Molecular and Behavioral Assessment of Melia azedarach extract in Parkinsonian rat models with novel nano-ethosomal gel formulation

Shivanjali Shinde[1], Anjali Lokhande[2], Nilima Shinde[3], Saloni Kamble[4], Vedanti Kadam[5], Ganesh More[6], Ganesh Mhaske[7], Shraddha Satkar[8], Sakshi Gholve[9], Yogita Bodhane[10], Vaishnavi Adkar[11], Pradip Maske[12], Mayuri Gurav[13]

[1,2,3,4,5,7,8,9,10,11,12,13] *IVM's Krishnarao Bhegade Institute of Pharmaceutical Education and Research, Talegaon Dabhade, Pune 410507.*

[6]*D.Y. Patil School of Pharmacy, D.Y. Patil University, Ambi, Talegaon Dabhade, Pune-410507.*

***Abstract*—Objectives: *Melia azedarach* Linn., a member of the Meliaceae family, is traditionally valued in Ayurveda for its medicinal qualities, characterized by its Tikta and Kashaya tastes, Sheeta (cooling) potency, and Ruksha (dry) nature. It is known for a wide range of pharmacological actions, making it beneficial in various health disorders. This study was designed to investigate the anti-Parkinsonian potential of an ethanolic leaf extract of *Melia azedarach* in animal models of Parkinson's disease induced by haloperidol and 6-hydroxydopamine (6-OHDA), along with the formulation of a nano-based delivery system for targeted treatment. Methods: Adult male Wistar rats (180–220 g) were randomly assigned to six groups (n=6), each subjected to a specific intervention. Haloperidol was used to induce catalepsy, while 6-OHDA was administered to mimic Parkinsonian symptoms. The experimental groups included a normal control (distilled water), a vehicle control (6-OHDA + saline), a disease control (6-OHDA alone), a standard group receiving Levodopa, and two test groups receiving *Melia azedarach* extract at 100 mg/kg and 200 mg/kg subcutaneously for 48 days. Behavioral assessments focused on catalepsy, muscle stiffness, and locomotor activity. Phytochemical profiling was carried out using UV and IR spectroscopy, while molecular docking was performed using AutoDock. SwissADME was employed to evaluate pharmacokinetic properties. A nano ethosomal gel was developed and characterized based on particle size, zeta potential, pH, drug content, texture, viscosity, and spreadability. Results: Computational studies identified flavonoids such as Nobiletin, Quercetin, and Kaempferol as key contributors the observed neuroprotective effects. These compounds showed higher binding affinity in molecular docking analyses than Levodopa. The 200 mg/kg dose of Melia azedarach significantly reduced haloperidol-induced catalepsy and improved motor function in the 6-OHDA model. Conclusion: *Melia azedarach* leaf extract demonstrated promising anti-Parkinsonian effects by improving motor deficits in experimental models. The nano ethosomal gel formulation enhances its potential for targeted brain delivery, suggesting therapeutic promise in Parkinson's disease management.**

***Index Terms*—Anti-Parkinson effects, Melia azedarach, Molecular interaction studies, Nano-based ethosomal formulation, Parkinson's disease experimental models.**

I. INTRODUCTION

Parkinson's disease (PD) is a progressive neurological disorder characterized by the degeneration of dopamine-producing neurons, leading to motor symptoms like tremors, rigidity, bradykinesia, and postural instability, as well as nonmotor symptoms such as dementia, soft speech, and difficulty swallowing. The condition is characterized by the buildup of misfolded α-synuclein proteins within Lewy bodies, leading to disruption of normal neuronal function. Muscle rigidity in PD results from an imbalance between excitatory cholinergic and inhibitory dopaminergic neurons due to degeneration of the substantia nigra, and oxidative stress plays a key role in neuronal degeneration and disease progression. While levodopa is the gold standard treatment, long-term use often leads to motor complications like dyskinesia and motor fluctuations, prompting the exploration of alternative or complementary therapies, including herbal remedies. For centuries, plants have been utilized in traditional medicine for their healing and therapeutic benefits. Melia azedarach

(Mahanimba), native to the sub-Himalayan region of India, is being researched for its potential benefits in managing Parkinson's disease (PD). Studies suggest it may help address key mechanisms involved in the disease. Its bioactive compounds could offer anti-inflammatory, antioxidant, and neuroprotective effects in PD management [1]-[8]. *Melia azedarach (Mahanimba*) is known for its diverse medicinal properties, including antiulcer, antipyretic, anti-fertility, anticancer, antiviral, wound healing, and hepatoprotective effects. According to Ayurveda, it has *Tikta* and *Kashaya rasa* (bitter and astringent tastes), *Sheeta veerya* (cooling potency), and *Ruksha guna* (dry quality). These properties make it useful for treating various health conditions [9], [10]. ROS production is a critical response of plants to abiotic stresses, causing oxidative damage. Reactive oxygen species (ROS) can directly damage membrane lipids, inactivate enzymes, and harm nucleic acids, which may ultimately result in cell death [11], [12]. *Melia azedarach* (*Mahanimba*) has been demonstrated in both experimental and clinical studies to exhibit a broad spectrum of therapeutic properties, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-ulcer, antipyretic, and cardioprotective effects. The leaves contain limonoids like nimbolinin, which provide antioxidant and anticancer benefits, while the flowers offer pharmacological effects such as astringent, diuretic, and anti-inflammatory actions. The fruits have purgative and anti-parasitic properties, while the seeds are employed in the treatment of rheumatism and skin conditions. A decoction of the bark helps alleviate symptoms of fever, including thirst, nausea, and weakness. These diverse properties make *M. azedarach* a valuable plant in traditional medicine [13]-[18]. *Melia azedarach* contains various bioactive compounds, including kaempferol 3-O-β-rutinoside, kaempferol 3-L-rhamno-D-glycoside, rutin, quercetin, nobiletin, and astragalin [14], [19], [20], [21]. Flavonoids provide neuroprotective effects by shielding against neurotoxin damage and reducing neuroinflammation. Their ability to cross the blood-brain barrier and favorable pharmacokinetics make them promising for treating neurological disorders. Flavonoids regulate key inflammatory pathways, such as NF-κB, MAPK, and Nrf2, which are crucial in the progression of neurological diseases [22], [23]. Given its traditional use in neurodegenerative disorders and antioxidant properties, *M. azedarach* was evaluated for its potential anti-Parkinson's effects in a neurotoxin-induced rat model. Herbal medicines have long been used for their healing properties, but poor absorption often limits their effectiveness, especially in older patients with Parkinson's disease. To address this, we developed a nano-ethosomal gel to enhance drug delivery, improving absorption and therapeutic outcomes. Transdermal formulations, which bypass first-pass metabolism and offer convenient self-administration, provide an effective alternative for Parkinson’s treatment, improving patient compliance and reducing dosing frequency [24]. Nano ethosomes are non-invasive drug delivery systems that enhance drug penetration through the skin and absorption into the systemic circulation. Composed of phospholipids and high concentrations of alcohol (ethanol and isopropyl alcohol), they are particularly effective for transcellular drug delivery, allowing drugs to efficiently cross the skin layers. Due to their high ethanol content, nano ethosomes can penetrate the stratum corneum, the outermost skin layer, with sizes ranging from 10 nm to several microns, offering versatile and effective skin-based drug delivery [25]-[27].

