



RESEARCH ARTICLE

# Pharmacological study on the neuroprotective and antioxidant potential of *Leucas aspera* (Willd.) Link methanolic extract

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## Abstract

The present study investigates the plant-based methanolic extract from *Leucas aspera* (Willd.) Link, which has been shown to enhance the antioxidant and neuroprotective properties against Parkinson's disease (PD). Phytoconstituents extracted from the plant possess potential antioxidant and therapeutic qualities, making it a medicinal herb. Antioxidant tests, including the Phosphomolybdenum assay, Free Radical Scavenging Assay (FRSA) and Free Radical Antioxidant Power assay (FRAP), were performed on methanolic extracts of *L. aspera*. The methanolic extract of the plant exhibited antioxidant activity against both aqueous and ethyl acetate extracts, as well as against Levodopa (L-DOPA), as evidenced by the results, which indicated a positive response towards its antioxidant potential. The gas chromatography-mass spectrometry (GC-MS) analysis of the plant extract revealed the presence of key bioactive ingredients, including 1-(1H-imidazol-2-yl)-2,2-dimethylpropan-1-one, cyclohexane carboxamide, ethyl tridecanoate, docosanoic acid ethyl ester, oleonic acid and squalene. These bioactive ingredients play a significant role in the plant's antioxidant and neuroprotective properties. For instance, 1-(1H-imidazol-2-yl)-2,2-dimethylpropan-1-one is known for its antioxidant activity, while oleonic acid and squalene have neuroprotective properties. The presence of catechins and epigallocatechins (EGCG) is directly involved in protecting neurons from excessive stress and oxidative damage. Histopathological analysis showing the neuroprotective activity of the *L. aspera*-induced sample at 500 mg/L compared to the Trichloroethylene (TCE)-induced group. Herbal formulations extracted from the *L. aspera* plant exhibit strong antioxidant properties, which enable them to chelate metals and demonstrate their medicinal value in reducing the progression of neurodegenerative disorders. The study implies support for the pharmacological utility of *L. aspera* plant extract in the development of neuroprotective medicines.

**Keywords:** antioxidant potential; flavonoids; neuroprotective activity; oxidative stress; phytoconstituents

## Introduction

In the era of a global population and the release of harmful gases into the environment, nature has always played a vital role in the extraction of naturally available therapeutic drugs from herbal plants. Extracting these novel compounds from plants as therapeutic agents for disease management is where phytopharmaceuticals act as neuroprotective agents (1). Over the years, traditional medicine has addressed several types of neurological dysfunction. The ongoing research on traditional medicines derived from plant extracts has demonstrated the potential therapeutic properties of secondary metabolites derived from plant material, with their ability to manage diseases with comparatively fewer side effects (2). For the record, over 80 % of the global population relies on traditional medicines as the primary means of disease management and illness therapy, as described by the World Health Organization (WHO) (3).

The rising occurrence of these neurological disorders due to improper diet intake in the population and a few genetic factorial-based studies have revealed the onset of neurodys function concerning Parkinson's disorder (4). Biochemical assays are established in the field of investigation involving blood serum levels

to detect neuromuscular dysfunction in the muscular system, which can lead to neuronal and cellular damage due to oxidative stress (5). The primary role of these secondary metabolites derived from the plant extract is to balance the imbalanced concentrations of radical ions in the cellular chain, leading to stress and neuronal loss (6). The imbalance between the plant extract molecule and the oxygen molecule, which causes oxidative stress, is associated with the occurrence of biological disturbances leading to the pathogenesis of neurological disorders (6).

The presence of free radical ions plays a crucial role in the mechanisms of several biological processes related to neurodegeneration and cellular signalling pathways (7). Medicinal plants are recognized for their antioxidant therapeutic potential in mitigating these free radical molecules, which can lead to excessive oxidative stress and result in neuronal loss and degeneration of healthy neurons (8). Flavonoid compounds derived from plant extracts are known to exhibit antioxidant properties with their lower molecular structure and weight, with the presence of potent bioactive molecules present in the flavonoid compound, with its strong hydrogen and carbon molecules attached in a hexyne and hexane structure containing catechins and epicatechin (EC), epigallocatechin (9).

