Phytochemical Characterization and Neuroprotective Potential of Belamcanda Chinensis Rhizome Extract: An HR-LCMS Based In Vitro Investigation

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Abstract—**Neurodegenerative disorders are among the leading causes of disability worldwide and are primarily associated with oxidative stress, neuroinflammation, and progressive neuronal loss. The search for natural neuroprotective agents has gained significant attention due to their potential to modulate multiple pathological pathways involved in neurodegeneration. The present study aimed to investigate the phytochemical composition and neuroprotective potential of the hydroalcoholic rhizome extract of Belamcanda chinensis through chromatographic fractionation, HR-LCMS profiling, and in vitro evaluation using SH-SY5Y neuronal cells. Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, tannins, glycosides, coumarins, saponins, carbohydrates, and gums and mucilage. Column chromatographic separation followed by TLC profiling resulted in the formation of two pooled fractions, PF1 and PF2. HR-LCMS analysis identified twenty major compounds in each fraction, including gallic acid, catechin, chlorogenic acid, caffeic acid, mangiferin, tectoridin, iridin, irigenin, tectorigenin, β-sitosterol, ursolic acid, ferulic acid, apigenin, hispidulin, betulin, and daucosterol.**

Neuroprotective activity was assessed against hydrogen peroxide-induced oxidative stress in SH-SY5Y cells using the MTT assay. Both fractions exhibited concentration-dependent cytoprotective effects. PF1 showed an IC50 value of 41.2 µg/mL, whereas PF2 demonstrated greater potency with an IC50 value of 34.7 µg/mL. At 100 µg/mL, PF2 restored cell viability to 89.1 ± 2.5%, indicating superior neuroprotective efficacy compared with PF1. Morphological assessment further confirmed the protective effects of both fractions against oxidative stress-induced neuronal damage.

The findings suggest that Belamcanda chinensis rhizome is a rich source of bioactive phytochemicals with significant neuroprotective potential. The observed activity may be attributed to the synergistic effects of flavonoids, phenolic acids, isoflavonoids, and triterpenoids identified in the extract. These results support the potential application of Belamcanda chinensis as a promising source of natural neuroprotective agents for the management of oxidative stress-associated neurodegenerative disorders.

Index Terms—**Belamcanda chinensis, Neuroprotection, HR-LCMS, SH-SY5Y cells, Phytochemicals, Oxidative stress, MTT assay.**

I. INTRODUCTION

1.1. Neurodegenerative Disorders and Therapeutic Challenges

Neurodegenerative disorders are progressive neurological conditions characterized by the gradual loss of neuronal structure and function, ultimately leading to cognitive impairment, memory loss, behavioral abnormalities, and motor dysfunction. Among these disorders, Alzheimer's disease and Parkinson's disease represent the most prevalent forms and are responsible for a substantial proportion of neurological disability worldwide. The increasing prevalence of these disorders is closely associated with population ageing and increased life expectancy, creating a significant socioeconomic and healthcare burden across both developed and developing countries [1].

The pathogenesis of neurodegenerative diseases is highly complex and involves multiple interconnected mechanisms, including oxidative stress, mitochondrial dysfunction, neuroinflammation, protein aggregation, and apoptotic cell death. Excessive generation of reactive oxygen species (ROS) can damage cellular proteins, lipids, and nucleic acids, resulting in progressive neuronal injury and functional decline. Mitochondrial abnormalities further exacerbate oxidative damage by impairing cellular energy metabolism and promoting free radical generation [2].

Despite considerable advances in neuroscience research, currently available therapeutic interventions remain largely symptomatic and provide limited protection against disease progression. Most approved drugs primarily improve cognitive or motor symptoms without effectively preventing neuronal degeneration. Consequently, there is growing interest in identifying novel neuroprotective agents capable of targeting multiple pathological pathways involved in neurodegeneration [3].

1.2. Medicinal Plants as Sources of Neuroprotective Agents

Natural products have long served as an important source of therapeutic agents and continue to contribute significantly to modern drug discovery. Medicinal plants are particularly attractive in neurodegenerative disease research because they contain structurally diverse phytochemicals capable of exerting antioxidant, anti-inflammatory, and cytoprotective effects. Unlike conventional drugs that often act on a single molecular target, plant-derived compounds frequently demonstrate multitargeted mechanisms of action [4].

