Qualitative and quantitative estimation, antioxidant and anti-Parkinson activities of *Kalanchoe lanceolata*: A novel medicinal plant

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ABSTRACT

The herb *Kalanchoe lanceolata*, which is widely used in traditional medicine, has showed promise in treating a range of ailments.The present project was arranged to study the antioxidant and anti-Parkinson efficacy of *Kalanchoe lanceolata*extracts against rotenone-induced Parkinson diseases in rats. Fine powder of the plant was extracted with methanol and then fractionated through various solvents with increasing order of polarity. Phytochemical screenings were done using standard protocols and *in-vitro* antioxidant activities of plant fractions were evaluated using different free radicals. *In-vivo* anti-Parkinson and oxidative dysfunction experiments were conducted in rats. Results revealed that various fractions possessed flavonoids, alkaloids, terpenoids saponins, tannin, anthraquinon, and phlobatanine, while terpeniods and alkaloids were absent in aqueous fraction. *In-vitro* antioxidant activities of various fractions of *Kalanchoe lanceolata* showed that methanol fraction has remarkable scavenging efficacy of 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and superoxide free radicals followed by chloroform fraction. Free radicals produced by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Hydrogen peroxide (H_2O_2), and hydroxyl free radicals were considerably scavenged by methanol fraction followed by ethyl acetate fractions. *In-vivo* study of animal model showed that methanol fraction has significant recovery effects at behavioural, physiological and biochemical level against rotenone induced Parkinson disease. *Kalanchoe lanceolata* has significantly improved rotenone-induced motor and nonmotor deficits (depression and cognitive impairments), increased antioxidant enzyme activity, and reduced neurotransmitter changes. It has been concluded from the present data that *Kalanchoe lanceolata*enhances neurotransmitter levels by alleviating oxidative stress and antioxidant enzyme activity, hence improving motor activity, cognitive functioning, and decreasing depressed behavior. These data suggest that *Kalanchoe lanceolata*may be a promising medicinal agent for reducing the risk and progression of Parkinson's disease.

Keywords: *Kalanchoe lanceolata*, Antioxidant, Anti-Parkinson, 2,2-Diphenyl-1-picrylhydrazyl, Motor activity

Introduction

Parkinson's disease (PD) is a clinical disorder that may be diagnosed. It has a wide range of etiologies and clinical symptoms [1]. The incidence of Parkinson's disease, a neurological disorder, is rapidly increasing globally, barring an infectious cause [2]. Parkinson's disease

(PD) is growing more and more common in the elderly people. its symptoms include memory loss, mobility issues, and sleep disturbances. Parkinson's disease (PD) is a complex neurological disorder characterized by the substantia nigra pars compacta (SNpc) losing dopaminergic neurons, which results in a dopamine shortage [3] and unintentional movements. Neurodegenerative illnesses, including Parkinson's disease (PD), are also mostly caused by mitochondrial malfunction [4]. Although its etiology is still largely understood, alterations in proteostasis, oxidative stress, and neuroinflammation are generally recognized as significant components contributing to the pathophysiology of PD [5,6]. Drugs combining levodopa and carbidopa, such as Sinemet, Paracopa, Rytary, and Duopa, are commonly used as approved therapies for PD motor symptoms [7,8]. However, long-term use of these medications can result in toxicity, depression, ulcers, and hypertension [9,10]. As a result of side effects of allopathic drugs in the current world, having a strong supplemental herbal therapeutic ingredient is essential [11,12]. Sometimes, specific genetic changes can result in the inheritance of Parkinson's disease (PD) [13]. It has been determined that the SNCA, LRRK2, and PARK2 genes increase the risk of Parkinsonism [14,15]. Exposure to several chemicals, such as pesticides and herbicides, has been associated with an increased risk of PD [16]. The risk of developing PD may be increased by head injuries, toxins, free radicals and other environmental factors [17,18]. When the body's free radicals and antioxidant levels are unbalanced, oxidative stress occurs, which can damage neurons in the brain and cause Parkinson's disease. The development of Parkinson's disease may be affected by mental illness [19,20]. Mitochondria are energy-producing structures in cells, and dysfunction of these structures may contribute to the evolution of Parkinson's disease [21–23]. Medicinal plant plays a crucial role in the alleviation of Parkinson diseases. Auddy et al., [24], reported that ayurvedic doctors in India recommended numbers of medicinal herbs to treat neurodegenerative conditions like Alzheimer's, Parkinson's, memory damage, nerve degeneration, and other neuronal disorders. Mucuna pruriens have anti-Parkinson and neuroprotective properties in animal models of Parkinson's disease [25]. Bhangale et al., [26] investigated the neuroprotective effects of Ficus religiosa (L.) leaf pet ether extract in Parkinson disease caused by 3-nitropropionic acid. Similarly, Tribulus terrestris [27], Myrica esulenta [28], Chromolaena odorata (L) [29], Sterculia guttata [30], Acorus calamus Linn [31], Datura metel [32], and Momordica dioica [33] were investigated for the treatment of Parkinsonian disorders. The succulent plant *Kalanchoe lanceolata*, often known as "Mother of Thousands" or "Bryophyllum lanceolatum," belongs to the Crassulaceae family. *Kalanchoe lanceolata* extracts was not testified with theirtherapeutic practice against neurodegenerative ailments. Consequently, the current study

was arranged to investigate phytochemical qualitative analysis of its chemical constituents, antioxidant activity and anti-Parkinson's activity of *Kalanchoe lanceolata* against rotenone induced rat model through behavioral and biochemical analysis.

