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Evaluation of Antioxidant and Neuroprotective Activity of *Moringa Oleifera* and *Plectranthus Amboinicus* Leaves Against Oxidative Stress- Induced Neurodegeneration

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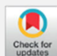

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Abstract: Neurodegenerative disorders are closely linked to oxidative stress, which causes damage to nerve cells and leads to memory and cognitive problems. The aim of this study was to evaluate the antioxidant and neuroprotective effects of *Moringa oleifera* and *Plectranthus amboinicus* leaf extract using in-vitro cell models. Fresh *Moringa oleifera* and *Plectranthus amboinicus* leaves were collected, authenticated, and extracted using the Soxhlet method, which produced a yield of 25.3%. The antioxidant activity of the extract was tested using DPPH and hydrogen peroxide scavenging assays. The results showed that the extract scavenged free radicals in a concentration-dependent manner. The IC_{50} values were found to be 87.31 µg/ml for the DPPH assay and 49.78 µg/ml for the hydrogen peroxide assay, indicating good antioxidant potential. Cell viability was studied using the MTT assay on Neuro-2A cells. The results confirmed that the extract was non-toxic at lower concentrations, demonstrating good cytocompatibility with neuronal cells. The neuroprotective effect of the extract was further supported by significant inhibition of acetylcholinesterase activity, suggesting a role in improving neurotransmitter function. In addition, the reactive oxygen species (ROS) assay showed that treatment with the extract significantly reduced intracellular ROS levels in hydrogen peroxide-treated cells, confirming its protective effect against oxidative stress. Overall, the study indicates that *Moringa oleifera* and *Plectranthus amboinicus* extract are a rich source of natural bioactive compounds with strong antioxidant and neuroprotective properties. Future studies should focus on identifying the active phytochemicals, understanding the molecular mechanisms involved, and confirming these effects using in-vivo animal models. This may help in the development of plant-based therapies for neurodegenerative diseases.

Keywords: *Moringa oleifera* and *Plectranthus amboinicus,* Antioxidant activity, Neuroprotection, Oxidative stress, Neurodegenerative disease.

1. INTRODUCTION:

1.1. Oxidative Stress:

Oxidative stress is the condition that results from the buildup of harmful substances called free radicals in the body. The body has its own protective system that keeps the amount of free radicals at a safe level. The free radicals are also known as reactive oxygen species (ROS). ROS are produced in the body as a result of normal body functions like breathing, digestion, and energy production. In a normal body, antioxidants help to neutralize the effects of ROS. But in cases where antioxidants are not sufficient to neutralize ROS, oxidative stress occurs. [1][2]. When the amount of ROS in the body gets too high, they start to damage critical parts of the cell. For instance, ROS damage fats in the cell membrane. As a result, the cell membrane becomes weak. ROS can also damage proteins in the body. Damage to proteins results in the alteration of enzyme functions. ROS can also damage DNA. DNA damage can cause genetic changes. All these changes cause the body to age. [2][3]

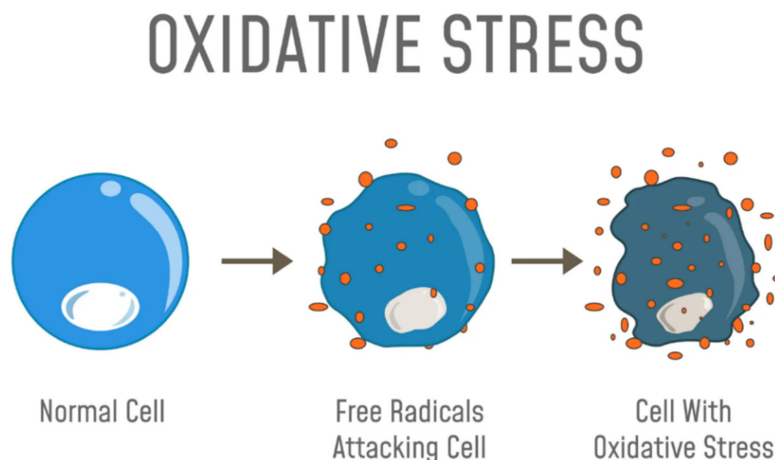


Fig. 1: Cells with oxidative stress

Oxidative stress is associated with various long-term diseases, including heart disease, diabetes, cancer, and brain disorders such as Alzheimer and Parkinson diseases. This is because the brain is more susceptible to oxidative damage due to its high oxygen requirement and low antioxidant defense capacity. [4][5]

Oxidative stress can be caused by various factors. These include air pollution, smoking, alcohol, radiation, infections, prolonged inflammation, mental stress, and unhealthy food habits. Consuming less fruit and vegetables can increase oxidative damage due to low antioxidant intake. [3][6]

The body has its defense mechanisms against oxidative stress in the form of antioxidants. These include antioxidants that are enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and antioxidants that are vitamins, such as vitamin C and E, and flavonoids and polyphenols, which are plant-based compounds and herbs. [2][6]

Reactive oxygen species are mainly produced in the mitochondria during the production of energy. At low levels, they are useful in the process of cell communication and immune response. However, problems arise when the production of ROS becomes excessive. [2][7]

Oxidative stress causes chronic inflammation in the body. When there is inflammation, the production of ROS increases, thus promoting further damage. This leads to continuous damage in the body. [4][5]

Oxidative stress also causes damage to the mitochondria. When the mitochondrial DNA and proteins are damaged, the production of energy is reduced, and the production of ROS increases. This

process plays an essential role in the process of aging and brain conditions such as stroke, Alzheimer's, and Parkinson's. [5][7]

Oxidative stress causes damage in the nervous system. It damages nerve cells through the degradation of the cell membrane, interference with calcium, and the activation of cell death. Nerve cells are very sensitive since they require high amounts of energy and cannot be repaired. Thus, oxidative stress plays a major role in neurodegeneration. [5][8]

The level of oxidative stress can be determined by using markers that include malondialdehyde, protein carbonyls, and 8-hydroxy-2′-deoxyguanosine, which are used to determine lipid peroxidation, protein damage, and DNA damage, respectively. Antioxidant levels can be determined by measuring the activity of antioxidant enzymes and the total antioxidant capacity. [3][6]

Oxidative stress can be managed by improving lifestyle and dietary habits. Antioxidants that can be obtained from fruits, vegetables, herbs, and spices can help in protecting cells against oxidative damage. [1][2][6]

1.2. Long-Term Complications of Oxidative Stress:

When oxidative stress occurs for a long time, it leads to permanent damage of cells. This damage occurs slowly and leads to aging and other diseases. [1][9]

Oxidative stress is responsible for various heart diseases, such as high blood pressure, blocked arteries, heart attack, and stroke. Oxidative stress damages blood vessels and increases cholesterol oxidation. [4][10]

In diabetes, oxidative stress is caused by increased glucose levels, leading to an increase in ROS. Oxidative stress damages insulin-producing cells and leads to other complications, such as nerve damage, kidney disease, and eye damage. [3][11]

