

Pharmacological Evaluation of the Antiparkinson Activity of Ethanolic Extract of *Bombax ceiba* Flowers in Experimental Models

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss, oxidative stress, neuroinflammation, and neurotransmitter imbalance. The present study evaluated the antiparkinsonian and neuroprotective potential of the ethanolic extract of *Bombax ceiba* flowers using in vitro and in vivo experimental models. The plant material was standardized, extracted with ethanol, and subjected to phytochemical screening and thin-layer chromatography. The ethanolic extract showed an 8.33% yield and contained alkaloids and flavonoids. Among the evaluated fractions, the ethanol:ethyl acetate fraction exhibited the highest DPPH radical-scavenging activity (83.89% inhibition at 500 µg/mL) with an IC₅₀ of 94.82 µg/mL, compared with 82.05 µg/mL for the standard. Acute oral toxicity evaluation according to OECD 423 indicated relative safety at lower doses, and 200 and 400 mg/kg doses were selected for pharmacological evaluation. Antiparkinsonian activity was assessed in Rotenone- and MPTP-induced PD models using Rotarod performance, neurotransmitter estimation, antioxidant enzymes, TNF-α, and histopathological examination. The ethanolic extract improved motor coordination, restored dopamine, serotonin, and noradrenaline levels, enhanced SOD and catalase activities, and reduced TNF-α levels. Histopathological findings demonstrated reduced neuronal degeneration and inflammatory changes, with greater neuroprotection at 400 mg/kg. Overall, the findings demonstrate significant antioxidant, neuroprotective, and antiparkinsonian potential of *Bombax ceiba* flowers.

Keywords: *Bombax ceiba*; Parkinson's disease; Neuroprotection; Antioxidant activity; Rotenone/MPTP model.

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1. Introduction

Natural products obtained from plants are important sources of biologically active compounds with therapeutic, nutritional, cosmetic, and industrial applications. Medicinal plants contain diverse phytoconstituents, including alkaloids, flavonoids, glycosides, tannins, terpenoids, steroids, phenolic compounds, and volatile oils, which contribute to their wide range of pharmacological activities and have supported the development of modern medicines and nutraceuticals [1,2]. The extraction, separation, purification, and identification of these phytoconstituents are important steps in phytochemical research. The efficiency of extraction depends on several factors, including solvent polarity, temperature, extraction time, particle size, solvent-to-material ratio, and the chemical nature of the constituents. Conventional and modern extraction techniques, followed by chromatographic and

spectroscopic methods, facilitate the isolation and characterization of biologically active compounds from medicinal plants [3-6].

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized predominantly by degeneration of dopaminergic neurons in the substantia nigra and subsequent reduction of dopamine levels in the striatum. Although the precise etiology of PD remains incompletely understood, several factors, including mitochondrial dysfunction, oxidative stress, neuroinflammation, abnormal protein accumulation, genetic factors, and environmental influences, contribute to its pathogenesis and progression [7-11]. Oxidative stress and impaired mitochondrial function promote excessive generation of reactive oxygen species, lipid and protein oxidation, depletion of endogenous antioxidant defenses, and neuronal damage [12,13].

Furthermore, α -synuclein aggregation, persistent neuroinflammation, excitotoxicity, and apoptosis contribute to progressive neuronal degeneration in PD [14,16].

The limitations associated with conventional therapeutic approaches and the multifactorial nature of neurodegeneration have increased interest in medicinal plants and naturally occurring bioactive compounds as potential neuroprotective agents. Phytochemicals such as polyphenols, flavonoids, alkaloids, and terpenoids have demonstrated antioxidant, anti-inflammatory, anti-apoptotic, and mitochondrial protective activities through modulation of multiple pathological pathways [17-20]. Several naturally occurring compounds, including quercetin, apigenin, chlorogenic acid, hydroxytyrosol, and schisandrin B, have demonstrated promising neuroprotective effects in experimental models by reducing oxidative stress, neuroinflammation, mitochondrial dysfunction, and neuronal damage [21-26]. Preclinical investigations further suggest that plant-derived compounds and herbal extracts may protect dopaminergic neurons and improve behavioral abnormalities associated with neurodegenerative disorders [27,28].

Among medicinal plants, *Bombax ceiba* Linn., commonly known as the Red Silk Cotton Tree or Semal, is a large deciduous tree widely distributed in tropical and subtropical regions of Asia. The plant has traditionally been used in various systems of medicine for the management of inflammation, gastrointestinal disorders, wounds, skin disorders, diabetes, and other ailments. Different parts of *Bombax ceiba*, particularly its flowers, contain several bioactive phytoconstituents, including flavonoids, phenolic compounds, tannins, glycosides, saponins, alkaloids, and terpenoids. These constituents are associated with diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and neuroprotective effects [29,30,31].

The antioxidant and neuroprotective properties of *Bombax ceiba* are of particular interest in the context of Parkinson's disease, where oxidative stress, neuroinflammation, mitochondrial dysfunction, and neuronal degeneration play important roles in disease progression. Experimental studies have reported beneficial effects of *Bombax ceiba* and its phytoconstituents in models of neurological disorders, supporting further investigation of its potential neuroprotective and anti-Parkinson activity [30,32,33]. Therefore, systematic phytochemical and pharmacological evaluation of *Bombax ceiba*, particularly its flowers, may provide valuable evidence regarding its potential as a natural source of neuroprotective agents and as a possible

complementary therapeutic approach for Parkinson's disease.

2. Materials and Methods

Materials

The study utilized *Bombax ceiba* flowers, ethanol, petroleum ether, chloroform, ethyl acetate, methanol, ferric chloride, H_2SO_4 , HCl, iodine, DPPH, ascorbic acid, phytochemical screening reagents, silica gel, TLC plates, and other required chromatographic solvents and reagents. Healthy adult Wistar rats were used as experimental animals, with Levodopa as the standard drug. Materials for biochemical and histopathological investigations included phosphate buffer, normal saline, formalin, paraffin wax, xylene, and hematoxylin-eosin stains. Major equipment included Soxhlet apparatus, rotary vacuum evaporator, UV-Visible spectrophotometer, centrifuge, homogenizer, Rotarod apparatus, microtome, and light microscope.