Materials and methods

Plant collection

The fully matured plant of *Kalanchoe lanceolata* was collected from local area of Bhopal (M.P.)after its proper scientific identification and its voucher specimens with Accession No. 119/Bot/Saf/145was placed at herbariumin the department of Botany, Safia College of Arts and Science, peer gate Bhopal. Running tap water was used to wash the *Kalanchoe lanceolata* inorder to clean from dust. *Kalanchoe lanceolata* was shed dried for three weeks before being processed intoa fine powder.

Extraction and fractionation

For one-week, fine plant powder was soaked in an 80% methanol solution before being filtered through Whatman 45 filter paper. The filtrate was evaporated separately using a rotary evaporatoruntil crude extracts are obtained from the filtrate.The crud extract was first diluted in distilled water to create an aqueous extract, which wasused to prepare different fractions on the basis of polarity. Different solvents, such as n-hexane,ethyl acetate, chloroform, and butanol, were used in the solvent-solvent partition technique toseparate the fractions. Different fractions were collected separately and evaporated using arotary vacuum evaporator. The dried fractions were stored at 4˚C for further investigations.

Phytochemical analysis

Various biochemical analyses protocol was used to assess the secondary metabolites present in *Kalanchoe lanceolata* various fractions. Standard procedures were used for each of the chemical tests forthe presence of tannins, saponins, flavonoids, terpenoids, alkaloids, phlobatannins, cardiac glycosides, couramins, and anthraquinone.

Total phenolic contents estimation

The Atala *et al*.,[34] methodology was to determine phenolic constituents. Samples from plant extract were combined with 10 ml of the folin-Ciocalteau reagent. The mixture was incubated for 10 min followed by the addition of 0.115 mg/ml Na_2CO_3. Ascorbic acid was used as a standard.OD was checked at 765 nm to measure the absorbance spectrum. Per g of dried material,the amount of total phenolic was calculated as mg of gallic acid equivalents (GAE).

Test to determine the presence of total flavonoids

Pompilio *et al*., [35] technique was used to determine the total constituents of flavonoids.According to this procedure 0.25 ml of each fraction and 15–250 g/ml rutin

concentrationswere used. A mixture of each fraction and rutin were mixed with 1.5 ml of deionized water and 5% NH_4NO_3 and incubated for 6 min. After incubation 0.2 ml $AlCl_3$ (w/v), 1.0 molar sodium hydroxide (NaOH) was added and incubated for 5 min. OD was calculated at 510 nm. Thedried fraction of rutin equivalent mg/g extract was used to compute the ratio of flavonoids.To ensure the correctness of the results, the experiment was repeated three times for eachsample.

***In-vitro* antioxidant activities**

Evaluation of plant extracts for DPPH radical scavenging activity

Various plant fractions were used to determine the DPPH's scavenging capacity of each fraction [36]. A stock solution of reagents was prepared by dissolving 0.006 mg of DPPH in 100 ml of methanol. 0.2 mlvarious plant fractions and ascorbic acid was mixed 2.8 ml DPPH solution and incubated for 30min. Using a spectrophotometer, the absorbance spectrum was recorded and calculated at 517 nm.According to the formula below, the percentage of DPPH inhibition was calculated as follows:

% of DPPH inhibition = [(Absorbance of DPPH-Absorbance of sample) / (Absorbance of DPPH)]. ×100

ABTS cation radical assay

The scavenging of ABTS free cation radicals were carriedout by making a minor modification to the protocol described by Brahmi *et al*., [37].7mM of ABTS reagent was mixed with 2.45 mM potassium per sulphate reagent. The combinationwas maintained in the dark for 8 hrs. After incubation 50% methanol solution was usedto dilute the sample, yielding absorbance 0.900 (0.02) at 745 nm. Each fraction was mixed with3.0 ml of the diluted reagent. The absorbance was up to 6 min using the formula when usingthe percent activity of different fractions;

Scavenging impact (%) = (Control Ab-Sample Ab) / (Control Ab) × 100

Superoxide radical scavenging activity. In this procedure each fraction of *Kalanchoe lanceolata* was combined with nicotinamide adenine dinucleotide reduced (NADH) solution and0.5 ml of Nitro Blue Trizol. PMS was added, and incubated at 25˚ for 15 min. Calculations ofthe absorbance spectrum was carried out at 530 nm [38].

Superoxide Scavenged% = [Absorbance of Control—Absorbance of Sample]/Absorbance of Control x 100

Assay for detection of H2O2 radicals reducing effect

Ascorbic acid was used as the standard, and different concentrations of each fraction with 0.4 ml H2O2 (50 mM phosphate buffer, pH 7.4) was used to test for the reduction of the H2O2

free radical. Phosphate buffer was used as a blank to measure the absorbance of mixtures at 230 nm [39]. Experiment was repeated in replicates to determine the hydrogen per oxide scavenging ability.

Hydrogen peroxide scavenging percentage = [Control absorbance−sample absorbance] /Control absorbance] *100

***In-vivo* anti-parkinson animal model**

Wistar rats male (200±50 gm) were residence in groups of fiveunder regulated humidity and temperature settings (25±2 °C, 55-65%). Rats were given regular rodent food and unlimited amounts of water. Prior to the experiments, rats spent 7 days becoming used to the lab environment. Between 8:00 and 15:00 hours, all studies were conducted in a room with no background noise. Each set of studies used a different group of rats (n=6). The Institutional Animal Ethics Committee (IAEC), established by the India's Ministry of Environment and Forests, located in New Delhito oversee and supervise the use of experimental animals, gave its approval to the animal experiments.

