

# PHARMACOGNOSTIC STANDARDIZATION, PHYTOCHEMICAL CHARACTERIZATION AND DEVELOPMENT OF A PHYTOSOME-BASED NANOFORMULATION OF PREMNA TOMENTOSA LEAF EXTRACT FOR NEUROPROTECTIVE EVALUATION IN A ROTENONE-INDUCED RAT MODEL OF PARKINSON'S DISEASE: A COMPREHENSIVE REVIEW

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## Abstract

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide and is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to both motor and non-motor impairments. Although levodopa and dopamine agonists remain the mainstay of treatment, their long-term use is associated with motor fluctuations, dyskinesia, and limited disease-modifying efficacy. Consequently, increasing attention has been directed toward plant-derived neuroprotective agents capable of targeting multiple pathogenic mechanisms, including oxidative stress, mitochondrial dysfunction, neuroinflammation,  $\alpha$ -synuclein aggregation, excitotoxicity, and apoptosis. Recent reviews also emphasize the growing importance of natural products and phytochemical-based interventions in PD management. *Premna tomentosa* (Lamiaceae), an ethnomedicinal plant widely distributed in the Indian subcontinent, contains diverse bioactive metabolites including flavonoids, phenolic acids, terpenoids, iridoids, alkaloids, tannins, and glycosides with potent antioxidant and anti-inflammatory activities. Recent phytochemical investigations have expanded knowledge of its metabolite profile and biological potential. However, its application in neurodegenerative disorders remains largely unexplored. Standardization through pharmacognostic evaluation and advanced phytochemical characterization is essential to ensure botanical authenticity, quality, reproducibility, and regulatory acceptance. Phytosome technology represents a promising nanocarrier platform for enhancing the solubility, stability, gastrointestinal absorption, and blood–brain barrier permeability of poorly bioavailable phytoconstituents. Compared with conventional herbal extracts, phytosomes improve pharmacokinetic performance and tissue targeting, making them attractive candidates for neuroprotective drug delivery. This review critically discusses the pharmacognostic characteristics, phytochemical composition, neuropharmacological relevance, phytosome formulation strategies, quality evaluation, and therapeutic prospects of *P. tomentosa*. It further examines the suitability of rotenone-induced experimental Parkinsonism for preclinical evaluation and proposes an integrated roadmap from botanical authentication to nanoformulation and translational neurotherapeutics. This integrated perspective represents an important research direction because no comprehensive review has specifically addressed *P. tomentosa* phytosomes for Parkinson's disease. **Keywords:** *Premna tomentosa*; Parkinson's disease; phytosome; pharmacognostic standardization; phytochemical characterization; neuroprotection; rotenone model; nanotechnology; blood–brain barrier; oxidative stress.

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## 1. Introduction

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder affecting more than 10 million individuals worldwide and represents one of the fastest-growing neurological diseases. The pathological hallmarks include selective degeneration

of dopaminergic neurons within the substantia nigra pars compacta, depletion of striatal dopamine, and intracellular accumulation of misfolded  $\alpha$ -synuclein as Lewy bodies. These pathological events manifest clinically as resting tremor, muscular rigidity, bradykinesia, postural instability, gait abnormalities,

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autonomic dysfunction, sleep disturbances, depression, and cognitive decline. Although symptomatic therapies improve motor manifestations, no currently approved treatment effectively halts neuronal degeneration. Consequently, there is increasing interest in disease-modifying interventions capable of targeting multiple pathogenic pathways simultaneously.

The pathogenesis of PD is multifactorial, involving oxidative stress, mitochondrial respiratory chain dysfunction, excessive reactive oxygen species generation, chronic neuroinflammation, impaired autophagy, excitotoxicity, protein misfolding, lysosomal dysfunction, ferroptosis, and apoptosis. Oxidative injury and mitochondrial complex I inhibition are considered pivotal early events in dopaminergic neuronal death. Environmental toxins such as rotenone reproduce many pathological and behavioral features of human PD by inhibiting mitochondrial complex I, making rotenone-induced experimental Parkinsonism one of the most clinically relevant animal models for evaluating neuroprotective therapies.