As the scientific community has focused on the validation and standardization of traditional medicines in recent decades, particularly those derived from conventional herbal sources extracted from plants (10). Alternative, safer and more accessible treatments are desperately needed as neurological illnesses are becoming more common worldwide, particularly in older populations. Due to its natural abundance and long-standing reputation for healing, herbal remedies present a promising path for the development of new drugs (11). Flavonoids, particularly catechins and epigallocatechins, have become recognized as bioactive compounds with significant neuroprotective and antioxidant properties among the wide variety of phytochemicals (12). These substances have molecular effects that alter oxidative pathways, lessen neuroinflammation and increase the lifespan of neurons (13).

*Leucas aspera* (Willd.) Link is a medicinal plant commonly used in traditional medical systems. In the field of neuropharmacology, the therapeutic potential of essential phytochemicals present in plant extracts has recently been demonstrated (14). Catechins, which are abundant in its methanolic extract, are known to interact with cellular receptors and enzymes that control apoptosis and oxidative stress (15). Since oxidative stress plays a significant role in brain damage in conditions such as Parkinson's and Alzheimer's, the antioxidant molecule present in *L. aspera* has been shown to have viable therapeutic properties (16). Its function as a natural neuroprotectant is further reinforced by its metal-chelating properties, which help prevent the production of free radicals triggered by metal ions (17). Such plant-based therapies could pave the way for more comprehensive and long-lasting approaches to neurodegenerative disease when combined with contemporary biomedical procedures (18).

## Materials and Methods

### Collection of plant material

The plant material, collected during the August–October flowering season, from Bengaluru, Karnataka, was authenticated at the Mahatma Gandhi Botanical Garden, located on the campus of the University of Agricultural Sciences' Gandhi Krishi Vigyan Kendra. This crucial step was carried out by a botanist from the Department of Horticulture, ensuring the accuracy and reliability of the plant sample. The authenticated plant sample was then deposited (Accession number USAB 5653) as *L. aspera* of the Lamiaceae family (19). A 500 g fresh leaf sample was shade-dried at room temperature, resulting in a sample yield of 80 g of dried powder in a 6:1 ratio for further Soxhlet extraction of the plant sample.

### Antioxidant activity

#### Ferric reducing antioxidant power assay

Ferric-reducing antioxidant power assay was carried out using methanolic plant extracts at different concentrations of *L. aspera* leaf sample in ( $\mu\text{g/mL}$ ) and mixed with sodium phosphate buffer at pH 6.6 and potassium ferricyanide solution. The solution was mixed thoroughly and incubated at 50 °C for 20 min and vortexed using a vortex apparatus. Before the absorbance measurement at 700 nm, a 10 % trichloroacetic acid solution was centrifuged at 3000 rpm for 10 min. The pellet was discarded by mixing the supernatant with ferric chloride and deionized water, using ascorbic acid as a standard to reference the sample concentrations. The reading was then analyzed using a UV-Vis spectrometer (20).

#### Phosphomolybdenum assay

The overall capacity of the total antioxidant present in the plant extract sample was evaluated using the Phosphomolybdenum method. Different aliquot concentrations of plant extract were measured and mixed with ammonium molybdate, sodium phosphate and sulfuric acid at a concentration of 4 mM. The antioxidant assay is based on a quantitative analysis in which the reduction of Mo (VI) to Mo (V) occurs at an acidic pH. The samples were incubated at 95 °C for 90 min. Samples were allowed to cool to room temperature and absorbance was measured at 695 nm using a UV-Vis spectrometer, with ascorbic acid as the standard reference (21).

#### Free radical scavenging assay

The radical scavenging capacity against DPPH (2,2-diphenyl-1-picrylhydrazyl) was assessed using the Free Radical Scavenging Assay (FRSA) method. Test samples, ranging from 10 to 70  $\mu\text{g/mL}$ , were prepared by combining them with a 1 mg/mL DPPH solution. The samples were derived from a stock solution diluted with methanol, ethyl acetate and an aqueous extract, resulting in concentrations ranging from 10 to 70  $\mu\text{g/mL}$  when diluted using methanol. Concomitant with blanks and a positive reference medication (L-DOPA), which were included for comparison. The solution underwent a 30 min incubation at 25 °C in the dark, followed by measurement of absorbance. A decrease in the DPPH solution's absorbance indicates an elevation in Radical Scavenging Capacity (RSC), following the previously known methodology (22).