Experimental design

Standard protocols of Parkinson disease induction were used for animal treatment. 30 male adultWister rat were randomly divided into 5 groups. The rats of control group received 0.5% N/saline p.o. every day. For three weeks, the disease control group received rotenone in 1% DMSO (5 mg/kg per body weight s.c.) after every two days. For 21 days, one hr prior to the administration of rotenone, various groups of tests were administered orally with *Kalanchoe lanceolata* methanol extract (200and 400 mg/kg b.w.) after 24 hrs. The standard control group animals also received, one hr beforerotenone, oral administration of a mixture of levodopa-carbidopa (200 mg/kg b.w.). After 21 days period, all animals weights were recorded. Throughout the experiment, all animals were treated humanely according to the international criteria approved by ethical committee for the Care andUse of Laboratory Animals. The animals were sacrificed by an overdose of xylazine and ketamineanesthesia at day 22 by cervical dislocation. Brain was removed, cleaned with phosphate bufferand kept for biochemical at -20° in freezer. Serum samples were collected through heart puncture.The collected serum was processed for various biochemical tests.

Evaluation parameters

Body weight and food intake. The rats body weights were recorded before the trialbegan and then every week before the behavioral assessments. The amount of food consumed each day was also recorded,

Open field test. For the time being, rats were assessed in the open field. The examination was carried out in a box (10 by 10 cm in size, with walls 40 cm high). It was processed forthree minutes and evaluated for latency to move and square number crossing.

Catalepsy test. To measure catalepsy, the bar test was used. A horizontal bar 9 cm tall and parallel to the floor was used in this test. The rats have their front paws in a semi-rearing position and were perched on the bar. A timer was used to time how long the first paw istaken off the bar. The time limit was set at three minutes.

Walk-on-Beam test. The 100 cm 2 box was placed, and the 100 cm beam wasinstalled up from the ground. On the beam, the rat had been set and was permitted to movefreely. Successfully across the rat, and an interval was obtained.

Evaluation through rotarod. We tested the rat's ability to coordinate its musclesusing the rotarod equipment. All rat groups latency to fall was monitored as a performance indicator throughout for 2 min rotation of the rod.

The footprint screening. The fore foot and back foot were coated with harmless red and green colors in order to assess the foot print activity. A 10 cm wide by 100 cm long run way was built. It was permissible for the rat to take steps on it. The white paper was spread out onthe runway for each rat. Measurements were made of the step's length, the bases widths and the claws gaps. Centimeters were used for all measurements.

Social interaction performance. A videotape was used to assess the social attraction.Three parameters were examined: (i) active interaction evaluation, (ii) inactive interaction assessment, and (iii) numbers of interactions. A common instrument for analyzing social interaction was the social connection box, which gave rats a regulated environment in whichthey could interact. It assumed the form of a transparent, rectangle box containing two sections divided by small apertures that permit rodents to communicate across the sides.

Intake of sucrose solution. The rats home cage was utilized to assess sucrose consumption activities. This investigation was carried out on an every day. Two like graded containers were kept in the cage. A 1% w/v sucrose solution was utilized in one bottle, and regular water was used in the other. To eliminate the possibility of confusing effects, the bottles were exchanged daily. The following formula was used to calculate data:

% Sucrose utilization = (Utilization of sucrose / Utilization of normal water) × 100

Hidden platform finding in water. In this study, rats have to find a platform in a pool that exists underneath water. A flattened-top cylindrical of 5 cm radius was positioned 3 cm underneath the water. To disguise the platform, milk was put in the water. The target quadrant was chosen. For the purpose of this experiment, the Water tub had been separated by four sections. Rats

from each quadrant were placed in the tank for a 2 min training session. There was a 15 min gapbetween the two studies. Following a workout session for 60 min, memory was evaluated.

Results

Phytochemical screening

Qualitative analysis. Phytochemical evaluation of an extract of *Kalanchoe lanceolata*exposed the existence of numerous phytoconstituents in the methanol extract flowed in differentfractions, i.e., n-hexane, chloroform, ethyl acetate, butanol and aqueous. Different phytochemicalswere present which are shown in the Table 1.

Table 1. Phytochemical analysis of various fractions of *Kalanchoe lanceolata*

Fractions	Flavonoids	Alkaloids	Tannins	Terpeniods	Saponins
Butanol	+Ve	+Ve	+Ve	+Ve	+Ve
Methanol	+Ve	+Ve	+Ve	+Ve	+Ve
N-hexane	+Ve	+Ve	+Ve	+Ve	+Ve
Chloroform	+Ve	+Ve	+Ve	+Ve	+Ve
Aqueous	+Ve	-Ve	+Ve	-Ve	+Ve
Ethyl Acetate	+Ve	+Ve	+Ve	+Ve	+Ve

Quantitative analysis of total flavonoids and phenolic compounds.

Total phenolic and total flavonoids contents. Folin‑Ciocalteau procedure was used to find the totalphenolic compounds in various fractions of *Kalanchoe lanceolata*. Table 2 revealed maximum concentrationof total phenolic contents in methanol fraction (280.46±1.01mg GAE/g) followed byButanol (176.33±2.01mg GAE/g), chloroform (110.24±1.06mg GAE/g), ethyl acetate (24.11±2.33mg GAE/g), n-Hexane (16.11±2.30mg GAE/g) and aqueous (11.35±3.01mg GAE/g)fractions respectively. Similarly maximum quantities of flavonoids were present in methanol fraction of *Kalanchoe lanceolata* followed by butanol, chloroform, ethyl acetate, n-Hexane and aqueous fractions.