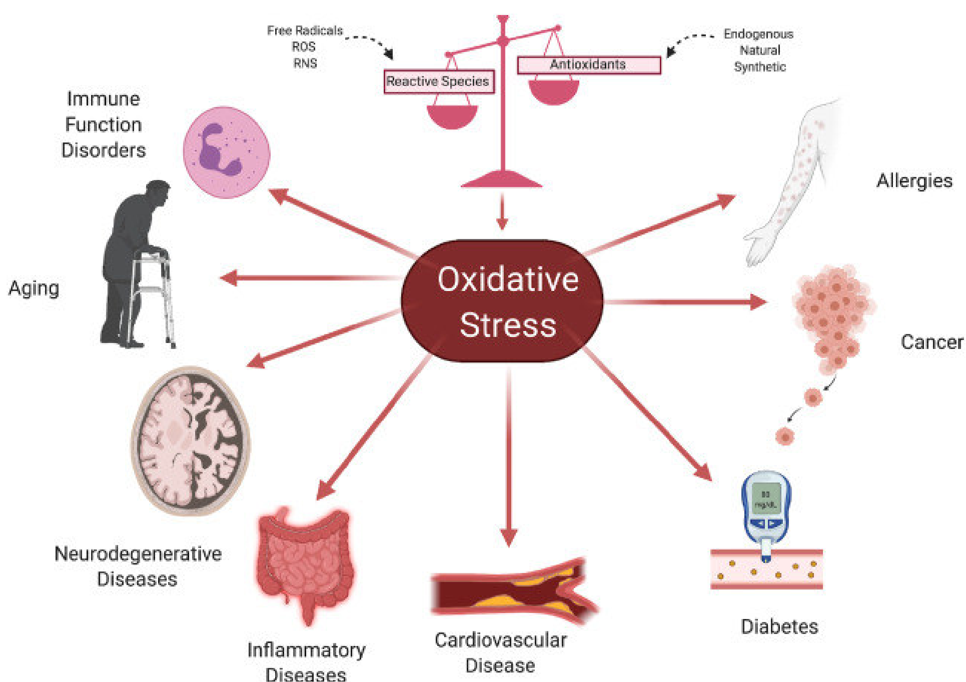


Fig. 2: Long-term complications of oxidative stress

Oxidative stress can lead to cancer because it damages DNA. The abnormal cells will start growing uncontrollably. [2][12]

When oxidative stress acts for a long time in the brain, it leads to diseases like Alzheimer's disease and Parkinson's disease. Oxidative stress results in nerve cell death, memory loss, and reduced brain functions. [5][8]

When oxidative stress acts for a long time in the body, it results in long-term inflammation. Long-term inflammation results in the production of more ROS. Long-term inflammation can lead to diseases like arthritis, asthma, and bowel disorders. [4][5]

Aging can be associated with oxidative stress. In old people, the ability to repair cells is impaired. The old have low immunity. The old are prone to diseases. [9][13]

When oxidative stress acts for a long time in the body, it can lead to fertility problems. In men, oxidative stress can lead to poor sperm quality. In women, oxidative stress can lead to hormonal imbalance and premature ovarian aging. [3][14]

1.3. Antioxidant Activity and Neuroprotection:

Antioxidants play an important role in protecting the body. They do this by eliminating free radicals. They stop harmful reactions from occurring, which can harm fats, proteins, and DNA. [2][6]

Oxidative stress can have a big effect on the brain. Antioxidants play an important role in protecting nerve cells. They do this by reducing oxidative stress, improving energy production, reducing inflammation, and protecting nerve cells from death. [5][7][8]

1.4. Bioactive Compounds with Antioxidant Activity:

Bioactive compounds are natural compounds found in plants, and they promote health in the body. Bioactive compounds include antioxidants, which protect the body from damage. [15][16]

Polyphenols and flavonoids help in reducing oxidative stress and inflammation in the body. Phenolic acids protect DNA and protein, while carotenoids protect membranes from damage. Vitamins C, E, and A help in enhancing antioxidant defense. Alkaloids, tannins, saponins, and glucosinolates also help in reducing free radical damage and enhancing antioxidant defense. [15][16]

1.5. Plant profile:

Moringa oleifera

a. Fig. 3: Leaves of Moringa oleifera

BOTANICAL NAME: Moringa oleifera Lam.

FAMILY: Moringaceae

BOTANICAL DESCRIPTION:

Moringa oleifera is a hardy perennial tree which sheds its leaves seasonally and grows to a height of 5-12 meters. The trunk is light grey, soft, and corky with fragile branches which droop. The leaves are light green, alternate, and arranged in three levels of leaflets, giving the impression of a tripinnate leaf. The flowers are pale yellow to creamy white, fragrant, and arranged in loose axillary racemes, with the main flowering season from March to June. The fruits are elongated, three-angled pods commonly referred to as drumsticks, each containing 10-20 rounded seeds with thin, winged papery structures.

TAXONOMICAL CLASSIFICATION:

- Kingdom: Plantae
- Clade: Angiosperms
- Clade: Eudicots
- Clade: Rosids
- Order: Brassicales
- **Family:** Moringaceae
- **Genus:** Moringa
- **Species:** Moringa oleifera

VERNACULAR NAMES:

- **Tamil:** முருங்கை (Murungai)
- **English:** Drumstick tree / Horseradish tree
- **Hindi:** सहजन (Sahjan)
- **Malayalam:** മുരിങ്ങ (Muringa)
- **Telugu:** మునగ (Munaga)

SYNONYMS:

- Moringa pterygosperma Gaertn.
- Hyperanthera moringa (Lam.) Vahl

PHYTOCHEMICAL CONSTITUENTS:

Alkaloids:

- Moringine
- Moringinine

Flavonoids:

- Quercetin
- Kaempferol
- Myricetin

Phenolic Acids:

- Gallic acid
- Chlorogenic acid
- Caffeic acid
- Ferulic acid

Glycosides:

- Niazirin
- Niaziminin
- Quercetin glycosides

Terpenoids and Sterols:

- β-Sitosterol
- Lupeol
- Ursolic acid

Other Constituents:

- Tannins
- Saponins
- Vitamins such as A, C, and E
- Minerals such as calcium, iron, and potassium

TRADITIONAL USES:

- The leaves and bark are used to treat fever

- It is used topically to treat inflammation and joint pain
- It is used to treat digestive problems such as diarrhea and ulcers
- It is used to treat respiratory problems such as cough, asthma, and bronchitis
- The leaves are used as a nutritional supplement
- The seeds are used to purify water

MEDICINAL USES:

- It is used as an antipyretic
- It is used to treat arthritis and rheumatism
- It is used to treat infections caused by bacteria and fungi
- It is used to treat diabetes by controlling the level of blood glucose
- It is used to treat oxidative stress
- It is used to treat neurological disorders such as brain functions

Plectranthus amboinicus

b. Fig. 4: Leaves of Plectranthus amboinicus

BOTANICAL NAME: Plectranthus amboinicus (Lour.) Spreng.

FAMILY: Lamiaceae

BOTANICAL DESCRIPTION:

Plectranthus amboinicus is a perennial, aromatic, and succulent herb that grows to a height of 30 to 90 cm. The plant has thick, fleshy, and highly branched stems, and the stems emit a strong oregano-like aroma. The simple, oppositely arranged, broadly ovate-shaped leaves of the plant are densely covered with soft hairs, giving the plant a velvety texture and crenate margins. The pale violet to bluish-purple flowers are arranged in spike-like inflorescences, and the flowering period is between October and February. The formation of seeds is rare in the plant, and the plant is propagated through vegetative means only.

TAXONOMICAL CLASSIFICATION:

- Kingdom: Plantae
- **Clade:** Angiosperms
- **Clade:** Eudicots
- **Clade:** Asterids
- **Order:** Lamiales
- **Family:** Lamiaceae
- **Subfamily:** Nepetoideae

- **Genus:** Plectranthus
- **Species:** Plectranthus amboinicus

VERNACULAR NAMES:

Tamil: கற்பூரவல்லி (Karpooravalli)

- **English:** Indian borage / Cuban oregano
- **Hindi:** पान अजवाइन (Paan Ajwain)
- **Malayalam:** പനിക്കൂർക്ക (Panikoorka)
- **Telugu:** వాము ఆకులు (Vaamu aakulu)

SYNONYMS:

- Coleus amboinicus Lour.
- Coleus aromaticus Benth.
- Plectranthus aromaticus Roxb.

