	4-Vinylphenol	C_8H_8O	120	3.24
5.	9.057	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	15.97

6.	9.265	Furandimethanol	$C_6H_8O_3$	128	0.34
7.	9.334	Benzeneacetic acid	$C_8H_8O_2$	136	1.52
8.	9.601	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134	0.71
9.	10.148	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	2.15
10.	10.474	Glutaric acid, 1-naphthyl tridecyl ester	$C_{28}H_{40}O_4$	440	1.68
11.	11.256	L-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	2.28
12.	11.815	2-Propenoic acid, 3-phenyl-	$C_9H_8O_2$	148	1.08
13.	11.913	1,3-Dioxan-4-one, 6-methyl-2-phenyl-, (2R-cis)-	$C_{11}H_{12}O_3$	192	0.70
14.	12.059	DL-Proline, 5-oxo-, ethyl ester	$C_7H_{11}NO_3$	157	4.85
15.	13.390	Phenol, 4-ethenyl-2,6-dimethoxy-	$C_{10}H_{12}O_3$	180	1.00
16.	13.853	Ethyl N-(o-anisyl)formimidate	$C_{10}H_{13}NO_2$	179	0.97
17.	15.724	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.70
18.	16.012	1-(2-Bromoethyl)pyrrolidin-2-one	$C_6H_{10}BrNO$	191	1.23
19.	16.264	Cardoltriene	$C_{21}H_{30}O_2$	314	0.94
20.	17.569	[1,2,4]-Triazolo[1,5-a]pyrimidin-7(3H)-one, 2-amino-5-isopropyl-	$C_8H_{11}N_5O$	193	0.32
21.	18.123	4,6-Diamino-O-cresol	$C_7H_{10}N_2O$	138	1.70
22.	19.060	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.38
23.	23.203	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	$C_{57}H_{104}O_6$	884	2.58
24.	23.747	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.04
25.	26.471	Glycidyl palmitate	$C_{19}H_{36}O_3$	312	0.31
26.	28.294	Galantamine	$C_{17}H_{21}NO_3$	287	3.38
27.	29.220	1-(4-Methoxyphenyl)-3,6-diazatricyclo[4.3.1.1(3,8)]undecan-9-ol	$C_{16}H_{22}N_2O_2$	274	6.42
28.	29.667	(4aS,8aS)-3-Methoxy-11-methyl-9,10,11,12-tetrahydro-4aH-benzo[2,3]benzofuro[4,3-cd]azepin-6(5H)-one/Galanthaminone/Narwedine	$C_{17}H_{19}NO_3$	285	0.47
29.	31.512	Ethyl 2-cyano-2-(9-fluorenylidene)acetate	$C_{18}H_{13}NO_2$	275	11.62
30.	31.851	5,6,8,9-Tetrahydrobenz(a)anthracen-11(10H)-one	$C_{18}H_{16}O$	248	1.05
31.	33.357	3b,4,5,6,7,7a-Hexahydrobenzo[b]fluoranthene	$C_{20}H_{18}$	258	1.39
32.	33.467	Phthalic acid, 4-methoxybenzyl methyl ester	$C_{17}H_{16}O_5$	300	0.47
33.	34.843	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	282	0.43

