Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 5s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Assessment of Antioxidant and Antimicrobial Activity of Leucas Aspera and its Application in Neuroprotection

Varsha Rani[1], Manjula Kannasandra Ramaiah[*1]

[1]Department of Biotechnology, School of Applied Sciences, REVA University, Bengaluru, Karnataka, India – 560064.

[*1]Professor, Department of Biotechnology, School of Applied Sciences, REVA University, Bengaluru, Karnataka, India.

***Corresponding Author:**

Email ID: manjula.kr@reva.edu.in

Cite this paper as: Varsha Rani, Manjula Kannasandra Ramaiah, (2025) Assessment of Antioxidant and Antimicrobial Activity of Leucas Aspera and its Application in Neuroprotection. *Journal of Neonatal Surgery*, 14 (5s), 388-395.

ABSTRACT

Some phytochemicals play a major role in protecting neuronal health. *Leucas aspera* exhibits beneficial effects in supporting neuroprotective activities. The major role of *Leucas aspera* extract was studied for antioxidant and antimicrobial activities. Phenols and flavonoids derived from the plant help in the scavenging activity of free radical ions by lowering the stress involved in increasing the levels of oxidative ions in the body and shielding cells from internal harm. This study focuses on evaluating the effects of *Leucas aspera* for its antioxidant activity by performing DPPH (2, 2-diphenyl-1-picrylhydrazyl), Nitrite assay using Nitric Oxide, and (2, 2'-azinobis-3-ethylbenzothiazoline-6sulphonic acid) ABTS assay. The obtained nitric oxide IC_{50} value was 89.60 (µg/ml). In addition, the *Leucas aspera* extract zone of inhibition was measured and antibacterial activity of the plant extract concerning *Bacillus subtilis* (0.469 µg/ml) *Staphylococcus aureus* (10 µg/ml), *Klebsiella pneumoniae* (9 µg/ml), *E. coli* (8.25 µg/ml), *Candida albicans* (7.5 µg/ml), *Penicillium chrysogenum* (8.0 µg/ml), *Aspergillus niger* (8.5 µg/ml). This suggests that polyphenols and flavonoids derived from *Leucas aspera* contain precursors that are potential metabolites responsible for the cure and management of the progress of neurodegenerative diseases. *Leucas aspera-derived* compounds from the chromatography technique are beneficial in analyzing bioactive compounds towards the neurodegenerative disorder.

Keywords: *flavonoid, metabolite, Neurodegenerative disease, Oxidative stress, polyphenol*

1. INTRODUCTION

Neurodegenerative disorders (NDD) are broadly described as a selective dopaminergic neuron loss and neuronal dysfunction in the peripheral and central nervous systems in the brain. Earlier research has shown that the development of such neurological dysfunctioning can only be managed then a cure, in the year 2019 globally around 50 million people were affected with NDD moreover the estimation of the cases affected with dementia and Parkinson's cases tends to increase by over 152 million cases by the year 2060 (1). In neurological disorders involving slowness in the body movement Parkinson's, Alzheimer's, and multiple sclerosis, the patients not only suffer from dementia (2) and neuronal loss but very severe cases involving major nerve damage in the susbtantia brain region leading to the behavioral and mental instability of the individual around the family members. Many lifestyle changes, inherited genetics, and rough impact of environmental factors are involved in the Etiology of NDD onset (3). In recent years, advanced medical research has turned the page of involving ancient herbal and natural compounds, particularly derived from herbal plants, which could potentially lead to the management and neuroprotection from nerve damage due to NDD (4).

Stress causing oxidation and neurodegenerative illnesses are caused by the overproduction of active oxygen species, causing hydroxyl, superoxide, nitric oxide, and hydrogen peroxide ions release. Despite the wide range of medications available to treat neurodegenerative illnesses, many of these conditions remain untreated due to their significant negative effects. According to studies, treating neurodegenerative illnesses lowers the brain's antioxidant defense system and produces more free radicals, which reduces oxidative stress (5). Nature has served as a reservoir of medicinal compounds, contributing significantly to the pharmacopeia of modern medicine. A wide variety of modern medications have natural sources, frequently influenced by their previous use in traditional medicine used by native people. Plants also produce antioxidants to combat oxidative stress induced by factors like light and oxygen. This makes them promising reservoirs of antioxidant molecules (6).