#### Thin Layer Chromatography (TLC)

Thin Layer Chromatography was performed using silica gel on the TLC glass plate. A silica gel slurry was prepared by mixing methanol and ethyl acetate in a specific solvent proportion and then poured into the chromatography apparatus. The glass plates are kept for overnight air-drying and heated at 100–120 °C for 1–2 hr to remove the moisture content and activate the adsorbent on the plate. The samples extracted after the chromatography run are collected into different fractions and tested for fluorescence under a UV light chamber, with labelling on the silica sheet using the standard quercetin solution. Further determination of the TLC was calculated using the retardation factor ( $R_f$ ) formula and the values for sample and standard run against the standard to determine the alkaloids and flavonoids present (23).

#### Gas Chromatography-Mass Spectrometry (GC-MS) analysis

##### Sample preparation

The sample was prepared using a wide range of fractions in a conical flask holding the methanolic extract. The procedure comprised using a magnetic stirrer with ethyl acetate, hexane, acetone and methanol as solvents concurrently. After obtaining and storing each fraction independently, the sample was analyzed using GC-MS analysis (24).

##### GC-MS analysis

The phytochemical screening of crude extracts from *L. aspera*, obtained from whole plants, underwent GC-MS analysis using an Agilent Technologies instrument (6890 N) coupled with a JOEL GCMATE II autosampler and a GC-MS setup. The conditions were as follows: a capillary column (624 ms; 30 m length, 0.32 mm diameter) operated in the electron ionization mode at 70 eV. Helium (99.999 %) served as the carrier gas, flowing at a continuous rate of 1.491 mL/min, with an injection volume of 1.0 mL. The injector was set to 140 °C and the ion source was set to 200 °C. The oven temperature was set to start at 45 °C. Spectra of mass were acquired at 70 eV (25).

### Histopathological staining of the hypothalamus region

Histopathological examination was carried out to compare the activity of the treated and untreated Wistar rat models with that of the control. Brain tissue samples from Wistar rats were collected. The hypothalamic area of the animals' brains was removed after they had been anesthetized with chloroform. For further histopathological staining, the tissue was kept in a 20 % formaldehyde solution. A tiny layer of brain tissue was sliced for additional examination using a thirty-micrometer coronal instrument (26).

### Results

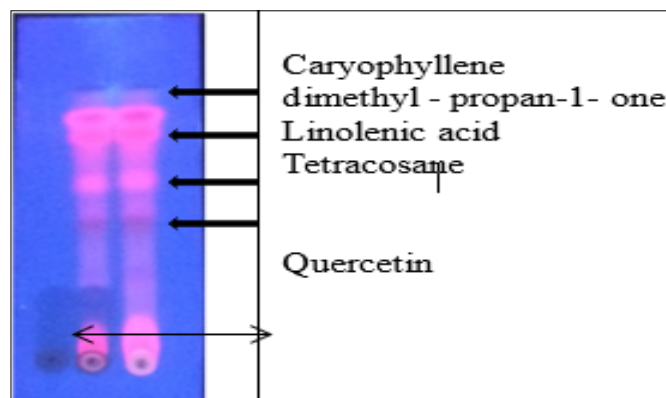
The antioxidant capacity of the *L. aspera* leaf extract for the Free Radical Antioxidant Power assay (FRAP) and Phosphomolybdenum assay was estimated in comparison with the L-DOPA, methanol and aqueous, ethyl acetate extract as the calculated IC<sub>50</sub> value of 1499.3 ± 0.9 µg/mL and the maximum activity concentration of Phosphomolybdenum reduction assay showed 0.056 µg/mL at 100 mg/mL concentration and FRSA of 46.36 ± 0.087 µg/mL of the plant extract with better radical scavenging activity against standard ascorbic acid, the study showed comparison to other solvents methanolic extract displayed better antioxidant property greater than aqueous and ethyl acetate extract (Fig. 1).