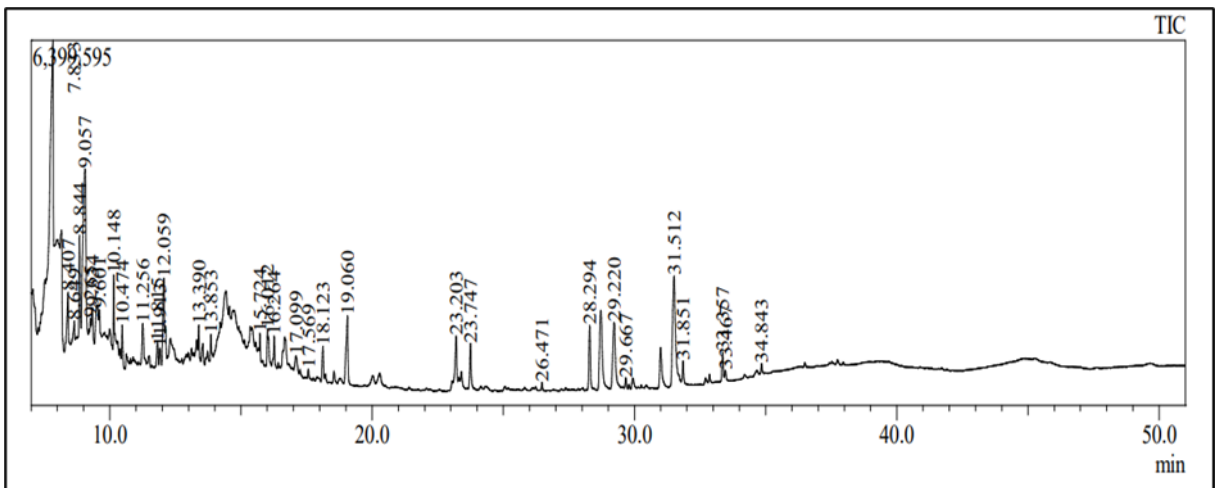

Figure 1: Total ion chromatogram (TIC) of *Z. rosea* leaf extract obtained by GC-MS

***Invitro* antioxidant activity**

The antioxidant potential of ZRLE was evaluated using DPPH radical scavenging and nitric oxide (NO) scavenging assays. In both models, the extract exhibited concentration-dependent inhibition, indicating its ability to neutralize both free radicals and reactive nitrogen species. The DPPH radical scavenging activity exhibited a concentration-dependent inhibition. Non-linear regression analysis using a constrained four-parameter logistic model demonstrated acceptable goodness-of-fit (R^2= 0.95-0.98). ZRLE demonstrated a moderate DPPH free radical scavenging activity with IC_{50} value of 15.13 µg/mL (95% CI: 13.52-17.15), compared with the standard ascorbic acid with IC_{50} value of 3.67µg/mL (95% CI: 3.12-4.26). (**Figure 2A**).

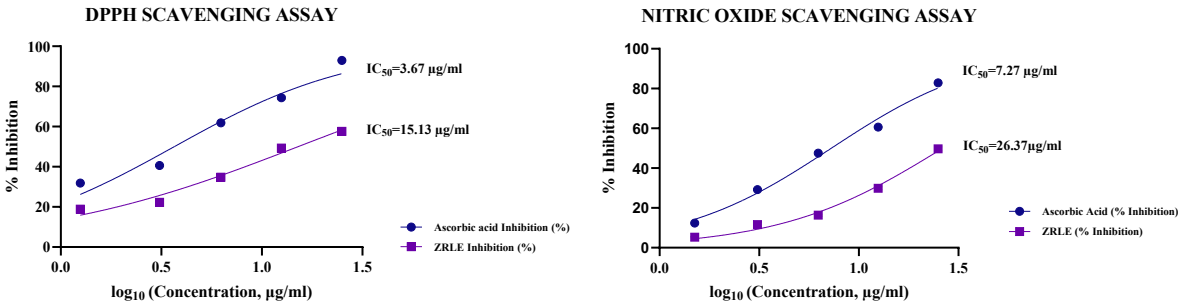

Figure 2: Antioxidant activity of *Z. rosea* leaf extract showing (A) DPPH radical scavenging activity and (B) nitric oxide scavenging activity.

Similarly, in the nitric oxide scavenging assay, ZRLE showed concentration-dependent inhibition of nitrite formation generated from SNP. Ascorbic acid exhibited strong NO scavenging activity with an IC_{50} of 7.27 µg/ml (95% CI: 6.77-7.82), whereas ZRLE exhibited moderate activity with IC_{50} of 26.37µg/ml (95% CI: 24.54-28.60) with goodness-of-fit, R^2=0.98-0.99 (**Figure 2B**).

Overall, the results indicate that the ZRLE possesses appreciable antioxidant activity, with stronger efficacy against DPPH radicals and nitric oxide radicals. These activities may be attributed to the presence of anti-oxidant phytoconstituents in *Z. rosea* leaves, that are capable of scavenging reactive species.

Acetylcholinesterase (AChE) inhibitory activity

ZRLE exhibited concentration-dependent inhibition of acetylcholinesterase (AChE) activity over the tested range of 1.5–25 µg/mL. The percentage inhibition increased progressively with increasing concentration, indicating effective suppression of AChE activity with increased concentration.