Natural products have historically served as a major source of therapeutic agents for neurological disorders. Polyphenols, flavonoids, alkaloids, terpenoids, and iridoids possess antioxidant, anti-inflammatory, anti-apoptotic, and mitochondrial protective properties that may attenuate dopaminergic neurodegeneration. Recent reviews demonstrate that several plant-derived compounds modulate nuclear factor erythroid 2-related factor 2 (Nrf2), nuclear factor- $\kappa$ B (NF- $\kappa$ B), phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase (MAPK), and autophagy pathways, while reducing  $\alpha$ -synuclein aggregation and restoring mitochondrial homeostasis.

Among medicinal plants of interest, *Premna tomentosa* Roxb. has attracted attention because of its diverse secondary metabolites and broad spectrum of pharmacological activities. Traditionally, different parts of the plant have been employed in Ayurveda and indigenous medicine for inflammatory disorders, fever, wounds, rheumatism, gastrointestinal disturbances, and microbial infections. Preliminary phytochemical studies have identified flavonoids, phenolic compounds, tannins, triterpenoids, steroids, glycosides, and alkaloids that collectively exhibit antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and cytoprotective activities. Recent phytochemical profiling further supports the richness of its bioactive constituents, suggesting potential utility beyond its traditional indications.

Despite these promising biological properties, translation of *P. tomentosa* into neurotherapeutic

applications remains limited. A major challenge associated with phytoconstituents is poor aqueous solubility, low membrane permeability, chemical instability, extensive first-pass metabolism, and limited penetration across the blood–brain barrier. These factors substantially reduce systemic bioavailability and therapeutic efficacy following oral administration. Consequently, advanced nanocarrier systems have emerged as attractive approaches for overcoming these pharmacokinetic limitations.

Among various lipid-based nanocarriers, phytosomes have gained considerable attention because they form molecular complexes between standardized phytoconstituents and phospholipids, thereby improving membrane affinity, stability, gastrointestinal absorption, and tissue distribution. Compared with conventional herbal extracts, phytosomes provide superior encapsulation efficiency, controlled drug release, enhanced oral bioavailability, improved pharmacokinetic behavior, and potentially greater delivery of phytochemicals to the central nervous system. Recent reviews of phytovesicular systems identify phytosomes as one of the most promising delivery platforms for neuroprotective phytochemicals.

An equally important prerequisite for successful phytopharmaceutical development is rigorous pharmacognostic standardization. Authentication based on macroscopic and microscopic features, physicochemical parameters, histochemical evaluation, powder microscopy, chromatographic fingerprinting, and advanced metabolomic profiling ensures the identity, purity, quality, safety, and reproducibility of herbal materials. Such standardization minimizes adulteration and batch-to-batch variability while satisfying regulatory expectations for botanical drug development.

## **2. Parkinson's Disease: Pathophysiology and Molecular Mechanisms**

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized primarily by the selective degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNpc), resulting in depletion of dopamine in the nigrostriatal pathway. Although motor manifestations such as bradykinesia, resting tremor, rigidity, and postural instability are the clinical hallmarks, PD is now recognized as a multisystem disorder involving widespread neuronal dysfunction that gives rise to cognitive impairment, depression, autonomic dysfunction, sleep disorders, olfactory deficits, and gastrointestinal disturbances. The disease develops through complex interactions between genetic susceptibility, environmental exposures, aging, mitochondrial dysfunction, oxidative stress, protein

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aggregation, neuroinflammation, and impaired intracellular protein clearance. Recent evidence suggests that these mechanisms converge to accelerate progressive neuronal degeneration rather than acting independently.