Methods

Standardization of Plant Material

The *Bombax ceiba* plant material was standardized by evaluating macroscopic and microscopic characteristics and pharmacopoeial parameters, including total ash, water-soluble ash, acid-insoluble ash, and moisture content, following Indian Pharmacopoeia (1996). Total ash was determined by incinerating 2 g of air-dried drug at $\leq 450^\circ C$. Water-soluble and acid-insoluble ash were determined by treating the ash with water and 2 M HCl, respectively, followed by filtration, ignition, and weighing. Moisture content was determined by loss on drying. [34]

Extraction of *Bombax ceiba* Flowers

Coarsely powdered *Bombax ceiba* flowers (95 g) were first macerated with 800 mL petroleum ether for 24 h at room temperature, filtered, and the marc was air-dried. The marc was then extracted with 800 mL ethanol in a Soxhlet apparatus at $60^\circ C$ for 24 h until exhaustion. The extract was filtered, concentrated under reduced pressure using a rotary vacuum evaporator, and approximately 50 mL of semisolid ethanolic extract was obtained and stored in an airtight container for further evaluation. [35]

Preliminary Phytochemical Screening

The ethanolic extract of *Bombax ceiba* was subjected to preliminary phytochemical screening for the detection of carbohydrates, alkaloids, saponins, tannins, terpenoids, glycosides, steroids, phenols, amino acids, flavonoids, and anthraquinones using standard qualitative chemical tests. Carbohydrates were identified by Molisch's test, alkaloids by Hager's test, saponins by the foam test, tannins and phenols by

ferric chloride test, terpenoids and steroids by Salkowski's test, glycosides by Keller–Killiani test, amino acids by ninhydrin test, flavonoids by ferric chloride and lead acetate tests, and anthraquinones by the benzene–ammonia test. [36,37]

Thin Layer Chromatography

TLC plates were prepared using silica gel-G slurry and activated at 105°C for 30 min. The extract was applied approximately 2 cm above the bottom of the plate using a capillary tube and allowed to dry. The developing chamber was saturated with the mobile phase for 30 min, after which the plate was developed until the solvent travelled approximately 10–15 cm. The plate was then removed, dried, and examined visually and under UV light using suitable visualization reagents such as vanillin–H₂SO₄ and methanolic ferric chloride. The R_f values of the separated spots were subsequently calculated. (Harborne, 1998; Stahl, 2005; Wagner, 1996)

Column Chromatography of Active Extract

The active extract was subjected to column chromatography for separation and purification of bioactive phytoconstituents. A cylindrical chromatographic column of 40 cm height and 3.5 cm diameter was packed with silica gel (60–120#) as the stationary phase using n-hexane. The extract was mixed with silica powder to obtain a free-flowing material and carefully loaded onto the column. Cotton was placed at the bottom and above the sample to maintain uniform packing and prevent disturbance during elution. The column was eluted isocratically using petroleum ether, chloroform, and ethyl acetate as mobile phases at a flow rate of 6–8 drops/min. Five fractions of 200 mL each were collected separately and dried at room temperature for further evaluation. [38,39]

Phytoconstituent Tests of Bioactive Fractions

The bioactive fractions obtained from column chromatography were subjected to preliminary phytochemical tests for the detection of carbohydrates, alkaloids, saponins, tannins, terpenoids, glycosides, steroids, phenols, amino acids, flavonoids, and anthraquinones. Carbohydrates were detected by Molisch's test, alkaloids by Hager's test, saponins by the foam test, tannins and phenols by ferric chloride test, terpenoids and steroids by Salkowski's test, glycosides by Keller–Killiani test, amino acids by ninhydrin test, flavonoids by ferric chloride and lead acetate tests, and anthraquinones by the benzene–ammonia test. The development of characteristic colours or precipitates was considered indicative of the respective phytoconstituents. [40,41]

In vitro DPPH Activity

The free radical scavenging activity of the extracts was evaluated using the DPPH assay, with ascorbic acid as the standard. The extracts were prepared in methanol

at 1 mg/mL and diluted to concentrations of 25, 50, 75, 100, 200, 300, and 400 µg/mL. Each dilution (1 mL) was mixed with 1 mL of DPPH solution in methanol and incubated for 30 min at room temperature. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The percentage of DPPH scavenging activity was calculated using the absorbance of the control and sample. Ascorbic acid solutions were treated similarly, and all samples were analyzed in triplicate. [42]

In vivo Study

Acute Oral Toxicity Study

The acute oral toxicity of the ethanolic extract of *Bombax ceiba* flowers was evaluated according to OECD Guideline 423 (Acute Toxic Class Method). [43] Healthy adult Wistar rats were fasted overnight with water provided ad libitum and administered the extract orally at different dose levels. Animals were observed during the first 4 h, periodically for 24 h, and subsequently for 14 days for behavioral, neurological, autonomic, and other signs of toxicity, including changes in skin, fur, eyes, salivation, tremors, convulsions, lethargy, sleep, coma, and mortality. Based on the toxicity findings, doses of 200 and 400 mg/kg body weight were selected for evaluation of antiparkinsonian activity.

Experimental Design

The animals were randomly divided into five groups, with six animals in each group. Group I served as the normal control and received normal saline without Parkinson's disease induction. Group II served as the disease control and received Parkinson's induction without treatment. Group III served as the standard control and received Levodopa following Parkinson's induction. Groups IV and V served as low- and high-dose test groups and received ethanolic extract of *Bombax ceiba* flowers at 200 and 400 mg/kg body weight, respectively, following Parkinson's induction. All treatments were administered orally once daily throughout the experimental period.

Induction of Parkinson's Disease

Parkinsonism was experimentally induced in Wistar rats using the Rotenone/MPTP-induced neurotoxicity model. [44] Rotenone was freshly prepared in a suitable vehicle and administered according to the experimental protocol. The induced animals exhibited characteristic Parkinsonian alterations, including muscular rigidity, tremors, bradykinesia, postural instability, and impaired motor coordination. Animals in the disease control, standard control, and test groups received Rotenone/MPTP, whereas the normal control group received normal saline.

Drug Treatment Schedule

The ethanolic extract of *Bombax ceiba* flowers and Levodopa were administered orally once daily throughout the experimental period. Group I received

normal saline, Group II received Rotenone/MPTP only, Group III received Levodopa, and Groups IV and V received the ethanolic extract at 200 and 400 mg/kg body weight, respectively. At the end of the experimental period, behavioral and biochemical parameters were evaluated.

Evaluation of Behavioral Parameters

Rotarod Test

The Rotarod test was used to evaluate motor coordination and muscular balance in experimental animals. [49] Animals were trained to remain on the rotating rod before testing. During the test, each rat was placed individually on the rotating rod, and the fall-off time was recorded in seconds. A reduction in fall-off time indicated impaired motor coordination and muscular strength, whereas increased retention time indicated improvement in motor performance following treatment.

