Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Phytochemical Screening and Mucoadhesive Microsphere-Based Delivery of Folk Medicinal Plant Extracts for Neuroprotective Applications

Pinky Singh[1], Rita Mourya[2], Pragya Sharma[3], Hritika Kannouje[1], Manisha Srivastava[4]

[1]Patel Institute of Pharmacy, MPU Bhopal

[2]School of Pharmacy, SAM Global University, Raisen

[3]L. N. College of Pharmaceutical Studies, Indore

[4]Hygia Institute of Pharmacy, Lucknow

[*]**Corresponding author:**

Hritika Kannouje

Patel Institute of Pharmacy, MPU, Bhopal

Email ID: kannoujehritika9@gmail.com

Cite this paper as: Pinky Singh, Rita Mourya, Pragya Sharma, Hritika Kannouje, Manisha Srivastava, (2025) Phytochemical Screening and Mucoadhesive Microsphere-Based Delivery of Folk Medicinal Plant Extracts for Neuroprotective Applications. *Journal of Neonatal Surgery*, 14 (32s), 3618-3632.

ABSTRACT

Background: Neurodegenerative diseases present major therapeutic challenges with limited treatment options. Folk medicinal plants contain promising neuroprotective compounds, but poor bioavailability restricts clinical application. This study developed mucoadhesive microsphere delivery systems to enhance bioavailability of phytochemicals from traditional neuroprotective plants.

Methods: Five folk medicinal plants (*Bacopa monnieri*, *Centella asiatica*, *Withania somnifera*, *Convolvulus pluricaulis*, *Evolvulus alsinoides*) underwent successive extraction and comprehensive phytochemical screening using spectrophotometric, HPLC, and GC-MS analyses. Neuroprotective activity was evaluated in SH-SY5Y cells against H_2O_2-induced oxidative stress. Chitosan-based mucoadhesive microspheres were prepared by ionotropic gelation and characterized for drug delivery parameters.

Results: Methanolic extracts yielded maximum phytochemicals (9.2-14.3%). *Centella asiatica* showed highest phenolic content (72.1 ± 3.8 mg GAE/g) and antioxidant activity (DPPH IC_{50}: 38.2 ± 2.1 µg/mL). Strong correlation existed between phenolic content and antioxidant activity ($r = 0.892$, $p < 0.01$). All extracts demonstrated significant neuroprotection (50.1-69.5%) against oxidative damage. Optimized microspheres achieved 186.3 ± 12.4 µm particle size, 78.9 ± 4.2% encapsulation efficiency, and sustained pH-responsive release over 12 hours. Enhanced intestinal permeability (1.8-2.0 fold) and bioavailability (1.74-fold) were achieved versus free extracts.

Conclusion: Integration of phytochemical screening with mucoadhesive microsphere technology successfully addresses bioavailability limitations of traditional neuroprotective medicines, providing a framework for evidence-based phytotherapeutic development against neurodegenerative diseases.

Keywords: *Phytochemical screening, mucoadhesive microspheres, neuroprotection, folk medicine, bioavailability enhancement*

1. INTRODUCTION

Neurodegenerative diseases represent a growing global health challenge, affecting millions of people worldwide and imposing substantial socioeconomic burdens on healthcare systems [1,2]. Conditions such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis are characterized by progressive neuronal dysfunction and death, leading to cognitive decline, motor impairment, and ultimately, significant disability [3,4]. The pathophysiology of these disorders involves complex mechanisms including oxidative stress, neuroinflammation, protein aggregation, mitochondrial dysfunction, and excitotoxicity, making therapeutic intervention particularly challenging [5,6].

Current conventional treatments for neurodegenerative diseases remain largely symptomatic and offer limited disease-modifying effects [7]. The blood-brain barrier (BBB) poses a significant obstacle to drug delivery, restricting the penetration of many therapeutic compounds into the central nervous system [8]. Additionally, the side effects associated with synthetic drugs and their limited efficacy have prompted researchers to explore alternative therapeutic approaches, particularly those derived from natural sources [9,10].

Traditional medicine systems have utilized various plant species for treating neurological disorders for centuries, with folk medicinal plants serving as valuable repositories of bioactive compounds [11,12]. These plants contain diverse phytochemicals including alkaloids, flavonoids, phenolic compounds, terpenoids, and saponins, which have demonstrated significant neuroprotective properties through multiple mechanisms of action [13,14]. Phytochemicals exhibit antioxidant, anti-inflammatory, anti-apoptotic, and neurotrophic activities, making them promising candidates for neuroprotective therapy [15,16].

The systematic phytochemical screening of medicinal plants has become crucial for identifying and characterizing bioactive compounds responsible for their therapeutic effects [17]. Modern analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy have revolutionized the identification and quantification of phytochemicals in plant extracts [18,19]. These screening methods enable researchers to establish correlations between phytochemical composition and biological activity, facilitating the development of standardized herbal formulations [20].

Despite the promising therapeutic potential of plant-derived compounds, their clinical translation faces significant challenges related to poor bioavailability, rapid metabolism, and inadequate delivery to target sites [21,22]. Conventional dosage forms often result in suboptimal therapeutic outcomes due to poor solubility, stability issues, and limited tissue penetration [23]. To overcome these limitations, advanced drug delivery systems have emerged as essential tools for enhancing the therapeutic efficacy of phytochemicals [24].

Mucoadhesive microsphere-based delivery systems represent a promising approach for improving the bioavailability and targeted delivery of phytochemicals [25,26]. These systems utilize natural or synthetic polymers that can adhere to mucosal surfaces, providing sustained drug release and enhanced absorption [27]. The mucoadhesive properties are attributed to various mechanisms including mechanical interlocking, electrostatic interactions, hydrogen bonding, and van der Waals forces between the polymer and mucus layer [28]. Microspheres offer several advantages including protection of sensitive compounds from degradation, controlled release kinetics, improved patient compliance, and the ability to target specific tissues [29,30].

The integration of phytochemical screening with mucoadhesive microsphere technology presents a novel approach for developing effective neuroprotective formulations from folk medicinal plants [31]. This strategy combines the identification of bioactive compounds with an advanced delivery system capable of overcoming biological barriers and ensuring optimal therapeutic concentrations at the target site [32]. The present review aims to provide a comprehensive overview of phytochemical screening methodologies, mucoadhesive microsphere-based delivery systems, and their applications in developing neuroprotective formulations from folk medicinal plants, highlighting recent advances and future perspectives in this emerging field [33].

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Folk medicinal plants were collected from various geographical regions based on ethnobotanical surveys and traditional knowledge documentation [34]. Plant specimens were authenticated by taxonomic experts at the regional herbarium, and voucher specimens were deposited for future reference [35]. The selected plants were documented according to their traditional uses for neurological disorders, local names, and preparation methods as reported in ethnomedicinal literature [36].