PHYTOCHEMICAL CONSTITUENTS:

Alkaloids:

- Found in trace amounts

Flavonoids:

- Quercetin
- Apigenin
- Luteolin

Phenolic Compounds:

- Rosmarinic acid
- Caffeic acid
- Ferulic acid

Terpenoids (Essential oil constituents):

- Carvacrol
- Thymol
- β-Caryophyllene
- γ-Terpinene
- p-Cymene

Sterols:

- β-Sitosterol

Other Constituents:

- Tannins
- Saponins
- Volatile oils
- Resins

TRADITIONAL USES:

- Used for the treatment of cough, cold, asthma, and bronchitis
- Used for the relief of digestive disorders such as indigestion, diarrhea, flatulence, etc.
- Traditionally used for the reduction of fever
- Applied topically for the treatment of wounds, burns, insect bites, etc.
- Used for the promotion of lactation
- Traditionally used as an antiseptic for oral, throat, etc.

MEDICINAL USES:

- Shows antimicrobial activity against bacteria and fungi
- Has anti-inflammatory properties, which relieve pain, etc.
- Works as an antioxidant
- Works as an expectorant, which removes mucus from the lungs
- Provides neuroprotective effects by reducing oxidative neuronal damage

2. LITERATURE REVIEW:

Moringa oleifera

1. **Worku B, Tolossa N. (2024).** ***A Review on the Neuroprotective Effect of Moringa oleifera.*** Moringa oleifera is also known as the drumstick tree. It has good natural compounds like flavonoids, phenols and thiocyanates. People use parts of the plant like leaves, roots, bark, flowers and seeds for health reasons. These compounds help protect brain cells. They do this by reducing inflammation. They also help control stress. They support neurotransmitters that help with memory and emotional balance. The review says Moringa oleifera might help prevent diseases that affect the brain. More studies, on humans are needed.
2. **Azlan UK, et al. (2023).** ***An Insight into the Neuroprotective and Anti-Neuroinflammatory Effects of Moringa oleifera.*** Neurodegenerative disorders have been linked with progressive neuronal injury, which is caused primarily by oxidative stress and inflammation. This article demonstrates that Moringa oleifera contains compounds that have the capacity to protect the brain from oxidative stress through the presence of compounds such as quercetin, chlorogenic acid, and moringin.
3. **Hashim FJ, et al. (2021).** ***Neuroprotective assessment of Moringa oleifera leaf extract in SH-SY5Y cells.*** This research was conducted to assess the neuroprotective potential of Moringa oleifera leaf extract in neuronal cell lines. The results revealed that the Moringa oleifera leaf extract significantly reduced reactive oxygen species and increased antioxidant defense in the cells. Compared to Panax ginseng, Moringa oleifera showed low toxicity but was efficient in providing neuroprotective potential. This indicates that Moringa oleifera leaves are efficient in providing safety and efficacy in protecting the neurons.
4. **Pareek A, et al. (2023).** ***Moringa oleifera: An updated comprehensive review.*** Moringa oleifera is commonly known as the "tree of life." This is because of the various medicinal properties of the plant. The plant has antioxidant, anti-inflammatory, anticancer, hepatoprotective, cardioprotective, and wound-healing properties. These properties are attributed to the flavonoid, vitamin, and terpenoid content of the plant.
5. **Vergara-Jimenez M, et al. (2017).** ***Bioactive compounds and neuroprotective benefits of Moringa oleifera.*** In this study, the role of bioactive compounds in the plant Moringa oleifera and the benefits derived from them were discussed. According to the findings, the bioactive compounds in the plant play a role in the regulation of significant signaling pathways, which are essential in the repair of tissues and the reduction of oxidative stress.
6. **Stohs SJ, Hartman MJ. (2015).** ***Review of safety and antioxidant mechanisms of Moringa oleifera.*** This review article discussed the safety profile and the antioxidant mechanisms of Moringa oleifera. Moringa leaf extract and seed extract have been proven to be safe for both human consumption and for use in animals. The antioxidant activity helps regulate blood sugar, lipid levels, as well as the protection of organs, especially the liver, heart, and kidneys, through flavonoids, polyphenols, and glucosinolates.
7. **Wedad S Alqahtani, Gadah Albasher. (2021).** ***Moringa oleifera Lam. extract rescues lead-induced oxidative stress, inflammation, and apoptosis in rat brain.*** Severe oxidative stress is induced in the brain of rats due to lead exposure. This study revealed that Moringa oleifera extract significantly reduced oxidative stress, inflammation, and apoptosis in the rat brain cortex. The protective

mechanisms were mediated through the enhancement of antioxidant enzyme activities and the regulation of the inflammatory response.

8. **Falguni Goel. (2025).** ***Exploring the therapeutic role of Moringa oleifera in neurodegeneration.*** Neurodegenerative diseases involve progressive neurodegeneration caused by oxidative stress and inflammation. This review paper indicates that Moringa oleifera has antioxidants and vital nutrients that safeguard neurons, improve memory, and support brain functions. Animal studies have proven that it has neuroprotective properties, and initial human studies have proven it is safe.
9. **Akinlolu A, et al. (2025).** ***Neuroprotective potentials of Moringa oleifera against cadmium-induced neurotoxicity.*** Exposure to cadmium has been known to induce oxidative stress and damage to the brain. This study showed that Moringa oleifera extract was effective in restoring antioxidant enzymes, which helped to reduce oxidative stress and damage to neurons.
10. **Acharya SK. (2025).** ***Neuroprotective role of Moringa oleifera in stress, anxiety, and neurodegenerative disorders.*** Moringa oleifera showed promising results in the protection against oxidative stress and inflammation caused due to neurological disorders. Moringa oleifera enhanced memory, regulated the level of neurotransmitters, reduced anxiety, and provided protection to neurons.
11. **Cuschieri A, Camilleri E, Blundell R. (2023).** ***Cerebroprotective effects of Moringa oleifera in ischemic stroke.*** This article is a systematic review and meta-analysis that included studies on animal models of ischemic stroke. Moringa oleifera supplementation decreased brain injury and oxidative stress, as well as increasing antioxidant enzymes. These results show the possible cerebroprotective activity of Moringa oleifera, but more studies have to be conducted to support the results.
12. **El-Sherbiny GM, et al. (2024).** ***Antibacterial, antioxidant, cytotoxicity, and phytochemical screening of Moringa oleifera leaves****.* Moringa oleifera leaf extract demonstrated significant antioxidant and antibacterial potential due to the high content of phenolic and flavonoid compounds, especially quercetin. The extract was found to be safe at moderate concentrations and has potential uses in the pharmaceutical and food industries.
13. **Onasanwo SA, et al. (2021).** ***Long-term consumption of Moringa oleifera enhances neurocognition.*** In this study, rats were subjected to long-term supplementation with Moringa oleifera, which enhanced their memory and learning. In the study, the rats showed reduced oxidative stress, increased antioxidant enzyme activity, and changes in the structure of the hippocampus.
14. **Sahu KK, et al. (2025).** ***Amelioration of cognitive deficit by Moringa oleifera flower extract.*** In the study of Sahu KK, the researchers were able to improve memory and learning in Alzheimer's disease using the Moringa oleifera flower extract. The extract was shown to decrease amyloid-beta levels, antioxidants, and brain tissue protection.
15. **Kashif Ali, et al. (2020).** ***Protective effects of Moringa oleifera against arsenic-induced oxidative stress.*** This article showed that Moringa oleifera leaf extract had the capacity to protect against oxidative stress caused by sodium arsenate. It also showed that the extract had the capacity to protect against developmental damage.
16. **Ji Liu, et al. (2020).** ***Antioxidant protection of Moringa oleifera flavonoids in vitro.*** Moringa oleifera flavonoids from the leaves of the plant showed protective effects against oxidative stress induced by hydrogen peroxide. The flavonoids increased antioxidant enzymes, inhibited lipid peroxidation, and inhibited apoptosis, thus demonstrating their strong protective effects.
17. **Igado OO, Olopade JO. (2017).** ***Review on neuroprotective effects of Moringa oleifera leaf extract.*** This review article was based on experimental evidence showing the neuroprotective effects of Moringa oleifera leaves. Its nutritional and antioxidant content is beneficial for the brain and could be useful in the management of neurodegenerative disorders.