#### Thin Layer Chromatography (TLC) analysis

Thin Layer Chromatography is a chromatography technique used to separate non-volatile mixtures. It is performed on a silica gel-coated plate. The resulting thin-layer chromatogram revealed a Retardation factor (Rf) value of 2.31, indicating the relative mobility of the compounds under the specified conditions. For component separation, a drop of plant extract was applied to the paper and the paper was then left in the solvent (Fig. 2).

#### GC-MS chromatography

The hydrodistillation of *L. aspera* leaves produced a colourless oil. GC-MS studies of the oil revealed the presence of 13 components that eluted between 22 and 66 min, accounting for 98.5 % of the oil (Table 1). The oil was mainly monoterpene. It was composed of

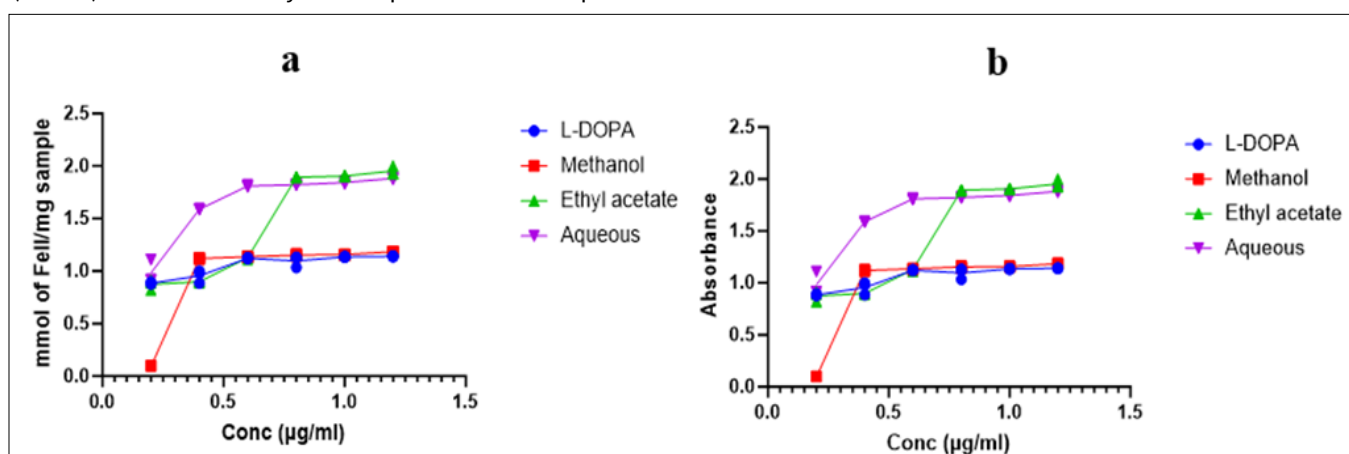


**Fig. 2.** Compound separation using thin-layer chromatography profiling of *L. aspera* extract.

caryophyllene (1.65 %), 2(1H)-naphthalenone, octahydro-4a, 5-dimethyl-(4 $\alpha$ ,5 $\alpha$ ,8 $\alpha$ ) (3.37%), phytol (4.18 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.34 %), hexadecanoic acid (4.75 %), 5,8-octadecadienoic acid (2.74 %), 1-(1H-imidazol-2-yl)-2,2-dimethylpropan-1-one (11.75 %), 1-(1H-imidazol-2-yl)-2,2-dimethylpropan-1-one (10.18 %), 13-tetradecynoic acid (4.16 %), tetracosane (6.52 %), squalene (3.36 %) and tetratetracontane (3.07 %) (Fig. 3, 4).