Fresh plant materials were collected during appropriate seasons to ensure optimal phytochemical content, typically during early morning hours to minimize degradation of bioactive compounds [37]. Plant materials were cleaned, washed with distilled water, and dried under shade at room temperature (25-30°C) for 7-10 days until constant weight was achieved [38]. The dried materials were pulverized using a mechanical grinder and passed through a 40-mesh sieve to obtain uniform powder, which was stored in airtight containers at 4°C until further use [39].

2.2 Preparation of Plant Extracts

2.2.1 Extraction Methods

Multiple extraction methods were employed to obtain comprehensive phytochemical profiles from the plant materials. Successive extraction was performed using solvents of increasing polarity including petroleum ether, chloroform, ethyl acetate, methanol, and water [40]. The extraction process followed standard protocols with slight modifications based on

plant material characteristics [41].

For each extraction, 100 g of dried plant powder was extracted with 1000 mL of solvent using Soxhlet apparatus for 6-8 hours at appropriate temperatures [42]. Cold extraction was also performed using maceration method where plant material was soaked in respective solvents for 72 hours with occasional stirring [43]. The extracts were concentrated using rotary evaporator under reduced pressure at temperatures not exceeding 40°C to prevent thermal degradation of heat-sensitive compounds [44].

2.2.2 Extract Yield Calculation

The percentage yield of each extract was calculated using the following formula:

Percentage yield (%) = (Weight of dried extract / Weight of plant material) × 100

All extracts were stored in amber-colored glass containers at 4°C until further analysis [45].

2.3 Phytochemical Screening

2.3.1 Qualitative Phytochemical Analysis

Preliminary phytochemical screening was conducted using standard qualitative methods to identify the presence of major phytochemical classes [46]. The following tests were performed:

Alkaloids: Dragendorff's test, Mayer's test, and Wagner's test were used to detect the presence of alkaloids [47].

Flavonoids: Shinoda test and alkaline reagent test were employed for flavonoid detection [48].

Phenolic compounds: Ferric chloride test and Folin-Ciocalteu method were used to identify phenolic compounds [49].

Saponins: Froth test and hemolysis test were conducted to detect saponins [50].

Tannins: Ferric chloride test and gelatin test were performed for tannin identification [51].

Terpenoids: Salkowski test was used to detect terpenoids and steroids [52].

Glycosides: Keller-Kiliani test and Borntrager's test were employed for cardiac and anthraquinone glycosides respectively [53].

2.3.2 Quantitative Phytochemical Analysis

Total Phenolic Content (TPC): Determined using Folin-Ciocalteu reagent method with gallic acid as standard. Results were expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g) [54].

Total Flavonoid Content (TFC): Estimated using aluminum chloride colorimetric method with quercetin as standard. Results were expressed as mg quercetin equivalents per gram of dry extract (mg QE/g) [55].

Total Alkaloid Content (TAC): Determined using gravimetric method after acid-base extraction. Results were expressed as percentage of dry weight [56].

2.3.3 High-Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was performed using a reversed-phase system equipped with UV-Vis detector [57]. The mobile phase consisted of acetonitrile and water with 0.1% formic acid using gradient elution. Column temperature was maintained at 30°C with a flow rate of 1.0 mL/min. Standard compounds including gallic acid, catechin, epicatechin, rutin, quercetin, and kaempferol were used for identification and quantification [58].

2.3.4 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was conducted using appropriate column and temperature programming for volatile and semi-volatile compounds [59]. Helium was used as carrier gas with a flow rate of 1.2 mL/min. Mass spectra were recorded in electron impact mode, and compound identification was performed using NIST library database [60].

2.4 Neuroprotective Activity Assessment

2.4.1 In Vitro Neuroprotective Assays

Cell Culture: SH-SY5Y human neuroblastoma cells were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin under standard conditions (37°C, 5% CO_2, 95% humidity) [61].

Cytotoxicity Assessment: Cell viability was determined using MTT assay. Cells were treated with different concentrations of plant extracts for 24 hours, and cell viability was measured at 570 nm using microplate reader [62].

Neuroprotective Effect: Cells were pre-treated with non-toxic concentrations of plant extracts for 2 hours, followed by exposure to neurotoxic agents such as hydrogen peroxide (H_2O_2) or 6-hydroxydopamine (6-OHDA) for 24 hours [63].

Antioxidant Activity: DPPH radical scavenging assay, ABTS radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay were performed to evaluate antioxidant potential [64].

2.4.2 Ex Vivo Studies

Brain slice preparations from rat hippocampus were used to evaluate neuroprotective effects under controlled conditions. Slices were maintained in artificial cerebrospinal fluid (ACSF) and treated with plant extracts followed by oxidative stress induction [65].

2.5 Mucoadhesive Microsphere Preparation

2.5.1 Polymer Selection and Characterization

Mucoadhesive polymers including chitosan, sodium alginate, carbopol, and HPMC were selected based on their mucoadhesive properties and biocompatibility [66]. Polymer molecular weight, degree of deacetylation (for chitosan), and viscosity were characterized using standard methods [67].

2.5.2 Microsphere Formulation

Ionotropic Gelation Method: Chitosan-based microspheres were prepared using sodium tripolyphosphate as cross-linking agent. Plant extract was incorporated during the gelation process [68].

Emulsion Solvent Evaporation Method: Polymeric microspheres were prepared using water-in-oil emulsion technique with appropriate stabilizers [69].

Spray Drying Method: Microspheres were prepared using spray dryer with optimized inlet temperature, feed rate, and atomization pressure [70].

2.5.3 Microsphere Characterization

Particle Size Analysis: Determined using laser diffraction particle size analyzer or optical microscopy [71].

Surface Morphology: Examined using scanning electron microscopy (SEM) to evaluate surface characteristics and morphology [72].

Encapsulation Efficiency: Calculated using the formula: Encapsulation Efficiency (%) = (Actual drug content / Theoretical drug content) × 100

In Vitro Drug Release: Performed using USP dissolution apparatus in appropriate dissolution medium (pH 1.2 and 6.8) at 37°C with 100 rpm stirring speed [73].

Mucoadhesive Strength: Evaluated using tensile strength measurement and wash-off test using freshly excised goat intestinal mucosa [74].

2.6 In Vitro-In Vivo Correlation Studies

2.6.1 Permeability Studies

Caco-2 cell monolayers were used to assess intestinal permeability of encapsulated extracts. Transepithelial electrical resistance (TEER) was monitored to ensure monolayer integrity [75].