Non-linear regression analysis using a constrained four-parameter logistic model demonstrated excellent goodness-of-fit (R^2=0.99 and 0.98) with standard galantamine exhibiting IC_{50} value of 1.17 µg/mL (95% CI: 1.14–1.21) and ZRLE with IC_{50} value of 15.77 µg/mL (95% CI: 14.67–17.04) (**Figure 3**). These findings indicate that although the leaf extract possesses appreciable anti-AChE activity, its potency is considerably lower than that of the galantamine.

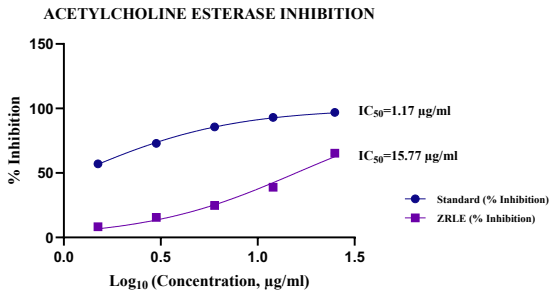

Figure 3: Acetyl Choline Esterase inhibitory activity of *Z. rosea* leaf extract

***In vitro* cytotoxicity of ZRLE on SH-SY5Y cells**

The cytotoxic potential of the ZRLE evaluated in SH-SY5Y cells using the MTT assay revealed a concentration-dependent reduction in cell viability after exposure to ZRLE. Lower concentrations maintained high cell viability, whereas higher concentrations exhibited moderate cytotoxic effects. The LC_{50} value was calculated using non-linear regression analysis using a constrained four-parameter logistic model and was found to be 83.38 µg/ml (R^2=0.99), indicating that the extract possesses a favourable safety profile in neuronal cells. Based on these findings, non-cytotoxic concentrations were selected for subsequent neuroprotection studies. The data representing cytotoxicity is represented in **Figure 4A** and morphological changes are represented in **Figure 4B.**

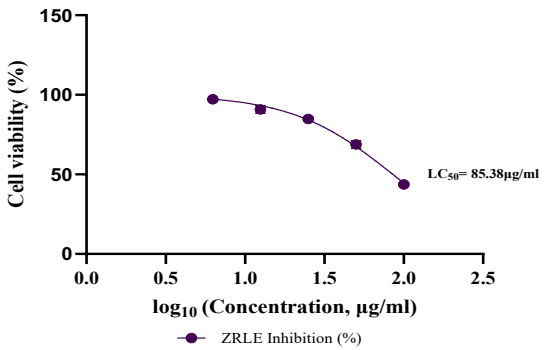

Figure 4A: Graphical representation depicting the neurotoxic effect of *Z. rosea* Leaf extract on SHSY5Y cells by MTT assay. All experiments were done in triplicates and LC_{50} values were determined by non-linear regression analysis using a constrained four-parameter logistic model.

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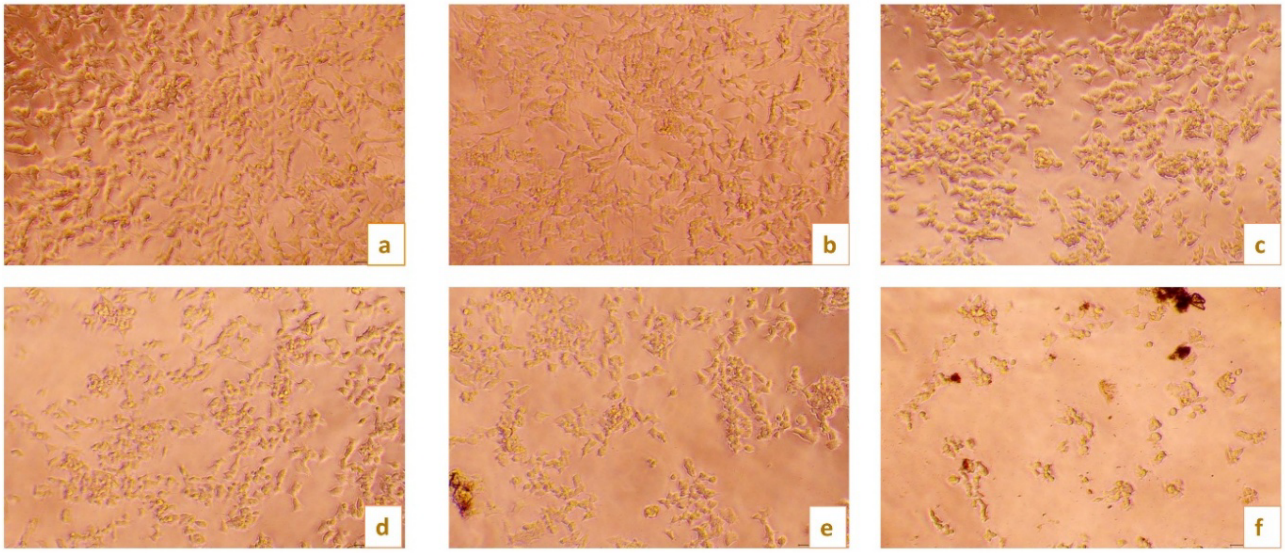