II. OBJECTIVES

1. The aim of this study was to investigate the anti-Parkinson’s activity of *Melia azedarach* leaves in vivo.
2. To develop nano formulation of the extract to achieve targeted drug therapy against Parkinson’s Disease.

III. MATERIAL AND METHODS

a) Collection of Plant Material:

Fresh leaves of *M. azedarach* were collected in September from the local area of Pune district, Maharashtra, India. The plant was authenticated at the Botanical Survey of India, Western Regional Centre, 7-Koregaon Road, Pune-411001 (Specimen No. SBSMA-1).

b) Animals:

Male Wistar rats, weighing between 180–220 g, were selected for the study and allowed to acclimate to the laboratory environment for one week. The rats were randomly assigned to six groups of six animals each

and housed in well-ventilated polypropylene cages with a 12:12 h light/dark cycle at 25 ± 2°C and 55–65% relative humidity. During the experiment, rats had free access to normal pellet diet and water. The experimental protocol was approved by the Institutional Animal Ethics Committee, in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

c) Drugs and chemicals:

The drugs used in the study were haloperidol (RPG Life Sciences Ltd., Gujarat, India), levodopa (Sun Pharma Laboratories Ltd., India), sodium pentobarbital (Anuh Pharma Limited, India), 6-OHDA hydrobromide, and ascorbic acid (Hardi Chem Enterprise, Ahmedabad, India). The chemicals used included ethanol AR and Tween 80 AR (New Neeta Chemicals, India). All chemicals were of analytical grade and were purchased from standard suppliers.

d) Preparation of Leaf Extract:

The leaves and seeds were collected, dried in the shade, and ground into a coarse powder. One thousand grams of the powdered plant material were placed in a stoppered container with 1000 ml of ethanol. The mixture was kept in contact with the solvent for 3 days at room temperature, with intermittent agitation to ensure complete dissolution of the soluble components. Afterward, the mixture was filtered, the marc was pressed, and the extract was clarified by filtration following standing. Once dried, the extract was measured, and the yield was computed using the specified formula.

$$\% \text{ of Extractive yield (w/w)} = \frac{\text{Weight of Dried Extract}}{\text{Weight of Dried Leaves Powder}} \times 100$$

The yield of the extract was found to be 28.6% (w/w).

e) UV-Vis Spectroscopy:

A Shimadzu 19000i UV-Vis spectrophotometer was used to perform UV-Visible spectroscopy. Ethanol was added to the sample to increase its volume from 100 µL to 3 ml. The sample was then scanned across the wavelength range of 200–800 nm.

f) IR Spectroscopy:

The FT/IR-4100 type A spectrophotometer 8000 series was used to perform IR spectroscopy. The ethanol extract from Melia azedarach was mixed with KBr salt, crushed into a thin pellet, and scanned between 4,000 and cm¯1 range [28].

g) Molecular Docking:

Several phytoconstituents were molecularly docked using AutoDock Vina, PubChem, and Biovia software. The binding affinities of Nobiletin, Quercetin, and Kaempferol towards dopaminergic and NMDA receptors were determined and compared with the standard drug, Levodopa.

h) Grouping of animals:

Neurodegeneration was induced in Wistar rats (n=30) using the neurotoxin 6-hydroxydopamine under anesthesia with sodium pentobarbital at a dose of 50 mg/kg. The rats were allowed to recover for 3 d and then, the animals were divided into six groups (n=6).

Catalepsy Model:

- Normal (NM): rats received oral doses of distilled water each day
- Vehicle Control (VC): Haloperidol (1mg/kg i.p.) +Saline (10mg/kg.p.o)
- Disease Control (DC): Haloperidol (1mg/kg i.p.)
- Standard: Haloperidol (1mg/kg i.p.) +Levodopa (6mg/kg p.o)
- Test 1: Haloperidol (1mg/kg i.p.) +Extract (100 mg/kg s.c)
- Test 2: Haloperidol (1mg/kg i.p.) +Extract (200 mg/kg s.c)

6-OHDA Model:

- Normal (NM): rats received oral doses of distilled water each day
- Vehicle Control (VC): 6-OHDA) +Saline (10mg/kg.p.o)
- Disease Control (DC): 6-OHDA (1mg/kg i.p.)
- Standard (STD): 6-OHDA+Levodopa (6mg/kg p.o)
- Test 1: 6-OHDA+Extract (100 mg/kg s.c)
- Test 2: 6-OHDA+Extract (200 mg/kg s.c)

The treatment period was 54 days.

i) Phytochemical evaluation:

After drying, the extracts were weighed. The presence or absence of various phytoconstituents, including triterpenoids, steroids, alkaloids, vitamins, tannins, glycosides, and flavonoids, was determined using standard test procedures [29].

j) Acute Oral Toxicity of the Extract:

The animals were randomly divided into five groups, with 10 rats in each group. Before beginning the treatment, the rats were fasted for six hours but had free access to water. Group I received only distilled water as a control. Groups II to V were given oral doses of the ethanolic extract of *Melia azedarach* at 1000, 2000, 3000, and 4000 mg/kg, respectively. All

administrations were carried out orally. The rats were observed continuously for 72 hours to monitor any signs of mortality [30].

k) Haloperidol Induced Catalepsy:

Haloperidol is an antipsychotic that blocks D2 receptors, leading to catalepsy in rodents, which includes symptoms like bradykinesia and rigidity. This condition mimics the motor impairments observed in Parkinson's disease, as it results from blocking D2 receptors in the nigrostriatal pathway. Haloperidol-induced catalepsy is commonly used as an animal model to examine motor dysfunction in Parkinson's disease and assess potential anti-Parkinsonian treatments [31]. Considering this, the haloperidol-induced catalepsy model was selected for the current research. The method outlined by Elliott and Close in 1990 was followed to assess anticataleptic activity. The animals were divided into six groups, with six rats in each group (n=6). Group I served as the normal group, Group II as the vehicle control, Group III as the disease control, Group IV received standard Levodopa (6 mg/kg, p.o.), and Groups V and VI were treated with *Melia azedarach* extract (100 and 200 mg/kg, p.o.), respectively. Catalepsy was measured through the standard bar test procedure. Catalepsy was induced using haloperidol (1 mg/kg, i.p.), and animals were examined every 30 minutes for a duration of 210 minutes. The time the rat maintained its forepaws extended on the elevated bar was considered the cataleptic score, with a maximum time limit of 5 minutes [32].

l) Induction of Parkinsonism by 6-OHDA:

The rats were secured in a stereotaxic apparatus and anesthetized with an intraperitoneal injection of sodium pentobarbital at 50 mg/kg. A unilateral injection of 6-OHDA (20 µg of 6-OHDA hydrobromide in 4 µL of 0.9% saline with 0.02 µg/mL of ascorbic acid) was delivered over a five-minute period, and the needle remained in place for an additional five minutes. A stainless-steel needle (0.28 mm outer diameter) was carefully inserted into the substantia nigra at the following coordinates relative to bregma: anterior/posterior: −4.8 mm; medial/lateral: −2.2 mm; ventral/dorsal: −7.2 mm, −3.5 mm. The wound was sealed with dental cement, and stainless-steel screws were used to secure the cranium. After surgery, each rat was returned to its home cage. Sham-operated animals received the same procedure, except that normal saline was injected in place of 6-OHDA. Following a 48-hour recovery period, the animals were treated with 6-OHDA once daily for 55 days, according to their respective groups [33], [34].

m) Locomotor Activity:

Spontaneous locomotor activity was recorded using a digital Actophotometer (Hicon Instrument, India) that was equipped with infrared-sensitive photocells. The testing chamber was ventilated, darkened, and designed to minimize light and sound disturbances. A computerized counter recorded the electric impulses generated by beam interruptions along the x or y axis. Each animal underwent testing for five minutes on days 15, 20, 25, 30, 35, 40, 45, 50, and 55 after the 6-OHDA injection. The results were documented as the number of counts within each five-minute interval [35].

n) Neurotransmitter Quantification by ELISA:

Striatal dopamine level was analyzed using commercial ELISA kits (PreClinBio Solutions, Pune) following standardized protocols with minor optimizations. For dopamine assessment, tissue homogenates were centrifuged (12,000×g, 15 min, 4°C) and supernatants were loaded in duplicate (50 µL/well) alongside 0-1000 pg/mL standards. Plates were incubated with biotinylated antigen (60 min, 37°C), washed (4× PBS-Tween), and developed with streptavidin-HRP (30 min) followed by TMB substrate (10 min). Absorbance was measured at 450 nm (Epoch 2 microplate reader), with neurotransmitter concentration calculated against respective standard curve (dopamine: $y = 0.0012x + 0.0285$, $R^2 = 0.9977$).

o) Development of Formulation (Nano-ethosomes):

M. azedarach-loaded ethosomes were prepared using the cold method. First, soya lecithin was carefully weighed and dissolved in the required amount of water. The drug and cholesterol were measured and dissolved in ethanol. The drug-ethanol-cholesterol mixture was gradually added to the lecithin solution while stirring thoroughly to ensure a consistent mixture. Propylene glycol was then added and mixed well. The resulting solution was sonicated or extruded to reduce the vesicle size. Finally, the mixture was stored in a glass vial in the refrigerator for future use (Table I) [36]-[41].

Table I. Formulation of ethosomes [36]-[41].

Ingredients	Quantity (%)
Drug	1
Soya Lecithin	4
Cholesterol	0.5
Ethanol	45
Propylene glycol	20
Water	Q. S

p) Preparation of ethosomal gel

Methyl- and propyl-paraben were added to 50 ml water and required quantity of Carbopol was added little by little and dispersed homogenously. Propylene glycol and EDTA were incorporated into the mixture mentioned above. To 25 ml of water, triethanolamine was added to maintain the desired pH. To the above gel, the prepared ethosomes was added and dispersed well to get the ethosomal gel (Table II) [42]-[44].

Table II. Preparation of Nano-Ethosomal Gel [42]-[44].

Sr. No	Ingredients	Ethosomal Gel (%)	Gel (%)
1	Ethosomes	1	-
2	*M. Azedarach* Extract	-	1
3	Carbopol	2	2
4	Propylene glycol	15	15
5	Sodium edentate	0.01	0.01
6	Propyl Paraben	0.001	0.001
7	Methylparaben	0.05	0.05
8	Triethanolamine	Q. S	Q. S
9	Water	Q. S	Q. S

q) In Vitro Skin Permeation Study

In vitro drug diffusion studies were performed using a locally fabricated Franz diffusion cell with an effective diffusion area of approximately 3.8 cm^2. Rat abdominal skin, carefully excised and prepared, was employed as the semi-permeable membrane, with the stratum corneum side facing the donor compartment. The receptor chamber was filled with 100 mL of phosphate buffer (pH 7.4) and maintained at 37 ± 0.5 °C to mimic physiological conditions. A fixed quantity of gel formulation (equivalent to 1 g) was applied evenly onto the skin in the donor compartment. The receptor solution was stirred continuously to ensure uniform drug distribution. At predetermined intervals between 30 and 270 minutes (up to 8 hours), 5 mL aliquots were withdrawn from the receptor chamber and replaced immediately with an equal volume of fresh buffer to maintain sink conditions. Samples were analysed spectrophotometrically at 275 nm to quantify drug release. All experiments were performed in triplicate, and the mean cumulative drug release (CDR%) was calculated and plotted as a function of time to compare the release profiles of Nano-Ethosomal Gel and Normal Gel formulations.

IV. RESULTS

a) Phytochemical Screening:

Phytochemicals screening of ethanolic extracts of *Melia azedarach* tested positive for alkaloids, tannins, phenols, flavonoids, terpenoids, steroids, glycosides, anthraquinones, and saponins, while reducing sugars were absent. Flavonoids, including Nobiletin, Quercetin, and Kaempferol, are identified as key phytochemicals potentially responsible for the antiparkinsonian activity of *Melia azedarach*.

b) UV visible spectrum analysis:

The qualitative UV-VIS spectrum of the ethanolic extract of *Melia azedarach* displayed several distinct peaks across the 200 to 800 nm wavelength range. Flavonoids showed a prominent Band II absorption peak between 270 and 295 nm.

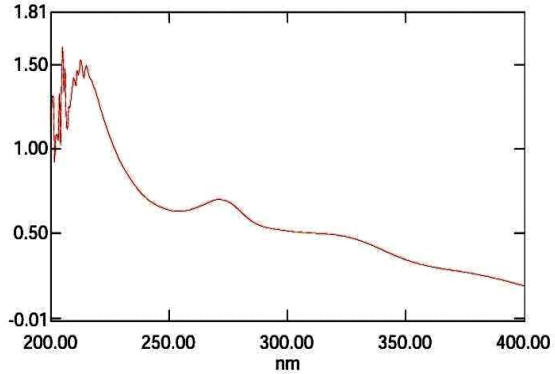


Fig No. 1: Ultra Violet-Visible Spectroscopy Analysis of ethanolic extract of *Melia azedarach.*

c) Infrared Spectroscopy analysis

The FT-IR spectrum (Fig. 1.2) revealed distinct peaks at 2372.01 cm^{-1} and 2345.02 cm^{-1}, indicating the presence of a C≡N nitrile group. Strong peaks at 1719.23 cm^{-1} and 1700.91 cm^{-1} corresponded to C=O bending vibrations. Peaks at 3713.26 cm^{-1} and 3691.09 cm^{-1} suggested the presence of a strong OH

bond. The FT-IR spectrum showed broad peaks at 1638.23 cm^{-1} and 1655.59 cm^{-1}, indicative of stretching vibrations of aromatic alkenes (C=C). These findings confirm the presence of flavonoids in the ethanolic extract.