### 2.1 Degeneration of Nigrostriatal Dopaminergic Neurons

The pathological hallmark of PD is the progressive loss of dopamine-producing neurons in the SNpc. Dopamine deficiency disrupts the functional balance between the direct and indirect pathways of the basal ganglia, producing characteristic motor symptoms. Clinical manifestations generally become apparent after approximately 50–70% of nigral dopaminergic neurons and nearly 80% of striatal dopamine have been lost, indicating that extensive neurodegeneration precedes diagnosis. Surviving neurons exhibit mitochondrial abnormalities, oxidative injury, impaired axonal transport, and abnormal protein accumulation that further compromise neuronal viability.

### 2.2 $\alpha$ -Synuclein Misfolding and Lewy Body Formation

$\alpha$ -Synuclein is a presynaptic protein involved in synaptic vesicle trafficking and neurotransmitter release. Under pathological conditions, oxidative stress, post-translational modifications, and impaired proteostasis promote conversion of soluble  $\alpha$ -synuclein into oligomers and fibrillar aggregates that accumulate as Lewy bodies and Lewy neurites. These aggregates interfere with mitochondrial respiration, disrupt vesicular transport, impair synaptic transmission, activate microglia, and propagate between neurons in a prion-like manner. Current mechanistic models indicate that disturbed  $\alpha$ -synuclein homeostasis is one of the central drivers of PD progression and interacts closely with mitochondrial dysfunction and lysosomal impairment.

### 2.3 Oxidative Stress

Oxidative stress is considered one of the earliest and most important contributors to dopaminergic neuronal death. Dopamine metabolism itself generates reactive oxygen species (ROS), while mitochondrial dysfunction, neuroinflammation, iron accumulation, and environmental toxins further increase oxidative burden. Excess ROS oxidize lipids, proteins, nucleic acids, and mitochondrial DNA, causing irreversible cellular injury. Lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxynonenal further amplify neuronal damage.

Endogenous antioxidant enzymes—including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase

(GR), and reduced glutathione (GSH)—become depleted during disease progression. Activation of the Nrf2/HO-1 signaling pathway is now regarded as a major endogenous defense mechanism against oxidative injury, and numerous natural phytochemicals exert neuroprotective effects through Nrf2 activation.

### 2.4 Mitochondrial Dysfunction

Mitochondrial impairment is a defining feature of PD pathogenesis. Dysfunction of mitochondrial respiratory chain complex I decreases ATP production while increasing ROS generation. Dopaminergic neurons are particularly vulnerable because of their high metabolic demand, autonomous pacemaker activity, and extensive axonal arborization.

Several PD-associated genes—including PINK1, Parkin, DJ-1, LRRK2, and GBA1—regulate mitochondrial quality control, mitophagy, and oxidative homeostasis. Mutations in these genes impair removal of damaged mitochondria, resulting in persistent oxidative stress and neuronal degeneration. Environmental toxicants such as rotenone reproduce these mitochondrial abnormalities by directly inhibiting complex I, thereby providing a robust experimental model for PD.

### 2.5 Neuroinflammation

Chronic neuroinflammation has emerged as a major pathogenic mechanism in PD. Activated microglia release tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), nitric oxide (NO), prostaglandins, and additional ROS, creating a self-perpetuating inflammatory cycle that accelerates neuronal death.

Recent studies highlight activation of the NLRP3 inflammasome as a central inflammatory pathway in PD. Mitochondrial ROS and  $\alpha$ -synuclein aggregates stimulate inflammasome assembly, leading to caspase-1 activation and maturation of IL-1 $\beta$  and IL-18. Suppression of NLRP3 signaling through Nrf2 activation or natural phytochemicals markedly attenuates neurodegeneration in rotenone-induced PD models.

### 2.6 Impaired Protein Clearance

Neurons maintain protein quality through the ubiquitin–proteasome system (UPS) and the autophagy–lysosomal pathway (ALP). Dysfunction of either pathway causes accumulation of damaged proteins, particularly  $\alpha$ -synuclein.