Figure 4B: Morphological assessment of SH-SY5Y cells. (a) Untreated control cells exhibiting normal neuronal morphology. (b–f) SH-SY5Y cells exposed to *Z. rosea* leaf extract at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL, respectively, showing concentration dependent morphological changes.

Neuroprotective effect of ZRLE against $AlCl_3$-induced toxicity

SH-SY5Y cells exposed to $AlCl_3$ resulted in significant neurotoxicity, with marked decrease in cell viability compared to untreated control cells. Morphological examination also revealed characteristics of neuronal damage, including cell shrinkage, reduced neurite outgrowth, and loss of cellular integrity as depicted in **Figure 5A**.

Pre-treatment with ZRLE significantly reduced $AlCl_3$-induced cytotoxicity in SH-SY5Y cells by exhibiting a substantial recovery in cell viability compared to the $AlCl_3$-treated group (**Figure 5B**). Maximum neuroprotective efficacy was exhibited by lower concentrations, restoring cell viability close to control. Phase-contrast microscopy data clearly indicated the severe cytotoxicity by $AlCl_3$, which was marked by cell rounding, shrinkage, cytoplasmic granulation, vacuolization, and detachment. ZRLE was able to provide protection against these toxic insults, with maximal protection at 25 µg/ml with residual vacuolization despite insensible loss of viability.

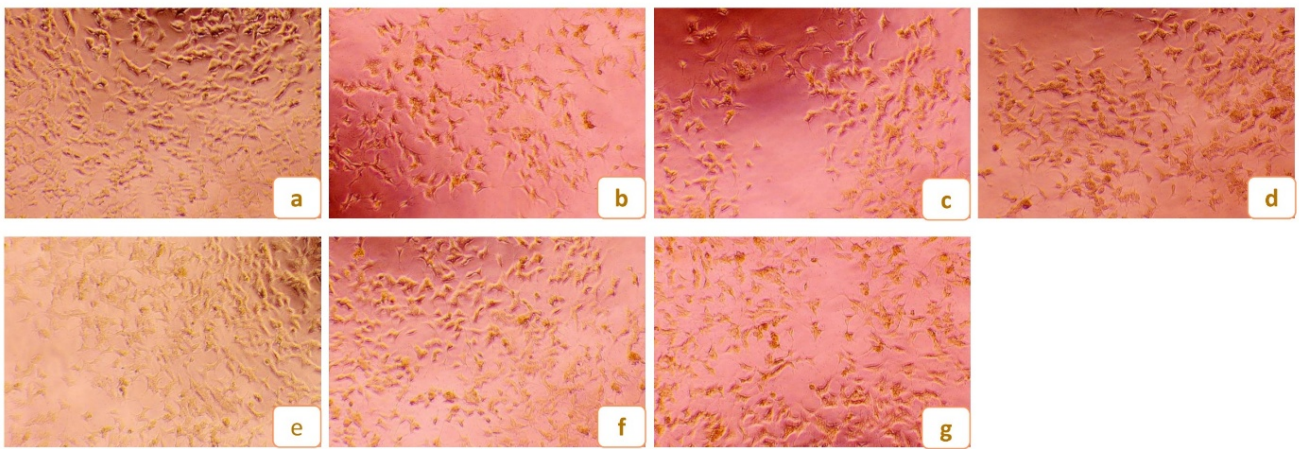

Figure 5A: **Morphological assessment of SH-SY5Y cells. (**a) Untreated control cells exhibiting normal morphology. (b) Cells exposed to Aluminium chloride (800 µM) showing cell shrinkage, rounding and reduced cell density (c–g) Cells pre-treated with the ethanolic *Z. rosea* leaf extract at increasing concentrations of 1.5, 3.1, 6.25, 12.5, and 25 µg/mL, respectively followed by $AlCl_3$ exposure, showing restoration of cell morphology