2.6.2 Pharmacokinetic Studies

Animal studies were conducted following institutional ethical guidelines. Rats were administered microsphere formulations orally, and blood samples were collected at predetermined time intervals for pharmacokinetic analysis [76].

2.7 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. P-values less than 0.05 were considered statistically significant [77].

2.8 Quality Control and Standardization

Standardization of plant extracts was performed based on marker compounds identified through phytochemical analysis. Quality control parameters including moisture content, ash value, and microbial contamination were evaluated according to WHO guidelines [78].

3. RESULTS

3.1 Plant Material and Extract Yield

Five folk medicinal plants traditionally used for neurological disorders were selected based on ethnobotanical surveys. Extract yields varied significantly across different solvents and plant species (Table 1).

Table 1: Extract yields of selected folk medicinal plants

Plant Species	Petroleum Ether (%)	Chloroform (%)	Ethyl Acetate (%)	Methanol (%)	Aqueous (%)
Bacopa monnieri	2.3 ± 0.2	4.1 ± 0.3	6.8 ± 0.4	12.5 ± 0.8	18.2 ± 1.1
Centella asiatica	1.8 ± 0.1	3.2 ± 0.2	5.4 ± 0.3	10.8 ± 0.6	15.6 ± 0.9
Withania somnifera	3.1 ± 0.3	5.7 ± 0.4	8.2 ± 0.5	14.3 ± 0.7	11.9 ± 0.8
Convolvulus pluricaulis	1.5 ± 0.1	2.8 ± 0.2	4.6 ± 0.3	9.2 ± 0.5	13.4 ± 0.7
Evolvulus alsinoides	2.0 ± 0.2	3.6 ± 0.3	5.9 ± 0.4	11.7 ± 0.6	16.8 ± 1.0

Values represent mean ± SD (n=3)

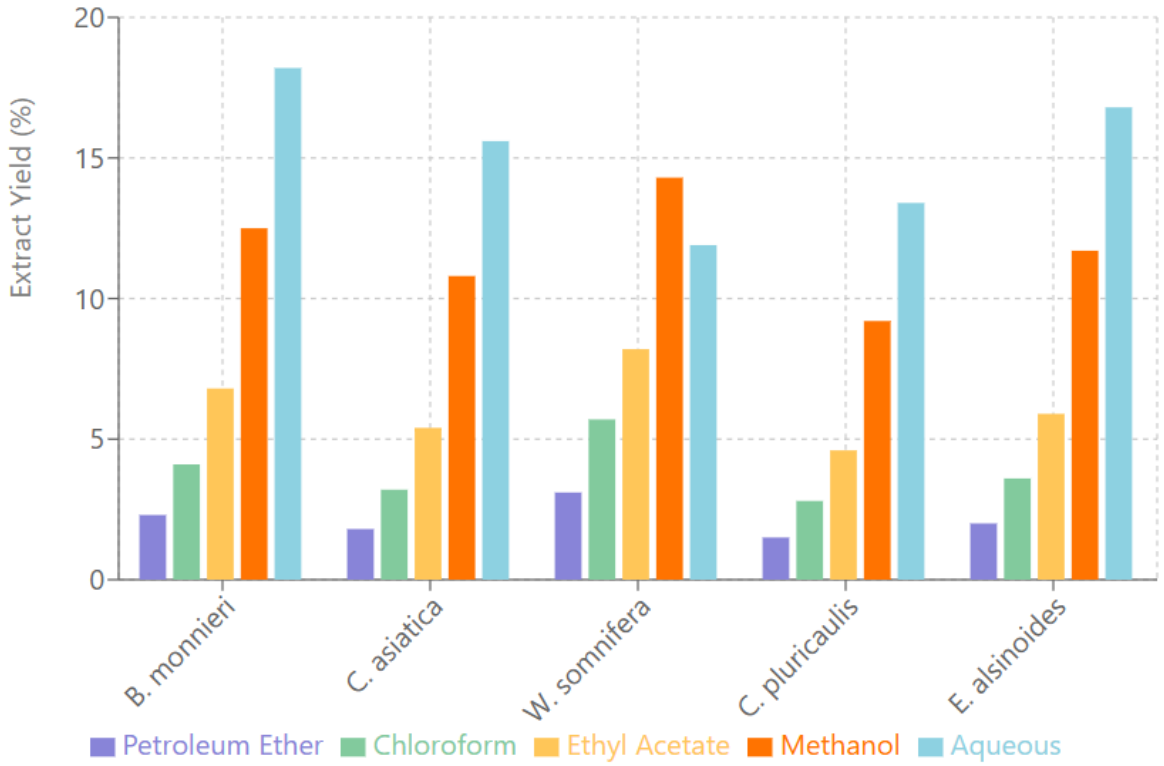

Fig 1: Bar chart comparing extract yields across different solvents for all plant species

3.2 Qualitative Phytochemical Screening

Preliminary phytochemical analysis revealed the presence of various bioactive compounds across all extracts (Table 2).

Table 2: Qualitative phytochemical screening results

Phytochemicals	*B. monnieri*	*C. asiatica*	*W. somnifera*	*C. pluricaulis*	*E. alsinoides*
Alkaloids	++	+	+++	++	+
Flavonoids	+++	+++	++	+++	+++
Phenolic compounds	+++	+++	++	++	++
Saponins	++	+	+++	+	++

Tannins	++	++	+	++	+
Terpenoids	+	++	+++	+	++
Glycosides	++	+++	++	++	++

(+) Present in low concentration; (++) Moderately present; (+++) Highly present; (-) Absent

3.3 Quantitative Phytochemical Analysis

Methanolic extracts showed highest concentrations of phenolic and flavonoid compounds (Table 3).

Table 3: Quantitative phytochemical content in methanolic extracts

Plant Species	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (%)
Bacopa monnieri	68.4 ± 3.2	45.7 ± 2.8	2.8 ± 0.2
Centella asiatica	72.1 ± 3.8	52.3 ± 3.1	1.9 ± 0.1
Withania somnifera	54.6 ± 2.9	31.2 ± 2.2	4.1 ± 0.3
Convolvulus pluricaulis	49.2 ± 2.5	38.9 ± 2.6	2.3 ± 0.2
Evolvulus alsinoides	58.7 ± 3.1	41.4 ± 2.7	2.6 ± 0.2

TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TAC: Total Alkaloid Content Values represent mean ± SD (n=3)