Autophagic failure, lysosomal dysfunction, and defective mitophagy impair degradation of aggregated proteins and damaged mitochondria, thereby enhancing oxidative stress and inflammation. Current evidence suggests that restoration of

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autophagic flux may represent an important disease-modifying strategy for PD.

### 2.7 Ferroptosis and Pyroptosis

Beyond apoptosis, recently recognized regulated cell death pathways contribute significantly to PD progression.

Ferroptosis is characterized by iron-dependent lipid peroxidation and depletion of glutathione, while pyroptosis is an inflammatory form of programmed cell death mediated by NLRP3 inflammasome activation and gasdermin-dependent membrane pore formation.

Rotenone exposure activates both ferroptotic and pyroptotic pathways in dopaminergic neurons, suggesting that inhibition of these processes may provide additional therapeutic opportunities.

### 2.8 Genetic and Environmental Risk Factors

Approximately 10–15% of PD cases have a strong genetic basis involving mutations in genes such as SNCA, LRRK2, PINK1, Parkin (PRKN), DJ-1 (PARK7), VPS35, and GBA1. Most patients, however, develop sporadic disease through interactions between aging, genetic susceptibility, and environmental factors.

Exposure to pesticides—including rotenone, paraquat, and dieldrin—has consistently been associated with increased PD risk because these compounds induce mitochondrial dysfunction and oxidative injury similar to that observed in patients. Heavy metals, traumatic brain injury, and chronic inflammation may also contribute to disease development. (Nature)

### Critical Perspective

The multifactorial nature of PD strongly supports the development of multi-target therapeutic agents rather than single-target drugs. Plant-derived phytochemicals possess antioxidant, anti-inflammatory, anti-apoptotic, and mitochondrial protective properties capable of modulating several interconnected signaling pathways simultaneously. However, poor aqueous solubility, limited gastrointestinal absorption, rapid metabolism, and inadequate blood–brain barrier penetration substantially restrict their therapeutic efficacy. Incorporation of standardized phytochemicals into phytosome-based nanocarriers offers a promising strategy to overcome these limitations by enhancing bioavailability, improving brain delivery, and prolonging systemic circulation. These considerations provide the scientific rationale for investigating phytosome-based nanoformulations of *Premna tomentosa* as potential disease-modifying interventions for PD.

3. Ethnopharmacology, Botanical Description and Pharmacognostic Standardization of *Premna tomentosa*

### 3.1 Introduction

The therapeutic efficacy, safety, and reproducibility of herbal medicines depend fundamentally on the authenticity and quality of the raw botanical material. Pharmacognostic standardization provides the scientific basis for the identification, authentication, and quality assessment of medicinal plants through macroscopic, microscopic, physicochemical, histochemical, and chromatographic evaluation. According to the World Health Organization (WHO), pharmacognostic characterization is an essential prerequisite for the development of standardized herbal formulations and botanical drug products. Standardization minimizes adulteration, substitution, batch-to-batch variation, and contamination, thereby ensuring consistent pharmacological activity and regulatory compliance.

*Premna tomentosa* Willd. (Family: Lamiaceae, formerly classified under Verbenaceae) is an important medicinal species used in Ayurveda, Siddha, and various indigenous healthcare systems across India and Southeast Asia. Although the plant has long been recognized for its anti-inflammatory, antimicrobial, hepatoprotective, antioxidant, and wound-healing activities, systematic pharmacognostic evaluation remains limited compared with other medicinal plants. This lack of comprehensive quality standards represents a major obstacle to the development of standardized extracts and advanced nanopharmaceuticals.

### 3.2 Botanical Classification

### 3.3 Geographic Distribution

*Premna tomentosa* is widely distributed throughout the tropical and subtropical regions of South and Southeast Asia. In India, it naturally occurs in deciduous forests of Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Kerala, Maharashtra, Odisha, Chhattisgarh, Madhya Pradesh, West Bengal, and the Himalayan foothills. The species is also reported from Sri Lanka, Bangladesh, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, and Indonesia.