Biochemical Estimation

At the end of the experimental period, animals were sacrificed under anesthesia and brain tissues were isolated, washed with ice-cold normal saline, and homogenized in phosphate buffer. The homogenate was centrifuged, and the resulting supernatant was used for estimation of biochemical parameters.

Estimation of Dopamine

Brain dopamine levels were estimated using standard biochemical assay procedures. [45] Changes in dopamine levels were assessed to evaluate dopaminergic neurodegeneration and the antiparkinsonian effect of treatment.

Estimation of Superoxide Dismutase

Superoxide dismutase (SOD) activity was estimated to assess the antioxidant defense status of brain tissue. [5] Changes in SOD activity were used to evaluate oxidative stress associated with Parkinsonian neurotoxicity.

Estimation of Catalase

Catalase activity was estimated using standard biochemical methods to assess endogenous antioxidant activity in brain tissue. [46]

Estimation of TNF- α

Tumor necrosis factor-alpha (TNF- α) levels were estimated by ELISA to assess neuroinflammatory changes associated with Parkinsonian neurodegeneration. [47]

Estimation of Serotonin and Noradrenaline

Serotonin and noradrenaline levels were estimated from brain homogenates using standard biochemical assay procedures. Alterations in these neurotransmitters were evaluated to assess neurochemical changes associated with Parkinsonian neurodegeneration.

Histopathological Examination

Following completion of the study, brain tissues were isolated under anesthesia, and the substantia nigra and

striatum were fixed in 10% formalin. The tissues were dehydrated using graded alcohol, cleared with xylene, embedded in paraffin wax, sectioned at approximately 5 μ m using a microtome, and stained with hematoxylin and eosin (H&E). [48] The stained sections were examined under a light microscope for neuronal degeneration, dopaminergic neuronal loss, cytoplasmic vacuolation, inflammatory infiltration, necrosis, and other structural abnormalities.

3. Results

Collection and Drying

Collection of *Bombax ceiba*: Healthy and mature leaves of *Bombax ceiba* were collected from natural/cultivated sources, inspected, cleaned, and washed with distilled water to remove dirt and contaminants.

Drying of *Bombax ceiba*: The cleaned leaves were shade-dried at room temperature or oven-dried at 40–50°C. The dried material was coarsely powdered and stored in an airtight container until further extraction and analysis.

Authentication

The collected *Bombax ceiba* plant material was authenticated by the Department of Botany, ased on its morphological characteristics.

Standardization of plants materials:

The physicochemical evaluation of *Bombax ceiba* extract showed a total ash value of 6%, acid insoluble ash value of 1.5%, water soluble ash value of 4%, and moisture content of 8%. These results indicate good purity, acceptable quality, and stability of the extract, confirming its suitability for further phytochemical and pharmacological investigations.

Extraction

Table 1: Yield of various extracts obtained from the whole plant powder

S r. N o.	Evaluat ion Paramet ers	Colo r	Nat ure	Percenta ge Yield (% W/W)
1.	Ethanol	Brow n	Semisolid and sticky	8.33

Preliminary Phytochemical Screening for Various Extracts

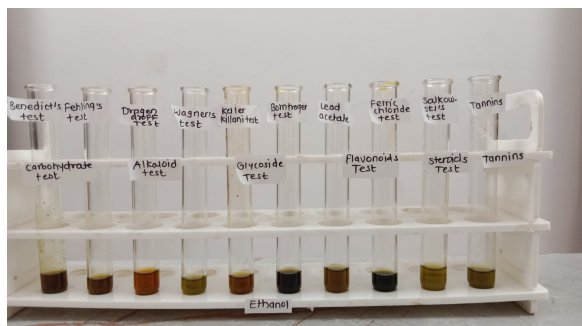


Figure 1: Preliminary Phytochemical Screening for Various Extracts

The ethanolic extract of *Bombax ceiba* was subjected to preliminary phytochemical screening, which revealed the presence of alkaloids as confirmed by positive results in Dragendorff's and Wagner's tests. However, carbohydrates, glycosides, flavonoids, steroids, tannins, and phenolic compounds were found to be absent. These findings indicate that the extract primarily contains alkaloidal constituents, which may contribute to its potential biological activity.

7.1 TLC for phytoconstituents

Table 2: TLC for phytoconstituents

Phytoconstituents	Mobility phase	Rf value	TLC plate image
1. Alkaloids	Toluene: methanol 9:1	0.35	

Preliminary Phytochemical Test of *Bombax ceiba*

The phytochemical screening of different fractions of *Bombax ceiba* revealed the presence of flavonoids in all fractions, indicating that they are the major phytoconstituents of the extract. Alkaloids were detected only in the n-hexane, ethanol:ethyl acetate, and ethanol fractions, while carbohydrates, glycosides, steroids, triterpenoids, and tannins/phenolic compounds were found to be absent in all fractions.

The widespread presence of flavonoids suggests that they may be primarily responsible for the observed biological activities of the extract. Among the fractions, the ethanol and ethanol:ethyl acetate fractions showed the presence of both flavonoids and alkaloids, indicating their potential for higher pharmacological activity.

TLC for phytoconstituents

Table 3: TLC for phytoconstituents

Phytoconstituents	Mobility phase	Rf value		
		n-Hexane fraction	Ethanol: Ethyl acetate fraction	Ethanol fraction
Alkaloid	n-hexane: ethanol 6:4	0.7	0.35	0.24
Flavonoid	n-Hexane: Ethanol 6:4	0.98	0.47	0.87

In vitro DPPH Activity

The DPPH assay demonstrated concentration-dependent antioxidant activity in all *Bombax ceiba* fractions. The ethanol:ethyl acetate fraction showed the highest activity, with 83.89% inhibition at 500 µg/mL, followed by the ethyl acetate and ethanol fractions, whereas the n-hexane fraction showed comparatively lower activity. The ethanol:ethyl acetate fraction exhibited an IC₅₀ of 94.82 µg/mL, which was comparable to the standard (82.05 µg/mL). The higher antioxidant activity of this fraction may be attributed to its greater content of phenolic and flavonoid constituents, indicating its potential as a potent free-radical-scavenging fraction.

