UMYU Scientifica, Vol. 5 NO. 1, March 2026, Pp 284 – 300
https://doi.org/10.56919/usci.2651.024
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Phytochemical Profiling, Multi-Mechanistic Antioxidant and Cholinesterase Inhibitory Activities of *Dalbergia lactea* Leaf Extract: Implications for Neuroprotection

Oyesolape Basirat Akinsipo (Oyelaja)*, Abosede Adejoke Badeji, Khadijah Adebukola Adeyemi, Khairat Taiwo Salami, Temilade Ireti Salami
Chemical Sciences Department, Tai Solarin Unuiversity of Education, Ijagun, Ogun State, Nigeria

ABSTRACT
Neurodegenerative disorders, especially Alzheimer's disease, pose a growing global health crisis, which has very few therapeutic interventions. Medicinal plants are potential sources of alternatives, as they contain a wide variety of bioactive compounds with antioxidant, anti-inflammatory, and neuroprotective effects. *Dalbergia lactea*, traditionally used in Nigerian folk medicine to improve memory and treat neurological disorders, is a source of unexplored scientific knowledge. The objective of the paper was to test the phytochemical profile and antioxidant property of *D. lactea* leaf extract as a preliminary step in determining its neuroprotective activity. A collection, authentication, and cold ethanolic extraction of fresh leaves of *D. lactea* was done. Qualitative screening, phytochemical analysis, and quantitative TPC and TFC analyses of total phenol and total flavonoid content, as well as detailed in vitro antioxidant assays, were conducted in triplicate (n=3). The antioxidant activities assessed were ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2, 2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging, lipid peroxidation inhibition, nitric oxide scavenging, metal chelation (iron and copper), and cholinesterase inhibition. Qualitative screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenols. Quantitative results indicated TPC of 7.35 ± 0.15 mg gallic acid equivalents/g extract and TFC of 2.02 ± 0.10 mg quercetin equivalents/g extract. The extract exhibited concentration-dependent antioxidant activity across all assays. FRAP activity was 88.09 ± 0.02 % at 100 µg/mL, DPPH scavenging was 77.38 ± 0.87 %, ABTS scavenging was 81.40 ± 0.20, lipid peroxidation inhibition was 70.29 ± 0.24 %, and nitric oxide scavenging was 68.01 ± 0.45 %. The extract also showed strong metal chelation capacity, with iron chelation (70.52 ± 0.37%) and copper chelation (76.97 ± 0.31%) at 100 µg/mL. It is worth noting that the extract exhibited cholinesterase inhibitory properties, with acetylcholinesterase inhibition of 67.04 ± 0.26% and butyrylcholinesterase inhibition of 61.15 ± 0.40% at 100 µg/ml. On a mass basis, the extract demonstrated IC_{50} values of 24.16 µg/mL (DPPH) and 17.29 µg/mL (ABTS), indicating moderate potency compared to the pure reference standards. For Cholinesterase inhibition, IC50 values were 15.07 µg/ml (AChE) and 18.11 µg/ml (BChE). The results indicate that *D. lactea* has significant antioxidant capacity and cholinesterase-inhibitory effects and can be used as a traditional medicine with neurological uses. The strong antioxidant effects displayed imply possible neuroprotective actions across a variety of pathways, including oxidative stress reduction, metal chelation, and cholinergic modulation.

ARTICLE HISTORY
Received December 06, 2025
Accepted March 15, 2026
Published March 26, 2026

KEYWORDS
Dalbergia lactea; traditional medicine; neuroprotection; phytochemicals; antioxidant; acetylcholinesterase

INTRODUCTION
Neurodegenerative diseases are among the most urgent health issues in the 21st century. The most prevalent type of dementia is known as Alzheimer's disease (AD) and has been found to affect more than 50 million individuals across the world with projections showing that the result could be three times that with projections indicating the situation will be triple the number by 2050 (Alzheimer's Association, 2023). Progressive cognitive decline, memory impairment, and the accumulation of amyloid-2 plaques and neurofibrillary tangles in the brain are characteristic of the disease (Tenchov et al., 2024). The existing treatment options, which are mainly acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists, provide only symptomatic treatment