Research has also shown the importance of natural herbal products, specifically extracts from plant compounds that have been found to exhibit unique medicinal properties, such as secondary metabolites and bioactive compounds beneficial in releasing oxidative stress and nerve damage. The phytocompounds present in the plant extract consist of flavonoids and phenol, which act as a therapeutic agent against neurological dysfunction (7). The efficacy of these compounds not only releases oxidative stress but also helps in neuronal inflammation causing damage to the brain region respectively microbial invasions are also involved in the study pattern to check the sensitivity of the plant extract against any fungal or bacterial strains to enhance the health benefits towards neurological mechanisms (8).

Flavonoids and phenols, medicinally derived compounds from plant extract, are known to have medicinal properties in treating neurological ailments. Flavonoid compounds are known for their rich quercetin content and kaempferol compounds. Leucas aspera, belonging to the Lamiaceae family, is a traditionally used plant for its herbal and medicinal properties in the management of neurological disorders (9). The antioxidants present in the leucas aspera plant extract have been shown to exhibit damage to nerve functioning. This study helps us to assess the antimicrobial and antioxidant qualities of *Leucas aspera* towards the neurodegenerative disorder, mitigating healthy brain and normal functioning (10). The major outcome of the research work aims to develop a therapeutic approach by utilizing natural medicinal resources to manage the neurological disorder causing neurodegeneration.

2. MATERIALS AND METHODS

2.1 Collection of Herbal Plant Material

Herbal plant sample leaves were obtained from the Kattigenahalli layout, Bengaluru, and authenticated by the Botanist from the Department of Horticulture, GKVK Campus Bengaluru, approved accession number UASB5653.

2.2 Plant extraction preparation

Whole plant leaf parts were collected and ground using a pester and mortar until a fine powdered form was achieved. The collected powder was extracted using Polar and Non-polar solvents, methanol, ethyl acetate, and aqueous, using the Soxhlet apparatus. The plant extract obtained after several cycles of the Soxhlet extracting procedure and stored maintaining the temperature below 50°C. Later, the sample extracted was preserved at -80°C until further use for analysis and characterization (11).

2.3 Qualitative phytochemical analysis

The extracted plant compound was tested for qualitative analysis to determine the phytochemical compounds present in the plant sample using the standard protocol described by (12) compound described in Table 1.

2.4 Assessment of Total Phenolic Content

The amount of total phenolic compound in the *Leucas aspera* was estimated using the phenol reagent Folin-Ciocalteu's. The assay involves oxidizing the phenolic compounds with an F-C reagent, forming a blue color compound. Taking standard as Gallic acid solution for the neutralisation comparison with the sample extracted, the various defined concentrations of the Folin-Ciocalteu's reagent and four milliliters (75 g/l) of sodium carbonate to create the calibration curve was taken. Incubation of 30 minutes at 20°C was maintained, and absorbance was taken at 765 nm (13).

2.5 Assessment of Total Flavonoid Content

The presence of bioactive flavonoid content in the plant extract was determined using the aluminum chloride spectrometry assay. Different concentration of the plant extract was taken, and 5% of the sodium nitrite and 10% aluminum chloride were allowed to mix at room temperature for 5 minutes, keeping quercetin as the standard solution. The spectrometry absorbance of the solution was determined at 510nm (14).

2.6. Scavenging Assay using DPPH Antioxidant Assay

The amount of antioxidant compound present in the herbal plant extract sample was estimated using light-sensitive radical scavenging assay. Different concentrations of the plant extract were taken, and 80mM of the DPPH 5 ml solution was mixed with the tubes resting for 30 minutes of resting incubation at room temperature, spectroscopy at 517nm under UV-Vis Spectrometer. Inhibition percentage was recorded and calculated using the formula taking ascorbic acid as standard (15).

2.7. ABTS Scavenging assay

The ABTS radical scavenging assay was used to determine the scavenging property of the plant's extract and measure the relative antioxidant capacity of the herbal plant extract to scavenge the free radicals. Different plant sample concentrations were combined with 0.238 mM ABTS radical solution and 10 mM PBS, maintaining the neutralizing pH 7.4 under the resting incubation period of 30 minutes at room temperature. Further absorbance was read at 734nm (16).

2.8 Assay for Scavenging Nitric Oxide

Radical scavenging assay for the nitrate ions involved in the reduction of nitrite into nitrogen oxide in the presence of Griess

reagent forming a stable compound, nitric oxide assay helps to determine the property of plant extract samples to scavenge the nitric oxide radicals. The mixture consists of 10Mm sodium nitroprusside and phosphate buffer at pH 7.4, with 0.5ml of Griess reagent at 25°C was incubated for 150 minutes. The spectrophotometry analysis of the plant sample was determined at 546nm (17).