Table 2. Total phenolic and flavonoid components various fractions

Sample	Total phenolic components as mg gallic acid equivalent(GAE mg/g extract)	Total flavonoids as mg rutin equivalent(mg/g extract)
Methanol	280.46±1.01	45.16±1.08
Butanol	176.33±2.01	34.12±1.34
Chloroform	110.24±1.06	30.32±1.06
Ethylacetate	24.11±2.33	12.16±0.94
n-Hexane	16.11±2.30	10.25±0.83
Aqueous	11.35±3.01	6.25±0.37

Antioxidants free radical scavenging assay

Kalanchoe lanceolata anti-free radical scavenging ability was checked using its various fractions as shown in Fig 2. The present finding showed that various fractions are effective in scavenging free radicals.The order of scavenging of DPPH, ABTS, H_2O_2 and superoxide were

methanol<ethyl acetate <butanol <chloroform however ascorbic acid was as a standard antioxidant.

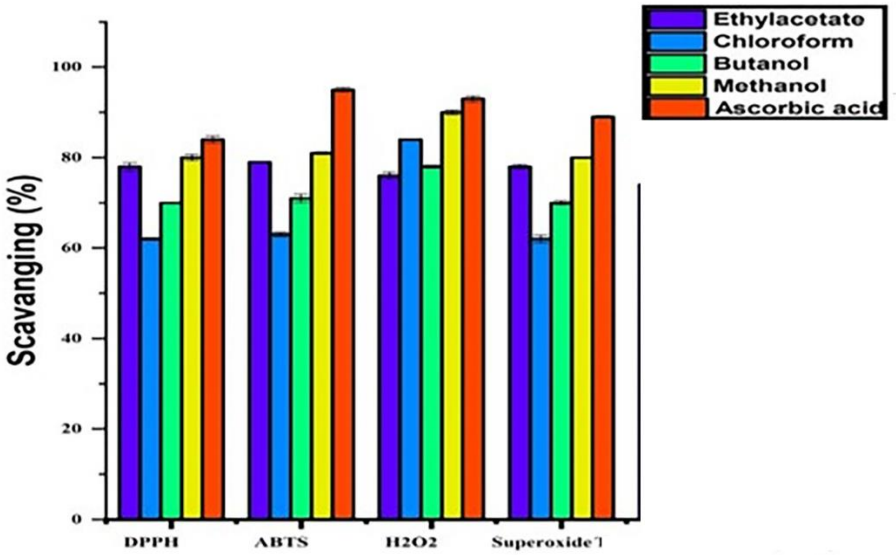

Fig 2. Antioxidants free radical scavenging ability of various fractions of *Kalanchoe lanceolata*

***In-vivo* anti-Parkinson activity**

Effects of *Kalanchoe lanceolata* on body weight. Body weight of experimental rats play a key rolein the anti-Parkinson activity of plant extracts. The animals body weight was determined inorder to assess their general health. 5 mg/kg body weight rotenone caused non-significantreduction in body weight of rats. *Kalanchoe lanceolata* 200 mg/kg and 400 mg/kg body weight treatment restored the decrease in the body weight of experimental rats and improved their general health condition dose dependently. Similarly, levodopa-carbidopa showed recovery effects ascompare of rotenone induced Parkinson diseased rats as shown in Table 3.

Table 3. Effects of *Kalanchoe lanceolata* on body weight (g) of PD model rats

Groups	After 7 days	After 14 days	After 21 days
Control	225.41±2.81	235.13±1.83	244.35±3.56
5 mg/kg Rotenone	208.22±2.28	198.48±2.36	186.83±2.45
5 mg/kg Rotenone +200 mg/kg L.carbidopa	220.33±3.33	222.83±4.64	224.31±3.32
5 mg/kg Rotenone +200 mg/kg*K. lanceolata*	212.18±1.91	219.65±2.45	220.03±3.43
5 mg/kg Rotenone +400 mg/kg *K. lanceolata*	221.28±2.67	223.65±2.33	232.53±2.48

Values are represented in Mean ± SEM (*n = 6*).

Effects *Kalanchoe lanceolata* on food intake The food intake of all experimental rats (*n = 6*) was measured to investigate the physical general condition of experimental rats. The normal controlgroup rats did not show any significant change. Rotenone induced Parkinson diseased

ratsshowed non-significant reduction in the intake of food which revealed complications in rats.The rats treated with 200 mg/kg and 400 mg/kg body weight *Kalanchoe lanceolata* methanol extract presented improvement and increased the food intake dose dependently; however, the rats administered with 200 mg/kg body weight L. carbidopa showed normal food intake as were shownin Table 4.

Table 4. Effects of *Kalanchoe lanceolata*on food intake (g) of PD model rats

Groups	After 7 days	After 14 days	After 21 days
Control	105.61±1.39	106.47±1.87	107.19±3.70
5 mg/kg Rotenone	100.47±3.72	93.30±2.63	85.89±2.36
5 mg/kg Rotenone +200 mg/kg L.carbidopa	104.09±5.51	104.73±2.40	105.33±4.44
5 mg/kg Rotenone +200 mg/kg*K. lanceolata*	102.47±5.07	102.83±4.32	103.13±2.26
5 mg/kg Rotenone +400 mg/kg *K. lanceolata*	104.76±3.56	105.23±3.72	106.14±2.38

Values are represented in Mean ± SEM (*n = 6*).