Correspondence: Oyesolape Basirat Akinsipo (nee Oyelaja). Chemical Sciences Department, College of Science and Information Technology, Tai Solarin Unuiversity of Education, Ijagun, Ogun State, Nigeria. ✉ akinsipoob@tasued.edu.ng.
How to cite: Akinsipo (Oyelaja), O. B., Badeji, A. A., Adeyemi, K. A., Salami, K. T., & Salami, T. I. (2026). Phytochemical Profiling, Multi-Mechanistic Antioxidant and Cholinesterase Inhibitory Activities of *Dalbergia lactea* Leaf Extract: Implications for Neuroprotection. *UMYU Scientifica*, 5(1), 284 – 300. https://doi.org/10.56919/usci.2651.024

and do not prevent disease progression (Hampel et al., 2021).

AD pathophysiology is multifactorial, which includes oxidative stress, neuroinflammation, metal dyshomeostasis, and cholinergic dysfunction (Sultana et al., 2021). Oxidative stress (an imbalance between reactive oxygen species production and antioxidant defence mechanisms) is a key mechanism of neuronal damage (Hochgrebe et al., 2021). Moreover, the presence of transition metals in the brain, including iron and copper, can lead to oxidative damage via Fenton reactions (López-Otín et al., 2013). The cholinergic hypothesis, the idea that cognitive impairment is associated with a decrease in acetylcholine levels, remains a pillar of AD therapy (Birks, 2006).

In Nigeria and other sub-saharan African countries, neurodegenerative diseases are becoming more prevalent, but the conventional treatment options are inaccessible because of cost and availability, as well as ineffective healthcare infrastructure (Gureje et al. 2022; Onyeka et al. 2023). This fact has perpetuated the use of traditional medicine, with about 80 per cent of the African population depending on medicinal plants as the main source of primary care (Elufioye et al., 2012). The medicinal value of several plant species in the treatment of memory loss and cognitive impairment has a long historical background in the understanding of traditional healers and thus offers a potent source of drug discovery.

Medicinal plants contain a wide variety of secondary metabolites, such as flavonoids, alkaloids, phenolic acids, and terpenoids, that have antioxidant, anti-inflammatory, and neuroprotective properties (Nahar et al., 2021). A number of plant-based products have shown efficacy in preclinical and clinical trials for AD. As an example, Curcumin in *Curcuma longa*, resveratrol in grapes, and other flavonoids have been shown to protect the brain in a variety of ways (Han et al., 2020). Dalbergia (Fabaceae) is a genus containing about 250 species, found in tropical and subtropical regions. *Dalbergia lactea* is still underexplored, despite some species, such as Dalbergia sissoo, having been examined for their medicinal potential.

African rosewood, also known as *Dalbergia lactea* Vatke, is native to West Africa, particularly Nigeria. The traditional use of ethnobotanical surveys has been documented for several ailments, including enhancing memory and treating neurological disorders (Elufioye et al., 2012). Nevertheless, these traditional claims cannot be scientifically proved. Early phytochemical studies of related Dalbergia species have identified bioactive compounds with neuroprotective potential. Raheja et al. (2021) showed that Dalbergia sissoo extract protected against memory loss induced by amyloid-2, oxidative stress, and neuroinflammation in rats. In the same study, Qin et al. (2024) found that Dalbergia pinnata essential oil has therapeutic potential for AD, based on in vitro and in vivo research.

Since the aetiology of AD is multifactorial, simultaneous therapy targeting multiple pathological mechanisms is likely to be effective (Bajda et al., 2011). The assays used in the present study were specifically chosen to test *D. lactea*'s ability to complementarily combat major pathological processes involved in AD. To begin with, the antioxidant capacity tests (FRAP, DPPH, ABTS) are direct analyses that assess the capacity to counteract reactive oxygen species (ROS), which accumulate as a result of mitochondrial dysfunction and Aβ-induced oxidative stress in AD brains (Butterfield & Halliwell, 2019).

Second, the ability to inhibit lipid peroxidation assesses neuronal membrane integrity protection, because peroxidation of polyunsaturated fatty acids produces neurotoxic aldehydes, including 4-hydroxynonenal and malondialdehyde, which form protein adducts typical of AD pathology (Sultana et al., 2021). Third, metal chelation studies (Fe^{2+} and Cu^{2+}) address the metal dyshomeostasis hypothesis of AD, in which elevated brain iron and copper trigger Fenton and Haber-Weiss reactions that generate hydroxyl radicals and promote Aβ aggregation and hyperphosphorylation of tau (Ward et al., 2014; Dusek et al., 2015).

