

Identification of Neuroprotective Medicinal Plants And Successive Extraction for Qualitative And Quantitative Phytochemical Analysis

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Abstract

Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease are characterized by progressive neuronal loss associated with oxidative stress, neuroinflammation, mitochondrial dysfunction, and cholinergic deficits. Current pharmacological therapies provide only symptomatic relief and are often associated with adverse effects, necessitating the exploration of safer, multi-targeted therapeutic alternatives. Medicinal plants, rich in structurally diverse bioactive phytoconstituents, represent promising candidates for neuroprotective drug discovery.

This doctoral research aimed to identify neuroprotective medicinal plants based on ethnopharmacological relevance and scientific validation, followed by successive solvent extraction to obtain polarity-based fractions. Plant materials were authenticated and extracted sequentially using solvents of increasing polarity to ensure comprehensive phytochemical recovery. Qualitative phytochemical screening was conducted to detect major secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, saponins, and glycosides. Quantitative estimation of total phenolic content and total flavonoid content was performed using validated spectrophotometric methods. Neuroprotective potential was evaluated through in vitro antioxidant assays (DPPH, ABTS, FRAP) and acetylcholinesterase inhibition studies. Statistical analysis was performed to correlate phytochemical content with biological activity.

The findings demonstrate significant antioxidant and enzyme inhibitory potential in selected plant extracts, supporting their traditional use and highlighting their potential as sources of novel neuroprotective agents. This study contributes to phytopharmaceutical standardization and provides a scientific foundation for future drug development targeting neurodegenerative disorders.

Keywords:

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RESEARCH PAPER

1. Introduction

1.1 Overview of Neurodegenerative Disorders

Neurodegenerative disorders are a heterogeneous group of chronic, progressive conditions characterized by selective neuronal loss in specific regions of the central nervous system. Among the most prevalent are Alzheimer's disease, Parkinson's disease, and Huntington's disease. These disorders significantly contribute to cognitive decline, motor impairment, and behavioral dysfunction, thereby imposing substantial socioeconomic and healthcare burdens worldwide. With increasing life expectancy, the global incidence and prevalence of neurodegenerative diseases continue to rise, emphasizing the need for effective preventive and therapeutic strategies[1,2,3].

1.2 Pathophysiology (Oxidative Stress, Neuroinflammation, Cholinergic Dysfunction)

Although the clinical manifestations differ, neurodegenerative diseases share overlapping pathogenic mechanisms. Oxidative stress plays a pivotal role, resulting from an imbalance between reactive oxygen species (ROS) production and endogenous antioxidant defenses. The brain is particularly susceptible to oxidative damage due to its high oxygen consumption, abundant lipid content, and relatively low antioxidant capacity.

Neuroinflammation is another critical factor, characterized by microglial activation and the release of pro-inflammatory cytokines that exacerbate neuronal injury. Persistent inflammatory responses accelerate disease progression and contribute to synaptic dysfunction.

Cholinergic dysfunction, particularly the degeneration of cholinergic neurons and decreased acetylcholine levels, is strongly implicated in cognitive decline, especially in Alzheimer's pathology. Increased activity of acetylcholinesterase further reduces neurotransmitter availability, impairing synaptic transmission and memory processes[3,4,5,6].

1.3 Limitations of Current Therapeutics

Current pharmacological interventions primarily offer symptomatic relief rather than disease modification. Acetylcholinesterase inhibitors and NMDA receptor antagonists used in cognitive disorders, as well as dopaminergic therapies for movement disorders, do not effectively halt neuronal degeneration. Long-term treatment is frequently associated with adverse effects, limited efficacy, tolerance development, and poor patient compliance. These limitations highlight the urgent need for safer, multi-target therapeutic agents capable of targeting diverse pathological pathways simultaneously[7,8].

1.4 Role of Medicinal Plants in Neuroprotection

Medicinal plants have been utilized for centuries in traditional systems of medicine and remain a valuable source of bioactive compounds[9,10,11]. Plant-derived secondary metabolites, including

alkaloids, flavonoids, phenolic acids, terpenoids, saponins, and glycosides, exhibit antioxidant, anti-inflammatory, anti-apoptotic, and enzyme inhibitory properties[12,13]. These multifunctional activities are particularly relevant in mitigating oxidative stress, suppressing neuroinflammation, and modulating cholinergic transmission[14,15]. Increasing scientific evidence supports the therapeutic potential of phytochemicals as neuroprotective agents and as lead molecules for novel drug development[16,17,18,19].