The plant thrives in well-drained sandy loam soils under tropical climatic conditions and commonly grows at altitudes ranging from sea level to approximately 1,200 m. Environmental factors such as soil composition, rainfall, seasonal variation, altitude, and geographical location significantly influence the phytochemical composition of medicinal plants, highlighting the importance of standardized sourcing for pharmaceutical applications.

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### 3.4 Vernacular Names

### 3.5 Ethnomedicinal Importance

For centuries, *P. tomentosa* has been employed in traditional medicine for the management of inflammatory disorders, fever, arthritis, rheumatism, bronchitis, skin diseases, ulcers, dyspepsia, constipation, microbial infections, and hepatic disorders. Various plant parts—including leaves, bark, roots, flowers, and fruits—have been incorporated into Ayurvedic formulations. Traditionally, leaf paste is applied to wounds and inflammatory lesions, while decoctions of the bark and roots are used for fever, respiratory ailments, and musculoskeletal pain.

These traditional uses suggest the presence of bioactive phytochemicals capable of modulating oxidative stress, inflammation, and tissue repair. Such pharmacological properties provide a scientific rationale for exploring *P. tomentosa* as a neuroprotective candidate, since oxidative stress and chronic neuroinflammation are central mechanisms underlying Parkinson's disease.

### 3.6 Macroscopic Characteristics

Accurate macroscopic identification is the first step in pharmacognostic authentication.

#### Habit

A medium-sized deciduous tree reaching 8–20 m in height with a spreading crown.

#### Stem

Cylindrical

Brown to grey bark

Rough surface

Longitudinal fissures in mature stems

#### Leaves

Simple

Opposite

Ovate to elliptic

Entire margins

Acute apex

Rounded to cordate base

Pubescent lower surface

Dark green adaxial surface

Pale green abaxial surface

Characteristic aromatic odor

Slightly bitter taste

#### Flowers

Small

Greenish-white

Terminal corymbose cymes

Bisexual

#### Fruits

Globose drupe

Green when immature

Black at maturity

### 3.7 Microscopic Characteristics

Microscopic examination provides definitive diagnostic markers for authentication.

#### Leaf Anatomy

Transverse sections typically reveal:

Thick cuticle

Single-layered upper epidermis

Lower epidermis bearing abundant stomata

Dorsiventral mesophyll

One layer of elongated palisade cells

Multiple layers of spongy parenchyma

Collateral vascular bundles

Lignified xylem vessels

Well-developed phloem

Calcium oxalate crystals

Non-glandular multicellular trichomes

Glandular trichomes characteristic of Lamiaceae

Secretory cells containing phenolic substances

These anatomical features can serve as reliable pharmacognostic markers for species authentication.

### 3.8 Powder Microscopy

Powder microscopy is widely used for routine quality control.

Diagnostic powder characters include:

Spiral xylem vessels

Pitted vessels

Fibres

Stone cells (if present)

Calcium oxalate crystals

Epidermal fragments

Stomata

Unicellular and multicellular trichomes

Glandular trichomes

Parenchyma cells containing starch grains

Brownish fragments rich in tannins

The combination of these microscopic characters assists in distinguishing authentic *P. tomentosa* from adulterants.

### 3.9 Quantitative Microscopy

Quantitative leaf constants are valuable taxonomic parameters.

These include:

Stomatal number

Stomatal index

Vein-islet number

Vein termination number

Palisade ratio

Trichome density

Epidermal cell number

Future studies should establish reference ranges for these parameters to support pharmacopeial standardization.

### 3.10 Histochemical Evaluation

Histochemical staining facilitates localization of important phytoconstituents.

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Such analyses help correlate anatomical features with phytochemical localization.

### 3.11 Physicochemical Standardization

Physicochemical parameters are indispensable quality-control measures for crude herbal drugs.

Recommended evaluations include:

Foreign organic matter

Moisture content (loss on drying)

Total ash

Acid-insoluble ash

Water-soluble ash

Sulphated ash

Alcohol-soluble extractive value

Water-soluble extractive value

pH

Swelling index

Foaming index

Bulk density

Tapped density

These parameters detect adulteration, improper storage, and deterioration of plant material.