Plectranthus amboinicus

18. **Bhatt P, Negi PS. (2012).** ***Antioxidant and antibacterial activities in the leaf extracts of Indian borage (Plectranthus amboinicus).*** In this study, the antioxidant and antibacterial activities of the solvent extracts of Plectranthus amboinicus leaves have been evaluated. The results revealed that the ethyl acetate extract contained the highest phenolic content along with strong free radical scavenging activity. Moreover, the acetone extract displayed good antibacterial activity against food-borne pathogens. Therefore, the P. amboinicus leaves have the potential to function as an antioxidant and an antimicrobial agent.

19. **Manurung K, Sulastri D, Zubir N, Ilyas S. (2020).** ***In silico anticancer activity and in vitro antioxidant potential of flavonoids in Plectranthus amboinicus.*** The flavonoids, including luteolin, quercetin, apigenin, rutin, and chrysoeriol, were isolated from the Plectranthus amboinicus leaves. The results of the study indicated the potential of the isolated flavonoids in the treatment of cancer through the strong interaction of the isolated flavonoids with cancer receptors using the molecular docking method.

20. **Nor Asiah binti Muhamad Nor, et al. (2025).** ***Phytochemical screening and antioxidative potentials of Plectranthus amboinicus leaves extract.*** This study evaluated methanolic and aqueous leaf extracts of Plectranthus amboinicus for their potential as natural antioxidants. The methanolic extract of Plectranthus amboinicus leaves had higher phenolic and flavonoid content and antioxidant activity compared to the aqueous extract.

21. **Suja S, Indhumathi T. (2013).** ***Protective role of Plectranthus amboinicus in aluminium-induced neurotoxicity.*** This study aimed to evaluate the neuroprotective activity of Plectranthus amboinicus against aluminum-induced neurotoxicity in rats. Aluminum-induced neurotoxicity resulted in cognitive impairment and oxidative stress, and administration of Plectranthus amboinicus reversed cognitive functions and antioxidant defense.

22. **Victor Arokia Doss D, Nithyanand P, Sowndarya R. (2016).** ***Antidepressant effect of Plectranthus amboinicus in chronic stress-induced rats.*** This study evaluated the antidepressant activity of Plectranthus amboinicus leaf extract in rats exposed to chronic unpredictable mild stress. Treatment significantly reduced depressive behavior and improved antioxidant status in brain tissue. The antidepressant effect was comparable to standard medication, indicating therapeutic potential.

23. **Santos Filipe M, et al. (2025).** ***Plectranthus amboinicus: A systematic review of traditional uses, phytochemistry, and therapeutic applications.*** This systematic review discussed the traditional uses and pharmacological effects of Plectranthus amboinicus. The plant demonstrated potent antioxidant, antimicrobial, anti-inflammatory, wound-healing, and anticancer activity. Polyphenols were found to be responsible for its activity, thus giving the plant its medicinal value.

24. **Jakubowska M, et al. (2023).** ***Phytochemical profile and antioxidant activities of Coleus amboinicus Lour.*** This study examined the chemical composition and antioxidant activity of Coleus amboinicus, which is grown in diverse geographical locations. The study found differences in phenolic compounds and antioxidant activity of the plant based on the location of cultivation. The study implies that environment affects the medicinal value of the plant.

25. **Kuljanabhagavad T, et al. (2024).** ***Free radical scavenging and antimicrobial activity of Coleus amboinicus-mediated iron oxide nanoparticles.*** In this research, iron oxide nanoparticles were synthesized using the extract of the stem of the Coleus amboinicus plant. These nanoparticles were highly effective in their antioxidant and antimicrobial activities, especially against oral pathogens.

26. **Irwan, et al. (2023).** ***Effects of enzymatic treatment on phenolic content and antioxidant activity of Plectranthus amboinicus extracts.*** In this study, the researchers used cellulase and pectinase enzymes to assist in the extraction of bioactive compounds from the leaves of Plectranthus

amboinicus. The results showed that the extracts obtained using cellulase had higher phenolic content, antioxidant, and anti-inflammatory activities. The method was efficient and eco-friendly.

27. **Vijayanand S, Thiruvengadam SR, Malathi R. (2023).** ***Comparative antioxidant activity of stem and leaf extracts of Plectranthus amboinicus.*** This study was done to compare the antioxidant activities of fresh and dried leaf extracts of Plectranthus amboinicus and the extracts of the stem of the same plant. Fresh stem extract had the maximum phenolic content and strong free radical scavenging activity. This shows that the medicinal value of the different parts of the plants differs.
28. **Kuppusamy P, et al. (2017).** ***GC–MS based metabolite profiling and antioxidant properties of Plectranthus amboinicus.*** The study was conducted to evaluate the chemical composition of various solvent extracts of the plant using GC-MS techniques. The study revealed that the methanol extract of the plant exhibited the highest antioxidant and antimicrobial activities due to the presence of compounds such as carvacrol and squalene, which contribute to the medicinal properties of the plant.
29. **Blacio S, et al. (2025).** ***Chemical composition and antioxidant activity of Plectranthus amboinicus essential oil.*** Essential oils of Plectranthus amboinicus leaves from different locations were obtained, and their chemical composition was observed to vary. The main components of the essential oil were germacrene D, α-humulene, and carvacrol. The antioxidant property was also observed to vary.
30. **Nor Asiah binti Muhamad Nor, et al. (2025).** ***Antioxidative potentials of Plectranthus amboinicus leaves.*** This study confirmed that the methanolic extract of Plectranthus amboinicus leaves had stronger antioxidative activity than its aqueous extract. This study proved the value of Plectranthus amboinicus as a natural antioxidative agent.

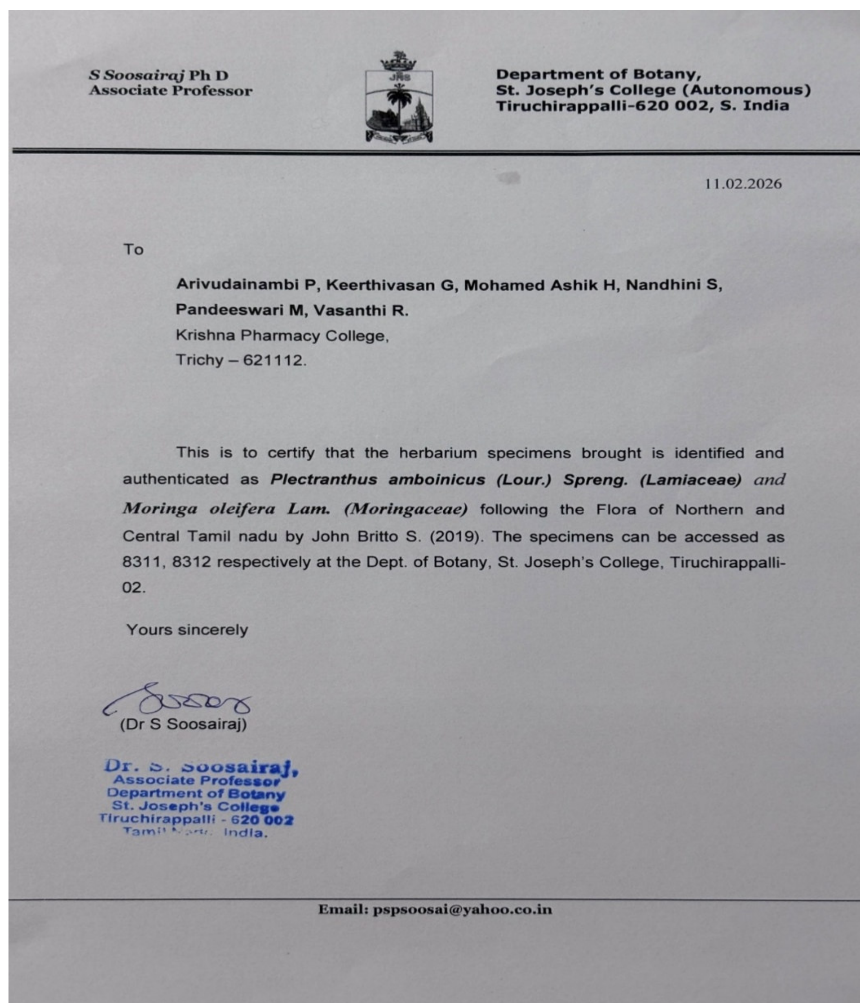
3. AIM:

To evaluate the antioxidant and neuroprotective potential of *Moringa oleifera* leaf extract against oxidative stress–induced neurodegeneration using in-vitro cellular models.