2.9 Antimicrobial assay

Herbal plant extract was evaluated using the antimicrobial assay, which was assessed utilizing the strains of fungi and bacteria by the agar well diffusion method. In particular, MRBA agar was used to culture *Aspergillus niger* (MTCC 872), *Penicillin chrysogenum* (MTCC 7420), *Staphylococcus aureus* (MTCC -96), *Klebsiella pneumoniae* (ATCC 13883) and *Escherichia coli* (MTCC 589) *Bacillus subtilis* (MTCC-5981). We examined three distinct methanol extract dosages (25µg/ml, 50µg/ml, and 75µg/ml) and contrasted them with ciprofloxacin, the positive control. Leaf extracts from *Leucas aspera* were assessed for their antibacterial activity (18).

2.10 Column chromatography

Column chromatography was performed to extract the plant leaf extract at different fractions to test the flavonoid compound present in the extract equivalent to quercetin. A glass column, shaped like a cylinder filled with stationary phase material such as silica gel, is slowly filled from the top with a liquid solvent known as the mobile phase, which moves downward due to the gravity of external pressure. This technique is employed to purify compounds from a mixture. Once the column is prepared, the sample is introduced at the top with a liquid solvent known as the mobile phase, which moves downwards due to gravity or external pressure. Chemicals from a mixture that interacts differently with the silica gel, as mobile phase and the stationary phase can be separated using this technique. As the sample moves through the column at varying degrees or varying speeds. As a result of this process, compounds are removed from the mixture. To ascertain their structures, the isolated chemicals are gathered as fractions and put through further investigation (19)

2.11 Statistical analysis

The statistical tool Graph Pad Prism 9 software and Excel were used for the analysis of two-way ANOVA. There were three replicates of each sample used in the experiment.

3. RESULTS

3.1 Qualitative Analysis of Phytocompounds:

Several Phytocompounds were found by performing qualitative analysis. The table represents bioactive compound extracts of *Leucas aspera* leaves after a comprehensive phytochemical screening (Table 1).

Table 1: Phytochemical analysis of *Leucas aspera*

Components	Methanol	Aqueous	Ethyl acetate
Carbohydrates	+	-	-
Alkaloids	-	-	-
Cardiac glycosides	+	-	-
Flavonoids	+	+	-
Phenol	+	+	-
Amino acid	-	+	-
Protein	+	-	+
Saponins	+	-	-
Tannins	-	-	-
Sterols	-	+	-
Terpenoids	+	+	-
Quinones	+	-	-
Oxalates	-	-	+

3.2 Total phenolic content Estimation

Quantitative analysis was performed to estimate the amount of polyphenolic content in *the Leucas aspera* plant extract using Folin-Ciocalteu's method. Phenolic compounds are found abundantly in most herbal plants for redox potential properties and antioxidant activity. Optical density was compared concerning different solvents of plant extract, keeping the standard solution as Gallic acid. The total phenol content was recorded at 650 nm under UV-Spectrometer (GAE/mg) representation of data was presented with the Gallic acid equivalent (fig 1).

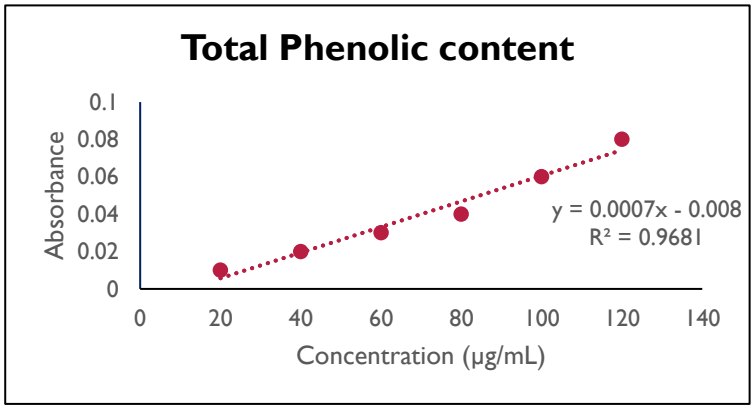

Fig 1: Total phenolic content present in *Leucas aspera* leaf extract

3.3 Total flavonoid Content Estimation

The flavonoid compound derived from *Leucas aspera* was determined to be 3.85 - 3.42 mg/g of ethyl acetate, which was determined in the methanolic extract, whereas the aqueous-based solvent was estimated as 3.23 mg/g, assessed in terms of quercetin equivalents per milligram (QAE). Significantly, the methanolic extract is represented in (fig 2).