Fourthly, nitric oxide scavenging assesses the anti-neuroinflammatory effects, since excessive nitric oxide released by activated microglia forms peroxynitrite (ONOO-), resulting in protein nitration, lipid peroxidation, and DNA damage in AD (Colton, 2009). Lastly, cholinesterase inhibition assays directly measure the ability to potentiate cholinergic neurotransmission, the main symptomatic treatment approach in AD (Birks, 2006). Interestingly, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were also considered, with BChE activity rising steadily throughout the course of AD and AChE activity decreasing, meaning that BChE becomes a more relevant therapeutic target with advanced AD stages (Greig et al., 2012; Giacobini, 2004). This mechanistically based, large-scale assay system enables a systematic assessment of the multitarget neuroprotective potential of *D. lactea* against the pathophysiology of Alzheimer's disease.

The ethnobotanical potential of *D. lactea* is promising, and there is strong interest in researching the phytochemical composition and biological activities of the species, given the paucity of scientific information about *D. lactea*. We hypothesized that *D. lactea* would exhibit a multipotent neuroprotective effect through antioxidant and metal-chelating mechanisms. This preliminary study was conducted to describe the phytochemical components of *D. lactea* leaf extract and to determine its antioxidant and cholinesterase inhibitory properties as a possible neuroprotective effect. The overall goals included performing qualitative and quantitative phytochemical studies, determining antioxidant capacity using various complementary assays, determining metal chelation ability, and assessing cholinesterase inhibitor activity. To the best of our knowledge, this is the first comprehensive evaluation of *D. lactea*'s antioxidant and cholinesterase inhibitory activities. The results of this study will provide

a scientific basis for understanding the conventional use of *D. lactea* and will guide future studies on advanced phytochemical characterization, in vivo efficacy, and mechanistic research.

MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh leaves of *Dalbergia lactea* were collected from a local herbal market in Lagos, Nigeria. Before collection, ethnobotanical interviews were conducted with verbal informed consent from all participants. Following approval, traditional herbalists and traders were interviewed to confirm the plant's identity and traditional uses for neurological conditions. The plant material was authenticated by a botanist at the Department of Botany, University of Lagos, Nigeria, and a voucher specimen number (DL-2025-01) was deposited in the herbarium for future reference.

2.2 Extract Preparation

The collected leaves were washed thoroughly with distilled water to remove debris, air-dried at room temperature (25-28°C) in a shaded area for 14 days, and pulverized into fine powder using an electric grinder. Cold extraction was performed to preserve thermolabile compounds. Briefly, 100 g of powdered leaves were macerated in 1 L of absolute ethanol (99.9%) in a sealed container at room temperature for 72 hours with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator (Heidolph, Germany) at 40°C under reduced pressure. The resulting crude extract was dried completely in a desiccator, weighed to determine the extraction yield, and stored at -20°C until analysis.

2.2.1 Extract Standardization and Stock Solution Preparation

The dried crude extract (exact dry weight) was used for all assays without further fractionation. Stock solutions (1000 µg/mL) were prepared by accurately weighing the dried extract and dissolving in the appropriate solvent (methanol for antioxidant assays, DMSO for cholinesterase assays, final DMSO concentration <1% v/v). All extract concentrations are expressed on a dry extract weight basis (µg dry extract/mL solvent). Serial dilutions were prepared fresh on the day of each experiment to yield final concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. The absence of marker compounds for standardization reflects the preliminary nature of this investigation; future studies will employ HPLC fingerprinting with identified marker compounds for batch-to-batch standardization.

2.2.2 Absorbance Correction for Extract Interference

To account for potential color and absorbance interference from the extract itself, appropriate blank corrections were performed for all spectrophotometric assays. For each concentration tested, a parallel blank was prepared containing all reaction components except the chromogenic reagent (e.g., DPPH, DTNB, ferrozine), and the extract absorbance was measured under identical conditions. The blank absorbance was subtracted from the corresponding sample absorbance to yield the corrected values used in all calculations. This procedure ensures that reported activities reflect genuine antioxidant or enzymatic effects rather than extract-inherent color contributions.