#### Hematoxylin and eosin-stained staining of the hypothalamic region

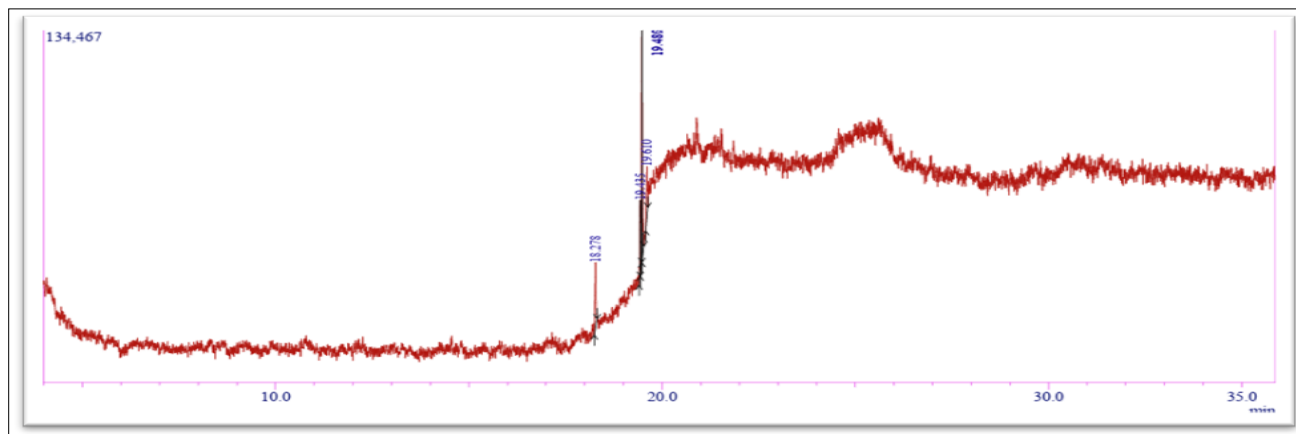
The hypothalamic brain region in the control group displayed normal, intact neurons well-organized with neuroglial cells, in comparison with the Trichloroethylene (TCE)-treated group. At the higher dose of 500 mg TCE, the induced region exhibited neurodegenerative changes, including neuronal shrinkage, vacuolation and clustering of the darkly stained nuclei of glial cells, resulting from the formation of reactive oxygen species that led to oxidative stress and neuroinflammation. However, the treated group with *L. aspera* extract demonstrated a significant protective role of the plant sample against neurodegeneration, characterized by better neuronal integrity and minimal disruption, highlighting the strong antioxidant potential of the plant extract and the importance of this research in the fields of neuroprotection and toxicology (Fig. 5).



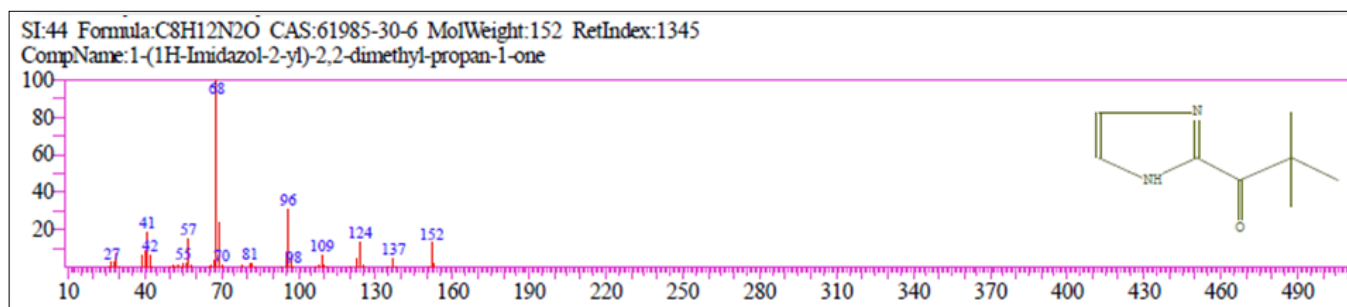
**Fig. 1.** a: Free radical antioxidant power assay (FRAP) of *Leucas aspera* leaf extract; b: Phosphomolybdenum activity of *L. aspera* leaf extract.

**Table 1.** Gas chromatography mass spectroscopy analysis of *L. aspera*

SL.No	R. Time	Area %	Height %	Name
1	18.278	14.57	10.36	ethyl tridecanoate
2	19.435	9.14	11.91	acetic acid, 2-methylbicyclo(2.2.1)hept-2-yl
3	19.480	39.36	36.62	cyclohexanecarboxamide, N, N-dimethyl-2-o
4	19.485	25.94	33.22	cyclohexanecarboxamide, N, N-dimethyl-2-o
5	19.610	10.99	7.89	docosanoic acid, ethyl ester
		100.00	100.00	