Flavonoids, phenolic acids, terpenoids, and glycosides have been widely reported to protect neuronal cells against oxidative stress-induced damage. These phytochemicals can scavenge free radicals, suppress neuroinflammation, regulate intracellular signaling pathways, and preserve mitochondrial function. Such activities contribute to improved neuronal survival and may help delay the progression of neurodegenerative disorders [5].

Increasing scientific evidence has demonstrated that phytochemical-rich medicinal plants possess significant neuroprotective potential in experimental models of Alzheimer's disease, Parkinson's disease, and other neurological disorders. Consequently, the exploration of medicinal plants remains an important strategy for the discovery of safer and more effective neuroprotective agents [6].

1.3. Belamcanda chinensis and Study Rationale

Belamcanda chinensis (L.) DC. (Family: Iridaceae), commonly known as Blackberry Lily, is an important medicinal plant traditionally used in East Asian medicine for the treatment of inflammatory and respiratory disorders. The rhizome is recognized as the principal medicinal part and contains a variety of bioactive constituents, including flavonoids, isoflavonoids, phenolic compounds, and triterpenoids [7].

Previous phytochemical investigations have identified compounds such as tectoridin, tectorigenin, iridin, irigenin, mangiferin, and related phenolic metabolites in the rhizome. Many of these compounds exhibit antioxidant and anti-inflammatory activities that are highly relevant to neuroprotection. However, despite extensive phytochemical characterization, the neuroprotective potential of Belamcanda chinensis remains insufficiently explored [8].

Therefore, the present study was undertaken to characterize the phytochemical constituents of the hydroalcoholic rhizome extract of Belamcanda chinensis using HR-LCMS and to evaluate the neuroprotective activity of its chromatographic fractions against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuronal cells. The study aims to establish a scientific basis for the potential application of this medicinal plant in the management of oxidative stress-associated neurodegenerative disorders.

Table 1. Botanical profile of Belamcanda chinensis

Parameter	Description
Botanical Name	Belamcanda chinensis (L.) DC.
Family	Iridaceae
Common Name	Blackberry Lily
Part Used	Rhizome
Habit	Perennial Herb
Distribution	China, Japan, Korea, India

II. MATERIALS AND METHODS

2.1. Plant Material and Extraction

Rhizomes of *Belamcanda chinensis* (L.) DC. were collected, authenticated, cleaned, shade-dried, and pulverized into coarse powder. The powdered material was subjected to hydroalcoholic extraction using ethanol and distilled water. The extract was filtered, concentrated under reduced pressure, and stored at 4°C until further use [9].

2.2. Preliminary Phytochemical Screening

The hydroalcoholic extract was subjected to preliminary phytochemical screening using standard qualitative procedures to identify major classes of secondary metabolites, including flavonoids, phenolic

compounds, tannins, glycosides, saponins, coumarins, carbohydrates, alkaloids, steroids, and proteins [10].

Figure 1. Belamcanda chinensis (L.) DC. plant and rhizome used in the present study.

2.3. Chromatographic Fractionation and TLC Analysis

The crude extract was fractionated by silica gel column chromatography using solvent systems of increasing polarity. Individual fractions were collected and monitored by thin-layer chromatography (TLC). Fractions exhibiting similar chromatographic profiles were pooled and designated as PF1 and PF2 for further phytochemical and biological evaluation [11].

2.4. HR-LCMS Analysis

The pooled fractions (PF1 and PF2) were analyzed using High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LCMS) for metabolite profiling. Compound identification was performed based on retention time, accurate mass measurements, fragmentation patterns, and comparison with available spectral databases and literature reports [12].

2.5. Cell Culture and Neuroprotective Evaluation

Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 [13].

Oxidative stress was induced using hydrogen peroxide (H_2O_2). Cells were pretreated with different concentrations of PF1 and PF2 prior to H_2O_2 exposure. Untreated cells served as the normal control, while H_2O_2-treated cells served as the oxidative stress control. Donepezil was used as the reference neuroprotective standard.