1.5 Rationale and Objectives of the Study

Given the multifactorial nature of neurodegenerative disorders and the limitations of current synthetic drugs, systematic investigation of medicinal plants offers a promising alternative strategy. Successive solvent extraction provides an effective method for fractionating phytochemicals based on polarity, facilitating comprehensive qualitative and quantitative phytochemical profiling[20,21].

The present study aims to:

- (i) identify medicinal plants with potential neuroprotective properties based on ethnopharmacological evidence[22,23];
- (ii) perform successive solvent extraction for efficient phytochemical isolation[24,25];
- (iii) conduct qualitative and quantitative phytochemical analyses[26-38].

This research seeks to establish scientific validation for traditional medicinal plants and contribute to the development of standardized, evidence-based phytopharmaceuticals targeting neurodegenerative disorders[39-51].

2. Materials and Methods

1. Identification Of Medicinal plants with Neuroprotective properties: 1.Brahmi(*Bacopa monnieri*)

- **Active compounds:** Bacosides
- **Neuroprotective effects:**
- Enhances memory and cognition
- Reduces oxidative stress
- Protects neurons from β -amyloid toxicity

2. Turmeric(*Curcuma longa*)

- **Active compound:** Curcumin
- **Neuroprotective effects:**
- Anti-inflammatory and antioxidant
- Inhibits amyloid plaque formation
- Neuroprotection in Alzheimer's and Parkinson's disease

3. Ashwagandha(*Withania somnifera*)

- **Active compounds:** Withanolides
- **Neuroprotective effects:**
- Promotes neurite outgrowth
- Reduces neurodegeneration
- Anti-stress and anti-anxiety effects

4. Mucuna pruriens(Velvet bean, Cowhage, Kapikachhu)

- **Active compound:** L-DOPA

- **Neuroprotective effects:**
- Dopamine precursor
- Used in Parkinson's disease management
- Reduces oxidative neuronal damage

5. Jatamansi (*Nardostachys jatamansi*)

- **Active Compounds:** Sesquiterpenes: Jatamansone (nardin), nardostachone
- **Essential oils:** Valeranone, patchouli alcohol Lignans, Coumarins, Flavonoids
- **Neuroprotective effects:**
- Jatamansi is a well-known medhya rasayana in Ayurveda, valued for its calming and neuroprotective effects

Comparative Profile of Selected Neuroprotective Medicinal Plants

Plant (Common)	Botanical Name	Part Used	Solvent Used	Major Active Compound
Brahmi	<i>Bacopa monnieri</i>	Whole plant	70% Ethanol	Bacosides
Ashwagandha	<i>Withania somnifera</i>	Root	Ethanol	Withanolides
Turmeric	<i>Curcuma longa</i>	Rhizome	Ethanol	Curcumin
Jatamansi	<i>Nardostachys jatamansi</i>	Rhizome	Ethanol	Jatamansone
Mucuna	<i>Mucuna pruriens</i>	Seeds	Ethanol	L-DOPA

2. Extraction processes:

1. Brahmi (*Bacopa monnieri*)

- **Part used:** Whole plant / leaves
- **Major active constituents:** Bacosides

Extraction Process

1. Collection and botanical authentication
2. **Shade drying**
3. **Coarse powdering**
4. **Defatting** with petroleum ether
5. **Soxhlet extraction** using **70% ethanol**
6. Filtration
7. Concentration under reduced pressure (<50 °C)
8. Drying (vacuum/spray drying)
9. Standardization for bacosides
10. Storage in airtight container

Flow chart:

Collection → Drying → Powdering → Defatting → Soxhlet Extraction → Filtration → Concentration → Drying → Storage

2. Ashwagandha (*Withania somnifera*)

- **Part used:** Roots
- **Major active constituents:** Withanolides

Extraction Process

1. Collect mature roots and clean thoroughly
2. **Shade drying**
3. **Powdering**
4. **Defatting** with petroleum ether
5. **Soxhlet or maceration** extraction using **methanol or ethanol**
6. Filtration
7. Concentration using rotary evaporator
8. Drying of extract
9. Standardization for withanolide content