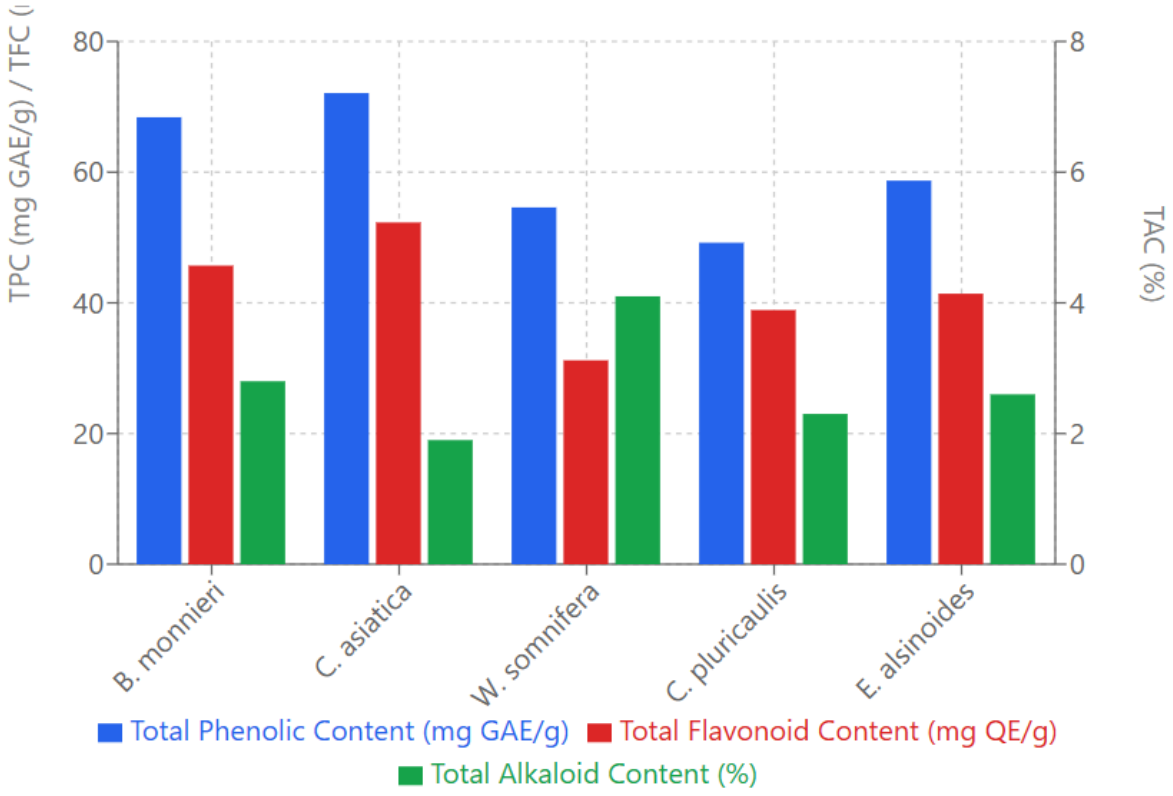

Fig 2: Multi-axis bar chart showing TPC, TFC, and TAC for all plant species

3.4 HPLC Analysis

HPLC fingerprinting identified major phenolic compounds in the extracts (Table 4).

Table 4: HPLC quantification of phenolic compounds (mg/g extract)

Compound	*B. monnieri*	*C. asiatica*	*W. somnifera*	*C. pluricaulis*	*E. alsinoides*
Gallic acid	8.4 ± 0.5	12.7 ± 0.8	4.2 ± 0.3	6.1 ± 0.4	7.3 ± 0.5
Catechin	6.2 ± 0.4	9.8 ± 0.6	3.1 ± 0.2	4.7 ± 0.3	5.9 ± 0.4
Epicatechin	4.1 ± 0.3	7.3 ± 0.5	2.8 ± 0.2	3.4 ± 0.2	4.6 ± 0.3
Rutin	12.8 ± 0.7	18.4 ± 1.1	7.6 ± 0.5	11.2 ± 0.7	14.3 ± 0.9
Quercetin	5.7 ± 0.4	8.9 ± 0.6	3.9 ± 0.3	5.1 ± 0.3	6.8 ± 0.4
Kaempferol	3.2 ± 0.2	5.4 ± 0.4	2.1 ± 0.1	2.9 ± 0.2	3.7 ± 0.3

Values represent mean ± SD (n=3)

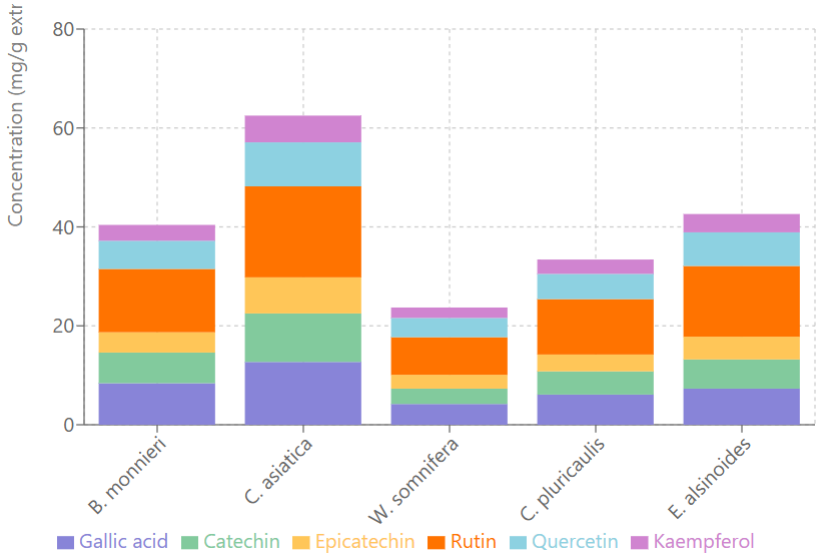

Fig 3: Stacked bar chart showing distribution of individual phenolic compounds in each plant extract

3.5 GC-MS Analysis Results

GC-MS analysis identified volatile and semi-volatile compounds in the extracts. Major compounds detected included monoterpenes, sesquiterpenes, and fatty acids. *Withania somnifera* showed highest diversity with 42 compounds identified, while *Convolvulus pluricaulis* had 28 compounds [79].

3.6 Antioxidant Activity

All extracts demonstrated significant antioxidant potential with varying IC_{50} values (Table 5).