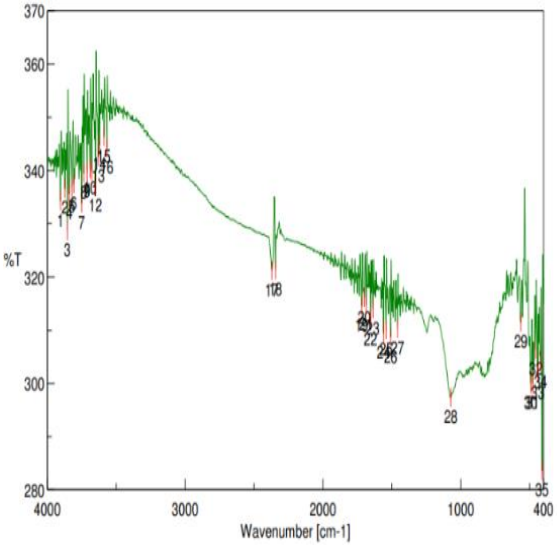


Fig No.2: Fourier-Transform Infrared Spectroscopy analysis of ethanolic extract of Melia azedarach.

Table No III. Infrared Spectroscopy analysis of ethanolic extract of Melia Azedarach

Extract	Peak Value	Functional Group	Functional Group Name
Ethanolic Extract of Melia Azedarach	2372.01 cm-1 2345.02 cm-1	C≡N	Nitril Group
	1719.23cm -1 1700.91 cm-1	C=O	Carbonyl group.
	3713.26 cm-1 3691.09 cm-1	OH bond	Alcohol group
	1638.23 cm-1 1655.59 cm-1	C=C	Aromatic Alkenes

d) Molecular Docking

Binding affinity of Standard (Levodopa) on NMDA Receptors

Table No IV. Binding affinity of Standard (Levodopa)

Mode	Affinity (kcal/mol)	Distance from best mode rmsd 1. B.	Distance from best mode rmsd u.b.
1	-7.3	0.000	0.000
2	-7.3	1.142	2.073
3	-7.2	1.662	2.508
4	-6.7	1.737	1.964
5	-6.3	2.184	6.142
6	-6.3	4.261	5.052
7	-6.3	3.352	5.559
8	-6.3	1.867	5.960
9	-6.3	3.574	6.363

(rmsd 1. b: Root Mean Square Deviation (RMSD) of the first binding pose)

Binding affinity of Flavonoid (Nobiletin) on NMDA Receptors

Table No V. Binding affinity of Nobiletin

Mode	Affinity (kcal/mol)	Distance from best mode rmsd 1. b.	Distance from best mode rmsd u.b.
1	-7.2	0.000	0.000
2	-7.1	2.750	9.021
3	-6.8	9.688	10.672
4	-6.8	9.701	10.082
5	-6.8	9.654	10.140
6	-6.7	4.801	9.960
7	-6.2	8.222	10.927
8	-5.7	8.820	11.736
9	-5.6	0.865	1.927

Binding affinity of Levodopa on Dopaminergic Receptors

Table No VI. Binding affinity of Levodopa

Mode	Affinity (kcal/mol)	Distance from best mode rmsd 1. b.	Distance from best mode rmsd u.b.
1	-7.2	0.000	0.000
2	-7.1	2.750	9.021
3	-6.8	9.688	10.672

4	-6.8	9.701	10.082
5	-6.8	9.654	10.140
6	-6.7	4.801	9.960
7	-6.2	8.222	10.927
8	-5.7	8.820	11.736
9	-5.6	0.865	1.927

Binding affinity of Flavonoid (Quercetin) on Dopaminergic Receptors

Table No VII. Binding affinity of Quercetin

Mode	Affinity (kcal/mol)	Distance from best mode rmsd 1. b.	Distance from best mode rmsd u.b.
1	-8.1	0.000	0.000
2	-8.0	2.166	7.484
3	-7.8	1.005	1.755
4	-7.7	2.624	1.418
5	-7.5	1.732	7.913
6	-7.5	1.744	7.565
7	-7.4	1.638	7.560
8	-7.4	3.405	7.831
9	-7.3	2.748	7.060

Binding affinity of Flavonoid (Kaempferol) on Dopaminergic Receptors

Table No VIII. Binding affinity of Kaempferol

Mode	Affinity (kcal/mol)	Distance from best mode rmsd 1. b.	Distance from best mode rmsd u.b.
1	-7.7	0.000	0.000
2	-7.7	0.004	1.164
3	-7.7	2.367	7.164
4	-7.3	1.907	7.709
5	-7.3	2.767	7.223
6	-7.2	1.732	7.387
7	-7.2	1.858	7.457
8	-7.0	2.730	7.151
9	-7.0	4.119	6.888

e) 2D and 3D Structure of standard (Levodopa) and Flavonoids (Nobiletin, Quercetin and Kaempferol)

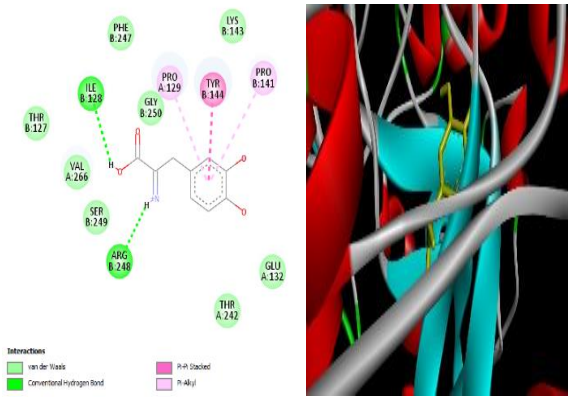


Fig No.3: 2D and 3D structure of Standard (Levodopa).