Table 4: IC₅₀ (µg/ml) value

Sr. No.	Sample Code	IC ₅₀ (µg/ml)
1	Methanol (Standard)	82.05 µg/ml
2	Ethanol: Ethyl acetate fraction	94.82 µg/ml

In Vivo Activity

Acute Oral Toxicity Study

The acute oral toxicity study of the ethanolic extract of *Bombax ceiba* flowers was conducted according to OECD Guideline No. 423. Animals were monitored for the first 4 h and periodically for 14 days. No significant toxicity was observed at lower doses, whereas mortality occurred at 1000 mg/kg on day 7 and at 2000 mg/kg on day 5, preceded by reduced locomotor activity, lethargy, and decreased food intake. Based on these findings, the extract was considered relatively safe at lower doses, and 200 and 400 mg/kg were selected for further pharmacological evaluation.

Effect of Ethanolic Extract of *Bombax ceiba* Flowers in Rotenone-Induced Parkinson's Disease Model

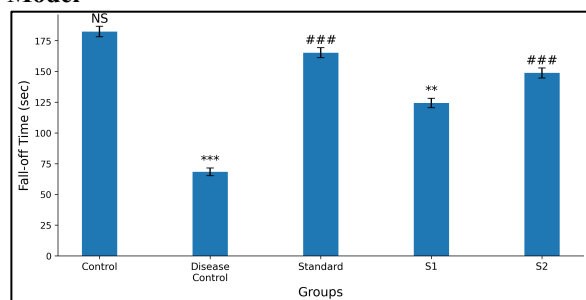


Figure 2: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Rotarod Performance in Rotenone-Induced Parkinson's Disease Model

Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Dopamine Level in Rotenone-Induced Parkinson's Disease Model

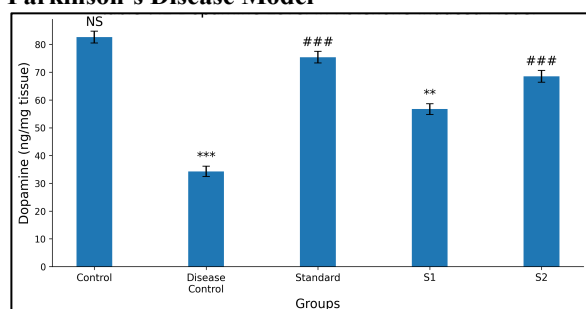


Figure 3: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Dopamine Level in Rotenone-Induced Parkinson's Disease Model

Effect of Ethanolic Extract of *Bombax ceiba* Flowers on SOD Level in Rotenone-Induced Parkinson's Disease Model

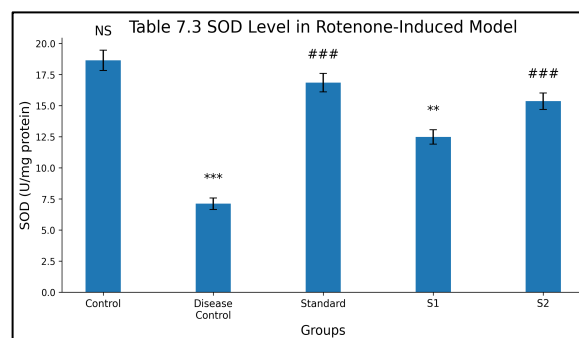


Figure 4: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on SOD Level in Rotenone-Induced Parkinson's Disease Model

Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Catalase Level in Rotenone-Induced Parkinson's Disease Model

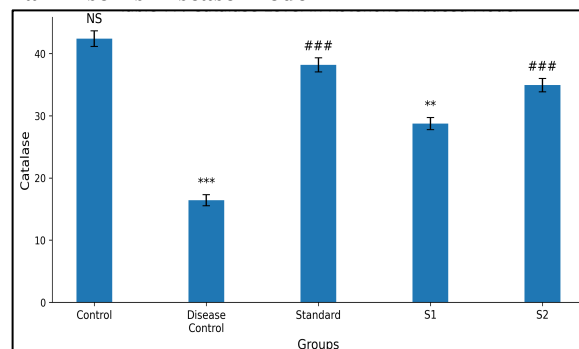


Figure 5: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Catalase Level in Rotenone-Induced Parkinson's Disease Model

Effect of Ethanolic Extract of *Bombax ceiba* Flowers on TNF- α Level in Rotenone-Induced Parkinson's Disease Model

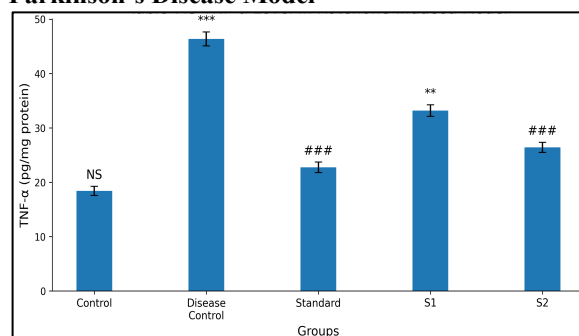


Figure 6: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on TNF- α Level in Rotenone-Induced Parkinson's Disease Model

Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Serotonin Level in Rotenone-Induced Parkinson's Disease Model

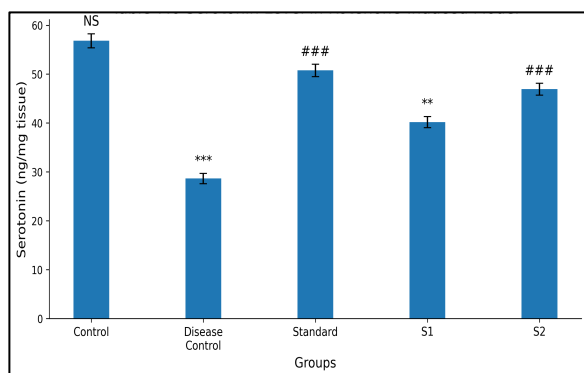


Figure 7: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Serotonin Level in Rotenone-Induced Parkinson's Disease Model
Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Noradrenaline Level in Rotenone-Induced Parkinson's Disease Model

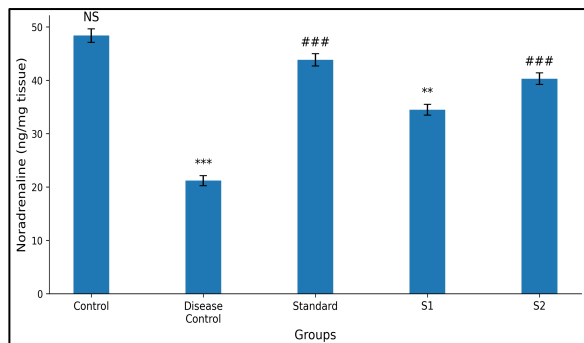


Figure 8: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Noradrenaline Level in Rotenone-Induced Parkinson's Disease Model
Effect of Ethanolic Extract of *Bombax ceiba* Flowers in MPTP-Induced Parkinson's Disease Model