Rotarod test and latency to cross the beam Behavioral markers play a crucial rolein the assessment of Parkinson activities of plant extracts. To measure muscular strength, arotarod test was repeatedly used. In comparison to the healthy control group rats, the rotenoneinduced Parkinson induced diseased control group rats revealed significant reduction($P<0.01$) and took less time of falling as compare to normal control group rats. Rats treatedwith 200 and 400 mg/kg body weight of *Kalanchoe lanceolata* showed significantly ($P<0.01$) lengthened time before falling. Similar results were inferred by the treatment of 200 mg/kg body L. carbidopaTable 5.

Coordination system plays an important role in Parkinson disease. A beam walking testwas to check motor and coordination states. The beam was 100 cm long. The latency to crossthe beam and time of falling was significantly ($P<0.01$) increased in the rats treated with rotenoneas compare to non-treated control group rats. 200 mg/kg and 400 mg/kg body weightadministration of *Kalanchoe lanceolata* significantly reduced ($P<0.01$) the time of falling dose dependently. Similarly, 200 mg/kg body weight L. carbidopa showed significant reduction ($P<0.01$) of timefalling as compare to rotenone induced rats as displayed in Table 5.

Table 5. Effects of *Kalanchoe lanceolata* on motor symptom of PD model rats (Rotarod test).

Groups	Time of falling (S)	Time of falling Crossing the Beam (S)
Control	56.13±1.47++	9.35±0.74++
5 mg/kg Rotenone	8.46±1.66**	38.23±0.58**
5 mg/kg Rotenone +200 mg/kg L.carbidopa	46.61±1.55++	14.50±0.57++

5 mg/kg Rotenone +200 mg/kg*K. lanceolata*	25.23±1.55++	23.16±0.74++
5 mg/kg Rotenone +400 mg/kg *K. lanceolata*	49.26±2.61++	11.61±0.87++

Values are represented in Mean ± SEM (*n = 6*)

**Significance from the control group at P<0.01 probability levels and

++Significance from the Rotenone group at P<0.01 probability level.

Open field test (OFT) and cataleptic condition test. An open-field test paradigmwas to track locomotors and ambulatory activities in rats (*n = 6*). Data revealed that rats treatedwith rotenone showed significantly increased (P<0.01) the latency to move while decreased the crossing of number of squares during this study during time period of 2 min.The rats treated with 200 mg/kg and 400 mg/kg body weight *Kalanchoe lanceolata* methanol extract and similarly 200 mg/kg body weight L. carbidopa significantly decreased (P<0.01) the time ofmovement and crossing of squares. An inclined plane or bar test was used to diagnose catalepsy.The percentage of cataleptic score measurement revealed that rotenone induced diseasedrats showed significant (P<0.01) increase in percent cataleptic score as compare to normalnon treated rats. The administration of 200 mg/kg and 400 mg/kg body weight of *Kalanchoe lanceolata* presented significant improvement (P<0.01) and reduced the score dose dependently. Rats treatedwith 200 mg/kg body weight L. carbidopa also significant (P<0.01) reversed the catalepticscoreTable 6.

Table 6. Effects of *Kalanchoe lanceolata* on open field test of PD model rats

Groups	Latency to move (s)	Number of squares to crossed (cm)	% Cataleptic score
Control	2.42±0.07++	158.73±3.95++	0.58++
5 mg/kg Rotenone	10.20±0.16**	51.37±2.98**	6.06**
5 mg/kg Rotenone +200 mg/kg L.carbidopa	3.75± 0.13++	139.55±3.49++	2.24++
5 mg/kg Rotenone +200 mg/kg*K. lanceolata*	7.16±0.08++	138.70±2.28++	3.22++
5 mg/kg Rotenone +400 mg/kg *K. lanceolata*	4.42± 0.13++	149.60±3.37++	2.85++

Values are represented in Mean ± SEM (*n = 6*)

**Significance from the control group at P<0.01 probability levels and

++Significance from the Rotenone group at P<0.01 probability level.

Footprint test. In this investigation, the rat's footprints were also collected to analyze their stride length and base width patterns of various experimental rats. 5 mg/kg bodyweight

Rotenone induced in rats caused altered stride and base patterns, by noticeably shorterforelimb and hind limb strides in comparison to control animals. The front base width and thehind base width both significantly decreased (P<0.01) and increase paw overlap as well. Rats treated with 200 mg/kg and 400 mg/kg body weight *Kalanchoe lanceolata* showed marked improvement (P<0.01) and reversed the changed caused by treatment of rotenone in rats. The standard drug 200 mg/kg body weight L. carbidopa showed significant (P<0.01) results as compare torotenone induced rats Table 7

Table 7. Effects of *Kalanchoe lanceolata* on walking by footprint test of PD model rats.

Groups	Stride length(cm) Forelimb	Stride length(cm) Hind limb	Base width(cm) Fore base	Base width(cm) Hind base	Paw overlap (cm)
Control	12.40±0.32++	14.16±0.30++	5.28±0.08++	5.68±0.06++	0.15±0.03++
5 mg/kg Rotenone	2.96±0.26**	4.50±0.21**	2.21±0.06**	2.46±0.04**	1.11±0.03**
5 mg/kg Rotenone +200 mg/kg L.carbidopa	11.70±0.24++	12.11±0.26++	5.11±0.09++	4.83±0.04++	0.28±0.03++
5 mg/kg Rotenone +200 mg/kg*K. lanceolata*	7.23±0.31++	8.42±0.18++	3.34±0.06+	3.06±0.07+	0.69±0.04+
5 mg/kg Rotenone +400 mg/kg *K. lanceolata*	12.50±0.48++	11.61±0.41++	5.01±0.02++	4.35±0.01++	0.36±.02++

Values are represented in Mean ± SEM (*n = 6*)

**Significance from the control group at P<0.01 probability levels and

++Significance from the Rotenone group at P<0.01 probability level.