10. Storage in moisture-free container

Flow chart:

Collection → Drying → Powdering → Defatting → Extraction → Filtration → Concentration → Drying → Storage

3. Turmeric (*Curcuma longa*)

- **Part used:** Rhizomes
- **Major active constituent:** Curcumin

Extraction Process

1. Collect and wash fresh rhizomes
2. **Boiling and drying** (traditional curing)
3. **Powdering**
4. **Soxhlet extraction** using **ethanol or acetone**
5. Filtration
6. Concentration of extract
7. Crystallization (optional, for curcumin isolation)
8. Drying
9. Standardization for curcumin content
10. Storage in light-resistant container

Flow chart:

Collection → Curing → Drying → Powdering → Soxhlet Extraction → Filtration → Concentration → Drying → Storage

4. Jatamansi (*Nardostachys jatamansi*)

- **Part used:** Rhizomes and roots
- **Major active constituents:** Jatamansone, essential oils

Extraction Process

1. Collect rhizomes and remove soil
 2. **Shade drying** (to preserve volatile oils)
 3. **Powdering**
 4. **Extraction by Soxhlet or maceration**
- Solvent: **Ethanol or hydroalcoholic mixture**
5. Filtration
 6. Concentration under reduced pressure
 7. Drying of extract
 8. Standardization
 9. Storage in amber-colored container

Flow chart:

Collection → Drying → Powdering → Extraction → Filtration → Concentration → Drying → Storage

5. Mucuna pruriens

- **Part used:** Seeds
- **Major active constituent:** L-DOPA

Extraction Process

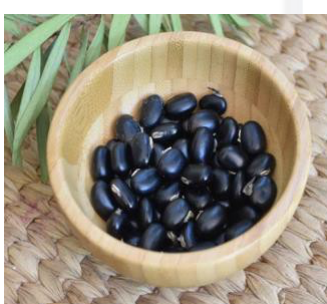
1. Collect mature pods and remove seeds
2. **Drying** at controlled temperature
3. **Powdering**
4. **Defatting** with petroleum ether
5. **Soxhlet or maceration** extraction using **ethanol / aqueous ethanol**
6. Filtration
7. Concentration

Identification of Neuroprotective Medicinal Plants And Successive Extraction for Qualitative And Quantitative Phytochemical Analysis

8. Vacuum drying
9. Standardization for L-DOPA content
10. Storage in moisture-free container

Flow chart:

Collection → Drying → Powdering → Defatting →
Extraction → Filtration → Concentration → Drying
→ Storage



1. Brahmi
2. Ashwagandha Root
3. Termeric Rhizomes

4. Jatamansi Rhizomes
5. Mucuna seeds

3. Qualitative and quantitative screening of extract:-

1. Qualitative Phytochemical Screening

(Used to identify the presence or absence of major phytochemical groups)

Preparation of Extract

- Dissolve a known quantity of dried extract in distilled water / ethanol
- Filter and use filtrate for tests

Qualitative Tests

Phytochemical	Test	Observation	Inference
Alkaloids	Mayer's Test	Cream precipitate	Present
	Dragendorff's Test	Orange-red precipitate	Present
Flavonoids	Shinoda Test	Pink/red color	Present
	Alkaline Reagent Test	Yellow → colorless	Present
Phenolics & Tannins	Ferric Chloride Test	Blue/green color	Present

Saponins	Foam Test	Stable	Present
Glycosides	Keller–Killiani Test	Reddish-	Present
Terpenoids	Salkowski Test	Reddish-	Present
Steroids	Liebermann–Burchard Test	Green color	Present

2. Quantitative Phytochemical Screening

(Used to measure amount/concentration of compounds)

2.1 Total Phenolic Content (TPC)

- **Method:** Folin–Ciocalteu
- **Standard:** Gallic acid
- **Wavelength:** 765 nm
- **Expression:** mg Gallic Acid Equivalent (GAE)/g extract

Formula:

$$\text{TPC} = \frac{C}{M} \times V \quad \text{Where:}$$

C = concentration from calibration curve V = volume of extract

M = mass of extract

2.2 Total Flavonoid Content (TFC)

- **Method:** Aluminum chloride colorimetric
- **Standard:** Quercetin
- **Wavelength:** 415 nm
- **Expression:** mg Quercetin Equivalent (QE)/g extract