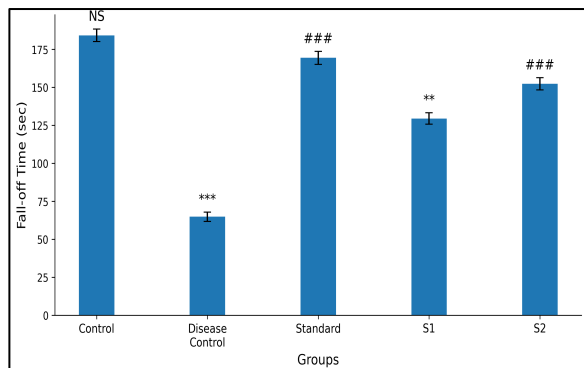


Figure 9: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Rotarod Performance in MPTP-Induced Parkinson's Disease Model
Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Dopamine Level in MPTP-Induced Parkinson's Disease Model

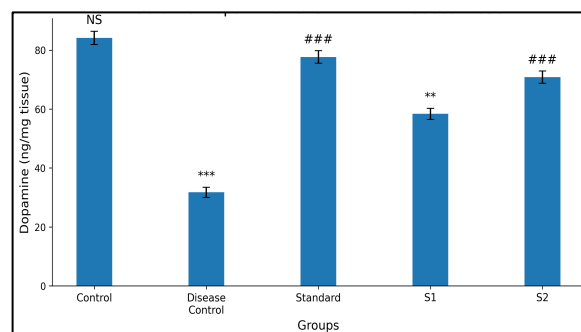


Figure 10: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Dopamine Level in MPTP-Induced Parkinson's Disease Model
Effect of Ethanolic Extract of *Bombax ceiba* Flowers on SOD Level in MPTP-Induced Parkinson's Disease Model

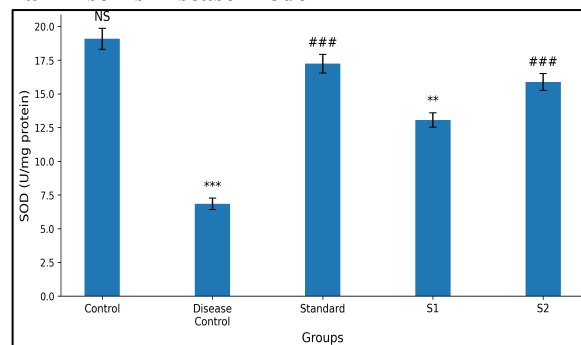


Figure 11: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on SOD Level in MPTP-Induced Parkinson's Disease Model
Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Catalase Level in MPTP-Induced Parkinson's Disease Model

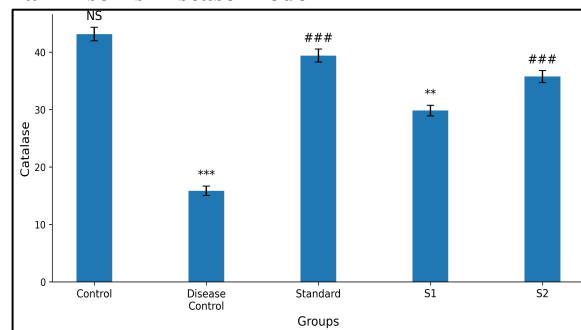


Figure 12: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Catalase Level in MPTP-Induced Parkinson's Disease Model
Effect of Ethanolic Extract of *Bombax ceiba* Flowers on TNF- α Level in MPTP-Induced Parkinson's Disease Model

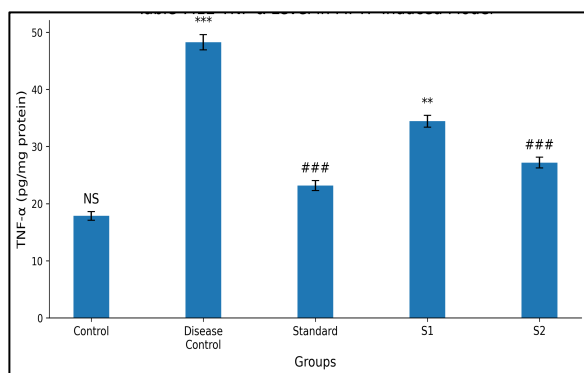


Figure 13: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on TNF- α Level in MPTP-Induced Parkinson's Disease Model

Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Serotonin Level in MPTP-Induced Parkinson's Disease Model

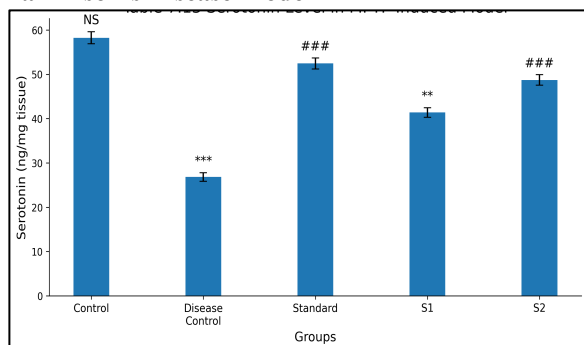


Figure 14: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Serotonin Level in MPTP-Induced Parkinson's Disease Model

Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Noradrenaline Level in MPTP-Induced Parkinson's Disease Model

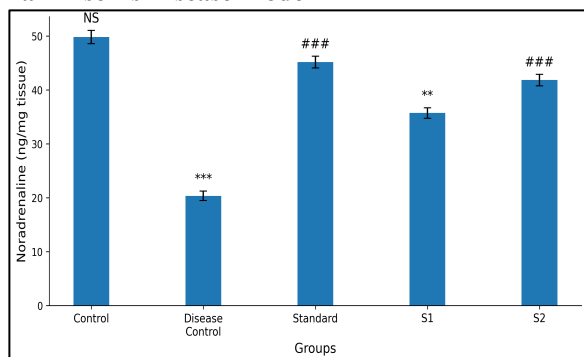


Figure 15: Effect of Ethanolic Extract of *Bombax ceiba* Flowers on Noradrenaline Level in MPTP-Induced Parkinson's Disease Model