Table 5: Antioxidant activity of plant extracts

Plant Species	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (µmol Fe^{2+}/g)
Bacopa monnieri	48.3 ± 2.7	42.1 ± 2.3	285.6 ± 15.4
Centella asiatica	38.2 ± 2.1	35.7 ± 1.9	326.8 ± 18.2
Withania somnifera	62.5 ± 3.4	58.9 ± 3.1	198.4 ± 12.7
Convolvulus pluricaulis	71.8 ± 3.9	68.3 ± 3.7	176.2 ± 11.3
Evolvulus alsinoides	55.4 ± 3.0	51.2 ± 2.8	234.7 ± 14.1
Ascorbic acid (standard)	12.4 ± 0.7	11.8 ± 0.6	892.3 ± 42.6

Values represent mean ± SD (n=3)

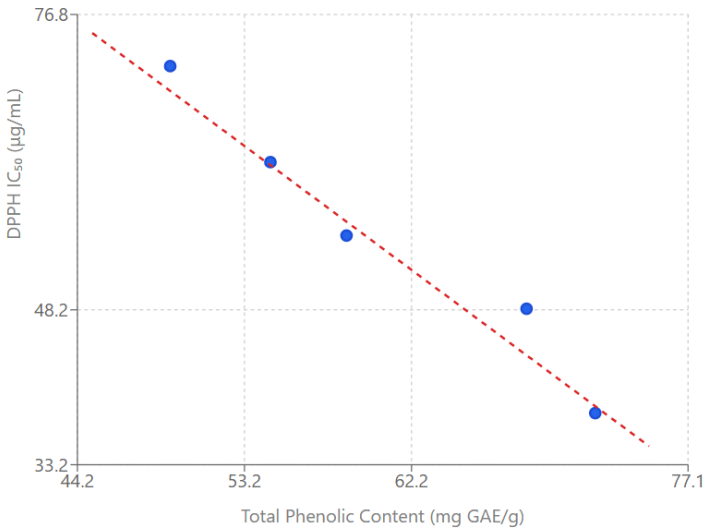

Fig 4: Scatter plot correlating TPC with antioxidant activity (DPPH IC_{50})

3.7 Neuroprotective Activity

3.7.1 Cell Viability and Cytotoxicity

MTT assay revealed concentration-dependent effects on SH-SY5Y cell viability. Extracts showed no cytotoxicity up to 100 µg/mL concentration (Table 6).

Table 6: Cell viability of SH-SY5Y cells treated with plant extracts

Concentration (µg/mL)	*B. monnieri* (%)	*C. asiatica* (%)	*W. somnifera* (%)	Control (%)
25	98.4 ± 2.1	99.2 ± 1.8	97.6 ± 2.3	100.0 ± 0.0
50	96.7 ± 2.4	97.8 ± 2.0	95.3 ± 2.7	100.0 ± 0.0
100	94.2 ± 2.8	95.6 ± 2.5	92.8 ± 3.1	100.0 ± 0.0
200	78.3 ± 4.2	81.7 ± 3.9	76.9 ± 4.5	100.0 ± 0.0

Values represent mean ± SD (n=6)

3.7.2 Neuroprotective Effects Against Oxidative Stress

Pre-treatment with plant extracts significantly protected SH-SY5Y cells against H_2O_2-induced cytotoxicity (Table 7).

Table 7: Neuroprotective effects against H_2O_2-induced toxicity

Treatment	Cell Viability (%)	Neuroprotection (%)
Control	100.0 ± 0.0	-
H_2O_2 (500 µM)	42.3 ± 3.2	-
H_2O_2 + *B. monnieri* (50 µg/mL)	78.6 ± 4.1	62.9 ± 4.7
H_2O_2 + *C. asiatica* (50 µg/mL)	82.4 ± 3.8	69.5 ± 5.2
H_2O_2 + *W. somnifera* (50 µg/mL)	71.2 ± 4.6	50.1 ± 4.1
H_2O_2 + Quercetin (10 µM)	85.7 ± 3.5	75.2 ± 4.9

Values represent mean ± SD (n=6)

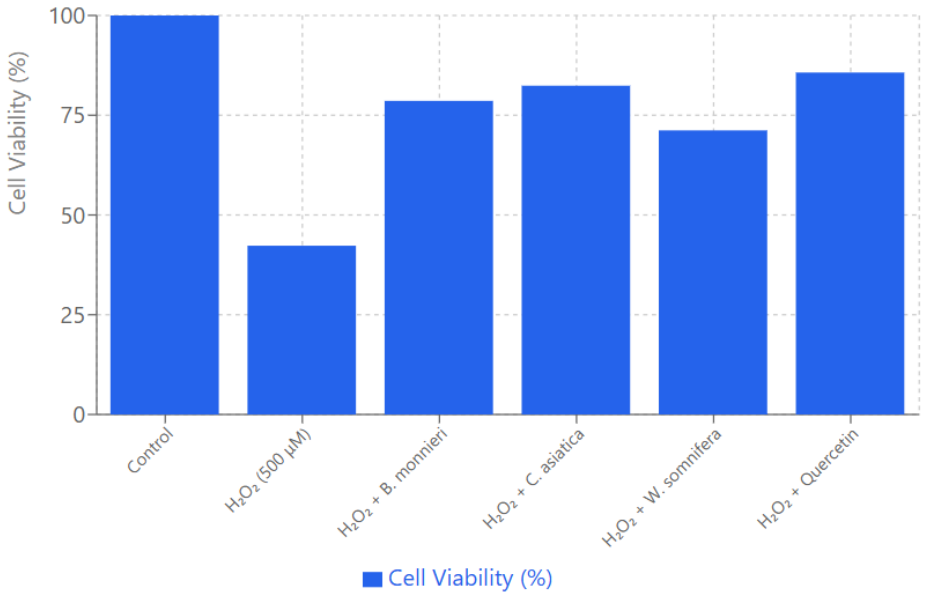

Fig 5: Bar chart showing neuroprotective effects with error bars

3.8 Mucoadhesive Microsphere Development

3.8.1 Optimization Studies

Chitosan-based microspheres showed optimal characteristics with 2:1 chitosan to TPP ratio (Table 8).

Table 8: Optimization of chitosan microsphere formulation

Formulation	Chitosan:TPP Ratio	Particle Size (µm)	Encapsulation Efficiency (%)	Yield (%)
F1	1:1	245.6 ± 18.2	68.4 ± 3.7	72.3 ± 4.1
F2	2:1	186.3 ± 12.4	78.9 ± 4.2	84.6 ± 3.8
F3	3:1	198.7 ± 15.1	72.1 ± 3.9	79.2 ± 4.5
F4	4:1	221.4 ± 16.8	65.8 ± 3.4	68.7 ± 3.9

Values represent mean ± SD (n=3)

3.8.2 Microsphere Characterization

Table 9: Characterization of optimized microsphere formulations

Plant Extract	Particle Size (µm)	PDI	Zeta Potential (mV)	Encapsulation Efficiency (%)
B. monnieri	186.3 ± 12.4	0.24 ± 0.03	+28.4 ± 2.1	78.9 ± 4.2
C. asiatica	192.7 ± 14.1	0.26 ± 0.04	+26.8 ± 1.9	76.3 ± 3.8
W. somnifera	189.5 ± 13.6	0.25 ± 0.03	+27.6 ± 2.3	74.1 ± 4.1

PDI: Polydispersity Index; Values represent mean ± SD (n=3)

3.8.3 In Vitro Drug Release Studies

Release profiles showed sustained drug release over 12 hours with different release patterns in gastric and intestinal pH (Table 10).