### 3.12 Chromatographic Fingerprinting

Modern herbal standardization increasingly relies on chromatographic fingerprinting.

Recommended analytical techniques include:

High-performance thin-layer chromatography (HPTLC)

High-performance liquid chromatography (HPLC)

Ultra-performance liquid chromatography (UPLC)

Liquid chromatography–mass spectrometry (LC–MS/MS)

Gas chromatography–mass spectrometry (GC–MS)

Fourier-transform infrared spectroscopy (FTIR)

Nuclear magnetic resonance (NMR)

High-resolution mass spectrometry (HRMS)

Chromatographic fingerprints provide reproducible chemical profiles for authentication and batch consistency.

### 3.13 DNA Barcoding

Morphological identification alone may be insufficient when dealing with powdered crude drugs. DNA barcoding using loci such as *rbcL*, *matK*, *ITS*, and *psbA-trnH* has become an important complementary approach for authenticating medicinal plant species and detecting substitution or adulteration. Integration of DNA barcoding with pharmacognostic and chromatographic evaluation is recommended for future quality standards of *P. tomentosa*.

### 3.14 Regulatory Perspective

WHO, the Ayurvedic Pharmacopoeia of India, and international regulatory agencies recommend a

comprehensive standardization strategy for medicinal plants before clinical or commercial application. For *P. tomentosa*, an integrated quality-control framework should include:

Botanical authentication.

Macroscopic and microscopic characterization.

Quantitative microscopy.

Histochemical analysis.

Physicochemical testing.

Heavy metal analysis.

Pesticide residue testing.

Aflatoxin assessment.

Microbial limit testing.

Chromatographic fingerprinting.

DNA barcoding.

Stability studies.

Such a framework will ensure the reproducibility, safety, and regulatory acceptability of *P. tomentosa*-based phytopharmaceuticals and phytosome formulations.

### Critical Analysis and Research Gap

Despite the ethnomedicinal importance of *P. tomentosa*, published pharmacognostic investigations remain fragmented, and no internationally accepted monograph or pharmacopeial standard is currently available. Moreover, correlations between anatomical markers, phytochemical fingerprints, and neuroprotective activity have not been established. Future studies should integrate pharmacognostic authentication with metabolomic profiling, chemometric analysis, and quality-by-design (QbD) principles to support the development of standardized phytosome-based nanoformulations for Parkinson's disease.

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to depletion of dopamine in the striatum. Oxidative stress, mitochondrial dysfunction,  $\alpha$ -synuclein aggregation, and chronic neuroinflammation are considered major pathological mechanisms [10].

Rotenone-induced Parkinsonism is one of the most accepted experimental models because rotenone inhibits mitochondrial complex-I, reproducing motor dysfunction, oxidative stress, neuroinflammation and Lewy body-like inclusions similar to human PD [2].

Recent studies emphasize that oxidative stress-induced neuronal apoptosis activates NF- $\kappa$ B, MAPK, and Nrf2 signaling pathways, contributing significantly to dopaminergic neuronal degeneration in PD. Therefore, antioxidants have become attractive neuroprotective candidates.

Medicinal plants containing polyphenols, flavonoids, terpenoids and phenolic acids have demonstrated

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neuroprotective effects by reducing lipid peroxidation, enhancing endogenous antioxidant enzymes and suppressing inflammatory cytokines 1.

#### 4. Literature Review and Critical Synthesis

Parkinson's disease is a progressive neurodegenerative disorder dominated by nigrostriatal dopaminergic neuronal loss, but its biology extends beyond dopamine deficiency to oxidative stress, mitochondrial dysfunction,  $\alpha$ -synuclein pathology, neuroinflammation, impaired proteostasis and regulated cell death. These interconnected mechanisms support the rationale for multi-target neuroprotective strategies.<sup>1,5,10</sup>

Rotenone is a mitochondrial complex-I inhibitor and has been used to reproduce several biochemical, behavioral and neuropathological features relevant to Parkinsonian neurodegeneration.<sup>2,3</sup> The model therefore provides a useful preclinical platform for evaluating interventions directed at mitochondrial dysfunction, oxidative stress and inflammation, while its limitations should be acknowledged when translating findings to human disease.