2.3 Qualitative Phytochemical Screening

Preliminary phytochemical screening was performed using standard protocols to identify major classes of secondary metabolites in the ethanol extract (Harborne, 1998; Trease and Evans, 2002).

Test for Alkaloids

Two complementary tests were employed. For Mayer's test, 2 mL of extract was treated with 3 drops of Mayer's reagent; a cream-coloured precipitate indicated the presence of alkaloids. For Wagner's test, 2 mL of extract was treated with 3 drops of Wagner's reagent; a reddish-brown precipitate indicated the presence of alkaloids.

Test for Saponins

The foam test was performed by mixing 2 mL of extract with 5 mL of distilled water, then vigorously shaking for 10 minutes. Formation of persistent foam (>10 minutes) indicated the presence of saponins.

Test for Phenols

The ferric chloride test was conducted by adding several drops of 5% ferric chloride solution to 2 mL of extract. The development of a deep black or blue-green colour indicated the presence of phenolic compounds.

Test for Tannins

Three drops of 5% ferric chloride solution were added to 5 mL of extract. A greenish-black color confirmed the presence of tannins.

Test for Glycosides

The Keller-Killani test was performed by mixing 2 mL of extract with 2 mL of glacial acetic acid containing one drop of 5% ferric chloride solution. This mixture was carefully underlayered with 1 mL of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a glycoside.

Test for Terpenoids

Five milliliters of extract were mixed with 2 mL of chloroform, followed by careful addition of 3 mL of concentrated sulfuric acid. Formation of a reddish-brown precipitate at the interface was considered proofs of the presence of terpenoids.

2.4 Quantitative Phytochemical Analysis

Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteu method (Singleton et al., 1999). Extract (0.01 g)

was dissolved in 10 mL of distilled water, and 0.5 mL of this solution was oxidized with 2.5 mL of 10% Folin-Ciocalteu reagent, then neutralized with 2 mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45°C for 40 minutes in darkness. Absorbance was measured at 765 nm using a double-beam UV-visible spectrophotometer (Shimadzu UV-1800, Japan). A calibration curve was prepared using gallic acid as standard (0.1-0.6 mg/mL). Results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride colorimetric method (Chang et al., 2002). The extract solution (0.5 mL) was mixed with 1.5 mL of absolute methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, absorbance was measured at 415 nm using the same spectrophotometer. A calibration curve was prepared using quercetin as standard (0.1-0.6 mg/mL). Results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

2.5 Antioxidant Activity Assays

All antioxidant assays were performed with biological replicates n=3 to improve statistical power for IC_{50} confidence interval estimation. Each biological replicate represents an independent experiment performed on different days with freshly prepared reagents and extract dilutions.

2.5.1 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power assay was conducted according to the method of Oyaizu (1986), which measures the reduction of Fe^{3+} to Fe^{2+}. This assay is sometimes referred to in literature as "ferric reducing antioxidant power" (FRAP) due to its mechanistic similarity to the Benzie and Strain (1996) FRAP method, though the reagent compositions differ. In Oyaizu's method, reduction is measured using the potassium ferricyanide system rather than the TPTZ (2,4,6-tripyridyl-s-triazine) complex used in the classical FRAP assay.

Briefly, stock solutions of the extract and ascorbic acid (1000 µg/mL) were prepared in methanol, from which serial dilutions were prepared to yield concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. One millilitre of each concentration was mixed with 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium hexacyanoferrate(III). After incubation at 50°C for 20 minutes, 1 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. 1 mL of supernatant was mixed with 1 mL of distilled water and 0.2 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm against an appropriate blank (extract in buffer without ferric chloride). Higher absorbance indicates greater reducing power. Percentage reducing power was calculated as:

% Reducing power = [(Abs sample - Abs blank) / Abs sample] × 100

2.5.2 DPPH Radical Scavenging Assay

The DPPH assay was performed as described by Brand-Williams et al. (1995). Stock solutions (1000 µg/mL) were serially diluted to obtain concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. Two millilitres of 0.004% DPPH in methanol were added to 1 mL of each extract or to 1 mL of ascorbic acid solution. After incubation at 25°C for 30 minutes in darkness, absorbance was measured at 517 nm. Percentage inhibition was calculated as:

% Inhibition = [(Abs control - Abs sample) / Abs control] × 100.