2.6. Cell Viability Assay

Neuroprotective activity was evaluated using the MTT assay. Following treatment, MTT solution was added and incubated to allow the formation of formazan crystals by viable cells. The crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured using a microplate reader. Cell viability was expressed as a percentage relative to the untreated control group [14].

2.7. Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by an appropriate post hoc test. Differences were considered statistically significant at $p < 0.05$.

III. RESULTS

3.1. Preliminary Phytochemical Screening

Preliminary phytochemical screening of the hydroalcoholic rhizome extract of *Belamcanda chinensis* revealed the presence of several biologically important secondary metabolites. The extract showed positive tests for flavonoids, tannins and phenolic compounds, saponins, glycosides, coumarins, carbohydrates, and gums and mucilage. In contrast, alkaloids, steroids and terpenoids, proteins, amino acids, fixed oils and fats, quinones, and resins were not detected. The predominance of phenolic and flavonoid constituents suggested significant antioxidant potential and justified further phytochemical characterization of the extract.

Table 2. Preliminary phytochemical screening of the hydroalcoholic rhizome extract of *Belamcanda chinensis*

Phytochemical Class	Result
Alkaloids	Absent
Flavonoids	Present
Tannins and Phenolic Compounds	Present
Saponins	Present
Steroids and Terpenoids	Absent
Glycosides	Present
Coumarins	Present
Carbohydrates	Present
Proteins	Absent
Amino Acids	Absent
Fixed Oils and Fats	Absent
Gums and Mucilage	Present

Quinones	Absent
Resins	Absent

3.2. TLC Profiling and Fraction Selection

Column chromatographic fractionation of the hydroalcoholic extract yielded twelve fractions (F1–F12). TLC analysis demonstrated distinct chromatographic patterns among the collected fractions. Based on similarity in Rf values and spot profiles, fractions F1–F6 were pooled as PF1, while fractions F7–F12 were combined to obtain PF2.

The observed TLC patterns indicated successful separation of phytochemical constituents into two chemically distinct pooled fractions suitable for subsequent HR-LCMS characterization and neuroprotective evaluation.

Table 3. TLC profiling and pooled fraction selection

Fraction	Elution Solvent System	Rf Value(s)	Pooled To
F1	100% n-Hexane	0.78, 0.65, 0.45	PF1
F2	n-Hexane:Ethyl acetate (95:5)	0.77, 0.64, 0.45	PF1
F3	n-Hexane:Ethyl acetate (90:10)	0.76, 0.63, 0.44	PF1
F4	n-Hexane:Ethyl acetate (80:20)	0.78, 0.65, 0.45	PF1
F5	n-Hexane:Ethyl acetate (70:30)	0.77, 0.64, 0.46	PF1
F6	n-Hexane:Ethyl acetate (50:50)	0.78, 0.65, 0.45	PF1
F7	100% Ethyl acetate	0.58, 0.39, 0.24	PF2
F8	Ethyl acetate:Methanol (95:5)	0.59, 0.40, 0.24	PF2
F9	Ethyl acetate:Methanol (80:20)	0.60, 0.41, 0.25	PF2
F10	Ethyl acetate:Methanol (70:30)	0.58, 0.39, 0.24	PF2
F11	100% Methanol	0.57, 0.38, 0.23	PF2
F12	100% Methanol (continued)	0.59, 0.40, 0.24	PF2

3.3. HR-LCMS Characterization of PF1

HR-LCMS analysis of PF1 revealed the presence of twenty major phytochemical constituents belonging to phenolic acids, flavonoids, isoflavonoids, glycosides, sterols, and triterpenoids. Major compounds identified included gallic acid, catechin, chlorogenic acid, caffeic acid, quercetin-3-O-glucoside, mangiferin, tectoridin, iridin, irigenin, β-sitosterol, and ursolic acid.

The presence of these metabolites indicates that PF1 is enriched with antioxidant and biologically active phytochemicals that may contribute to neuronal protection against oxidative stress.