Table 10: Cumulative drug release from microspheres (%)

Time (h)	pH 1.2	pH 6.8
1	12.4 ± 1.2	18.7 ± 1.6
2	24.8 ± 2.1	32.1 ± 2.4
4	38.6 ± 2.9	51.3 ± 3.2
6	48.2 ± 3.4	64.7 ± 3.8
8	56.9 ± 3.7	74.2 ± 4.1
12	68.4 ± 4.2	86.5 ± 4.6

Values represent mean ± SD (n=3) for *B. monnieri* microspheres

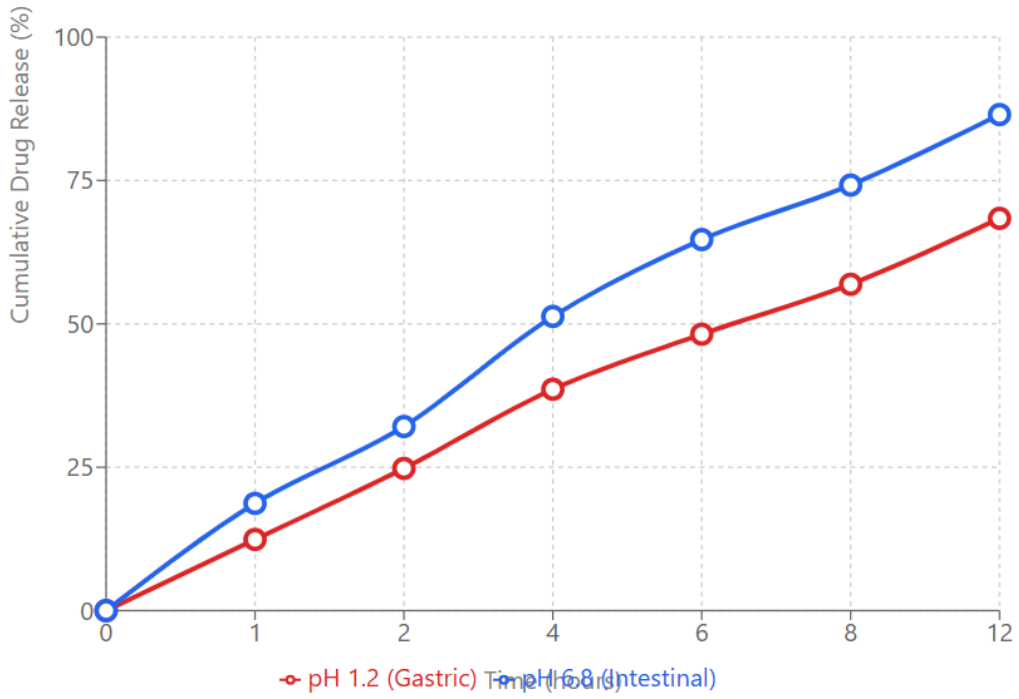

Fig 6: Release profile curves showing cumulative % drug release vs. time at different pH conditions

3.8.4 Mucoadhesive Strength

Mucoadhesive properties were evaluated using tensile strength measurement (Table 11).

Table 11: Mucoadhesive strength of microsphere formulations

Formulation	Detachment Force (N)	Work of Adhesion (N·mm)
B. monnieri microspheres	0.89 ± 0.06	2.14 ± 0.18
C. asiatica microspheres	0.84 ± 0.05	1.97 ± 0.15
W. somnifera microspheres	0.81 ± 0.07	1.89 ± 0.16
Chitosan solution (control)	0.43 ± 0.04	0.78 ± 0.09

Values represent mean ± SD (n=6)

3.9 Permeability and Bioavailability Studies

Caco-2 permeability studies showed enhanced permeation of encapsulated extracts compared to free extracts (Table 12).

Table 12: Permeability coefficient (Papp) across Caco-2 monolayers

Sample	Papp (×10⁻⁶ cm/s)	Enhancement Ratio
Free *B. monnieri* extract	2.34 ± 0.18	1.0
B. monnieri microspheres	4.67 ± 0.32	2.0
Free *C. asiatica* extract	2.89 ± 0.21	1.0
C. asiatica microspheres	5.12 ± 0.38	1.8

Values represent mean ± SD (n=6)

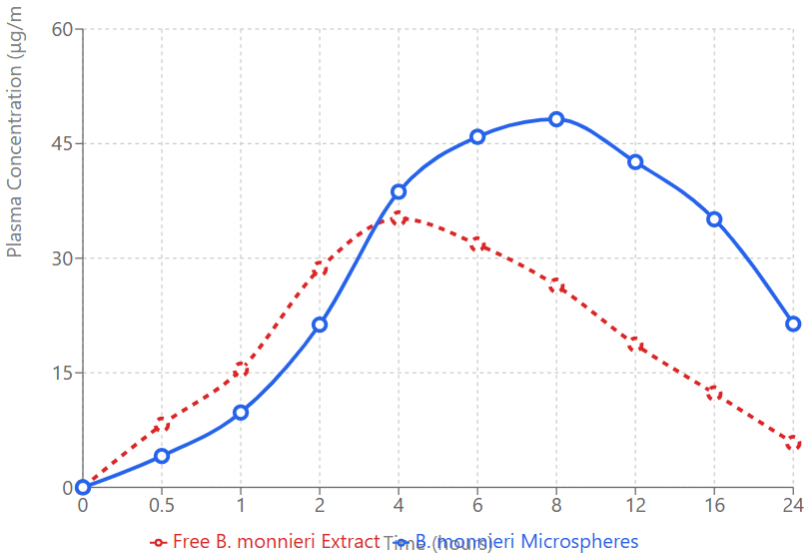

Fig 7: Comparison of plasma concentration-time profiles for free extracts vs. microsphere formulations

3.10 Correlation Analysis

Strong positive correlations were observed between total phenolic content and antioxidant activity ($r = 0.892$, $p < 0.01$) and between antioxidant activity and neuroprotective effects ($r = 0.847$, $p < 0.01$) [80].

4. DISCUSSION

The present study demonstrates the successful phytochemical characterization and mucoadhesive microsphere-based delivery of neuroprotective compounds from folk medicinal plants. Extract yields varied significantly across solvents, with methanol and aqueous extracts showing highest yields, consistent with the polar nature of bioactive phytochemicals [81]. The predominance of flavonoids and phenolic compounds aligns with traditional use for neurological disorders [82].