4. OBJECTIVE:

- To collect, authenticate, and prepare *Moringa oleifera* and *Plectranthus amboinicus* leaf material for experimental studies.
- To obtain bi-plant extract using Soxhlet extraction method.
- To evaluate the antioxidant activity of the extract using DPPH radical scavenging assay.
- To assess free radical scavenging activity using the hydrogen peroxide (H_2O_2) method.
- To determine the cytocompatibility of the extract in Neuro-2A cells using the MTT assay.
- To study the effect of the extract on neurotransmitter release through acetylcholinesterase (AChE) inhibition assay.
- To evaluate the neuroprotective effect of the extract against H_2O_2-induced oxidative stress by ROS fluorescent assay in Neuro-2A cells.
- To perform statistical analysis of experimental data using GraphPad Prism software.

5. COLLECTION AND AUTHENTICATION:



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This is to certify that the herbarium specimens brought is identified and authenticated as ***Plectranthus amboinicus (Lour.) Spreng. (Lamiaceae)*** *and* ***Moringa oleifera Lam. (Moringaceae)*** following the Flora of Northern and Central Tamil nadu by John Britto S. (2019). The specimens can be accessed as 8311, 8312 respectively at the Dept. of Botany, St. Joseph's College, Tiruchirappalli-02.

Yours sincerely

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6. METHODOLOGY:

6.1. SOXHLET EXTRACTION:

Soxhlet extraction is a very useful tool for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from particular interfering substances. Sample preparation of environmental samples has been developed for decades using a wide variety of techniques. Solvent extraction of solid samples, which is commonly known as solid–liquid extraction (also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid sample pretreatment. Conventional Soxhlet extraction remains as one of the most relevant techniques in the environmental extraction field.

Materials Required:

Ethanol was purchased from Merk, USA. Whatman No.1 filter paper was purchased from Millipore, USA.

Procedure:

1. Test sample can be fresh or dried. It needs to be crushed, using a pestle and mortar, to provide a greater surface area.
2. The test sample should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 20 g of thyme in a 25- x 80-mm thimble).
3. All equipment should be too assembled. Build a rig using stands and clamps to support the extraction apparatus.

4. Following this, the Ethanol is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle.
5. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor.
6. The side arm is lagged with glass wool.
7. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser.
8. The condensate then drips into the reservoir containing the thimble.
9. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again.
10. The process should run for a total of 8 hours.
11. Once the extraction set up, it can be left to run without direct supervision.
12. It is not advised to leave the equipment completely alone due to the mix of running water and an electrical appliance, so a technician or other lab user should be made aware.
13. The equipment can be turned off.

6.2. DPPH ASSAY:

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure 1, below, shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals (1). The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

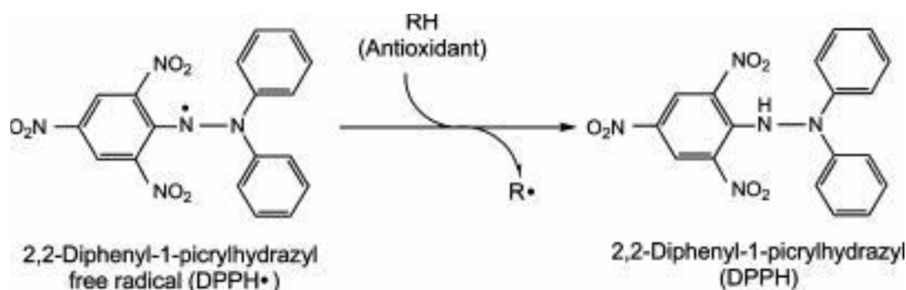

Fig. 5: DPPH Reaction

Material required:

0.1mM DPPH solution, Ascorbic acid, Methanol

mM DPPH Solution:

Dissolve 39 mg of DPPH in 100 ml of methanol and store at -20° C until needed.

Ascorbic acid (Standard):

1mg/ ml of Ascorbic acid

Procedure:

1. Briefly, prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of Sample at different concentration (500, 250, 100, 50, and 10 µg/ml).

2. The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes.
3. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference).
4. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity.
5. The capability of scavenging the DPPH radical can be calculated by using the following formula.
6. DPPH scavenging effect (% inhibition) = [(absorbance of control- absorbance of reaction mixture)/absorbance of control] X 100

6.3 HYDROGEN PEROXIDE SCAVENGING METHOD:

Principle:

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H2O2 can probably react with Fe2+, and possibly Cu2+ ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Material required:

Hydrogen peroxide solution and Sodium phosphate buffer.

Procedure:

Ability of plant extracts to scavenge hydrogen peroxide was estimated according to the method reported by Ruch et al. with minor modification. A solution of hydrogen peroxide (43 mM) is prepared in phosphate buffer (1 M pH 7.4). Different concentration of sample (500, 250, 100, 50 and 10 μg/ml) was added to a hydrogen peroxide solution (0.6 ml, 43 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating % inhibition as above. % inhibition = [(Control- Test)/control] ×100.

6.4 MTT ASSAY:

Principle:

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystal which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader.

Materials required:

Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), DMEM medium, 1X PBS, (India). 96 well tissue culture plate and wash beaker were from Tarson (India).

Procedure:

Cell culture:

Neuro 2a (Mouse Neuroblastoma Cell) Cell line were purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 μg/ml penicillin and 100 μg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C**.**

MTT assay:

The Test sample was tested for *in vitro* cytotoxicity, using Neuro 2a cells by MTT assay. Briefly, the cultured Neuro 2a cells were harvested by trypsinization and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 μL) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the Test sample in a serum-free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After incubation, MTT (10 μL of 5 mg/ml) was added to each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 μL) was aspirated off the wells and washed with 1X PBS (200 μl). Furthermore, to dissolve formazan crystals, DMSO (100 μL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA).

Formula: Cell viability % = Test OD/Control OD X 100

6.5. AChE ASSAY:

Material required:

Acetyl thiocholine iodide (ATCI), 5, 5- dithiobis (2- nitrobenzioc) acid (DTNB), sodium phosphate buffer, enzyme solution and test sample.

Procedure:

The modified method of Ellman et al. (1961) was used. Electric eel acetylcholinesterase was used, while acetyl thiocholine iodide (ATCI) was used as a substrate of the reaction. 5, 5- dithiobis (2-nitrobenzioc) acid (DTNB) was used for the measurement of AChE activity. In this procedure, 150 μl of 0.1 M sodium phosphate buffer (P^H 8.0), 10 μl test compound (TP) solution (in ethanol), and 20 μl enzyme solution (0.09 units/ml) were mixed and incubated for 15 min at 25^OC. 10 μl of DTNB (10 mM) was then added and reaction was initiated by the addition of substrate (10 μl of ATCI, 14 mM solution). The hydrolysis of the ATCI can be measured by the formation of the colored product 5- thio-2-nitrobenzoate anion formed by the reaction of DTNB and thiocholine, which is released by the hydrolysis of enzyme. The formation of the coloured product was measured at 410 nm wave length after 10 min. Physostigmine, a standard AChE inhibitor, was used as positive control, which was dissolved in ethanol. Percent acetylcholinesterase inhibition was calculated using following formula.

$$\text{Percentage inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

6.6. ROS ASSAY:

Reactive oxygen species (ROS) such as superoxide and hydrogen peroxide are continually produced during metabolic processes. ROS generation is normally counterbalanced by the action of antioxidant enzymes and other redox molecules. However, excess ROS can lead to cellular injury in the form of damaged DNA, lipids, and proteins.