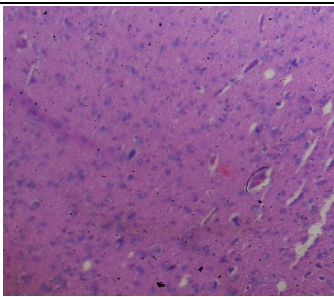
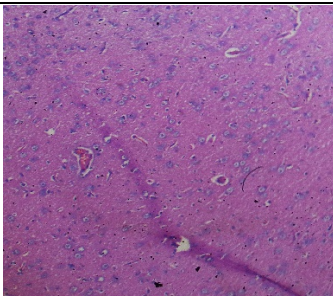
Histopathological Analysis

Rotenone-Induced Parkinson's Disease Model

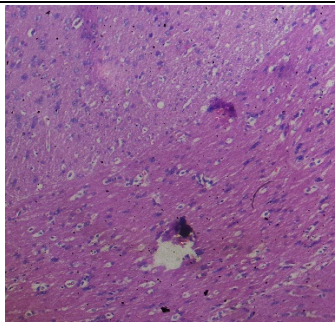
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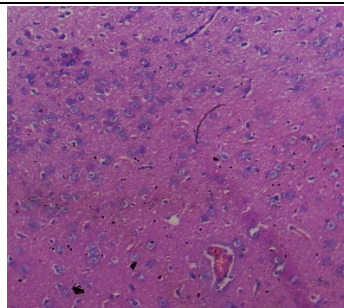
N		
o		
1		Brain sections of normal control animals showed normal histological architecture of substantia nigra and striatum regions. Neurons appeared intact with well-defined cellular boundaries and normal arrangement of dopaminergic neurons. No signs of neuronal degeneration, inflammatory cell infiltration, necrosis, or cytoplasmic vacuolation were observed.
	Normal Control	
2		Brain sections of disease control animals exhibited severe pathological alterations

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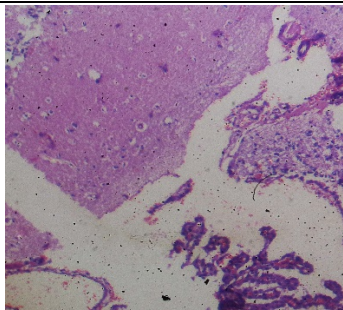
		characterized by marked neuronal degeneration, shrinkage of neurons, cytoplasmic vacuolation, inflammatory cell infiltration, and necrosis in substantia nigra and striatum regions. Significant loss of dopaminergic neurons and disruption of normal neuronal architecture were observed due to Rotenone-induced neurotoxicity.			demonstrated preservation of dopaminergic neurons with minimal cytoplasmic vacuolation and reduced necrotic changes when compared with disease control group.
				Standard	
	4				Brain sections of animals treated with ethanolic extract of Bombax ceiba flowers at dose of 200 mg/kg showed moderate protection against Rotenone-induced neurodegeneration. Reduced inflammatory infiltration, mild neuronal degeneration, and partial restoration of neuronal arrangement were observed.
	Disease Control				
3		Animals treated with Levodopa showed considerable restoration of normal neuronal architecture with reduced neuronal degeneration and inflammatory changes. The brain sections			

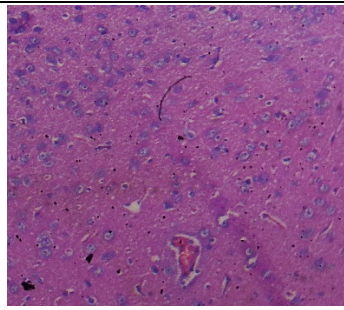
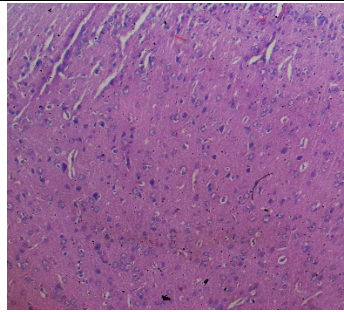
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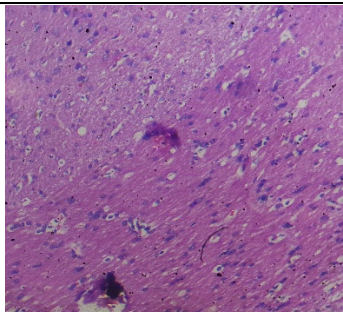
		compared with disease control group.
	Treatment 200 mg/kg	
5		Animals treated with ethanolic extract of Bombax ceiba flowers at dose of 400 mg/kg exhibited significant neuroprotective effect with near normal neuronal architecture. Marked reduction in neuronal degeneration, inflammatory cell infiltration, cytoplasmic vacuolation, and necrosis was observed. Preservation of dopaminergic neurons and improved structural integrity of brain tissue were evident when compared with disease control group.

	Treatment 400 mg/kg	
	MPTP-Induced Parkinson's Disease Model	
S r N o	10 X Image	Descriptio n
1		Brain sections of normal control animals showed normal histological architecture of substantia nigra and striatum regions with intact neuronal cells and normal arrangement of dopaminergic neurons. No pathological abnormalities such as neuronal degeneration, inflammatory infiltration, necrosis, or cytoplasmic vacuolation were observed.
	Normal Control	

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2		Brain sections of disease control animals exhibited severe neurodegenerative changes induced by MPTP administration. Marked neuronal degeneration, cytoplasmic vacuolation, inflammatory cell infiltration, necrosis, and loss of dopaminergic neurons were observed in substantia nigra and striatum regions. Significant disruption of normal neuronal architecture was evident in the disease control group.
	Disease Control	

3		Treatment with Levodopa showed significant protection against MPTP-induced neuronal damage. Brain sections demonstrated restoration of neuronal architecture with reduced inflammatory changes, minimal necrosis, and preservation of dopaminergic neurons when compared with disease control group.
	Standard	
4		Animals treated with ethanolic extract of Bombax ceiba flowers at dose of 200 mg/kg showed moderate neuroprotective activity. Reduced neuronal

		degeneration, mild inflammatory infiltration, and partial restoration of neuronal organization were observed compared with disease control animals.
	Treatment 200 mg/kg	
5		Brain sections of animals treated with ethanolic extract of Bombax ceiba flowers at dose of 400 mg/kg exhibited marked neuroprotective effect with near normal neuronal arrangement and preservation of dopaminergic neurons. Significant reduction in neuronal degeneration, cytoplasmic vacuolation, necrosis, and

		inflammatory infiltration was observed when compared with disease control group.
	Treatment 400 mg/kg	

4. Discussion

The present study entitled “Pharmacological Evaluation of the Antiparkinson Activity of Ethanolic Extract of Bombax ceiba Flowers in Experimental Models” was undertaken to evaluate the neuroprotective and antiparkinson potential of the ethanolic flower extract using various in vitro and in vivo experimental approaches. The study included collection, authentication, physicochemical evaluation, phytochemical screening, antioxidant activity, acute toxicity study, behavioral assessment, biochemical estimation, and histopathological analysis. The physicochemical evaluation of the extract revealed acceptable quality parameters with total ash value 6%, acid insoluble ash 1.5%, water soluble ash 4%, and moisture content 8%, confirming purity and stability of the extract. The percentage yield of ethanolic extract was found to be 8.33% w/w with semisolid sticky brown appearance. Preliminary phytochemical screening confirmed the presence of alkaloids and flavonoids, while TLC analysis further validated the presence of alkaloids and flavonoids with characteristic Rf values. The antioxidant activity was evaluated using DPPH assay. Among all fractions, the ethanol:ethyl acetate fraction exhibited maximum antioxidant activity with 83.89% inhibition at 500 µg/mL and IC₅₀ value of 94.82 µg/mL, indicating strong free radical scavenging potential.