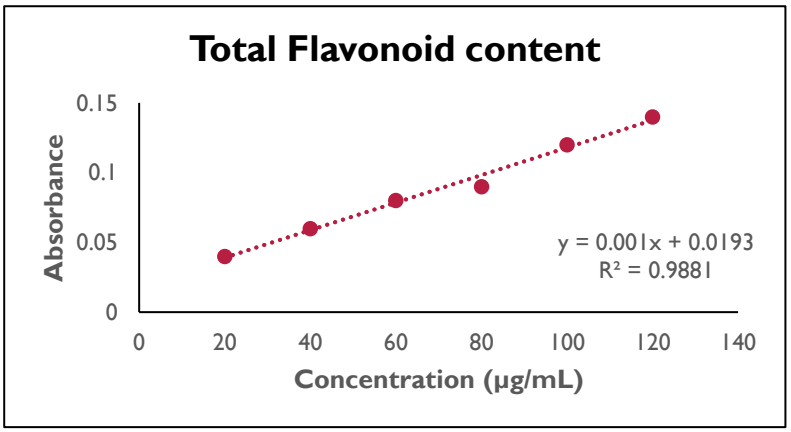

Fig 2: Total flavonoid content present in *Leucas aspera* leaf extract

3.4 1,1diphenyl-2-picryl-hydrazyl (DPPH) antioxidant assay

The assay for DPPH radical scavenging assay was carried out comparing methanolic, moderately polar solvent ethyl acetate, and aqueous extract of *Leucas aspera* and found that methanolic extract has better inhibition properties compared with the two polar solvents. The DPPH compound contains radicals that help in neutralizing the free radical ions inhibiting oxidative stress. The formation of a yellow color at the endpoint of the reaction post 30 minutes of incubation indicates the activation of the hydrogen atom. The radical scavenging activity increases with the increase in the concentration of the sample, keeping ascorbic acid as a standard. The samples after the incubation duration were measured and recorded at the absorbance 517nm, and the IC_{50} values were calculated and found to be 19.94 µg/ml in methanol and 10.09 µg/ml for aqueous extract (fig 3 (A) compared with the standard curve (fig 3(D).

3.5 Assay for Nitric Oxide Scavenging Assay

The scavenging activity of the nitrate ion present in the sample was determined by performing the test for radical scavenging assay. Inhibition in the nitric oxide concentration concerning *Leucas aspera* of the methanolic extract was found to be greater than aqueous and ethyl acetate extract, taking ascorbic acid as standard. The scavenging percentage IC_{5O} calculated was

calculated to be 89.60 47 µg/mL and 63.29 µg/mL aqueous extract (fig 3 (B) compared with the standard curve (fig 3(D).

3.6 ABTS (2,2 azobis-(3-ethylbenzothiozoline-6-sulphonic acid) assay

The Scavenging of ABTS radical's activity of *Leucas aspera* concerning different solvents was measured, keeping ascorbic acid as the standard. Among all three extracts, methanolic extract exhibited a better percentage of inhibition properties than ethyl acetate and aqueous extract. The absorbance of the sample was measured at 734nm, and the IC_{50} values were calculated and found to be 48.03 µg/ml in methanol and 34.65 µg/ml for aqueous extract (fig 3 (C) compared with the standard curve (fig 3(D).