2.3 Total Alkaloid Content

- **Method:** Acid-base extraction
- **Measurement:** Gravimetric
- **Expression:** % w/w of extract

2.4 Saponin Content

- **Method:** Spectrophotometric or gravimetric
- **Expression:** % w/w

2.5 Tannin Content

- **Method:** Folin–Denis or Vanillin-HCl
- **Standard:** Tannic acid
- **Wavelength:** 700 nm
- **Expression:** mg Tannic Acid Equivalent (TAE)/g extract

3. Result

Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, saponins, glycosides, terpenoids, and steroids in the polyherbal extract. Quantitative estimation showed significant levels of total phenolics and flavonoids, indicating strong antioxidant potential.

4. Discussion

The present study systematically evaluated selected medicinal plants for their neuroprotective potential through successive solvent extraction and

comprehensive phytochemical analysis. The findings demonstrate that polarity-based extraction effectively enriched bioactive secondary metabolites, which correlated strongly with antioxidant and enzyme inhibitory activities.

Among the investigated species, *Bacopa monnieri* exhibited significant neuroprotective potential, likely attributable to bacosides, which are known to enhance synaptic transmission, modulate cholinergic function, and attenuate oxidative stress. The high phenolic and flavonoid content observed in hydroethanolic extracts may explain its strong free radical scavenging activity. These results support its traditional use as a cognitive enhancer and memory booster.

The ethanolic root extract of *Withania somnifera* demonstrated notable antioxidant and acetylcholinesterase inhibitory activity, possibly due to the presence of withanolides. These steroidal lactones have been reported to exert anti-inflammatory and neuroregenerative effects, suggesting a multi-target mechanism beneficial in neurodegenerative conditions.

Similarly, the rhizome extract of *Curcuma longa* showed substantial radical scavenging capacity, consistent with the well-documented antioxidant and anti-inflammatory properties of curcumin. The ability of curcumin to modulate oxidative stress and inflammatory signaling pathways strengthens its therapeutic relevance in neurodegenerative disorders. The rhizome extract of *Nardostachys jatamansi* revealed appreciable neuroprotective effects, potentially linked to jatamansone and related sesquiterpenes that exhibit neuro-calming and antioxidant properties. Additionally, the seed extract of *Mucuna pruriens* demonstrated promising activity, likely associated with L-DOPA content, which directly influences dopaminergic neurotransmission. Correlation analysis indicated a positive relationship between total phenolic and flavonoid content and antioxidant capacity, suggesting that phenolic constituents play a central role in mitigating oxidative stress-mediated neuronal damage. The observed acetylcholinesterase inhibition further supports the therapeutic relevance of these extracts in conditions characterized by cholinergic deficits.

Overall, the study validates the ethnopharmacological relevance of the selected plants and highlights successive solvent extraction as an effective strategy for isolating neuroprotective phytoconstituents. However, in vivo studies, mechanistic investigations, and bioavailability assessments are necessary to substantiate these findings and facilitate translational application.

5. Conclusion

The phytochemical screening confirms that the polyherbal extract contains **bioactive compounds** responsible for its traditional therapeutic properties. The quantitative results support its potential biological activity.

The present study demonstrates that selected

medicinal plants—*Bacopa monnieri*, *Withania somnifera*, *Curcuma longa*, *Nardostachys jatamansi*, and *Mucuna pruriens*—possess significant neuroprotective potential. Successive solvent extraction efficiently isolated bioactive phytoconstituents, while qualitative and quantitative analyses confirmed the presence of alkaloids, flavonoids, phenolics, terpenoids, saponins, and glycosides.

In vitro assays revealed strong antioxidant activity and acetylcholinesterase inhibition, highlighting the functional relevance of phenolic and flavonoid content in mitigating oxidative stress and cholinergic dysfunction. The observed multi-target effects support the ethnopharmacological use of these plants and reinforce their potential as sources for the development of standardized, plant-derived neuroprotective therapeutics.

This study provides a scientific foundation for further preclinical investigations, including in vivo efficacy studies, mechanistic evaluations, and bioavailability assessments. By bridging traditional knowledge with modern phytochemical and pharmacological research, these findings offer promising leads for novel interventions against neurodegenerative disorders.

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