Acute oral toxicity study carried out according to OECD guideline 423 demonstrated that the extract was safe at lower doses. Based on toxicity findings, doses of 200 mg/kg and 400 mg/kg were selected for pharmacological studies. The antiparkinson activity was evaluated using Rotenone-induced and MPTP-induced Parkinson's disease models. Behavioral studies revealed that treatment with ethanolic extract significantly improved rotarod performance and motor coordination compared to disease control animals. Biochemical estimations demonstrated that the extract significantly restored dopamine, serotonin, noradrenaline, SOD, and catalase levels while reducing TNF-α levels in a dose-dependent manner. The higher dose (400 mg/kg) showed better protective

effect and results comparable to standard Levodopa treatment. Histopathological studies of brain tissue demonstrated severe neuronal degeneration and inflammatory changes in disease control groups, whereas treatment with ethanol extract showed marked neuroprotection with restoration of neuronal architecture and preservation of dopaminergic neurons, particularly at 400 mg/kg dose. Overall, the study demonstrated significant antioxidant, neuroprotective, and antiparkinson activity of ethanol extract of *Bombax ceiba* flowers, suggesting its potential therapeutic role in the management of Parkinson's disease.

5. Conclusion

The present investigation confirmed that the ethanol extract of *Bombax ceiba* flowers possesses significant antiparkinson and neuroprotective activity in experimental models of Parkinson's disease. The extract exhibited appreciable antioxidant activity and contained important phytoconstituents such as alkaloids and flavonoids, which may be responsible for its pharmacological effects. In both Rotenone-induced and MPTP-induced Parkinson's disease models, the ethanol extract significantly improved behavioral parameters, restored neurotransmitter levels, enhanced antioxidant enzyme activity, and reduced inflammatory markers. Histopathological studies also demonstrated protection against neuronal degeneration and preservation of normal brain architecture. The higher dose (400 mg/kg) showed more pronounced neuroprotective effects and produced results comparable to standard Levodopa treatment. The findings suggest that the antiparkinson activity of *Bombax ceiba* flowers may be attributed to their antioxidant and anti-inflammatory properties. Therefore, the plant may serve as a promising natural therapeutic candidate for the management of Parkinson's disease. However, further studies are required to isolate the active constituents and elucidate the exact molecular mechanisms responsible for its neuroprotective activity.

6. References

1. Zhu Y, Ouyang Z, Du H, Wang M, Wang J, Sun H, Kong L, Xu Q, Ma H, Sun Y. New opportunities and challenges of natural products research: When target identification meets single-cell multiomics. *Acta Pharmaceutica Sinica B*. 2022 Nov 1;12(11):4011-39.
2. Wang H, He Y, Jian M, Fu X, Cheng Y, He Y, Fang J, Li L, Zhang D. Breaking the bottleneck in anticancer drug development: efficient utilization of synthetic biology. *Molecules*. 2022 Nov 2;27(21):7480.
3. Butler MS. The role of natural product chemistry in drug discovery. *Journal of natural products*. 2004 Dec 28;67(12):2141-53.
4. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. *Nature reviews Drug discovery*. 2005 Mar 1;4(3):206-20.
5. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese medicine*. 2018 Apr 17;13(1):20.
6. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese medicine*. 2018 Apr 17;13(1):20.
7. Collaborators GB. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*. 2015;385(9963):117-71..
8. Yildiz-Unal A, Korulu S, Karabay A. Neuroprotective strategies against calpain-mediated neurodegeneration. *Neuropsychiatric Disease and Treatment*. 2015 Feb 5:297-310.
9. Klemann CJ, Martens GJ, Sharma M, Martens MB, Isacson O, Gasser T, Visser JE, Poelmans G. Integrated molecular landscape of Parkinson's disease. *npj Parkinson's Disease*. 2017 Apr 10;3(1):14.
10. Aarsland D, Batzu L, Halliday GM, Geurtsen GJ, Ballard C, Ray Chaudhuri K, Weintraub D. Parkinson disease-associated cognitive impairment. *Nature reviews Disease primers*. 2021 Jul 1;7(1):47.
11. Trist BG, Hare DJ, Double KL. Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. *Aging cell*. 2019 Dec;18(6):e13031.
12. Przedborski S, Vila M, Jackson-Lewis V. Series Introduction: Neurodegeneration: What is it and where are we?. *The Journal of clinical investigation*. 2003 Jan 1;111(1):3-10.
13. Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends in immunology*. 2008 Aug 1;29(8):357-65.
14. Rabiei Z, Solati K, Amini-Khoei H. Phytotherapy in treatment of Parkinson's disease: a review. *Pharmaceutical biology*. 2019 Jan 1;57(1):355-62.
15. Corona JC. Natural Compounds for the Management of Parkinson's Disease and Attention-Deficit/Hyperactivity Disorder. *BioMed research international*. 2018;2018(1):4067597.