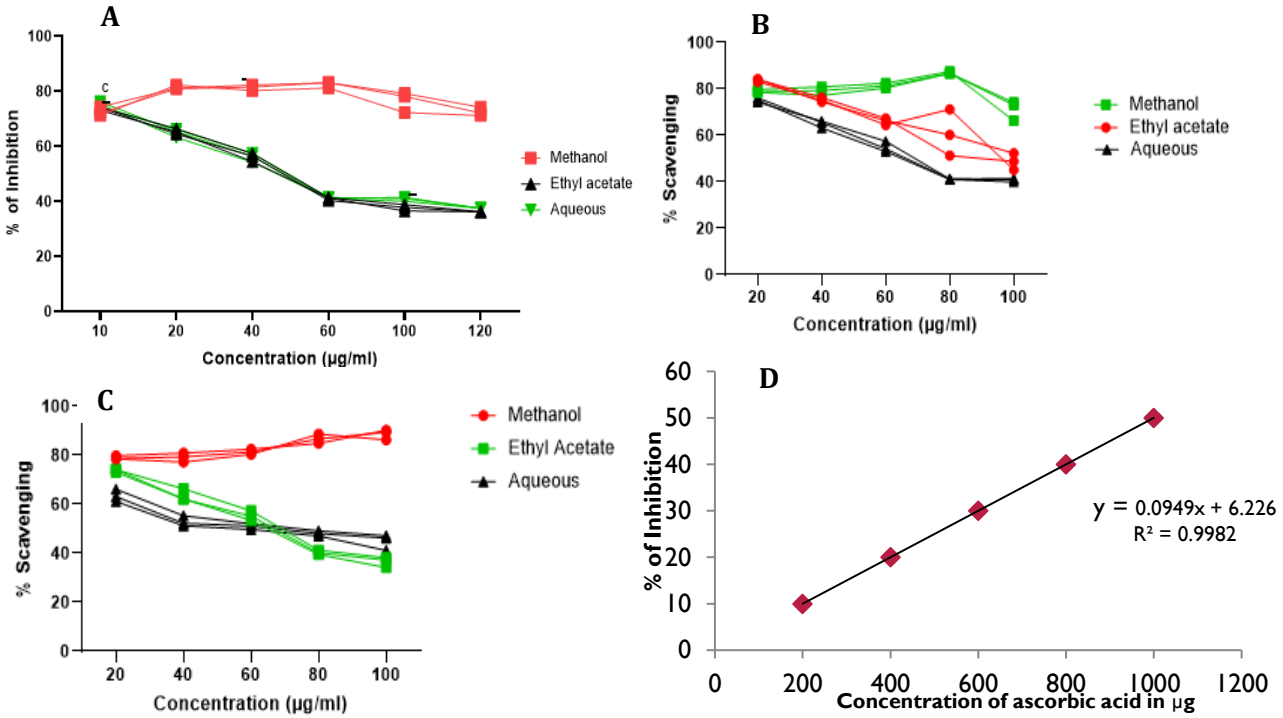

Figure 3: *Leucas aspera* leaf extract activity (A) DPPH Scavenging Assay comparison of different extracts of *leucas aspera* in methanol, ethyl acetate, and aqueous (B) Nitric oxide scavenging activity of extract (C) ABTS scavenging activity of the extract (D) Standard curve for ascorbic acid

3.7 Antimicrobial assay

From the *Leucas aspera*, three extracts and samples were tested for antimicrobial activity against the bacterial and fungal strains. Each isolate underwent sub-culturing on Nutrient Agar plates to eliminate adherent plant metabolites from the mycelia and was subsequently stored at 4 ºC for further investigations. The identified *Leucas aspera* strains' ability to combat bacteria was examined by targeting both Gram-negative (*Klebsiella pneumoniae)* and gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacterial species (Table 2).

Table 2: In Vitro Anti-bacterial and Anti-Fungal activity of Plant extract, and measurement of Diameter for the Zone of Inhibition (mm) against the bacterial and fungal strain.

Sample	Solvent	Bacterial Strain				Fungal Strain		
		Gram -positive		Gram -negative		Gram -positive		Gram -negative
		B. subtilis	*S. aureus*	*K. pneumoniae*	*E. coli*	*C. albicans*	*P. chrysogenum*	*A. niger*
Leaf	Methanol	10 ± 0.6^{a}	8.0 ± 0.7^{c}	9.0 ± 0.5^{b}	8.25 ± 0.8^{b}	7.5 ± 0.6^{b}	8.0 ± 0.9^{c}	8.5 ± 0.9^{c}
Leaf	Aqueous	9.25 ± 0.3^{b}	6.25 ± 0.28^{b}	7.25 ± 0.36^{b}	7.38 ± 0.8^{a}	8.0 ± 0.3^{b}	7.8 ± 0.3^{b}	8.2 ± 0.7^{c}

All three fungal isolates displayed antimicrobial activity, with notable efficacy against *Candida albicans* (7.5 ± 0.6) compared to other isolates. The isolate *Penicillium chrysogenum* exhibited antimicrobial activity with an inhibitory concentration measuring 8.0 ± 0.9 in the methanolic extract and 8.5 ± 09 in the aqueous extract. Additionally, the positive control, Ciprofloxacin, demonstrated inhibition of the fungi, producing an inhibitory concentration with a diameter of 11.0mm. These findings underscore the antimicrobial potential of the isolated *Leucas aspera* plant extract against fungal and bacterial strains, particularly in their activity with *Candida albicans*, and highlight the efficacy of *Penicillium chrysogenum* in different solvent extracts. The positive control further validated the inhibitory effects observed in the experimental isolates (fig 4).