Excess reactive oxygen species must be promptly eliminated from the cell by a variety of antioxidant defense mechanisms. Cellular antioxidant enzymes and other redox molecules serve to counterbalance ROS generated in the cell. (Cell Biolabs, Inc)

Materials required:

DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), Total reactive oxygen species (ROS) kit, Invitrogen (USA), Trypsin EDTA 1X PBS was from Himedia, (India). 6 well tissue culture plates and wash beakers were from Tarson (India), Fluorescent Imaging System, (ZOE, Bio-Rad, USA).

Procedure:

Cell Treatment:

Fish were treated for 3 days at different concentrations and then anesthetized. Primary cells were isolated from 0.1 g of tissue, homogenized, and treated with 500 µL of Trypsin-EDTA. The mixture was filtered and the volume was adjusted to 10 mL using sterile PBS. It was then centrifuged at 1500 RPM for 5 minutes. The supernatant was discarded, and the pellet was resuspended in DMEM medium. The cell suspension was then seeded into a 96-well plate.

ROS Assay:

The sample was tested for ROS production using Neuro 2a. Briefly, The Test sample was tested for *in vitro* cytotoxicity, using Neuro 2a cells by MTT assay. Briefly, the cultured Neuro 2a cells were harvested by trypsinization and pooled in a 15 ml tube. Then, the cells were plated at a density of $1×10^5$ cells/ml cells/well (200 µL) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C and cell were Pre-treated with H2O2 and treated with IC50 of test sample 520µg/ml. 1 ml of ROS assay buffer was added followed by 100 µl of 1X ROS assay staining solution was added to the wells and mixed gently. Then the plate was incubated for 60 minutes in a 37°C incubator with 5% CO_2. After the incubation period, the production of ROS was evaluated immediately by a fluorescence imaging system (ZOE, BIO-RAD).

7. RESULT AND DISCUSSION:

The present study showed that *Moringa oleifera* and *Plectranthus amboinicus* leaf extract has strong antioxidant and neuroprotective properties. The Soxhlet extraction gave a yield of 25.3%, indicating the presence of many useful phytochemicals. Antioxidant tests showed good free radical and hydrogen peroxide scavenging activity, with IC50 values of 87.31 µg/ml and 49.78 µg/ml. Cell viability studies confirmed that the extract was safe to Neuro-2A cells at lower doses, with an MTT IC50 of 520 µg/ml. The extract also showed strong acetylcholinesterase inhibition and reduced intracellular ROS levels, confirming its protective effect against oxidative stress–induced neuronal damage.

7.1. SOXHLET EXTRACTION

Fig. 6: Soxhlet Extraction

Sample in grams - 5.07g

YIELD %= YIELD IN GRAM/AMOUNT OF SAMPLE LOADEDX100

YIELD %= 5.07/20 X100

YIELD %= 25.3%

7.2. DPPH ASSAY:

A. OD Value at 517 nm:

Control Mean OD value: 2.019

Table 1

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	2.019	2.019	2.019
2.	500 µg/ml	0.678	0.685	0.696
3.	250 µg/ml	0.699	0.702	0.707
4.	100 µg/ml	0.715	0.721	0.725
5.	50 µg/ml	0.729	0.734	0.739
6.	10 µg/ml	0.745	0.756	0.776
7.	Ascorbic acid	0.08	0.099	0.089

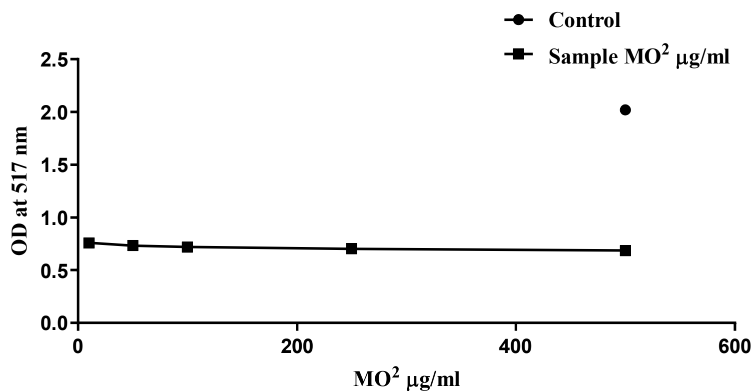

Fig. 7: OD Value at 517 nm

B. Percentage of inhibition:

Table 2

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Ascorbic acid	96.034	95.094	95.592	95.573
2.	500 µg/ml	66.419	66.072	65.527	66.006
3.	250 µg/ml	65.379	65.230	64.983	65.197
4.	100 µg/ml	64.586	64.289	64.091	64.322
5.	50 µg/ml	63.893	63.645	63.398	63.645
6.	10 µg/ml	63.101	62.556	61.565	62.407

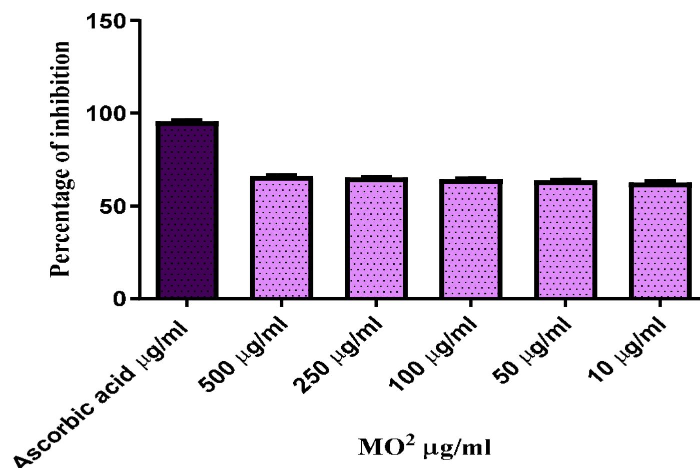

Fig. 8: Percentage of inhibition

C. IC50 Value of tested sample: 87. 31 µg/ml

Table 3

log(inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	1.941
HillSlope	-1.456
IC50	87.31
95% CI (profile likelihood)	
LogIC50	1.813 to 2.062
HillSlope	-2.168 to -1.008
IC50	65.03 to 115.3
Goodness of Fit	
Degrees of Freedom	13
R squared	0.9059
Sum of Squares	1825
Sy.x	11.85
Number of points	
# of X values	15
# Y values analyzed	15

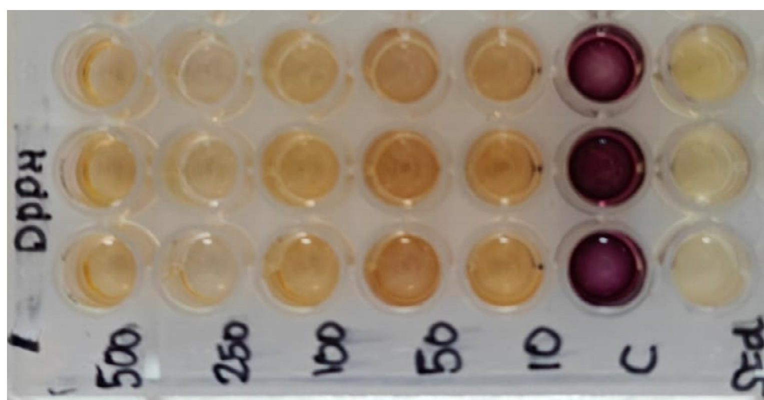

Fig. 9: DPPH- MO^2

7.3. HYDROGEN PEROXIDE SCAVENGING ASSAY:

A. OD Value at 230 nm:

Control Mean OD value: 1.117

Table 4

S. No	Tested sample concentration (µg/ml)	OD Value at 230 nm (in triplicates)		
1.	Control	1.121	1.119	1.113
2.	500 µg/ml	0.149	0.149	0.142
3.	250 µg/ml	0.158	0.153	0.162
4.	100 µg/ml	0.196	0.191	0.197
5.	50 µg/ml	0.265	0.267	0.270
6.	10 µg/ml	0.395	0.398	0.391
7.	Ascorbic acid	0.071	0.074	0.071