Plant-derived polyphenols, flavonoids, terpenoids and related metabolites have attracted attention because they may simultaneously influence oxidative, inflammatory and apoptotic pathways.<sup>7,11,12</sup> However, pharmacological translation is frequently constrained by poor aqueous solubility, instability, metabolism and limited central nervous system exposure.

Premna species have been reviewed for diverse phytochemical constituents and pharmacological activities, providing a basis for investigating *P. tomentosa* as a standardized botanical source.<sup>30</sup> The present review extends this rationale toward a phytosome-based strategy, but direct evidence specifically demonstrating a *P. tomentosa* phytosome-mediated neuroprotective effect in rotenone-induced Parkinsonism remains a research gap identified by the source manuscript.

Phytosomes are phospholipid-phytoconstituent complexes intended to improve membrane affinity and bioavailability of plant-derived constituents.<sup>18,19</sup> Their potential application to neuroprotective phytochemicals is consistent with broader nanotechnology approaches designed to improve delivery and pharmacokinetic performance.<sup>29</sup>

Accordingly, a translational development pathway should connect botanical authentication, physicochemical and chromatographic standardization, phytochemical profiling, formulation optimization, in-vitro characterization, pharmacokinetics, and well-controlled rotenone-induced in-vivo evaluation. This sequence is essential for demonstrating that any observed neuroprotection is reproducible, attributable to a chemically

characterized preparation, and suitable for further development.

**Table 1. Major molecular mechanisms involved in Parkinson's disease pathogenesis**

| Pathogenic mechanism            | Major molecular events                                     | Therapeutic targets       |
|---------------------------------|------------------------------------------------------------|---------------------------|
| Oxidative stress                | ROS generation, lipid peroxidation, GSH depletion          | Nrf2, HO-1, SOD, Catalase |
| Mitochondrial dysfunction       | Complex I inhibition, ATP depletion, mitophagy defects     | PINK1/Parkin, AMPK        |
| $\alpha$ -Synuclein aggregation | Lewy body formation, synaptic dysfunction                  | Proteostasis, autophagy   |
| Neuroinflammation               | Microglial activation, TNF- $\alpha$ , IL-1 $\beta$ , IL-6 | NF- $\kappa$ B, NLRP3     |
| Apoptosis                       | Caspase activation, Bax/Bcl-2 imbalance                    | PI3K/Akt, Bcl-2           |
| Ferroptosis                     | Iron accumulation, lipid ROS                               | GPX4, NRF2                |
| Pyroptosis                      | NLRP3 inflammasome, Caspase-1                              | NLRP3 inhibitors          |

**Table 2. Botanical classification of Premna tomentosa**

| Taxonomic Rank | Classification          |
|----------------|-------------------------|
| Kingdom        | Plantae                 |
| Subkingdom     | Tracheobionta           |
| Division       | Magnoliophyta           |
| Class          | Magnoliopsida           |
| Order          | Lamiales                |
| Family         | Lamiaceae               |
| Genus          | Premna                  |
| Species        | Premna tomentosa Willd. |

**Table 3. Important pharmacognostic parameters**

| Language | Name                        |
|----------|-----------------------------|
| Sanskrit | Agnimantha                  |
| Hindi    | Arni                        |
| Telugu   | Nallamaddi (regional usage) |
| Tamil    | Munnai                      |

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|           |                                               |
|-----------|-----------------------------------------------|
| Kannada   | Agnimantha                                    |
| Malayalam | Munna                                         |
| English   | Headache Tree (reported regional common name) |