Social interaction test. To track depressive-like behavior, a social interaction test was performed. 5 mg/kg body weight administration of rotenone in rats caused significant reduction (P<0.01) in social integrations as compared to non-treated normal control rats. Treatment of 200 mg/kg and 400 mg/kg body weight *Kalanchoe lanceolata* showed marked improvement in social behavior and significantly increased (P<0.01) the number of interactions, both active and passive. Similarly, 200 mg/kg body weight L. carbidopa markedly (P<0.01) boosted thequantity of interactions, both active and passive, in comparison to animals that had received rotenone injections Table 8.

Table 8. Effects of *Kalanchoe lanceolata* on PD model rats of social interaction test

Groups	No of interaction	Active interaction	Passive interaction
Control	16.50±0.619++	22.00±0.447++	16.16±0.37++
5 mg/kg Rotenone	3.33±0.421**	05.66±0.494**	1.11±0.48**
5 mg/kg Rotenone +200 mg/kg L.carbidopa	12.23±0.307++	20.16±0.307++	14.50±0.56++
5 mg/kg Rotenone +200 mg/kg*K. lanceolata*	8.13±0.307++	13.83±0.307++	3.16±0.41++
5 mg/kg Rotenone +400 mg/kg *K. lanceolata*	11.41±0.317++	19.06±0.207++	15.30±0.34++

Values are represented in Mean ± SEM (*n = 6*)

**Significance from the control group at P<0.01 probability levels and

++Significance from the Rotenone group at P<0.01 probability level.

Sucrose consumption test. The inability to enjoy the pleasure that sugar provides was described as anhedonia in rats. Rotenone-treated rats consumed significantly less(P<0.01) sucrose solution than the untreated normal control rats. However, after 24 hrs, 48hrs, and 72 hrs, rats treated with 200 mg/kg and 400 mg/kg body weight *Kalanchoe lanceolata* as well as 200mg/kg body weight consumed significantly more (P<0.01) sucrose solution as compare rotenone treated rats as shown in Table 9.

Table 9. Effects of *Kalanchoe lanceolata* on PD model rats of Depressive-like symptom

Groups	% Sucrose consumption		
	24h	48h	72h
Control	60.8 ± 0.2++	24.5 ± 0.3++	16.1 ± 0.4++
5 mg/kg Rotenone	13.2 ± 0.1**	55.1 ± 0.5++	50.2 ± 0.5**
5 mg/kg Rotenone +200 mg/kg L.carbidopa	50.4 ± 0.3++	34.7 ± 0.2++	28.5 ± 0.9++
5 mg/kg Rotenone +200 mg/kg*K. lanceolata*	31.0 ±0.9++	31.6 ± 0.8++	28.5 ± 0.9++
5 mg/kg Rotenone +400 mg/kg *K. lanceolata*	55.0 ±1.1++	26.2 ± 0.5++	27.6 ± 0.8++

Values are represented in Mean ± SEM (*n = 6*)

**Significance from the control group at P<0.01 probability levels and

++Significance from the Rotenone group at P<0.01 probability level.

Discussion

Due to their efficiency, sustainability, and local accessibility, herbal medicines are increasingly being to treat pathogenic disorders [39]. Due to the drawbacks of the currently available synthetic medications, innovative pharmacological therapeutic agents derived from plants have been discovered [40].In the current experiment, the phytochemical composition, antioxidant

capacity, and anti-Parkinson activity of the medicinal plant *Kalanchoe lanceolata* were assessed. Qualitative phytochemical analysis of various fractions revealed that alkaloids, tannins, glycosides, saponins, and flavonoids were all present in the plant methanol crude extract. In the crude plant extract of *Kalanchoe lanceolata*, other phytochemicals including steroids, terpenoids, and saponins were also discovered.Previous research has suggested that plant extracts include a variety of phytochemicals,including steroids, terpenoids, flavonoids, saponins, and alkaloids. These phytochemicals are vital for both the prevention and treatment of infectious diseases, as well as serving as free radical scavengers. Quantitative assessment showed that methanol fraction of *Kalanchoe lanceolata* plant extract showed maximum quantity of total phenolic and flavonoids contents as compare to other fractions. Similar reports were inferred by other studies.During current studies, the various solvent fractions of *Kalanchoe lanceolata* exhibited antioxidant activity measured by DPPH, ABTS and H2O2 scavenging methods. In DPPH, the value of methanolextracts was highest; in ABTS, butanol showed the highest percentage of inhibition, but in H2O2, ethyl acetate shows the highest percentage of scavenging activity. Previous studies have demonstrated that antioxidant behavior is one of the most frequently identified biological functions of biologically active chemicals that reduce the effects of oxidative stress.The extract of *Kalanchoe lanceolata* at 200 mg/kg and 400 mg/kg body weight dose level, recovered thebody weight and food intake as compared the disease control group.The time of falling on rotarod and sucrose consumption is minimum in rotenone treatedgroup as compared to normal control group. The plant *Kalanchoe lanceolata* shows positive results in rotarod and sucrose consumption tests. The earlier research showed that the rotenone group,period of falling was substantially shorter than control rats. Similar finding has been obtainedduring the investigation of quercetin pre and post-supplementation against rotenone inducedParkinson disease which has been significantly prolonged the period of falling. The earlier research also showed that rats given rotenone took significantly less sucrose solution than untreated rats. In contrast, groups given quercetin supplements drank greater quantities of sucrose solution over 24 hrs, 48 hrs, and 72 hrs than did the rotenone-only group [41].Latency to cross beam and cataleptic score is minimum in normal non treated controlgroup rats as compared to disease control rats. Rotenone caused sluggishness in motion due torigidity of muscle. *Kalanchoe lanceolata* (200 gm/kg and 400 mg/kg b.w) showed significant recovery effects in rats. Our study findings are consistent with earlier research which showed that rotenone injected rats showed a significantly higher cataleptic score and latency to walk on beam when compared to control rats. When compared to the group receiving only rotenone injections, the curcumin+rotenone group significantly