On NMDA Receptor

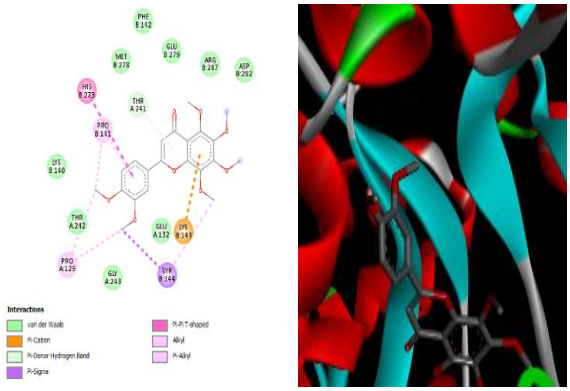


Fig No.4: 2D and 3D structure of Flavonoid (Nobiletin)

On NMDA Receptors

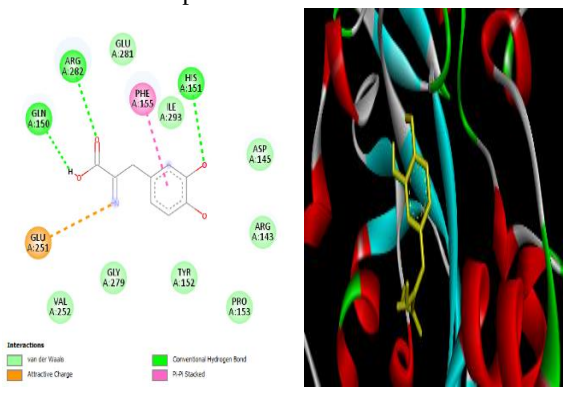


Fig No.5: 2D and 3D structure of Standard (Levodopa)

On Dopaminergic Receptors

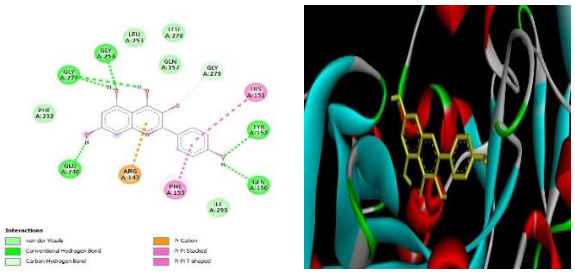


Fig No.6: 2D and 3D structure of Kaempferol.

On Dopaminergic Receptors

f) Acute Toxicity

The *M. azedarach* extract was found to be safe at all tested dosages, with no mortality observed even at a dose of 4000 mg/kg when administered orally. Based on this, a therapeutic dose of 400 mg/kg was established, with 200 mg/kg used as the higher dose and 100 mg/kg as the lower dose for further administration.

g) Effect of *M. azedarach* extract on Haloperidol induced catalepsy

When comparing the cataleptic score of animals treated with a vehicle to those given higher doses of *M. azedarach* extract (200 mg/kg) over an extended period of time, there was a significant (p< 0.001) reduction. When 100 mg/kg of M. azedarach extract was administered, no distinguishable effect was seen. Compared to the vehicle-treated group, treatment with levodopa (6 mg/kg) significantly (P < 0.001) decreased the duration of catalepsy (Figure no.7).

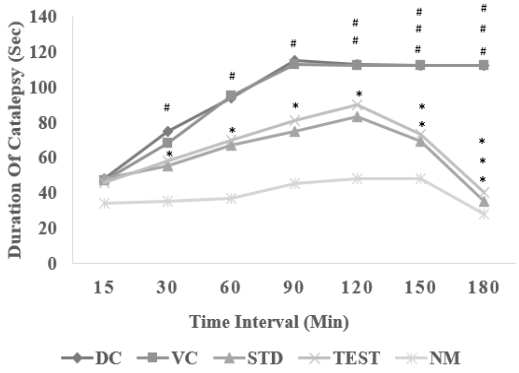


Fig.7: Effect of *M. azedarach* extract on Haloperidol induced catalepsy (Graphpad Prism V10).

h) The Effects of *M. azedarach* extract on 6-OHDA Induced Parkinson's Disease

Total locomotor activity in the 6-OHDA-treated group was significantly reduced (p < 0.001) compared to the vehicle-treated group. Administration of *M. azedarach* at a dose of 200 mg/kg resulted in a significant (p < 0.001) increase in locomotor activity from day 20 to day 55, compared to the 6-OHDA-treated control animals. In contrast, the 100 mg/kg dose of *M. azedarach* did not produce a significant effect. Levodopa (6 mg/kg) also significantly (p < 0.001) increased locomotor activity (Figure 8).

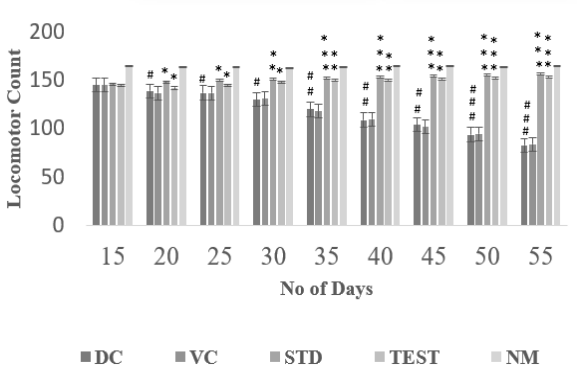


Fig.8: The Effects of *M. azedarach* extract on 6-OHDA Induced Parkinson's Disease (Graphpad Prism V10).

i) Neurotransmitter Quantification by ELISA

Group	Dopamine (pg/ml)
Normal Control	186.58 ± 2.50
Vehicle Control	161.35 ± 2.15
Disease Control	157.50 ± 2.92$^{\#}$
Levodopa	222.58 ± 0.00***
T-1 (100 mg/kg)	197.25 ± 3.50**
T-2 (200 mg/kg)	201.40 ± 4.20**

Statistical Notation: Values expressed as mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001 vs. Disease Control; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001 vs. Normal; T-1 = 100 mg/kg extract; T-2 = 200 mg/kg extract. (one-way ANOVA + Tukey's test) *

The extract demonstrated neurorestorative effects in a 6-OHDA-induced Parkinson's model, with the 200 mg/kg dose (T-2) showing comparable efficacy to Levodopa. T-2 restored dopamine levels which was lower than Levodopa. These results suggest that *M. azedarach* targets dopaminergic pathways offering a supportive and primary therapeutic option for Parkinson's therapy. The nano-ethosomal formulation may further enhance its translational potential by improving bioavailability.

j) ADME Properties (SwissADME)

Table No. IX ADME Properties

Properties (Nobiletin)	Pharmacokinetics
GI absorption	High
BBB permeant	No
P-gp Substrate	No
CYP1A2 inhibitor	No
CYP2C9 Inhibitor	Yes
CYP2D6 inhibitor	No
CYp3A4 inhibitor	Yes
Log P	3.96

k) Evaluation of Nano-ethosomal Gel

Table No. X Evaluation of Nano-ethosomal Gel

Sr. No	Evaluation Parameters	Results (Test Gel)	Results (Normal Gel)
1	Particle Size	162.5 nm	4873.6 nm
2	Zeta Potential	-20.7 mV	-33.1 mV
3	Polydispersity Index	0.337	0.554
4	pH	5.07	5.75
5	Viscosity	4259 cps	4681 cps
6	Content Uniformity	92%	85%
7	Texture	Smooth	Smooth
8	Spreadability	42 mm	52 mm

Particle Size Graphs (HORIBA)

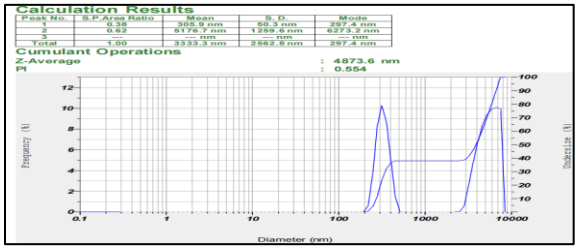


Fig.10: Normal Gel.