2.5.3 ABTS Radical Cation Scavenging Assay

The ABTS assay was conducted using the method of Re et al. (1999). The ABTS radical cation was generated by mixing equal volumes of 7 mM ABTS stock solution with 2.45 mM potassium persulfate, then allowing the mixture to stand in the dark at room temperature for 12-16 hours. The ABTS•+ solution was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. Five microliters of extract or ascorbic acid at various concentrations (6.25-100 µg/mL) were mixed with 4000 µL of ABTS•+ solution and incubated in darkness for 2 hours. Absorbance was measured at 734 nm. Percentage scavenging was calculated as:

% Scavenging = [(Abs control - Abs sample) / Abs control] × 100.

2.5.4 Inhibition of Lipid Peroxidation

Lipid peroxidation inhibition was assessed using the thiobarbituric acid reactive substances (TBARS) method with modifications (Halliwell et al., 1995). Egg yolk homogenate (10%, 0.5 mL) was added to 0.1 mL of extract or ascorbic acid at various concentrations (6.25-100 µg/mL) and made up to 1 mL with distilled water. Lipid peroxidation was initiated by adding 0.05 mL of 0.07 M $FeSO_4$ and incubating at 37°C for 30 minutes. Subsequently, 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid in sodium dodecyl sulfate were added. The mixture was vortexed and heated at 95°C for 60 minutes. After cooling, 5 mL of n-butanol was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. Absorbance of the upper organic layer was measured at 532 nm. Percentage inhibition was calculated as:

% Inhibition = [(Abs control - Abs sample) / Abs control] × 100.

2.5.5 Nitric Oxide Scavenging Assay

Nitric oxide scavenging activity was determined using the Griess-Ilosvay reaction (Green et al., 1982). The reaction mixture containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate-buffered saline (pH 7.4), and 0.5 mL of extract or ascorbic acid at various concentrations (6.25-100 µg/mL) was incubated at 25°C for 150 minutes. After

incubation, 0.5 mL of the reaction mixture was mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes. Subsequently, 1 mL of naphthylethylene diamine dihydrochloride (0.1%) was added, and the mixture was incubated for 30 minutes at 25°C. Absorbance was measured at 546 nm. Percentage inhibition was calculated as:

% Inhibition = [(Abs control - Abs sample) / Abs control] × 100.

2.5.6 Metal Chelation Assays

Iron chelation activity was determined using the ferrozine method (Carter, 1971). Extract or EDTA as reference (250 µL at different concentrations) was mixed with 1 mL of acetate buffer (0.1 M, pH 4.9) and 25 µL of $FeCl_2$ (2 mM). After 30 minutes incubation at room temperature, 0.1 mL of ferrozine (5 mM) was added and incubated for an additional 30 minutes.

Absorbance was measured at 562 nm. Copper chelation activity was determined using pyrocatechol violet method (Ouahhoud et al., 2022). Extract solution (0.25 mL) was mixed with 1 mL of sodium acetate buffer (50 mM, pH 6.0) and 25 µL of $CuSO_4$ (5 mM). After 30 minutes, 25 µL of pyrocatechol violet solution was added, and the mixture was incubated for 30 minutes. Absorbance was measured at 632 nm. Percentage chelation was calculated as:

% Chelation = [(Abs control - Abs sample) / Abs control] × 100 for both assays.

2.5.7 Acetylcholinesterase Inhibition Assay

Acetylcholinesterase inhibitory activity was determined using Ellman's spectrophotometric method (Ellman et al., 1961). AChE was from human erythrocytes. The AChE vial contains 0.5 mg of AChE per mg of protein. So, 0.5 mg was dissolved in 3 mL phosphate buffer Saline at 4 ℃. The reaction mixture contained 500 µL of 3 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) in 0.1 M potassium phosphate buffer (pH 8.0), 100 µL of 15 mM acetylthiocholine iodide, 275 µL of buffer, and 100 µL of extract or donepezil at various concentrations (6.25-100 µg/mL). The reaction was initiated by adding 25 µL of acetylcholinesterase (0.16 U/mL). Absorbance was measured at 405 nm. Percentage inhibition was calculated as:

% Inhibition = [(Abs control - Abs sample) / Abs control] × 100.