reduced cataleptic scores and latency to walk on beam [41].In open field test latency to move rotenone induced Parkinson diseased group showed higher movement than normal control group rats but the number of crossing of squares islow. *Kalanchoe lanceolata*(200 gm/kg and 400 mg/kg b.w) showed significant improvement as compare torotenone group rats. Calabrese *et al*., [42] reported similar results during movement in theopen field.5 mg/kg body weight injection caused abnormality in stride length of both fore limb and hind limb as well as the base width of both fore and hind limp as compared to normal control group. *Kalanchoe lanceolata* treatment revealed positive effects on rotenone-induced rats footprint test. The outcomes of this education were in agreement with Madiha *et al*. [41] which present edreduced walking outlines and reduced progress distance in rotenone-treated rats.

Conclusion

The methanol extract showed significant results in improving the various parameters of oxidative dysfunctions and Parkinson disease may be due to the presence of bioactive metabolites.Furthermore, mechanisms of action of these metabolites are encouraged to be investigated.

References

1. Bucur M, Papagno C (2023) Deep brain stimulation in Parkinson disease: a meta-analysis of the longterm neuropsychological outcomes. Neuropsychology review 33: 307–346.
2. Bloem BR, Okun MS, Klein C (2021) Parkinson’s disease. The Lancet 397: 2284–2303.
3. Kalia LV, Lang AE (2015) Parkinson’s disease. The Lancet 386: 896–912.
4. Moradi Vastegani S, Nasrolahi A, Ghaderi S, Belali R, Rashno M, et al. (2023) Mitochondrial Dysfunction and Parkinson’s Disease: Pathogenesis and Therapeutic Strategies. Neurochemical Research: 1–24.
5. De Lazzari F, Bubacco L, Whitworth AJ, Bisaglia M (2018) Superoxide radical dismutation as new therapeutic strategy in Parkinson’s disease. Aging and disease 9: 716.
6. Chang K-H, Chen C-M (2020) The role of oxidative stress in Parkinson’s disease.Antioxidants 9: 597.
7. Chopade P, Chopade N, Zhao Z, Mitragotri S, Liao R, et al. (2023) Alzheimer’s and Parkinson’s disease therapies in the clinic. Bioengineering & Translational Medicine 8: e10367.
8. Funayama M, Nishioka K, Li Y, Hattori N (2023) Molecular genetics of Parkinson’s

disease: Contributions and global trends. Journal of Human Genetics 68: 125–130.

9. Liu H, Bai Y, Huang C, Wang Y, Ji Y, et al. (2023) Recent progress of electrospun herbal medicine nanofibers. Biomolecules 13: 184.
10. Li T, Le W, Jankovic J (2023) Linking the cerebellum to Parkinson disease: an update. Nature Reviews Neurology: 1–10
11. Schapira AH, Jenner P (2011) Etiology and pathogenesis of Parkinson's disease. Movement disorders 26: 1049–1055.
12. Sharma P, Mittal P (2023) Paraquat (herbicide) as a cause of Parkinson's Disease. Parkinsonism & Related Disorders: 105932.
13. Zayed J, Ducic S, Campanella G, Panisset J, Andre P, et al. (1990) Environmental factors in the etiology of Parkinson's disease. The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques 17: 286–291.
14. Warner TT, Schapira AH (2003) Genetic and environmental factors in the cause of Parkinson's disease. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society 53: S16–S25.
15. Reeve A, Simcox E, Turnbull D (2014) Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing research reviews 14: 19–30.
16. Hindle JV (2010) Ageing, neurodegeneration and Parkinson's disease. Age and ageing 39: 156–161.
17. Jenner P, Jenner P (1991) Oxidative stress as a cause of Parkinson's disease. Acta Neurologica Scandinavica 84: 6–15.
18. Hwang O (2013) Role of oxidative stress in Parkinson's disease. Experimental neurobiology 22: 11.
19. Ferrari CC, Tarelli R (2011) Parkinson's disease and systemic inflammation. Parkinson's disease 2011.
20. Collins LM, Toulouse A, Connor TJ, Nolan YM (2012) Contributions of central and systemic inflammation to the pathophysiology of Parkinson's disease. Neuropharmacology 62: 2154–2168.
21. Khusnutdinova E, Gilyazova I, Ruiz-Pesini E, Derbeneva O, Khusainova R, et al. (2008) A mitochondrial etiology of neurodegenerative diseases: evidence from Parkinson's disease. Annals of the New York Academy of Sciences 1147: 1–20.
22. Chahardoli A, Sharifan H, Karimi N, Kakavand SN. Uptake, translocation, phytotoxicity, and hormetic effects of titanium dioxide nanoparticles (TiO2NPs) in Nigella arvensis L. Sci Total Environ (2022). Feb 1; 806(Pt 3):151222. Epub 2021 Oct

26.