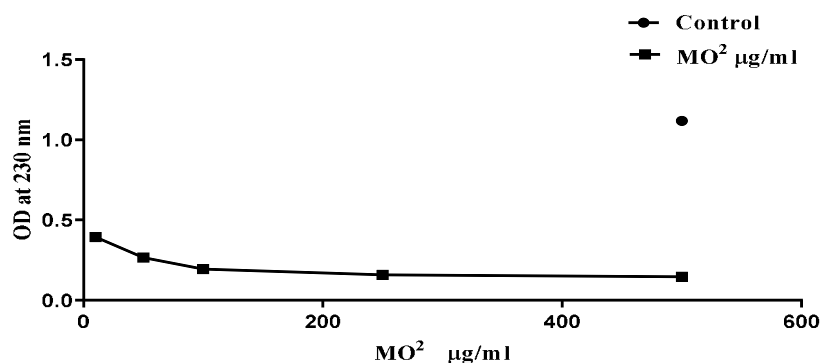

Fig. 10: OD Value at 230nm

B. Percentage of inhibition:

Table 5

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value
1.	Ascorbic acid	93.6437	93.3751	93.6437	93.5542
2.	500 µg/ml	86.6607	86.6607	87.2874	86.8696
3.	250 µg/ml	85.855	86.3026	85.4969	85.8848

4.	100 μg/ml	82.453	82.9006	82.3635	82.5724
5.	50 μg/ml	76.2757	76.0967	75.8281	76.0668
6.	10 μg/ml	64.6374	64.3688	64.9955	64.6673

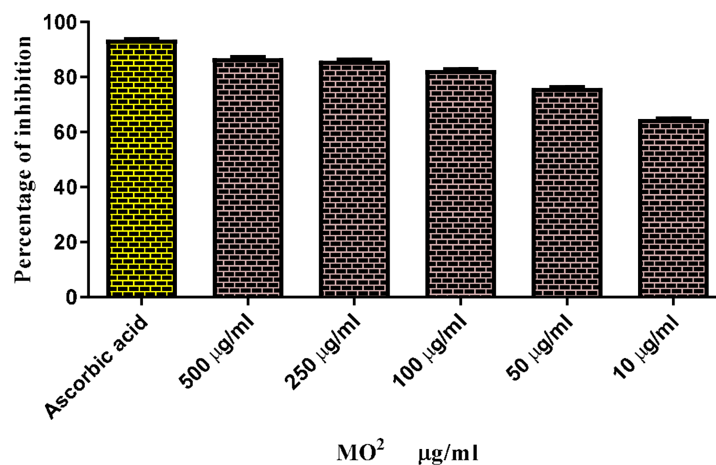

Fig. 11: percentage of inhibition

C. IC50 Value of tested sample: 49.78 μg/ml

Table 6

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.697
HillSlope		-2.136
IC50		49.78
Std. Error		
LogIC50		0.009829
HillSlope		0.1129
95% Confidence Intervals		
LogIC50		1.676 to 1.718
Hill Slope		-2.380 to -1.892
IC50		47.40 to 52.27
Goodness of Fit		
Degrees of Freedom		13
R square		0.9969
Absolute Sum of Squares		64.46
Sy.x		2.227
Number of points		
Analyzed	3	15

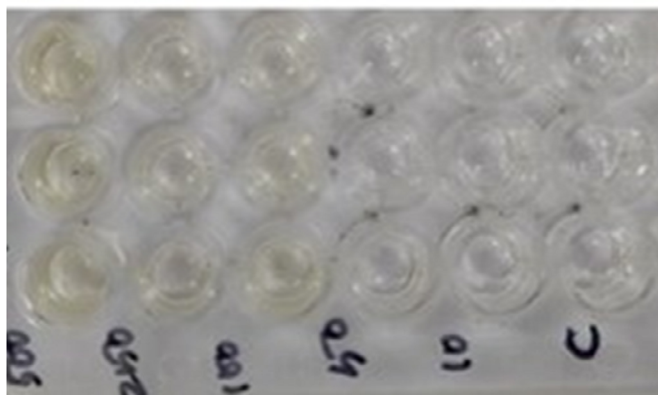

Fig. 12: H2O2- MO²

7.4. MTT ASSAY:

D. OD Value at 570 nm:

Table 7

S. No.	Tested sample concentration (μg/ml)	OD value at 570 nm (in triplicates)		
1	Control	0.896	0.897	0.898
2	1000 μg/ml	0.487	0.479	0.481
3	900 μg/ml	0.532	0.526	0.529
4	800 μg/ml	0.557	0.568	0.568
5	700 μg/ml	0.572	0.601	0.589
6	600 μg/ml	0.632	0.636	0.64
7	500 μg/ml	0.663	0.674	0.684
8	400 μg/ml	0.711	0.701	0.706
9	300 μg/ml	0.755	0.767	0.766
10	200 μg/ml	0.798	0.785	0.79
11	100 μg/ml	0.812	0.805	0.819

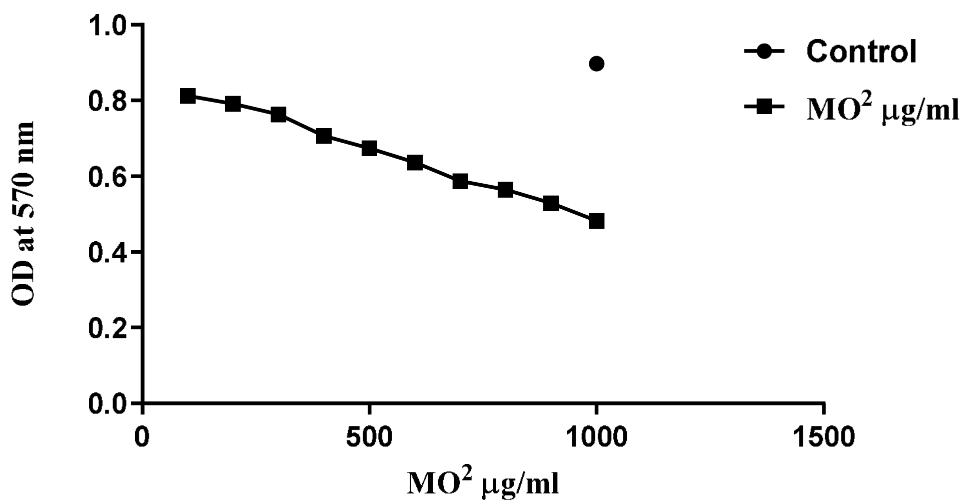

Fig. 13: OD Value at 570nm

B. Cell Viability (%):

Table 8

S. No.	Tested sample concentration (μg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
1	Control	100	100	100	100
2	1000 μg/ml	54.353	53.400	53.563	53.772
3	900 μg/ml	59.375	58.640	58.909	58.975
4	800 μg/ml	62.165	63.322	63.252	62.913
5	700 μg/ml	63.839	67.001	65.590	65.477
6	600 μg/ml	70.536	70.903	71.269	70.903
7	500 μg/ml	73.996	75.139	76.169	75.101
8	400 μg/ml	79.353	78.149	78.619	78.707
9	300 μg/ml	84.263	85.507	85.301	85.024
10	200 μg/ml	89.063	87.514	87.973	88.183
11	100 μg/ml	90.625	89.744	91.203	90.524