Table 4. HR-LCMS identified compounds in PF1

Sr. No.	Compound Name	Retention Time (min)	Molecular Formula	Molecular Weight (g/mol)
1	Gallic acid	3.45	$C_7H_6O_5$	170.12
2	3,4-Dihydroxybenzoic acid	4.82	$C_7H_6O_4$	154.12
3	Catechin	6.78	$C_{15}H_{14}O_6$	290.27
4	Chlorogenic acid	8.24	$C_{16}H_{18}O_9$	354.31
5	Belamcanone A	9.67	$C_{18}H_{16}O_7$	344.32
6	Caffeic acid	10.35	$C_9H_8O_4$	180.16
7	Irilin D	12.89	$C_{23}H_{24}O_{11}$	476.43
8	Quercetin-3-O-glucoside	14.52	$C_{21}H_{20}O_{12}$	464.38
9	Tectoridin	16.34	$C_{22}H_{22}O_{11}$	462.40
10	Iridin	17.91	$C_{24}H_{26}O_{13}$	522.46
11	Iristectorin A	19.78	$C_{23}H_{24}O_{12}$	492.43
12	Belamcanoside B	20.45	$C_{25}H_{28}O_{14}$	552.48
13	Mangiferin	21.67	$C_{19}H_{18}O_{11}$	422.34
14	Dichotomitin	23.12	$C_{17}H_{14}O_8$	346.29
15	Irigenin	25.89	$C_{18}H_{16}O_8$	360.32
16	Tectorigenin	27.34	$C_{16}H_{12}O_6$	300.26
17	Irisflorentin	28.91	$C_{17}H_{14}O_7$	330.29
18	Belamcanin C	30.67	$C_{19}H_{18}O_6$	342.34
19	β-Sitosterol	34.52	$C_{29}H_{50}O$	414.71
20	Ursolic acid	36.78	$C_{30}H_{48}O_3$	456.70

3.4. HR-LCMS Characterization of PF2

HR-LCMS profiling of PF2 resulted in the identification of twenty major metabolites. The fraction was rich in phenolic acids, flavonoids, isoflavonoids, phytosterols, and triterpenoids. Major constituents included vanillic acid, p-coumaric acid, ferulic acid, hispidulin, apigenin, chrysoeriol, irigenin, tectorigenin, β-sitosterol, betulin, betulone, and ursolic acid.

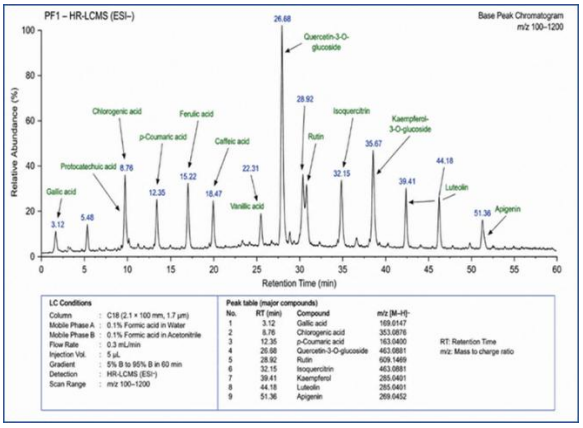

Figure 2. HR-LCMS chromatogram of PF1

Compared with PF1, PF2 demonstrated greater enrichment of flavonoids and phenolic compounds associated with antioxidant and cytoprotective activities, suggesting enhanced neuroprotective potential.

Table 5. HR-LCMS identified compounds in PF2

Sr. No.	Compound Name	Retention Time (min)	Molecular Formula	Molecular Weight (g/mol)
1	4-Hydroxyacetophenone	8.72	$C_8H_8O_2$	136.15
2	Vanillic acid	10.45	$C_8H_8O_4$	168.15
3	p-Coumaric acid	12.89	$C_9H_8O_3$	164.16
4	Belamcanquinone A	15.34	$C_{16}H_{14}O_4$	270.28
5	Ferulic acid	16.78	$C_{10}H_{10}O_4$	194.18
6	Hispidulin	18.92	$C_{16}H_{12}O_6$	300.26
7	Apigenin	20.15	$C_{15}H_{10}O_5$	270.24
8	Chrysoeriol	21.67	$C_{16}H_{12}O_6$	300.26
9	Belamcanaldehyde	23.45	$C_{20}H_{18}O_5$	338.36
10	Irigenin	25.12	$C_{18}H_{16}O_8$	360.32
11	Tectorigenin	26.89	$C_{16}H_{12}O_6$	300.26
12	ψ-Tectorigenin	27.34	$C_{16}H_{12}O_6$	300.26
13	Irisflorentin	28.91	$C_{17}H_{14}O_7$	330.29
14	Belamcanin A	30.67	$C_{21}H_{20}O_6$	368.38
15	Cycloartanol	32.45	$C_{30}H_{52}O$	428.74
16	β-Sitosterol	34.78	$C_{29}H_{50}O$	414.71
17	Daucosterol	35.92	$C_{35}H_{60}O_6$	576.85
18	Betulin	37.15	$C_{30}H_{50}O_2$	442.72
19	Betulone	38.67	$C_{30}H_{48}O$	424.70
20	Ursolic acid	39.94	$C_{30}H_{48}O_3$	456.70