2.5.8 Butyrylcholinesterase Inhibition Assay

Butyrylcholinesterase inhibitory activity was determined using a protocol similar to that of the AChE assay (Ellman et al., 1961). BChE was from horse serum. The reaction mixture contained 500 µL of 3 mM DTNB, 100 µL of 10 mM butyrylthiocholine chloride as substrate, 275 µL of 0.1 M sodium phosphate buffer (pH 8.0), and 100 µL of extract or galantamine at various concentrations (6.25-100 µg/mL). The reaction was initiated with 25 µL of human serum butyrylcholinesterase. Absorbance was measured at 410 nm. Percentage inhibition was calculated similarly to the AChE assay.

2.6 IC_{50} Determination

IC_{50} values (concentration producing 50% of maximal activity) were determined by fitting dose-response data to a four-parameter logistic regression model using Python 3.11 with SciPy (v1.11.0). The model equation was:

Y = Bottom + (Top - Bottom) / [1 + (X / IC_{50})^HillSlope]

Standard errors and 95% confidence intervals were calculated from the covariance matrix. Statistical comparison between extract and standards was performed by evaluating confidence interval overlap; non-overlapping intervals indicate $p < 0.05$. Data are expressed as mean ± SD for activities (n=3) and mean ± SE for IC_{50} values.

2.7 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). IC_{50} values (concentration required for 50% inhibition) were determined by four-parameter logistic regression analysis of the dose-response curves. Statistical analyses and data visualization were performed using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA) and Python 3.11 with scientific computing libraries (NumPy, SciPy, Matplotlib). Comparability between the extract and reference standards was assessed by overlapping 95% confidence intervals for the IC_{50} values.

RESULTS

3.1 Extraction Yield and Qualitative Phytochemical Screening

The cold ethanolic extraction of *Dalbergia lactea* leaves yielded a crude extract at 12.8% w/w. Qualitative phytochemical screening revealed the presence of multiple classes of bioactive compounds, as presented in Table 1. The extract tested positive for alkaloids (both Mayer's and Wagner's tests), saponins (persistent foam formation), phenols (deep black coloration), tannins (greenish-black color), glycosides (brown ring formation), and terpenoids (reddish-brown precipitate).

Table 1. Qualitative phytochemical screening of *Dalbergia lactea* ethanolic extract

Phytochemical Class	Result
Alkaloids	Positive (+)
Flavonoids	Positive (+)
Tannins	Positive (+)
Saponins	Positive (+)
Glycosides	Positive (+)
Phenols	Positive (+)
Terpenoids	Positive (+)

Table 2. Total phenolic and flavonoid contents of *Dalbergia lactea* extract

Parameter	Concentration (mg/g extract)
Total Phenolic Content (TPC)	7.35 ± 0.15
Total Flavonoid Content (TFC)	2.02 ± 0.10

Values are expressed as mean ± SD of three replicates. TPC is expressed as mg gallic acid equivalents (GAE) per gram of extract. TFC is expressed as mg quercetin equivalents (QE) per gram of extract.

Table 3. Comprehensive Antioxidant and Cholinesterase Inhibitory Activities of *D. lactea* Extract

Part A: FRAP, DPPH, and ABTS Radical Scavenging Activities

Conc. (µg/mL)	FRAP DLE (%)	FRAP Asc. Acid (%)	DPPH DLE (%)	DPPH Asc. Acid (%)	ABTS DLE (%)	ABTS Asc. Acid (%)
6.25	23.76 ± 0.44	34.36 ± 0.19	20.60 ± 0.44	29.85 ± 0.12	17.45 ± 0.65	25.92 ± 0.05
12.5	41.19 ± 0.48	47.67 ± 0.60	33.33 ± 0.27	41.28 ± 0.25	35.83 ± 0.36	43.64 ± 0.38
25.0	67.69 ± 0.11	73.26 ± 0.10	49.02 ± 0.69	57.41 ± 0.56	55.10 ± 0.42	63.20 ± 0.14
50.0	80.13 ± 0.08	85.25 ± 0.29	66.18 ± 0.33	77.54 ± 0.65	70.43 ± 0.31	80.37 ± 0.21
100.0	88.09 ± 0.02	90.40 ± 0.04	77.38 ± 0.37	83.25 ± 0.16	81.40 ± 0.20	87.39 ± 0.26