**Fig. 3.** GC-MS analysis of the methanolic extract of *L. aspera*.



**Fig. 4.** Peak for 1-(1H-Imidazol -2-yl)-2,2-dimethyl-propan-1.

## Discussion

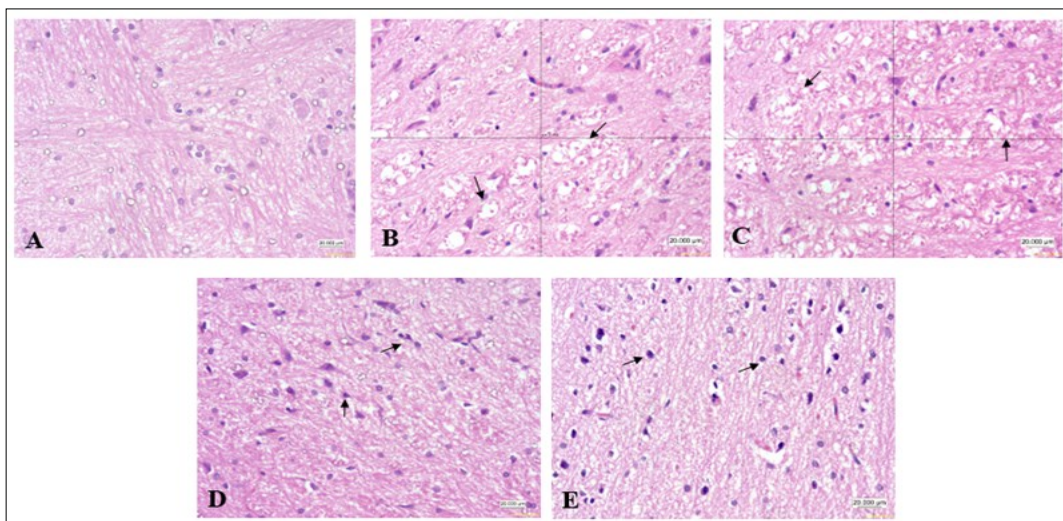
The current study aids in understanding the potential capacity of *L. aspera* methanolic extract (LAME), which exhibits notable antioxidant activity in several *in vitro* tests. These tests, including FRAP, Phosphomolybdenum and FRSA, are widely accepted and used in the field of photochemistry to measure the antioxidant potential of plant extracts. LAME also contains significant amounts of total phenolics and flavonoids, further supporting its antioxidant properties. These findings align with previous phytochemical and antioxidant studies on *L. aspera*, which indicate that leaf extracts exhibit detectable FRAP/Phosphomolybdenum activity and contain phenolics, flavonoids, terpenoids and other secondary metabolites (27,21).

The investigation aims to determine the potential neuroprotective benefits of LAME observed in cellular models of neurotoxicity. These benefits include the preservation of membrane integrity, decreased intracellular reactive oxygen species and enhanced neuronal survival. The study suggests that the polyphenolic components of LAME likely mediate these benefits. Flavonoids and related polyphenols have been shown to protect neurons by directly scavenging reactive oxygen species (ROS), enhancing endogenous antioxidant defences and regulating inflammation and stress-signalling pathways associated with neurodegeneration. The potential for LAME's antioxidant activity to result in cellular neuroprotection is explained by these broad pathways, offering hope for its therapeutic application.

Comparing quantitative activity, several recent and geographically diverse studies of *L. aspera* report similar antioxidant potency but point out solvent- and part-dependent variations. For instance, acetone or methanol leaf extracts frequently exhibit higher radical-scavenging or reducing-power values than hexane extracts and young leaves may contain higher concentrations of phenolic and flavonoids. The extraction decisions and assay results in this work are supported by the fact that we used methanol for Soxhlet

extraction, a method commonly used for the extraction of plant compounds and the reported total phenolic/flavonoid content is consistent with studies that found methanolic/acetone fractions to be particularly active (28, 29).