23. Frazier Epicurioua A., Rajendra Pandurang Patil Chandrakant Mane, Sanaei Daryoush, Asiri Fahad, Seo Seong S., et al. Environmental exposure and nanotoxicity of titanium dioxide nanoparticles in irrigation water with the flavonoid luteolin. May 2023. RSC Advances 13(21):14110–14118.
24. Auddy B., Ferreira M., Blasina F., Lafon L., Arredondo F., Dajas F., et al. (2003). Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. Journal of ethnopharmacology, 84(2–3), 131–138.
25. Dhanasekaran M., Tharakan B., & Manyam B. V. (2008). Antiparkinson drug–Mucuna pruriens shows antioxidant and metal chelating activity. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 22(1), 6–11.
26. Bhangale J. O., & Acharya S. R. (2016). Anti-Parkinson activity of petroleum ether extract of Ficus religiosa (L.) leaves. Advances in Pharmacological and Pharmaceutical Sciences, 2016.
27. Saleem U., Chauhdary Z., Raza Z., Shah S., Rahman M. U., Zaib P., et al. (2020). Anti-Parkinson's Activity of Tribulus terrestris via Modulation of AChE, α-Synuclein, TNF-α, and IL-1β. ACS omega, 5 (39), 25216–25227
28. Kabra A., Baghel U. S., Hano C., Martins N., Khalid M., & Sharma R. (2020). Neuroprotective potential of Myrica esulenta in Haloperidol induced Parkinson's disease. Journal of Ayurveda and integrative medicine, 11(4), 448–454.
29. Eze F. N., &Jayeoye T. J. (2021). Chromolaena odorata (Siam weed): A natural reservoir of bioactive compounds with potent anti-fibrillogenic, antioxidative, and cytocompatible properties. Biomedicine & Pharmacotherapy, 141, 111811.
30. Dhaliya Salam A., Pillai P. G., & Senthilkumar D. (2022). Anti parkinson activity of the ethanolic extract of sterculia guttata in wistar rats using different models. Journal of Pharmaceutical Negative Results, 10415–10424.
31. Sharma P., Jain D. K., Jain N. K., Jain A., Bhadoria U. S., Paliwal P., et al. (2022). Anti-parkinson's potential of acorus calamus linn: a review. Journal of Pharmaceutical Negative Results, 2540–2547.
32. Lawal B. A., Ayipo Y. O., Adekunle A. O., Amali M. O., Badeggi U. M., Alananzeh W. A., et al. (2023). Phytoconstituents of Datura metel extract improved motor coordination in haloperidol-induced cataleptic mice: Dual-target molecular docking

and behavioural studies. Journal of Ethnopharmacology, 300, 115753.

33. Shaik R. (2023). Anti-parkinsonian effect of momordica dioica on haloperidol induced parkinsonism in wistar rats. Journal of Pharmaceutical Negative Results, 69–81.
34. Atala E., Va´squez L., Speisky H., Lissi E., &Lo´pez-Alarco´n C. (2009). Ascorbic acid contribution toORAC values in berry extracts: An evaluation by the ORAC-pyrogallol red methodology. Food Chemistry,113(1), 331–335.
35. Pompilio M., Ierides I., &Cacialli F. (2022). Biomimetic approaches to “transparent” photovoltaics: currentand future applications. Molecules, 28(1), 180.
36. Brahmi F., Mechri B., Dhibi M., & Hammami M. (2013). Variations in phenolic compounds and antiradicalscavenging activity of Olea europaea leaves and fruits extracts collected in two different seasons.Industrial Crops and Products, 49, 256–264.
37. Shen Y., Zhang H., Cheng L., Wang L., Qian H., & Qi X. (2016). In vitro and in vivo antioxidant activity ofpolyphenols extracted from black highland barley. Food Chemistry, 194, 1003–1012.
38. Khan R. A., Khan M. R., Sahreen S., & Ahmed M. (2012). Evaluation of phenolic contents and antioxidantactivity of various solvent extracts of Sonchus asper (L.) Hill. Chemistry Central Journal, 6(1), 1–7.
39. Ahmed M. R., Shaikh M. A., Haq S. H. I. U., & Nazir S. (2018). Neuroprotective role of chrysin in attenuatingloss of dopaminergic neurons and improving motor, learning and memory functions in rats. InternationalJournal of Health Sciences, 12(3), 35. Alcantara, S. (2022). Parkinson’s Disease. DHRProceedings, 2(S2).
40. Calabrese C., Gregory W. L., Leo M., Kraemer D., Bone K., & Oken B. (2008). Effects of a standardizedBacopa monnieri extract on cognitive performance, anxiety, and depression in the elderly: a randomized,double-blind, placebo-controlled trial. The journal of alternative and complementary medicine,14(6), 707–713.
41. Madiha S., & Haider S. (2019). Curcumin restores rotenone induced depressive-like symptoms in animalmodel of neurotoxicity: assessment by social interaction test and sucrose preference test. MetabolicBrain Disease, 34, 297–308.
42. Calabrese C., Gregory W. L., Leo M., Kraemer D., Bone K., & Oken B. (2008). Effects of a standardizedBacopa monnieri extract on cognitive performance, anxiety, and depression in the elderly: a randomized,double-blind, placebo-controlled trial. The journal of alternative and complementary medicine,14(6), 707–713