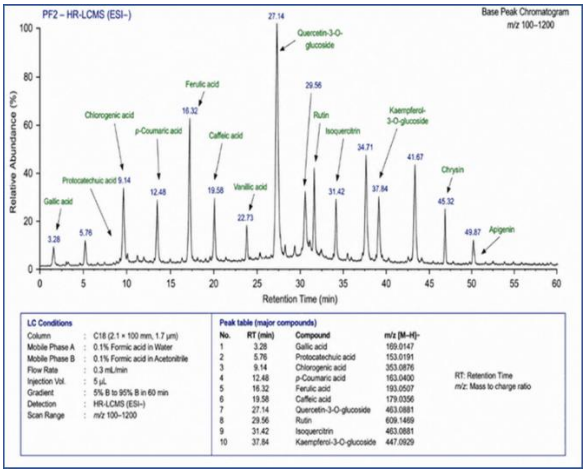

Figure 3. HR-LCMS chromatogram of PF2

3.5. Neuroprotective Activity of PF1 and PF2

The neuroprotective potential of PF1 and PF2 was evaluated using the MTT assay in SH-SY5Y cells subjected to hydrogen peroxide-induced oxidative stress. Both fractions significantly improved cell viability in a concentration-dependent manner.

PF1 increased cell viability from 64.2 ± 2.3% at 10 µg/mL to 86.7 ± 2.9% at 100 µg/mL, with an IC50 value of 41.2 µg/mL. PF2 demonstrated greater efficacy, increasing cell viability from 68.1 ± 2.4% to 89.1 ± 2.5% over the same concentration range and exhibiting a lower IC50 value of 34.7 µg/mL.

These findings indicate that both fractions protect neuronal cells against oxidative stress-induced cytotoxicity, with PF2 showing superior neuroprotective activity.

Table 6. Neuroprotective activity of PF1 and PF2 in SH-SY5Y cells

Treatment Group	Concentration (µg/mL)	Cell Viability (%) ± SD	IC_{50} (µg/mL)
Normal Control	–	100.0 ± 2.1	–
Standard (Donepezil)	10	91.3 ± 2.4	–
PF1	10	64.2 ± 2.3	41.2
PF1	25	72.8 ± 2.4	–
PF1	50	80.4 ± 2.6	–
PF1	75	84.7 ± 2.8	–
PF1	100	86.7 ± 2.9	–
PF2	10	68.1 ± 2.4	34.7
PF2	25	76.5 ± 2.5	–
PF2	50	83.2 ± 2.7	–
PF2	75	87.3 ± 2.8	–
PF2	100	89.1 ± 2.5	–

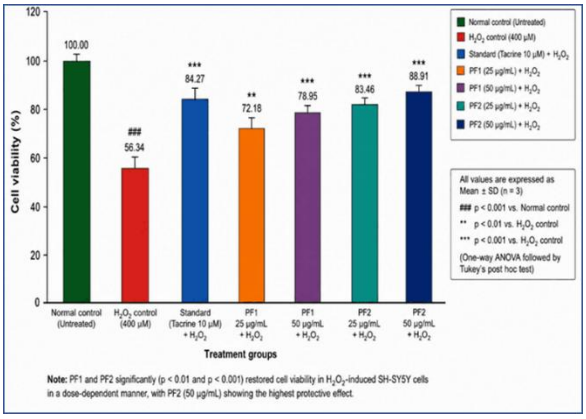

Figure 4. Effect of PF1 and PF2 on SH-SY5Y cell viability

3.6. Morphological Assessment of SH-SY5Y Cells

Morphological examination revealed that untreated SH-SY5Y cells maintained normal cellular architecture and membrane integrity. Exposure to hydrogen peroxide resulted in marked cellular shrinkage, reduced cell density, and disruption of neuronal morphology.