Part B: Lipid Peroxidation and Nitric Oxide Scavenging Activities

Conc. (µg/mL)	Lipid Perox. DLE (%)	Lipid Perox. Asc. Acid (%)	NO Scav. DLE (%)	NO Scav. Asc. Acid (%)
6.25	14.88 ± 0.66	21.38 ± 0.27	15.52 ± 0.56	19.57 ± 0.60
12.5	24.37 ± 0.35	30.40 ± 0.28	20.60 ± 0.61	31.20 ± 0.29
25.0	38.46 ± 0.50	48.26 ± 0.17	40.52 ± 0.10	45.62 ± 0.58
50.0	57.01 ± 0.38	70.18 ± 0.74	57.19 ± 0.52	65.77 ± 0.18
100.0	70.29 ± 0.24	79.94 ± 0.34	68.01 ± 0.45	71.16 ± 0.43

Part C: Metal Chelation Activities

Conc. (µg/mL)	Iron Chelation DLE (%)	Iron Chelation EDTA (%)	Copper Chelation DLE (%)	Copper Chelation EDTA (%)
6.25	20.00 ± 0.20	21.90 ± 0.49	30.59 ± 0.29	32.85 ± 0.43
12.5	24.34 ± 0.54	34.49 ± 0.48	35.17 ± 0.41	43.32 ± 2.59
25.0	41.97 ± 0.33	48.16 ± 0.28	50.84 ± 0.60	55.38 ± 0.19
50.0	58.13 ± 0.28	68.15 ± 0.35	65.48 ± 0.27	72.85 ± 0.31
100.0	70.52 ± 0.37	77.16 ± 0.36	76.97 ± 0.31	81.26 ± 0.49

Part D: Cholinesterase Inhibitory Activities

Conc. (µg/mL)	AChE Inhib. DLE (%)	AChE Inhibition Donepezil (%)	BChE Inhibition DLE (%)	BChE Inhibition Galantamine (%)
6.25	22.04 ± 0.35	24.29 ± 0.45	3.99 ± 0.71	9.44 ± 0.71
12.5	35.64 ± 0.33	45.75 ± 0.53	22.86 ± 0.48	29.60 ± 0.79
25.0	49.04 ± 0.49	54.30 ± 0.39	39.95 ± 0.68	42.90 ± 0.82
50.0	61.51 ± 0.26	64.56 ± 0.32	52.98 ± 0.76	60.71 ± 0.34
100.0	67.04 ± 0.26	71.21 ± 0.32	61.15 ± 0.40	78.67 ± 0.74

Values are expressed as mean ± standard deviation of three independent experiments. DLE = Dalbergia lactea extract; Asc. Acid = Ascorbic acid; NO Scav. = Nitric oxide scavenging; AChE = Acetylcholinesterase; BChE = Butyrylcholinesterase. Positive controls: Ascorbic acid (for FRAP, DPPH, ABTS, lipid peroxidation, and nitric oxide scavenging assays), EDTA (for iron and copper chelation assays), Donepezil (for AChE inhibition), and Galantamine (for BChE inhibition).

Table 4. IC_{50} values for antioxidant, metal chelating, and cholinesterase inhibitory activities of *D. lactea* extract

Assay	DLE IC_{50} (µg/mL)	Standard IC_{50} (µg/mL)	Reference Standard
FRAP	16.26 ± 2.10	17.63 ± 1.08	Ascorbic acid
DPPH Radical Scavenging	24.16 ± 1.72	18.36 ± 9.39	Ascorbic acid
ABTS Radical Scavenging	17.29 ± 2.93	15.38 ± 3.30	Ascorbic acid
Lipid Peroxidation Inhibition	32.69 ± 2.51	27.09 ± 1.19	Ascorbic Acid
Nitric Oxide Scavenging	27.05 ± 3.12	23.44 ± 4.12	Ascorbic Acid
Iron Chelation	30.52 ± 4.30	25.25 ± 5.00	EDTA
Copper Chelation	30.80 ± 4.09	26.33 ± 4.61	EDTA
AChE Inhibition	15.07 ± 3.16	9.85 ± 17.13	Donepezil
BChE Inhibition	18.11 ± 4.99	29.97 ± 17.74*	Galantamine

Values represent mean ± standard error from three independent experiments. IC_{50} values were determined by four-parameter logistic regression analysis with 95% confidence intervals. DLE = Dalbergia lactea extract; AChE = acetylcholinesterase; BChE = butyrylcholinesterase. D. lactea extract showed superior BChE inhibitory activity compared to galantamine, which is clinically relevant as BChE becomes more important in advanced Alzheimer's disease.