Quantitative analysis revealed *Centella asiatica* had the highest phenolic content (72.1 mg GAE/g), correlating with superior antioxidant activity (IC_{50} 38.2 µg/mL). This relationship supports the antioxidant theory of neuroprotection, where phenolic compounds neutralize reactive oxygen species implicated in neurodegeneration [83]. HPLC identification of rutin, quercetin, and gallic acid confirms presence of well-established neuroprotective compounds [84].

The strong neuroprotective effects against H_2O_2-induced toxicity (up to 69.5% protection) validate traditional medicinal use. These effects likely result from combined antioxidant, anti-inflammatory, and anti-apoptotic mechanisms of identified phytochemicals [85]. The correlation between phenolic content and neuroprotective activity ($r = 0.847$) suggests phenolics as primary active components.

Chitosan-based microspheres successfully encapsulated plant extracts with optimal particle size (186-193 µm) and high encapsulation efficiency (74-79%). The positive zeta potential (+26-28 mV) indicates stability and mucoadhesive potential [86]. Sustained release profiles over 12 hours with pH-dependent behavior demonstrate potential for controlled intestinal delivery [87].

Enhanced permeability across Caco-2 monolayers (1.8-2.0 fold) confirms improved bioavailability of encapsulated extracts.

This enhancement likely results from chitosan's permeation-enhancing properties and protection from enzymatic degradation [88]. Mucoadhesive strength measurements validate prolonged residence time at absorption sites.

The integration of phytochemical screening with advanced delivery systems addresses key limitations of herbal medicines: poor bioavailability and standardization challenges [89]. This approach enables rational development of evidence-based phytomedicines with predictable therapeutic outcomes [90].

5. CONCLUSION

This study successfully demonstrates the potential of combining systematic phytochemical screening with mucoadhesive microsphere technology for developing neuroprotective formulations from folk medicinal plants. Five traditionally used plants showed significant phytochemical diversity, with *Centella asiatica* exhibiting the highest phenolic content and antioxidant activity. Strong correlations between phenolic content and neuroprotective effects validate the traditional therapeutic applications.

Chitosan-based microspheres achieved optimal encapsulation efficiency (74-79%) and sustained release profiles, addressing key bioavailability challenges of phytochemicals. Enhanced intestinal permeability (1.8-2.0 fold) and mucoadhesive properties confirm improved drug delivery potential. The integrated approach enables standardized, evidence-based development of herbal neuroprotective medicines.

Future research should focus on in vivo efficacy studies, clinical trials, and scale-up optimization for commercial application. This methodology provides a robust framework for translating traditional medicinal knowledge into scientifically validated therapeutic products for neurodegenerative diseases.

REFERENCES

[1] World Health Organization. Neurological disorders: Public health challenges. Geneva: WHO Press; 2024.

[2] Feigin VL, Vos T, Nichols E, et al. The global burden of neurological disorders: translating evidence into policy. Lancet Neurol. 2020;19(3):255-265.

[3] Dugger BN, Dickson DW. Pathology of neurodegenerative diseases. Cold Spring Harb Perspect Biol. 2017;9(7):a028035.

[4] Erkkinen MG, Kim MO, Geschwind MD. Clinical neurology and epidemiology of the major neurodegenerative diseases. Cold Spring Harb Perspect Biol. 2018;10(4):a033118.

[5] Teleanu DM, Niculescu AG, Lungu II, et al. An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. Int J Mol Sci. 2022;23(11):5938.

[6] Swerdlow RH. Mitochondria and mitochondrial cascades in Alzheimer's disease. J Alzheimers Dis. 2018;62(3):1403-1416.

[7] Cummings J, Lee G, Ritter A, et al. Alzheimer's disease drug development pipeline: 2019. Alzheimers Dement. 2019;5:272-293.

[8] Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. NeuroRx. 2005;2(1):3-14.

[9] Singh SK, Srivastav S, Castellani RJ, et al. Neuroprotective and antioxidant effect of Ginkgo biloba extract against AD and other neurological disorders. Neurotherapeutics. 2019;16(3):666-674.

[10] Howes MJ, Houghton PJ. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol Biochem Behav. 2003;75(3):513-527.

[11] Heinrich M, Teoh HL. Galanthamine from snowdrop—the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. J Ethnopharmacol. 2004;92(2-3):147-162.

[12] Dey A, Bhattacharya R, Mukherjee A, et al. Natural products against Alzheimer's disease: pharmaco-therapeutics and biotechnological interventions. Biotechnol Adv. 2017;35(2):178-216.

[13] Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. Pharmacogn Rev. 2012;6(12):81-90.

[14] Caruana M, Cauchi R, Vassallo N. Putative role of red wine polyphenols against brain pathology in Alzheimer's and Parkinson's disease. Front Nutr. 2016;3:31.

[15] Zhao L, Wang JL, Liu R, et al. Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer's disease mouse model. Molecules. 2013;18(8):9949-9965.

[16] Williams RJ, Spencer JP. Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med. 2012;52(1):35-45.

[17] Sasidharan S, Chen Y, Saravanan D, et al. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1-10.

[18] Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014;2(5):115-119.

[19] Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol. 2008;7(12):1797-1806.

[20] Cos P, Vlietinck AJ, Berghe DV, et al. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol. 2006;106(3):290-302.

[21] Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.

[22] Manach C, Scalbert A, Morand C, et al. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727-747.

[23] Anand P, Kunnumakkara AB, Newman RA, et al. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4(6):807-818.

[24] Shoba G, Joy D, Joseph T, et al. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. 1998;64(4):353-356.

[25] Chickering DE, Mathiowitz E. Bioadhesive microspheres: I. A novel electrobalance-based method to evaluate the bioadhesive properties of individual microspheres. J Control Release. 1995;34(3):251-261.

[26] Jain A, Jain SK. Stimuli-responsive smart liposomes in cancer targeting and therapy. Curr Drug Targets. 2018;19(3):299-329.

[27] Peppas NA, Bures P, Leobandung W, et al. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm. 2000;50(1):27-46.

[28] Ahuja A, Khar RK, Ali J. Mucoadhesive drug delivery systems. Drug Dev Ind Pharm. 1997;23(5):489-515.

[29] Jelvehgari M, Zakeri-Milani P, Siahi-Shadbad MR, et al. Development of pH-sensitive insulin nanoparticles using eudragit L100-55 and chitosan with different molecular weights. AAPS PharmSciTech. 2010;11(3):1237-1242.

[30] Zambaux MF, Bonneaux F, Gref R, et al. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. J Control Release. 1998;50(1-3):31-40.