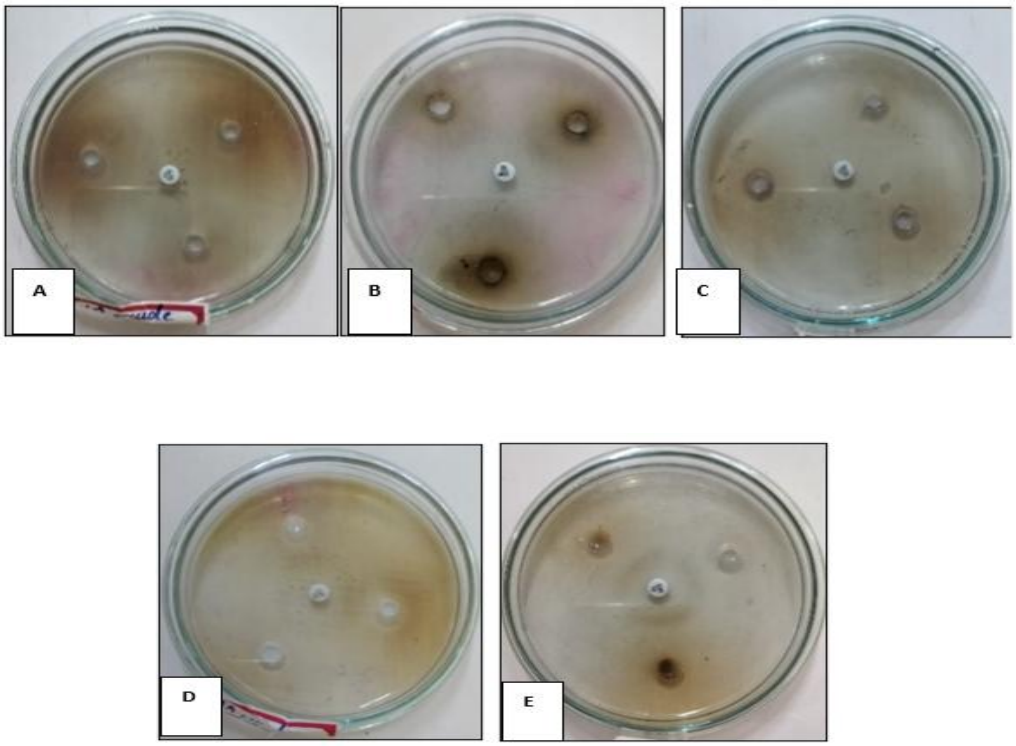

Fig 4: Antimicrobial activity (zone of inhibition) of *Leucas aspera* leaves methanolic extract, the diameter of zone of inhibition (mm) labeled as A, B, C, D, E concerning (A) *Bacillus subtilis*, (B) *Staphylococcus aureus*, (C) *Klebsiella pneumoniae*, (D) *E. coli* (E) *Candida albicans* measurements were taken at a concentration of 20% (w/v) DMSO as negative control while 10% (w/v) Ciprofloxacin was used as positive control.

3.8 Column Chromatography

The pure plant extract fractions were taken and applied to the silica gel column in which the plant extract was run. Two active fractions were taken on the TLC. The active fraction of (Rf 2.31). The active components were collected and concentrated, and the compounds were analyzed.

4. DISCUSSION

The research highlights the significant role of phytochemicals extracted from the methanolic extract of the *Leucas aspera* plant sample, which has been shown to exhibit neuroprotective activity. Reactive oxidative stress and mitochondrial dysfunction are the key factors mitigating neuronal loss and nerve damage (20). The finding in the study indicates that *Leucas aspera* methanolic extract exhibits higher antioxidant properties and antimicrobial activity towards the scavenging of free radical ions present. As evidence of the study implies, the IC_{50} value of the DPPH and Nitric oxide, ABTS assay exhibited concerning methanolic extract is 19.94, 89.60, and 48.30 (µg/mL). These results not only exhibit the higher scavenging ability of the extract but also show the potential bioactive compounds present in the leaves to release the oxidative stress causing neurodegeneration (21, 22).

The higher antioxidant properties of the herbal plant extract and the anti-microbial property of the *Leucas aspera* extract also help in enhancing its therapeutic values concerning the infection against any bacterial or fungal strains (23-28). The study showed an assessment of the microbial study for *Bacillus subtilis* and *Staphylococcus aureus* a significant inhibitory effect, exhibiting natural potential to fight against the bacterial strain of methanolic extract. The existence of secondary metabolites

and bioactive compounds present in the extract helps in neuronal inflammation and symptomatic disability concerning the disorder. *Leucas aspera* extract acts as a multifactorial approach towards neuroprotection, considering antimicrobial and antioxidant properties. Future research can explore the mechanism of the individual compounds targeting the specific gene involved in the development of neurological dysfunction, which could act as a cure for neurodegenerative diseases.