16. Cho B, Kim T, Huh YJ, Lee J, Lee YI. Amelioration of mitochondrial quality control and proteostasis by natural compounds in Parkinson's disease models. *International journal of molecular sciences*. 2019 Oct 21;20(20):5208.
17. Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *European journal of pharmacology*. 2013 Jan 5;698(1-3):6-18.
18. Jellinger KA. Basic mechanisms of neurodegeneration: a critical update. *Journal of cellular and molecular medicine*. 2010 Mar;14(3):457-87.
19. E. Salińska, W. Danysz, and J. W. Łazarewicz, "e role of excitotoxicity in neurodegeneration," *Folia Neuro pathologica*, vol. 43, no. 4, pp. 322–339, 2005.
20. Khan A, Jahan S, Imtiyaz Z, Alshahrani S, Antar Makeen H, Mohammed Alshehri B, Kumar A, Arafah A, Rehman MU. Neuroprotection: Targeting multiple pathways by naturally occurring phytochemicals. *Biomedicines*. 2020 Aug 12;8(8):284.
21. Wang X, Liang XB, Li FQ, Zhou HF, Liu XY, Wang JJ, Wang XM. Therapeutic strategies for Parkinson's disease: the ancient meets the future—traditional Chinese herbal medicine, electroacupuncture, gene therapy and stem cells. *Neurochemical research*. 2008 Oct;33(10):1956-63.
22. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. *Nature Reviews Immunology*. 2022 Nov;22(11):657-73.
23. Calabrese V, Cornelius C, Dinkova-Kostova AT, Calabrese EJ, Mattson MP. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. *Antioxidants & redox signaling*. 2010 Dec 1;13(11):1763-811.
24. Calabrese V, Cornelius C, Dinkova-Kostova AT, Calabrese EJ. Vitagenes, cellular stress response, and acetylcarnitine: relevance to hormesis. *Biofactors*. 2009 Mar;35(2):146-60.
25. Calabrese V, Cornelius C, Mancuso C, Barone E, Calafato S, Bates T, Rizzarelli E, Kostova AT. Vitagenes, dietary antioxidants and neuroprotection in neurodegenerative diseases. *Front Biosci*. 2009 Jan 1;14(3):76-97.
26. Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Giuffrida Stella AM. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. *Nature reviews neuroscience*. 2007 Oct;8(10):766-75.
27. Han C, Guo L, Yang Y, Li W, Sheng Y, Wang J, Guan Q, Zhang X. Study on antrodia camphorata polysaccharide in alleviating the neuroethology of PD mice by decreasing the expression of NLRP3 inflammasome. *Phytotherapy Research*. 2019 Sep;33(9):2288-97.
28. Wang C, Nguyen T, Yang X, Mellick GD, Feng Y. Phytochemical investigation of *Asarum sieboldii* var. *seoulense* and the phenotypic profiles of its constituents against a Parkinson's Disease olfactory cell line. *Bioorganic & Medicinal Chemistry Letters*. 2023 Aug 15;92:129386.
29. Kumari N, Radha, Puri S, Kumar M, Natta S, Dhumal S, Raman P, Rajalingam S, Singh J. Exploring the Therapeutic Potential of *Bombax ceiba* L. Flowers as Source of Functional Food Development: A Review. *Chemistry & Biodiversity*. 2025 Nov;22(11):e00247.
30. Khan A, Ullah F, Alkreathy HM, Ahmed M, Khan RA. Phytochemical screening, antioxidant and anti-Parkinson activities of *Berula erecta*: A novel medicinal plant. *PLoS One*. 2024 Nov 15;19(11):e0305751.
31. Huang SK, Hsieh CY, Fu PW, Lee CJ, Domingo GC, Alimboyoguen AB, Lin PY, Hung LC, Roxas TJ, De Castro-Cruz KA, Tsai PW. Phytochemical constituent analysis, antioxidative effect, and anti-inflammatory activity of *Bombax ceiba* flowers. *Biointerface Res. Appl. Chem*. 2023 Oct;13(5):408.
32. Li X, Gao D, Paudel YN, Li X, Zheng M, Liu G, Ma Y, Chu L, He F, Jin M. Anti-Parkinson's disease activity of *Sanguangprou vaninii* extracts in the MPTP-induced zebrafish model. *ACS chemical neuroscience*. 2022 Jan 19;13(3):330-9.
33. Nipurte A, Thawkar B, Kale M. Antiparkinson activity of the Ethanolic Extract from *Holarrehena antidysentrica* Leaves test in Swiss albino mice. *Research Journal of Pharmacy and Technology*. 2025 Nov 1;18(11):5281-7.
34. *Indian Pharmacopoeia*, 1996
35. Umdale S, Mahadik R, Otari P, Gore N, Mundada P, Ahire M. Phytochemical composition, and antioxidant potential of *Frerea indica* Dalz.: A critically endangered, endemic and monotypic genus of the Western Ghats of India. *Biocatalysis and agricultural biotechnology*. 2021 Aug 1; 35:102080.
36. Khandelwal, 2005.
37. Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., & Karki, N. (2020). Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. *Tribhuvan University Journal*, 35(2), 1-11.

38. Stahl, 2005.
39. Cojocaru, Sabina Ioana, et al. "Studies regarding the chromatographic separation of some Chelidonium majus L. alkaloids using different solvent systems." *Journal of Experimental and Molecular Biology* 11.4 (2010).
40. Sardjono RE, Khoerunnisa F, Musthopa I, Akasum NS, Rachmawati R. Synthesize, characterization, and anti-Parkinson activity of silver-Indonesian velvet beans (*Mucuna pruriens*) seed extract nanoparticles (AgMPn). In *Journal of Physics: Conference Series* 2018 May 1 (Vol. 1013, No. 1, p. 012195). IOP Publishing.
41. Bizzarri BM, Martini A, Serafini F, Aversa D, Piccinino D, Botta L, Berretta N, Guatteo E, Saladino R. Tyrosinase mediated oxidative functionalization in the synthesis of DOPA-derived peptidomimetics with anti-Parkinson activity. *RSC advances*. 2017;7(33):20502-9.
42. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*. 2022 Feb 16;27(4):1326.
43. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. *Journal of the American Pharmaceutical Association*. 1957;46(3):208–209.
44. Carlsson A, Lindqvist M, Magnusson T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature*. 1957;180:1200.
45. Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques*. 6th ed. Philadelphia: Churchill Livingstone Elsevier; 2008.
46. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron*. 2003 Sep 11;39(6):889-909.
47. Sharma DR, Wani WY, Sunkaria A, Kandimalla RJ, Verma D, Cameotra SS, Gill KD. Quercetin protects against chronic aluminum-induced oxidative stress and ensuing biochemical, cholinergic, and neurobehavioral impairments in rats. *Neurotoxicity research*. 2013 May;23(4):336-57.
48. Mittal P, Dhankhar S, Chauhan S, Garg N, Bhattacharya T, Ali M, Chaudhary AA, Rudayni HA, Al-Zharani M, Ahmad W, Khan SU. A review on natural antioxidants for their role in the treatment of Parkinson's disease. *Pharmaceuticals*. 2023 Jun 21;16(7):908.
49. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*. 1984;21(2):130–132.