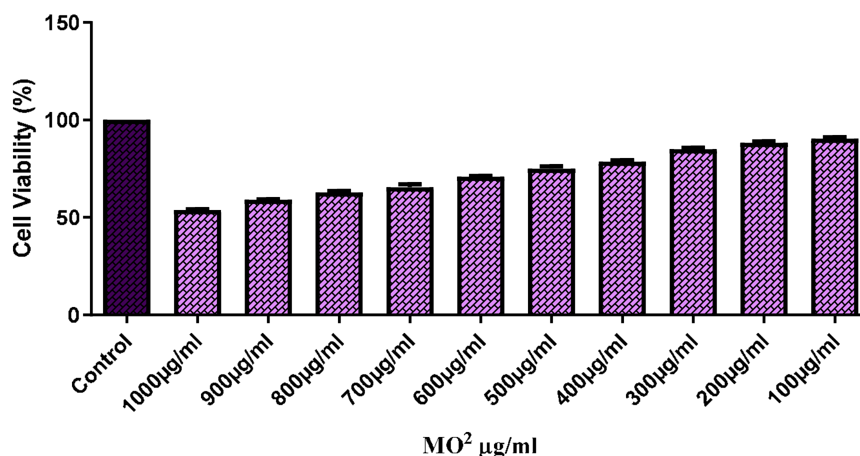

Fig. 14: Percentage of cell viability

C. IC50 Value of tested sample: 520.0 μg/ml

Table 9

log(inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	2.731
HillSlope	-3.236
IC50	520.0
95% CI (profile likelihood)	
LogIC50	2.714 to 2.748
HillSlope	-3.646 to -2.875
IC50	517.9 to 559.3
Goodness of Fit	
Degrees of Freedom	28
R squared	0.9763
Sum of Squares	764.7

Sy.x	5.226
Number of points	
# of X values	30
# Y values analyzed	30

7.5. AChE ASSAY:

A. OD Value at410 nm

Control Mean OD value: 1.941

Table 10

S. No	Tested sample concentration (μg/ml)	OD Value at 410 nm (in triplicates)		
1.	Blank	1.937	1.939	1.947
2.	500 μg/ml	0.148	0.150	0.161
3.	250 μg/ml	0.179	0.181	0.198
4.	100 μg/ml	0.233	0.250	0.262
5.	50 μg/ml	0.428	0.457	0.461
6.	10 μg/ml	0.730	0.754	0.788

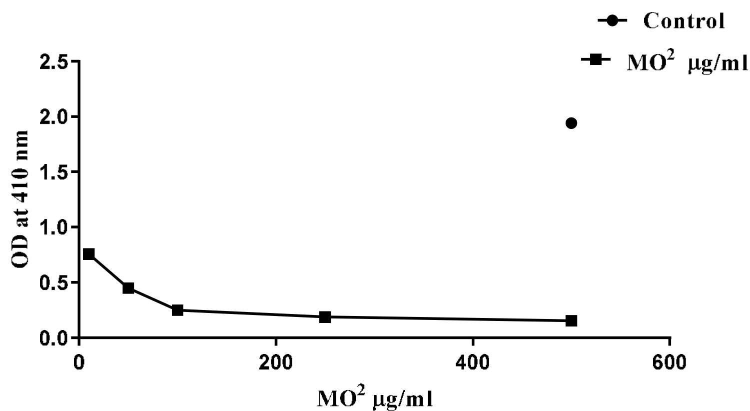

Fig. 15: OD Value at 410nm

B. Percentage of inhibition:

Table 11

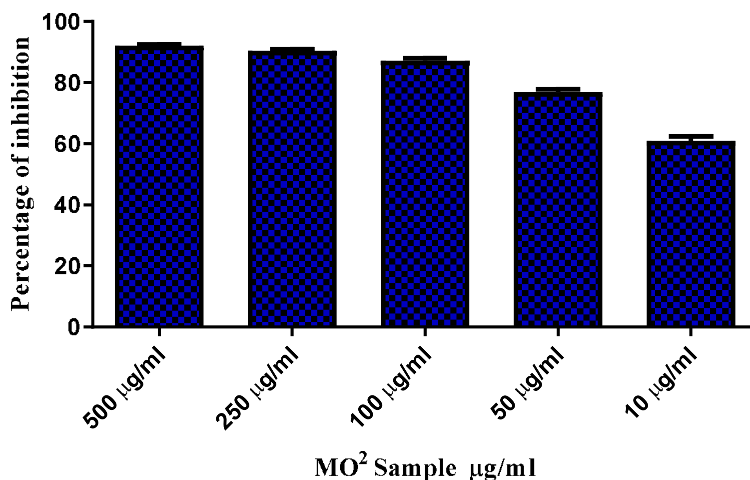

Fig. 16: Percentage of inhibition

C. IC50 Value of tested sample: 49.38 µg/ml

Table 12

log(inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	1.694
HillSlope	-2.346
IC50	49.38
Std. Error	
LogIC50	0.01338
HillSlope	0.1914
95% Confidence Intervals	
LogIC50	1.665 to 1.722
HillSlope	-2.759 to -1.932
IC50	46.20 to 52.77
Goodness of Fit	
Degrees of Freedom	13
R square	0.9935
Absolute Sum of Squares	134.0
Sy.x	3.210
Number of points	
Analyzed	15

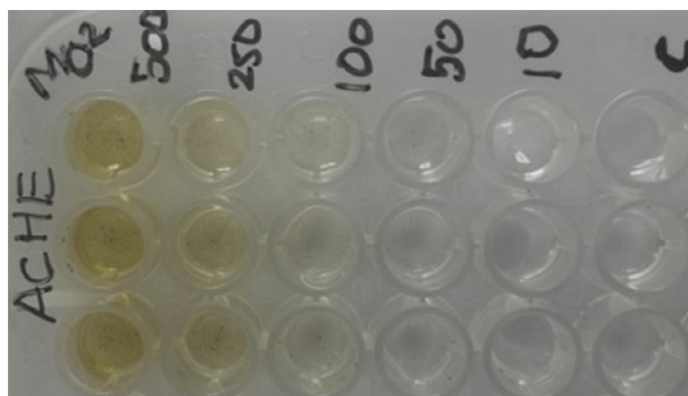

Fig. 17: AChE – MO^2

7.6. ROS ASSAY:

GROUP	
CONTROL	
H2O2 INDUCED	
H2O2 INDUCED MO^2 TREATED WITH IC50 VALUE (520.0 μg/ml)	

Fig. 18: ROS Assay

8. CONCLUSION:

This study shows that *Moringa oleifera* and *Plectranthus amboinicus* leaf extract has strong antioxidant and neuroprotective effects during oxidative stress. The extract was able to remove free radicals and hydrogen peroxide, showing good antioxidant activity. Cell viability tests confirmed that the extract was non toxic to Neuro-2A cells at the tested doses, proving it is safe for neuronal cells. The extract also reduced acetylcholinesterase activity, which helps maintain normal neurotransmitter levels. This is important for protecting brain function and reducing the risk of neurodegenerative

diseases. In addition, the extract lowered the level of reactive oxygen species inside cells exposed to hydrogen peroxide, showing that it protects nerve cells from oxidative damage. Overall, the results suggest that MO^2 contain useful natural compounds that reduce oxidative stress and support brain health. These findings support the traditional use of *Moringa oleifera* and *Plectranthus amboinicus* as a natural neuroprotective agent. However, more research is needed to confirm these benefits. Future studies should explain how the extract works at the molecular level and identify the active compounds responsible for its effects. Animal studies are also required to confirm its safety and effectiveness before it can be developed for treating neurodegenerative diseases.

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