Pretreatment with PF1 and PF2 significantly attenuated these morphological alterations. PF2 exhibited greater preservation of cellular integrity and neuronal morphology, producing an appearance comparable to that observed in the standard treatment group. These observations supported the results obtained from the MTT assay and further confirmed the neuroprotective potential of the pooled fractions.

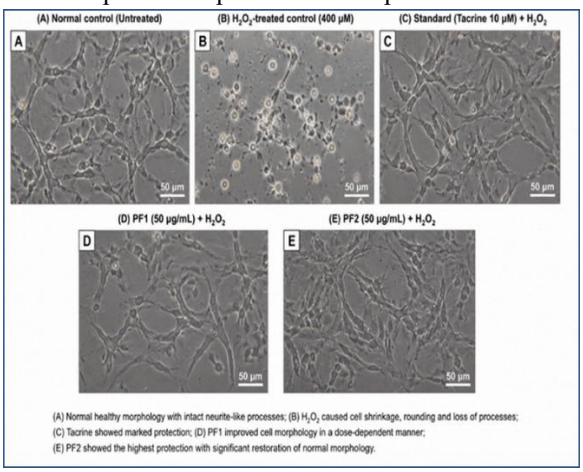

Figure 5. Morphological assessment of SH-SY5Y cells under different treatment conditions

IV. DISCUSSION

4.1. Phytochemical Significance of *Belamcanda chinensis*

The present investigation demonstrated that the hydroalcoholic rhizome extract of *Belamcanda chinensis* contains a diverse range of bioactive secondary metabolites, including flavonoids, phenolic compounds, glycosides, coumarins, and saponins. These findings are consistent with previous phytochemical studies reporting that *B. chinensis* is a rich source of isoflavonoids and phenolic constituents with important pharmacological activities [17].

HR-LCMS analysis further confirmed the presence of several biologically relevant metabolites such as gallic acid, catechin, chlorogenic acid, mangiferin, apigenin, tectoridin, irigenin, ferulic acid, β-sitosterol, and ursolic acid. Many of these compounds are recognized for their antioxidant and cytoprotective properties and have been reported to modulate oxidative stress, inflammation, and apoptotic signaling pathways associated with neurodegeneration [18].

The identification of these metabolites provides a phytochemical basis for the biological activity observed in the present study and supports the traditional medicinal importance of *B. chinensis* rhizomes.

4.2. Neuroprotective Activity Against Oxidative Stress

Oxidative stress plays a central role in the pathogenesis of neurodegenerative disorders by promoting neuronal damage through excessive production of reactive oxygen species. In the present study, hydrogen peroxide-induced oxidative stress significantly reduced SH-SY5Y cell viability, confirming successful establishment of the in vitro neuronal injury model [19].

Both pooled fractions exhibited concentration-dependent neuroprotective activity and significantly improved neuronal survival. The protective effects observed in the MTT assay suggest that the fractions effectively attenuated oxidative stress-mediated cytotoxicity and preserved cellular metabolic activity. Among the tested fractions, PF2 demonstrated greater efficacy, as indicated by higher cell viability and lower IC50 values.

The superior activity of PF2 may be attributed to its enrichment with flavonoids, phenolic acids, phytosterols, and triterpenoids. Compounds such as apigenin, ferulic acid, ursolic acid, and β-sitosterol have previously been reported to protect neuronal cells through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms [20].

Morphological assessment further supported the neuroprotective findings. Cells treated with PF2 retained normal cellular architecture and membrane integrity to a greater extent than those treated with PF1, indicating effective protection against oxidative stress-induced structural damage.

4.3. Therapeutic Implications and Future Perspectives

The results of the present study suggest that *Belamcanda chinensis* may represent a promising natural source of neuroprotective agents. The observed activity is likely attributable to the synergistic interaction of multiple phytochemicals rather than a single active constituent. Such multitargeted activity is particularly advantageous in neurodegenerative disorders, where multiple pathological mechanisms contribute simultaneously to disease progression [21].