The characterization of certain chemicals in *L. aspera* (GC-MS) and the connection of extract activity with enzyme-inhibitory and anti-inflammatory activities that supplement antioxidant mechanisms have been initiated by recent phytochemical profiling and bioactivity research (including 2023–2025 publications). These activities refer to LAME's ability to inhibit specific enzymes and reduce inflammation, which are additional mechanisms that contribute to its neuroprotective potential. The inference that the combined effects of terpenoids, phenolics and flavonoids in LAME can operate in a multimodal manner to minimize oxidative damage and downstream inflammatory neurotoxicity—a desirable profile for potential neuroprotective agents is strengthened by these more recent investigations (29, 30). Histopathological studies, in comparison with the control and drug-induced groups, showed protective and normal neuronal and glial structures. As the results in the TCE-induced group displayed vacuolation and neuroinflammation, this led to the degenerative morphology of the neurons. The treatment group demonstrated a significant potential neuroprotective role of the neurons, as the plant sample exhibits strong antioxidant properties against damaged neuronal chains (30). While the current data are promising, translating these findings into *in vivo* neuroprotection and therapeutic relevance is necessary. To move leads toward preclinical development, several recent reviews highlight the specific gaps in natural-product neuroprotectants and suggest integrating phytochemical profiling, pharmacokinetics and target-specific molecular research. Such follow-up research will support the idea that LAME could be produced therapeutically to lessen brain damage caused by oxidative stress. This potential for LAME to be a part of a therapeutic solution for neurodegenerative



**Fig. 5.** Histopathology analysis of the hypothalamic region showing -A: Control group; B: Trichloroethylene induced at 250 mg/L; C: Trichloroethylene induced at 500 mg/L; D: *L. aspera* induced at 250 mg/L; E: *L. aspera* induced at 500 mg/L.

diseases should inspire and motivate future research in this area.

## Conclusion

The current study investigated the antioxidant and neuroprotective properties of *L. aspera* methanolic extract, a plant widely used in conventional and medicinal therapies. Numerous *in vitro* and *in vivo* studies have demonstrated that the extract exhibits significant antioxidant activity, primarily due to its rich phytochemical content, which comprises mainly flavonoids, phenolics and other secondary metabolites. These compounds play a crucial role in scavenging free radicals and mitigating oxidative stress, a significant contributor to neuronal damage and the development of neurodegenerative disorders. The considerable role of *L. aspera* in combating oxidative stress provides reassurance and confidence in its potential as a neuroprotective agent. In neuroprotective studies, the methanolic extract of *L. aspera* has demonstrated protective properties against neurotoxic compounds, reducing neuronal cell death and maintaining cellular integrity. The extract significantly improved cell viability in oxidative stress-induced brain models, suggesting that it may help mitigate neurotoxicity. These findings indicate that the extract can maintain or restore neuronal function under stressful conditions, potentially by altering signalling pathways associated with oxidative stress, inflammation and cell death. Specifically, the extract shows promise in preventing the onset or progression of neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease and Huntington's disease. Furthermore, studies conducted in the lab revealed that the methanolic extract treatment increased the activity of endogenous antioxidant enzymes, which are naturally occurring enzymes in the body that help neutralize harmful free radicals. These findings support the hypothesis that *L. aspera*'s neuroprotective benefits are closely linked to its antioxidant properties, which combat oxidative damage, a key factor in the development of several neurodegenerative diseases, including Parkinson's. In conclusion, the methanolic extract of *L. aspera* has demonstrated potent neuroprotective and antioxidant properties, validating its long-standing use in the management of neurological disorders. In addition to confirming the plant's therapeutic properties, the study offers prospects for more pharmacological investigation and the potential development of phytopharmaceuticals. The potential of *L. aspera* for the

development of neuroprotective drugs is a promising and forward-looking aspect of this research, offering optimism for the future of neuroprotection.

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## Authors' contributions

VR conceptualized the review on medicinal plant, conducted the initial literature search and done all the experiments, VR and SJP contributed to data compilation, figure preparation and assisted with drafting the manuscript. MKR provided application insights on neuroprotective methodology and antioxidant studies protocols. SJP and MKR provided critical review of the applications studied on the plant work. SJP supervised the review process and contributed to critical revisions. MKR co-supervised the study, reviewed the final draft for scientific accuracy and ensured consistency across all sections. All the authors have contributed equally concerning the conceptualization of the study, data collection for the experimental study, design, analysis, interpretation of the results obtained and manuscript preparation. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** The study carried out was approved by the Institutional Animal Ethics Committee (IAEC) of Karnataka College of Pharmacy Bangalore (Reg. No- 1564/PO/Re/S/11/CPCSEA).

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