5. CONCLUSION

Leucas aspera is used as a medicinal plant that has traditionally been used in various systems of therapeutic medicine, including Ayurveda, for its potential health benefits. Antioxidant analysis of the extracted herbal formulation was evaluated to determine the inhibit oxidation, which can help in protecting normal cells from external radical causing stress and damage. Antioxidant results would indicate the potency of the extract in terms of its ability to scavenge free radicals or reduce oxidized compounds. The bioactive compounds derived from plants are mainly beneficial in neurological disorder management. The compounds have the potential to mitigate free radicals. Additionally, antioxidant activity is just one aspect of the potential health benefits of *Leucas aspera*, and future research is often required to understand its mechanism of therapeutic action involved in the management and cure for the neurological dysfunctioning. This study suggests the potential efficacy of *Leucas aspera* for antioxidant potential concerning its beneficial role in the control and management of the neurodegenerative causes.

6. ACKNOWLEDGEMENT

We would like to acknowledge REVA University for their Research facility and constant support to carry out the research work.

7. CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

[1] Roy, U. B., Keservani, R. K., Kesharwani, R. K., Jyothi, S. R., Akhila, A., Dakshayini, P. N., & Patil, S. J. (2024). Axonal pathology in traumatic brain injury: An overview. In R. N. Chaurasia, S. Ohia, & D. Bagchi (Eds.), *A review on diverse neurological disorders: Pathophysiology, molecular mechanisms, and therapeutics* (1st ed.). Elsevier. https://doi.org/10.1016/B978-0-323-95735-9.00045-0

[2] Collaborators, D. F. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: An analysis for the Global Burden of Disease Study 2019. *The Lancet. Public Health, 7*(2). https://doi.org/10.1016/S2468-2667(21)00249-8

[3] Delic, V., Beck, K. D., Pang, K. C. H., et al. (2020). Biological links between traumatic brain injury and Parkinson's disease. *Acta neuropathol commun, 8*(45). https://doi.org/10.1186/s40478-020-00924-7

[4] Lobine, D., Sadeer, N., Jugreet, S., Suroowan, S., Keenoo, B. S., Imran, M., Venugopala, K. N., Ibrahim, F. M., Zengin, G., & Mahomoodally, M. F. (2021). Potential of medicinal plants as neuroprotective and therapeutic properties against amyloid-β-related toxicity and glutamate-induced excitotoxicity in human neural cells. *Current Neuropharmacology*, *19*(9). https://doi.org/10.2174/1570159X19666210412095251

[5] Olufunmilayo, E. O., B. M., & Holsinger, R. M. (2023). Oxidative stress and antioxidants in neurodegenerative disorders. *Antioxidants, 12*(2), 517. https://doi.org/10.3390/antiox12020517

[6] Chaachouay, N., & Zidane, L. (2024). Plant-derived natural products: A source for drug discovery and development. *Drugs and Drug Candidates, 3*(1), 184-207. https://doi.org/10.3390/ddc3010011

[7] Kennedy, D. O., & Wightman, E. L. (2011). Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition, 2*(1), 32. https://doi.org/10.3945/an.110.000117

[8] Teleanu, R. I., Chircov, C., Grumezescu, A. M., Volceanov, A., & Teleanu, D. M. (2019). Antioxidant therapies for neuroprotection—A review. *Journal of Clinical Medicine, 8*(10), 1659. https://doi.org/10.3390/jcm8101659

[9] Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A., & Jaremko, M. (2019). Important flavonoids and their role as a therapeutic agent. *Molecules, 25*(22), 5243. https://doi.org/10.3390/molecules25225243

[10] Kolgi, R. R., Haleshappa, R., Sajeeda, N., Keshamma, E., Karigar, C. S., & Patil, S. J. (2021). Antioxidant and anticancer properties of ethanol extracts of *Leucas aspera*. *Asian Journal of Biological and Life Sciences, 10*(1), 165-171.

[11] Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy & Bioallied Sciences, 12*(1), 1.

https://doi.org/10.4103/jpbs.JPBS*17*519

[12] Haleshappa, R., Patil, S. J., & Murthy, S. M. S. (2021). Phytochemical analysis, in vitro evaluation of antioxidant and free radical scavenging activity of *Simarouba glauca* seeds. *Advances in Pharmacology and Pharmacy, 9*(1), 01-08. https://doi.org/10.13189/app.2021.090101

[13] Perez, M., Dominguez-López, I., & Lamuela-Raventos, R. M. (2023). The chemistry behind the Folin–Ciocalteu method for the estimation of (poly)phenol content in food: Total phenolic intake in a Mediterranean dietary pattern. *Journal of Agricultural and Food Chemistry, 71*(46). https://doi.org/10.1021/acs.jafc.3c04022

[14] Pandey, B., & Meena, R. (2015). Estimation of total phenolic and flavonoid contents in some medicinal plants and their antioxidant activities. *Nepal Journal of Science and Technology, 15*(1), 53–60. https://doi.org/10.3126/njst.v15i1.12010

[15] Haleshappa, R., Patil, S. J., Usha, T., & Murthy, S. M. (2020). Phytochemicals, antioxidant profile and GCMS analysis of ethanol extract of *Simarouba glauca* seeds. *Asian Journal of Biological and Life Sciences, 9*(3), 379-385.