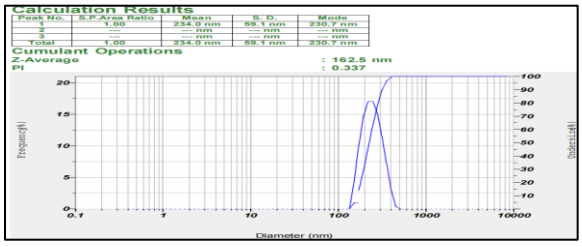


Fig.9: Test Gel

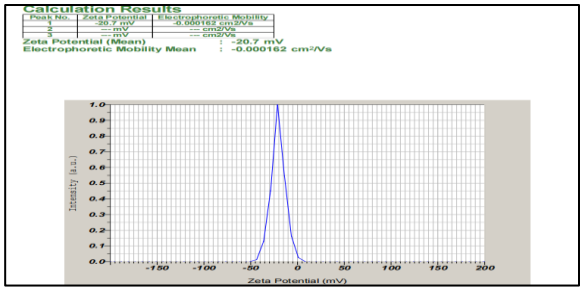


Fig.11: Zeta Potential (Test Gel).

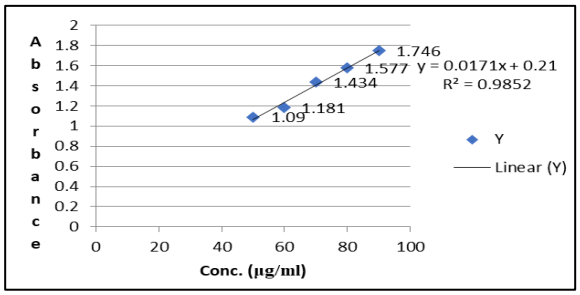
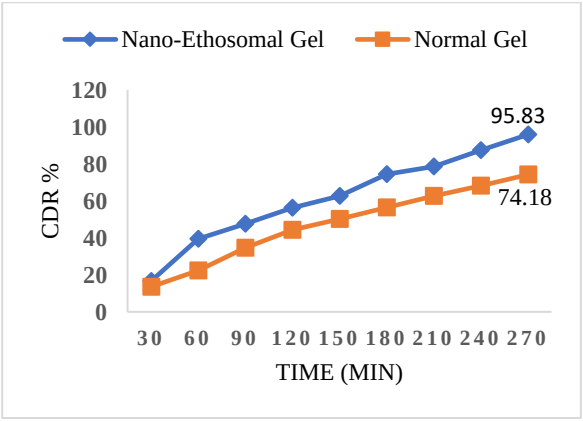


Fig.12: Calibration Curve.

In Vitro Skin Permeation Study



The release profile demonstrated a gradual increase in drug diffusion over time for both formulations. However, the Nano-Ethosomal Gel consistently showed a higher cumulative drug release compared to the Normal Gel at all time points, indicating enhanced permeation characteristics. At 30 minutes, the Nano-Ethosomal Gel showed a CDR of 18.23%, slightly higher than the Normal Gel (12.45%). At 150 minutes, CDR reached 66.89% for the Nano-Ethosomal Gel, compared to 55.26% for the Normal Gel. By 270 minutes, the Nano-Ethosomal Gel achieved a maximum CDR of 95.83%, surpassing the 74.18% observed for the Normal Gel. The enhanced release profile of the Nano-Ethosomal Gel can be attributed to the presence of ethosomal vesicles, which may improve the penetration and diffusion of the drug

through the membrane due to their nano-sized structure and high ethanol content.

V. DISCUSSION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra, leading to motor symptoms such as tremors, rigidity, bradykinesia, and postural instability. The pathogenesis of PD involves oxidative stress, mitochondrial dysfunction, and the accumulation of misfolded α-synuclein proteins. Current treatments, such as levodopa, provide symptomatic relief but are associated with long-term complications like dyskinesia and motor fluctuations. This study aimed to evaluate the anti-Parkinson's activity of *Melia azedarach* Linn. and develop a nano-formulation to enhance its therapeutic potential through targeted drug delivery. To mimic Parkinsonian symptoms in animal models, haloperidol and 6-hydroxydopamine (6-OHDA) were employed. Haloperidol, a D2 receptor antagonist, induces catalepsy, rigidity, and bradykinesia by blocking dopaminergic neurotransmission in the nigrostriatal pathway, replicating the motor deficits seen in PD. The 6-OHDA model, on the other hand, involves the unilateral injection of this neurotoxin into the substantia nigra, leading to selective degeneration of dopaminergic neurons. This model replicates the progressive neuronal loss observed in PD and allows for the evaluation of neuroprotective and neurorestorative agents. In the 6-OHDA model, locomotor activity (Fig. 8) was severely reduced in the disease control group (e.g., 120 ± 15 counts), while *Melia azedarach* (200 mg/kg) treatment significantly restored activity to 280 ± 20 counts by day 55 ($p < 0.001$), nearing Levodopa's effect (300 ± 25 counts). The 100 mg/kg dose showed minimal improvement (150 ± 10 counts). Similarly, in haloperidol-induced catalepsy (Fig. 7), the 200 mg/kg extract reduced cataleptic duration from 240 ± 12 sec (disease control) to 80 ± 8 sec ($p < 0.001$), outperforming the 100 mg/kg dose (200 ± 15 sec). The results demonstrated that *Melia azedarach* extract (200 mg/kg) significantly reduced haloperidol-induced catalepsy and improved locomotor activity in the 6-OHDA model. These findings align with the neurochemical data from the ELISA analysis, which revealed a dose-dependent restoration of striatal dopamine level. The neurochemical data from the ELISA analysis revealed that the extract's effects on striatal dopamine level was moderate compared to Levodopa. The plant contains bioactive compounds such as kaempferol, quercetin, rutin, and nobiletin, which offer neuroprotection by inhibiting dopamine-metabolizing enzymes, reducing oxidative stress, enhancing antioxidant defenses, and suppressing neuroinflammation. The presence of flavonoids was confirmed by a prominent Band II absorption peak between 270 and 295 nm, while FT-IR analysis revealed characteristic peaks for nitrile (C≡N), carbonyl (C=O), hydroxyl (OH), and aromatic alkene (C=C) groups, with key peaks at 2372.01 cm^{-1}, 1719.23 cm^{-1}, and 1638.23 cm^{-1}. Molecular docking studies indicated that the binding affinities of the active phytoconstituents were superior to that of the standard drug, Levodopa, with binding affinities of -7.3 for Levodopa and -7.2 for nobiletin on NMDA receptors. For dopaminergic receptors, Levodopa, quercetin, and kaempferol showed binding affinities of -7.2, -8.1, and -7.7, respectively, suggesting that flavonoids may contribute to the anti-Parkinson's activity. In PD, the loss of dopaminergic neurons leads to an imbalance between glutamate and dopamine, exacerbating neurodegeneration. Phytoconstituents like Nobiletin may help to restore dopamine-glutamate balance by blocking NMDA receptors, quercetin may be responsible for activation of pro-survival kinases enhancing mitochondrial biogenesis and kaempferol may prevent α-synuclein fibril deposition, reducing inflammation protecting the blood-brain barrier [45]-[47]. These findings indicate that flavonoids from *Melia azedarach* may be promising therapeutic agents for PD. ADME Properties were studied by using SwissADME software which showed that the compound has high gastrointestinal absorption and is permeable to the blood-brain barrier (BBB).