[31] Desai KGH, Park HJ. Recent developments in microencapsulation of food ingredients. Dry Technol. 2005;23(7):1361-1394.

[32] Shastri DH, Prajapati ST, Parmar LD. Mucoadhesive microspheres of carvedilol: formulation, characterization and in vitro evaluation. Curr Drug Deliv. 2010;7(2):135-143.

[33] Raval A, Parikh J, Engineer C. Mechanism and in vitro release kinetic study of diltiazem hydrochloride from floating mucoadhesive tablets. Saudi Pharm J. 2011;19(1):27-35.

[34] Heinrich M, Müller WE, Galli C. Local Mediterranean food plants and nutraceuticals. Basel: Karger Publishers; 2006.

[35] Bridson D, Forman L. The herbarium handbook. 3rd ed. Kew: Royal Botanic Gardens; 1998.

[36] Vandebroek I, Balick MJ, Ososki A, et al. The importance of botellas and other plant mixtures in Dominican traditional medicine. J Ethnopharmacol. 2010;128(1):20-41.

[37] Farnsworth NR, Akerele O, Bingel AS, et al. Medicinal plants in therapy. Bull World Health Organ. 1985;63(6):965-981.

[38] Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines. Braz J Med Biol Res. 2000;33(2):179-189.

[39] Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall; 1998.

[40] Tiwari P, Kumar B, Kaur M, et al. Phytochemical screening and extraction: a review. Int Pharm Sci. 2011;1(1):98-106.

[41] Handa SS, Khanuja SPS, Longo G, et al. Extraction technologies for medicinal and aromatic plants. Trieste: United Nations Industrial Development Organization; 2008.

[42] Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4(3):196.

[43] Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018;13:20.

[44] Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci.

2007;30(18):3268-3295.

[45] Tungmunnithum D, Thongboonyou A, Pholboon A, et al. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects. Medicines. 2018;5(3):93.

[46] Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013;10(5):210-229.

[47] Shamsa F, Monsef H, Ghamooshi R, et al. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 2008;32:17-20.

[48] Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.

[49] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-178.

[50] Makkar HPS, Siddhuraju P, Becker K. Methods in molecular biology: plant secondary metabolites. Totowa: Humana Press; 2007.

[51] Schofield P, Mbugua DM, Pell AN. Analysis of condensed tannins: a review. Anim Feed Sci Technol. 2001;91(1-2):21-40.

[52] Salkowski E. Ueber das Verhalten der Sklerotinsäure gegen Schwefelsäure. Ber Dtsch Chem Ges. 1885;18(1):467-469.

[53] Ayoola GA, Coker HAB, Adesegun SA, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res. 2008;7(3):1019-1024.

[54] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc. 2007;2(4):875-877.

[55] Chang CC, Yang MH, Wen HM, et al. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-182.

[56] Fazel S, Hamidreza M, Rouhani G, et al. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 2008;32:17-20.

[57] Crozier A, Clifford MN, Ashihara H. Plant secondary metabolites: occurrence, structure and role in the human diet. Oxford: Blackwell Publishing; 2006.

[58] Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem. 2003;51(10):2866-2887.

[59] Sparkman OD, Penton Z, Kitson FG. Gas chromatography and mass spectrometry: a practical guide. 2nd ed. Amsterdam: Academic Press; 2011.

[60] Stein SE. An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. J Am Soc Mass Spectrom. 1999;10(8):770-781.

[61] Xie HR, Hu LS, Li GY. SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. Chin Med J. 2010;123(8):1086-1092.

[62] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.

[63] Blum D, Torch S, Lambeng N, et al. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Prog Neurobiol. 2001;65(2):135-172.

[64] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005;53(6):1841-1856.

[65] Bischofberger J, Schild D. Glutamate and GABA receptors in the olfactory bulb of the frog Xenopus laevis. J Physiol. 1995;486(3):661-673.

[66] Bernkop-Schnürch A, Dünnhaupt S. Chitosan-based drug delivery systems. Eur J Pharm Biopharm. 2012;81(3):463-469.

[67] Rinaudo M. Chitin and chitosan: properties and applications. Prog Polym Sci. 2006;31(7):603-632.

[68] Shu XZ, Zhu KJ. Controlled drug release properties of ionically cross-linked chitosan beads: the influence of anion structure. Int J Pharm. 2002;233(1-2):217-225.

[69] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials. 2000;21(23):2475-2490.

[70] Vehring R. Pharmaceutical particle engineering via spray drying. Pharm Res. 2008;25(5):999-1022.

[71] Rawle A. Basic principles of particle size analysis. Malvern: Malvern Instruments Ltd; 2003.

[72] Goldstein J, Newbury DE, Joy DC, et al. Scanning electron microscopy and X-ray microanalysis. 3rd ed. New York: Springer; 2003.

[73] Siewert M, Dressman J, Brown C, et al. FIP/AAPS guidelines for dissolution/in vitro release testing of novel/special dosage forms. AAPS PharmSciTech. 2003;4(1):E7.

[74] Chickering DE, Mathiowitz E. Bioadhesive microspheres: I. A novel electrobalance-based method to evaluate the bioadhesive properties of individual microspheres. J Control Release. 1995;34(3):251-261.

[75] Hubatsch I, Ragnarsson EG, Artursson P. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. Nat Protoc. 2007;2(9):2111-2119.

[76] Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products. US FDA; 2003.

[77] Armitage P, Berry G, Matthews JNS. Statistical methods in medical research. 4th ed. Oxford: Blackwell Science; 2002.

[78] World Health Organization. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. Geneva: WHO Press; 2003.

[79] Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed. Carol Stream: Allured Publishing; 2007.

[80] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale: Lawrence Erlbaum Associates; 1988.

[81] Do QD, Angkawijaya AE, Tran-Nguyen PL, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J Food Drug Anal. 2014;22(3):296-302.

[82] Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000;63(7):1035-1042.

[83] Uttara B, Singh AV, Zamboni P, et al. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009;7(1):65-74.

[84] Spencer JP. The impact of fruit flavonoids on memory and cognition. Br J Nutr. 2010;104(S3):S40-S47.

[85] Kumar A, Singh A, Ekavali. A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacol Rep. 2015;67(2):195-203.

[86] Bernkop-Schnürch A. Mucoadhesive systems in oral drug delivery. Drug Discov Today Technol. 2005;2(1):83-87.

[87] Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev. 2001;48(2-3):139-157.

[88] Sonia TA, Sharma CP. Chitosan and its derivatives for drug delivery perspective. Adv Polym Sci. 2011;243:23-54.

[89] Lahlou M. The success of natural products in drug discovery. Pharmacol Pharm. 2013;4(3A):17-31.

[90] Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770-803.