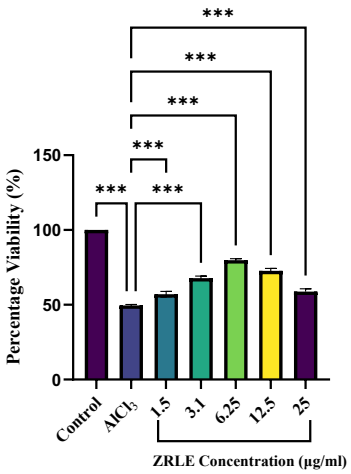

Figure 5B: **Graphical representation depicting the neuroprotective effect of *Z. rosea* leaf extract on SHSY5Y cells by MTT assay**. All experiments were done in triplicates and results represented as Mean+/- SD. One-way ANOVA and Dunnett's test were performed to analyze data. ***p< 0.001 compared to Aluminium Chloride induced groups.

DISCUSSION

Neurodegenerative disorders including Alzheimer's disease (AD) are characterized by progressive neuronal loss due to oxidative stress, metal toxicity, mitochondrial dysfunction, and apoptotic signalling[34]. Aluminium exposure has been associated to the pathogenesis of neuro degenerative disorders due to their ability to induce oxidative stress, disrupt calcium homeostasis, and promote amyloidogenesis, thus leading to neurodegeneration [35].

The presence of diverse phytoconstituents, including alkaloids, fatty acids, and antioxidant compounds, along with the galantamine- a clinically approved acetylcholinesterase inhibitor used in the AD management, and related alkaloids, highlights the therapeutic potential of *Z. rosea* in neurodegenerative diseases complementing previous reports that primarily focused on bulb-derived constituents [18,19,36,37].

Cytotoxicity evaluation in SH-SY5Y cells using MTT assay has demonstrated that the ZRLE was able to exhibit an acceptable safety profile, with an LC_{50} value indicating low inherent toxicity at biologically relevant concentrations. This finding is critical for neuroprotection studies, as excessive cytotoxicity can confound interpretation of protective effects. In order to ensure that the observed neuroprotective effects were due to actual cellular protection and not the artefacts associated with reduced cell survival, non-toxic concentrations were used in subsequent neuroprotective studies in an aluminium chloride- induced neurotoxic model[38].

Pre-treatment with ZRLE dramatically reduced aluminium-induced neuronal damage, as evidenced by improved cell viability and preservation of normal cell morphology. Interestingly, maximal neuroprotection was exhibited by *Z. rosea* at lower concentrations, suggesting a hormetic dose-response relationship, wherein optimal concentrations confer maximum protection whereas higher doses may induce mild cellular stress. Similar neuroprotective patterns have been reported for several plant-derived antioxidants and alkaloids[39–41].

The antioxidant assays further support the neuroprotective mechanism of the extract. ZRLE exhibited concentration-dependent scavenging of both DPPH radicals and nitric oxide, suggesting its ability to neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative stress plays a central role in neuronal degeneration by damaging lipids, proteins, and nucleic acids; therefore, antioxidant activity is a key contributor to neuroprotection[42]. The presence of phenolics, flavonoids, and other redox-active constituents in the leaf extract may attribute to the antioxidant activity exhibited by the ZRLE[43].

Cholinergic dysfunction is a key hallmark in AD pathology, and thus AChE inhibitors remain the mainstay of symptomatic treatment[44]. The inhibitory activity exhibited against AChE by the ZRLE, the enzyme that breakdown acetylcholine in the synaptic clefts, suggests the presence of bioactive compounds capable of enhancing cholinergic neurotransmission, further supporting its neuroprotective potential.

CONCLUSION

Overall, the results suggest the multi target neuroprotective mechanism of *Z. rosea* by attenuation of oxidative stress, preservation of cellular integrity, and modulation of cholinergic activity. Although the present study findings are limited to an *in vitro* model, they provide preliminary evidence supporting the therapeutic potential of *Z. rosea* leaves as a promising source of neuroprotective agents. Future studies focusing on mechanistic investigations, isolation and in vivo validation in AD models are warranted to substantiate the therapeutic potential of *Z. rosea*.

ETHICAL ISSUES

None to be declared

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CONFLICT OF INTERESTS

Authors declare no conflict of interests

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