[16] Cano, A., Maestre, A. B., & Arnao, M. B. (2022). ABTS/TAC methodology: Main milestones and recent applications. *Processes, 11*(1), 185. https://doi.org/10.3390/pr11010185

[17] Boora, F., Chirisa, E., & Mukanganyama, S. (2013). Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. *Journal of Food Processing, (1)*. https://doi.org/10.1155/2014/918018

[18] Sreedharan, S., Gothe, A., Aier, K., Kirankumar, S. V., Kumar, K. P., & Patil, S. J. (2020). Bioactive molecules and antimicrobial studies of *Rhus semialata* seeds. *Research Journal of Medicinal Plants, 13*(1), 10-17.

[19] Daf, A. N., et al. (2023). Comparison of different solvent and extraction methods for isolation of flavonoids compound from leaves of *Clerodendrum infortunatum* Linn. *Journal of Pharmacognosy and Natural Products, 8*(8). https://www.researchgate.net/publication/371721735

[20] Rahman, M. A., & Islam, M. S. (2013). Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract. *Asian Pacific Journal of Tropical Biomedicine, 3*(4), 273. https://doi.org/10.1016/S2221-1691(13)60062-3

[21] Vasudha, K., Archana, D., Mutyalamma, B., & Kishori, B. (2019). Phytochemical screening, antimicrobial, and antioxidant activities of root and leaf extracts of *Leucas aspera*. *Asian Journal of Pharmaceutical and Clinical Research, 12*(3), 141–147. https://doi.org/10.22159/ajpcr.2019.v12i3.29085

[22] Bairagi, J. H., Haritha, G., Yadav, L., Garg, S., Rani, V., Pulipati, S., Kolgi, R. R., Pundir, R., & Patil, S. J. (2023). To study of *Artemisia nilagirica* leaves for their antithyroid, oxidative and antihyperglycemic properties. *Journal of Advanced Zoology, 44*(S4), 40–51.

[23] Jamkhande, P. G., Wattamwar, A. S., Pekamwar, S. S., & Chandak, P. G. (2014). Antioxidant, antimicrobial activity and in silico PASS prediction of *Annona reticulata* Linn. root extract. *Beni-Suef University Journal of Basic and Applied Sciences, 3*(2), 140-148. https://doi.org/10.1016/j.bjbas.2014.05.008

[24] Premalatha, S. J., & Patil, S. J. (2022). Isolation and characterization of pharmacological endophytic fungi from *Cassia fistula* leaves. *Journal of Pharmaceutical Negative Results, 13*(Spl. 10), 3594-3597.

[25] Jyothi, S. R., Malathi, H., & Patil, S. J. (2023). Efficacy of Graviola seed extract (*Annona muricata*: Annonaceae) on E-Cadherin gene regulation and cytotoxicity in MDA-MB-231 cell lines. *Asian Journal of Pharmaceutics, 17*(1), 38-42.

[26] Keservani, R. K., Shelke, S. J., Gawali, V., Gaviraj, E. N., Binorkar, S. V., Rane, S. S., Sarvadnya, A. A., & Patil, S. J. (2024). Anti-Alzheimer effect of *Ammannia baccifera* whole plants ethanolic extract. *International Journal of Zoological Investigations, 10*(2), 671-678.

[27] Giri, S., Jamade, P. S., Pendakur, B., Sanjotha, G., Manawadi, S., Binorkar, S. V., Rao, N. S., & Patil, S. J. (2024). Anticancer, antidiabetic, antioxidant properties and phytoconstituents of efficacy of methanolic extract of *Euphorbia milii* leaves. *African Journal of Biological Sciences, 6*(6), 5419-5429.

[28] Devika, S. N. C., Keerthana, M., Dsouza, M. R., Patil, S. J., & Premalatha, S. J. (2024). Comparative in vitro study of the antidiabetic, anti-inflammatory, and antioxidant potential of *Piper cubeba*, *Piper betle*, and *Piper nigrum*. *The Bioscan, 19*(10-S1), 238-249.