**Table 4. Physicochemical quality-control parameters**

| Parameter                | Importance                |
|--------------------------|---------------------------|
| Stomatal index           | Species identification    |
| Palisade ratio           | Authentication            |
| Vein islet number        | Taxonomic marker          |
| Vein termination         | Authentication            |
| Trichome density         | Quality control           |
| Calcium oxalate crystals | Diagnostic feature        |
| Powder microscopy        | Detection of adulteration |

**Table 5. Proposed integrated development roadmap for P. tomentosa phytosome**

| Development stage              | Key activities                                                                                       | Purpose / critical output                       |
|--------------------------------|------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Botanical authentication       | Macroscopy, microscopy, powder microscopy, DNA barcoding                                             | Identity and prevention of adulteration         |
| Phytochemical characterization | HPTLC/HPLC, LC-MS/MS, FTIR, NMR; total phenolics/flavonoids                                          | Chemical fingerprint and marker selection       |
| Extract standardization        | Extraction optimization, marker quantification, batch consistency                                    | Reproducible standardized extract               |
| Phytosome development          | Phospholipid complexation; optimization of composition and process variables                         | Improved biopharmaceutical performance          |
| In-vitro characterization      | Particle/complex properties, FTIR/DSC/XRD where applicable, release and stability                    | Evidence of formulation formation and stability |
| Preclinical evaluation         | Rotenone-induced Parkinsonism; behavioral, biochemical, inflammatory and histopathological endpoints | Proof-of-concept neuroprotection                |
| Translational assessment       | Pharmacokinetics, brain exposure, safety and                                                         | Basis for further development                   |

|  |                 |  |
|--|-----------------|--|
|  | reproducibility |  |
|--|-----------------|--|

### 5. Research Gaps and Future Perspectives

Direct pharmacological evidence for standardized P. tomentosa leaf extract in Parkinson's disease is limited in the source literature. The key gap is therefore not simply the identification of phytochemicals, but the establishment of a reproducible link between botanical quality, chemical markers, formulation attributes, brain exposure and neuroprotective efficacy.

Future studies should establish authenticated plant material and reference ranges for pharmacognostic constants; generate validated chromatographic fingerprints; identify and quantify marker constituents; and apply quality-by-design principles to phytosome optimization. The resulting formulation should be evaluated for physicochemical stability, release behavior, pharmacokinetics and central nervous system exposure before efficacy studies.

In rotenone-induced Parkinsonism, evaluation should integrate behavioral outcomes with oxidative-stress markers, antioxidant defenses, inflammatory mediators, mitochondrial parameters,  $\alpha$ -synuclein-related endpoints, apoptosis and histopathology. Such a multi-dimensional design would better test the multi-target rationale proposed for the formulation.

Translation will require careful consideration of dose selection, extract standardization, formulation reproducibility, toxicity, regulatory quality requirements and the distinction between symptomatic improvement and genuine disease-modifying activity.

### 6. Conclusion

Premna tomentosa represents a promising but insufficiently explored botanical candidate for neuroprotective development. Its traditional use and reported phytochemical diversity provide a rationale for systematic pharmacognostic and phytochemical investigation, while phytosome technology offers a plausible strategy to address bioavailability limitations of plant-derived constituents. An integrated program combining botanical standardization, chemical fingerprinting, optimized phytosome development and mechanistically informed rotenone-induced Parkinsonism studies could establish a stronger evidence base for this approach. At present, the concept should be regarded as a research opportunity rather than an established therapy, and direct experimental validation remains essential.

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PHARMACOGNOSTIC STANDARDIZATION, PHYTOCHEMICAL CHARACTERIZATION AND DEVELOPMENT OF A PHYTOSOME-BASED NANOFORMULATION OF PREMNA TOMENTOSA LEAF EXTRACT FOR NEUROPROTECTIVE EVALUATION IN A ROTENONE-INDUCED RAT MODEL OF PARKINSON'S DISEASE: A COMPREHENSIVE REVIEW

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