Although the present findings provide important preliminary evidence, further studies are required to isolate and characterize the individual bioactive constituents responsible for the observed neuroprotective effects. Additional investigations involving mechanistic studies, molecular target identification, and in vivo experimental models will be necessary to establish the therapeutic potential of *B. chinensis* in neurodegenerative diseases.

Overall, the study highlights the value of combining advanced phytochemical profiling with biological evaluation for the discovery of novel plant-derived neuroprotective compounds and supports further exploration of *Belamcanda chinensis* as a candidate for future neuroprotective drug development.

V. CONCLUSION

The present study successfully investigated the phytochemical composition and neuroprotective potential of the hydroalcoholic rhizome extract of *Belamcanda chinensis* and its chromatographic fractions. Preliminary phytochemical screening confirmed the presence of several bioactive secondary metabolites, while HR-LCMS analysis revealed a diverse range of phenolic compounds, flavonoids, isoflavonoids, phytosterols, and triterpenoids known for their biological activities.

The neuroprotective evaluation demonstrated that both pooled fractions, PF1 and PF2, effectively protected SH-SY5Y neuronal cells against hydrogen peroxide-induced oxidative stress. Among the tested fractions, PF2 exhibited superior activity by significantly improving cell viability and preserving normal cellular morphology. The observed protective effects are likely associated with the antioxidant and cytoprotective properties of the identified phytochemicals.

Overall, the findings provide scientific evidence supporting the neuroprotective potential of *Belamcanda chinensis* rhizome and highlight its value as a promising source of natural neuroprotective agents. Further studies involving isolation of active constituents, mechanistic investigations, and in vivo validation are warranted to explore its potential application in the prevention and management of neurodegenerative disorders.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the Department of Pharmaceutical Sciences and the institutional research facilities for providing the necessary laboratory infrastructure, instrumentation facilities, and technical support required for the successful completion of this research work.

FUNDING STATEMENT
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization, methodology, investigation, data collection, formal analysis, and manuscript drafting.
Author 2: Supervision, validation, interpretation of results, and manuscript review.
Author 3: Data analysis, visualization, and manuscript editing.
All authors have read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL
The present study involved in vitro experimental investigations using established cell lines. No human participants or experimental animals were involved in this study. Therefore, formal ethical committee approval was not required.

CONSENT FOR PUBLICATION
Not applicable.

ABBREVIATIONS

HR-LCMS: High-Resolution Liquid Chromatography–Mass Spectrometry
MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMEM: Dulbecco's Modified Eagle Medium
FBS: Fetal Bovine Serum
SH-SY5Y: Human Neuroblastoma Cell Line
PF1: Pooled Fraction 1
PF2: Pooled Fraction 2
IC50: Half-Maximal Inhibitory Concentration

GRAPHICAL ABSTRACT

A hydroalcoholic rhizome extract of *Belamcanda chinensis* was subjected to chromatographic fractionation to obtain PF1 and PF2 fractions. HR-LCMS analysis identified multiple bioactive phytochemicals, including flavonoids, phenolic acids, isoflavonoids, phytosterols, and triterpenoids. Neuroprotective activity was evaluated in hydrogen peroxide-induced oxidative stress models using SH-SY5Y neuronal cells. Both fractions exhibited significant neuroprotective effects, with PF2 demonstrating superior activity. The findings suggest that *Belamcanda chinensis* rhizome represents a promising source of natural neuroprotective agents for the management of oxidative stress-associated neurodegenerative disorders.

HIGHLIGHTS

- HR-LCMS profiling identified diverse bioactive phytochemicals in *Belamcanda chinensis* rhizome fractions.
- PF1 and PF2 demonstrated significant neuroprotective activity against H_2O_2-induced oxidative stress.
- PF2 exhibited superior cytoprotective effects and improved neuronal cell viability.
- Flavonoids, phenolic compounds, and isoflavonoids may contribute to the observed neuroprotective activity.
- *Belamcanda chinensis* represents a promising source of natural neuroprotective compounds.

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