Parkinson's disease, primarily affecting individuals over 60, requires drug therapies with enhanced bioavailability, compatibility and reduced dosing frequency for personalized treatment. To address this, we have developed a nano ethosomal gel, which can efficiently cross the blood-brain barrier (BBB) due to its unique structure and small particle size. These nano-carriers, composed of phospholipids and ethanol, improve drug permeation and stability, ensuring more effective brain-targeted delivery. This

makes ethosomal formulations promising for treating neurological disorders like Parkinson's disease [48]. The average particle size of ethosomes varies between 136 and 230 nm [49]. Formulated Nano ethosomal gel was evaluated, all parameters were observed within range. The nano-formulation has a particle size of 162.5 nm, a zeta potential of -20.7 mV, and a polydispersity index of 0.337, indicating good stability and uniformity. The pH is 5.07, and the viscosity is 4259 cps. Content uniformity is 94%, with a smooth texture and spreadability of 42 mm.

VI. CONCLUSION

In view of the above facts, we are concluding that ethanolic extract of *Melia Azedarach* plant showed a promising effect in animals with Parkinson's disease. *M. azedarach* is commonly administered orally for various therapeutic purposes, but its poor bioavailability often limits the effectiveness of the treatment. To address this issue, a novel ethosomal drug delivery system has been developed, offering ease of self-application and enhanced permeability due to the inclusion of ethanol. This system is expected to provide superior performance compared to conventional transdermal formulations. In conclusion, the new delivery system is designed to improve permeability, leading to better absorption and ultimately increased bioavailability and also compatibility in Parkinson disease patients.

REFERENCES

[1] G. DeMaagd and A. Philip, "Parkinson's disease and its management: part 1: disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis," P T, vol. 40, no. 8, pp. 504–532, 2015.

[2] D. T. Dexter and P. Jenner, "Parkinson disease: from pathology to molecular disease mechanisms," Free Radic. Biol. Med., vol. 62, pp. 132–144, 2013, doi: 10.1016/j.freeradbiomed.2013.03.018.

[3] P. Rizek, N. Kumar, and M. S. Jog, "An update on the diagnosis and treatment of Parkinson disease," CMAJ, vol. 188, no. 16, pp. 1157–1165, 2016, doi: 10.1503/cmaj.151179.

[4] A. S. Alsabah, Antihyperlipidemic drugs, 2019, doi: 10.13140/RG.2.2.19407.30886.

[5] V. Dias, E. Junn, and M. M. Mouradian, "The role of oxidative stress in Parkinson's disease," J. Parkinsons Dis., vol. 3, no. 4, pp. 461–491, 2013, doi: 10.3233/JPD-130204.

[6] J. E. Ahlskog and M. D. Muenter, "Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature," Mov. Disord., vol. 16, no. 3, pp. 448–458, 2001, doi: 10.1002/mds.1093.

[7] B. R. Thanvi and T. Lo, "Long term motor complications of levodopa: clinical features, mechanisms, and management strategies," Postgrad. Med. J., vol. 80, no. 948, pp. 452–458, 2004, doi: 10.1136/pgmj.2003.013912.

[8] S. Vekariya, K. Nishteswar, B. R. Patel, and M. Nariya, "Evaluation of analgesic and antipyretic activities of Mahanimba (Melia Azedarach Linn.) leaf and root powder," Ayu, vol. 37, no. 2, pp. 140–144, 2016, doi: 10.4103/Ayu.AYU_69_15.

[9] D. Kar, P. Ghosh, P. Suresh, S. Chandra, and D. Paul, "Review on phyto-chemistry & pharmacological activity of Melia Azedarach," Int. J. Exp. Res. Rev., vol. 28, pp. 38–46, 2022, doi: 10.52756/IJERR. 2022.V28.006.

[10] K. C. Chunekar, Bhavaprakasha Nighantu of Bhavamishra: Guduchyadi Varga, 2nd ed., Varanasi: Chaukhambha Bharati Academy, 2010, pp. 317.

[11] S. Silva, G. Pinto, B. Correia, O. Pinto-Carnide, and C. Santos, "Rye oxidative stress under long term Al exposure," J. Plant Physiol., vol. 170, pp. 879–889, 2013, doi: 10.1016/j.jplph.2012.11.011.

[12] M. C. Dias, G. Pinto, and C. Santos, "Acclimatization of micropropagated plantlets induces an antioxidative burst: A case study with Ulmus minor Mill.," Photosynthetica, vol. 49, pp. 259–266, 2011, doi: 10.1007/s11099-011-0031-2.

[13] M. M. Azam, O. R. A. N. M. Mamun, N. M. Towfique, M. K. Sen, and S. Nasrin, "Pharmacological potentials of Melia Azedarach L-A review," Am. J. BioSci., vol. 1, pp. 44–49, 2013, doi: 10.11648/j.ajbs.20130101.16.

[14] Q. Kanwal, H. Ishtiaq, H. Siddiqui, and A. Javaid, "Antimicrobial activity screening of isolated flavonoids from Azadirachta indica leaves," J. Serb. Chem. Soc., vol. 76, pp. 1525–1533, 2011.

[15] P. C. Sharma, M. B. Yelne, and T. J. Dennis, Database on Medicinal Plants Used in Ayurveda,

New Delhi: Central Council for Research in Ayurveda and Siddha, 2001, pp. 389–406.

[16] P. K. Warrier, V. P. K. Nambiar, and C. Ramankutty, Indian Medicinal Plants: A Compendium of 500 Species, Hyderabad: Orient Longman Ltd., 1995, pp. 10–14.

[17] M. Rani, P. Suhag, R. Kumar, R. Singh, and S. B. Kalidhar, "Chemical component and biological efficacy of Melia azedarach stems," J. Med. Aromat. Plant Sci., vol. 21, pp. 1043–1047, 1999.

[18] K. Dhiman, Sacred Plants and Their Medicinal Uses, Delhi: Daya Publishing House, 2003, pp. 125–127.

[19] C. P. Khare, Encyclopedia of Indian Medicinal Plants, New York: Springer, 2007, pp. 305–306.

[20] J. Zeng, R. J. Ma, L. Wang et al., "Chemical constituents from the leaves of Melia azedarach," Nat. Prod. Res., vol. 33, no. 19, pp. 2860–2863, 2019, doi: 10.1080/14786419.2018.1501690.

[21] S. Mickymaray, "Efficacy and mechanism of traditional medicinal plants and bioactive compounds against clinically important pathogens," Antibiotics (Basel), vol. 8, no. 4, p. 257, 2019, doi: 10.3390/antibiotics8040257.

[22] M. Taghizadieh et al., "Neuroprotective effects of flavonoids, phytonutrients, and neurological disorders," in Neuroprotective Effects of Flavonoids, Academic Press, 2023, pp. 133–200, doi: 10.1016/B978-0-12-824467-8.00006-4.

[23] A. Hamsalakshmi, A. M. Alex, M. A. Marappa, S. Joghee, and S. B. Chidambaram, "Therapeutic benefits of flavonoids against neuroinflammation: A systematic review," Neuroinflammation, vol. 30, p. 111–136, 2022.

[24] S. Gangwar, S. Singh, and G. Garg, "Ethosomes: A novel tool for drug delivery through the skin," J. Pharm. Res., vol. 3, no. 4, pp. 688–691, 2010.

[25] S. Navaneethan, C. N. Somashekhar, and V. Y. Harshitha, "Formulation and evaluation of nano ethosomes containing rasagiline mesylate for the effective management of Parkinsonism," Int. J. Pharm. Res. Appl., vol. 7, no. 3, pp. 2112–2128, 2022.

[26] H. Jain, J. Patel, K. Joshi, P. Patel, and U. M. Upadhyay, "Ethosomes: A novel drug carrier," Int. J. Clin. Pract., vol. 7, no. 1, pp. 1–4, 2011.

[27] D. Patel and P. Bhargava, "Ethosomes-A phyto drug delivery system," Adv. Res. Pharm. Biol. Sci., vol. 2, pp. 1–8, 2012.

[28] T. Shimamura et al., "Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives: Inter-laboratory evaluation study," Anal. Sci., vol. 30, no. 7, pp. 717–721, 2014.

[29] K. R. Khandelwal, "Preliminary phytochemical screening," in Practical Pharmacognosy: Techniques and Experiments, Pune: Nirali Prakashan, 2008, pp. 149–156.

[30] V. Ravichandran, B. Suresh, M. N. Sathishkumar, K. Elango, and R. Srinivasan, "Antifertility activity of hydroalcoholic extract of Ailanthus excelsa (Roxb): An ethnomedicine used by tribals of Nilgiris region in Tamil Nadu," J. Ethnopharmacol., vol. 112, no. 1, pp. 189–191, 2007.

[31] C. A. M. Clark, L. A. R. Sacrey, and I. Q. Whishaw, "Righting elicited by novel or familiar auditory or vestibular stimulation in the haloperidol-treated rat: Rat posturography as a model to study anticipatory motor control," J. Neurosci. Methods, vol. 182, no. 2, pp. 266–271, 2009.

[32] P. J. Elliott and S. P. Close, "Neuroleptic-induced catalepsy as a model of Parkinson's disease I. Effect of dopaminergic agents," J. Neural Transm. Park. Dis. Dement. Sect., vol. 2, no. 2, pp. 79–89, 1990.

[33] S. Wang, L. F. Hu, Y. Yang, J. H. Ding, and G. Hu, "Studies of ATP-sensitive potassium channels on 6-hydroxydopamine and haloperidol rat models of Parkinson's disease: Implications for treating Parkinson's disease," Neuropharmacology, vol. 48, no. 7, pp. 984–992, 2005.

[34] R. Deumens, A. Blokland, and J. Prickaerts, "Modeling Parkinson's disease in rats: An evaluation of 6-OHDA lesions of the nigrostriatal pathway," Exp. Neurol., vol. 175, no. 2, pp. 303–317, 2002.

[35] D. S. Reddy and S. K. Kulkarni, "Possible role of nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging- and dizocilpine-induced learning impairment," Brain Res., vol. 799, no. 2, pp. 215–229, 1998.

[36] Y. Rao, F. Zheng, X. Zhang, J. Gao, and W. Liang, "In vitro percutaneous permeation and skin accumulation of finasteride using vesicular ethosomal carriers," AAPS PharmSciTech, vol. 9, pp. 860–865, 2008.

[37] X. F. Grace, K. Suganya, and S. Shanmuganathan, "Development of Terminalia chebula loaded ethosomal gel for transdermal drug delivery," Asian J. Pharm. Sci. Clin. Res., vol. 11, no. 6, pp. 2455–3891, 2018.
[38] S. Patel, "Ethosomes: A promising tool for transdermal delivery of drugs," Pharmainfo.net, vol. 5, no. 3, pp. 1–8, 2007.
[39] A. Sheer and M. Chauhan, "Ethosomes as vesicular carrier for enhanced transdermal delivery of ketoconazole—Formulation and evaluation," J. Pharm. Cosmet., vol. 1, no. 3, pp. 1–14, 2011.
[40] R. H. Guy, "Ethosomes and recent approaches in transdermal drug delivery system," Int. J. Pharm., vol. 6, pp. 112–116, 1985.
[41] M. Mezei and V. Gulasekharam, "Liposomes—a selective drug delivery system for the topical route of administration," Life Sci., vol. 26, pp. 1473–1477, 1980.
[42] N. K. Jain, Advances in controlled and novel drug delivery, 1st ed. New Delhi, India: CBS Publishers & Distributors, 2001, pp. 428–451.
[43] E. Touitou, "Composition of applying active substances to or through the skin," U.S. Patent 5,540,934, 1998.
[44] B. W. Barry, "Is transdermal drug delivery research still important today?" Drug Discov. Today, vol. 6, pp. 967–971, 2001.
[45] S. Kumar and A. K. Pandey, "Chemistry and biological activities of flavonoids: An overview," Sci. World J., vol. 2013, Art. no. 162750, 2013.
[46] N. Braidy et al., "Neuroprotective effects of citrus fruit-derived flavonoids, nobiletin and tangeretin in Alzheimer's and Parkinson's disease," CNS Neurol. Dis. Drug Targets, vol. 16, no. 4, pp. 387–397, 2017.
[47] M. Ay et al., "Molecular mechanisms underlying protective effects of quercetin against mitochondrial dysfunction and progressive dopaminergic neurodegeneration in cell culture and MitoPark transgenic mouse models of Parkinson's disease," J. Neurochem., vol. 141, no. 5, pp. 766–782, 2017.
[48] A. Abubakr, A. Othman, and M. Salah, "Ethosomes: A potential nanocarrier for transdermal drug delivery," ERU Res. J., vol. 2, pp. 161–176, 2023.
[49] A. C. Paiva-Santos et al., "Ethosomes as nanocarriers for the development of skin delivery formulations," Pharm. Res., vol. 38, no. 6, pp. 947–970, 2021.