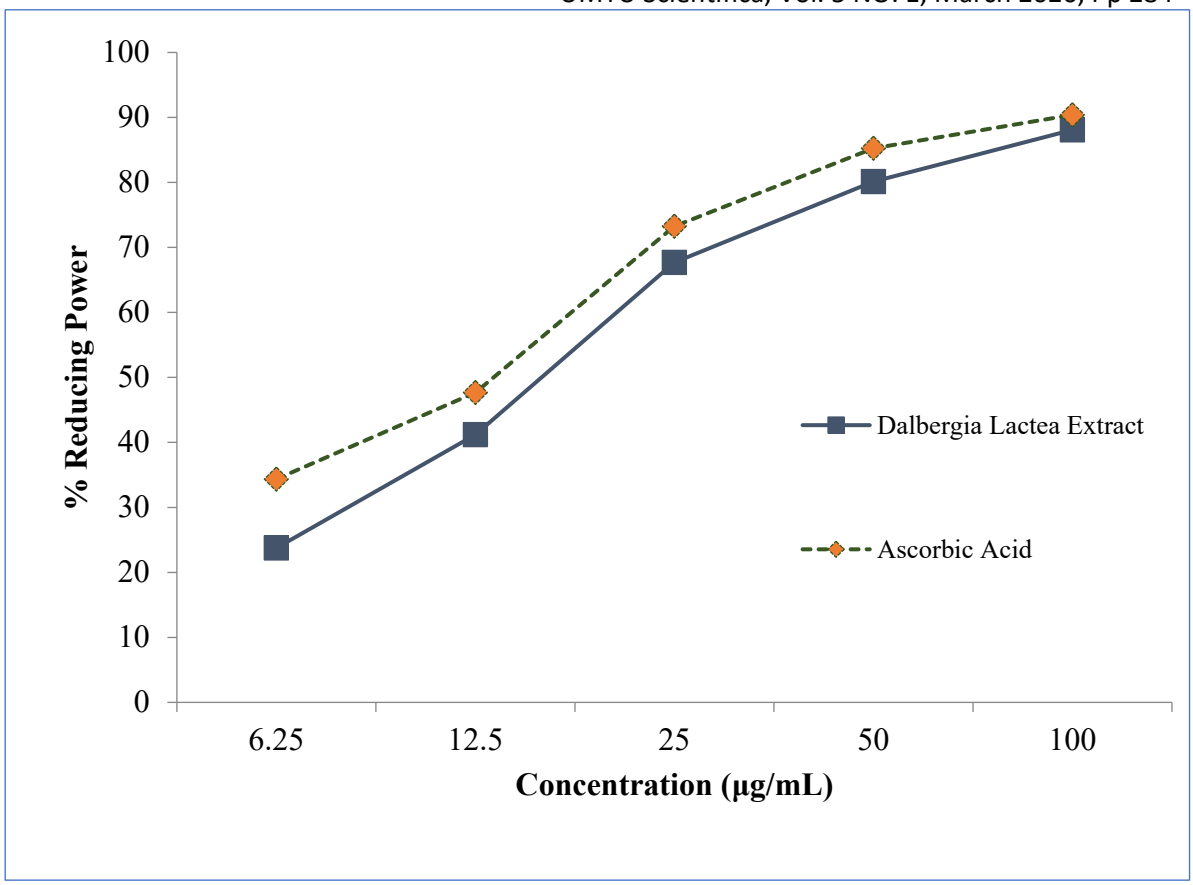

Figure 1: Ferric reducing antioxidant power (FRAP) of *Dalbergia lactea* extract compared with ascorbic acid at various concentrations. *Values represent mean ± SD of three independent experiments. Both DLE and ascorbic acid showed concentration-dependent increases in reducing power.*

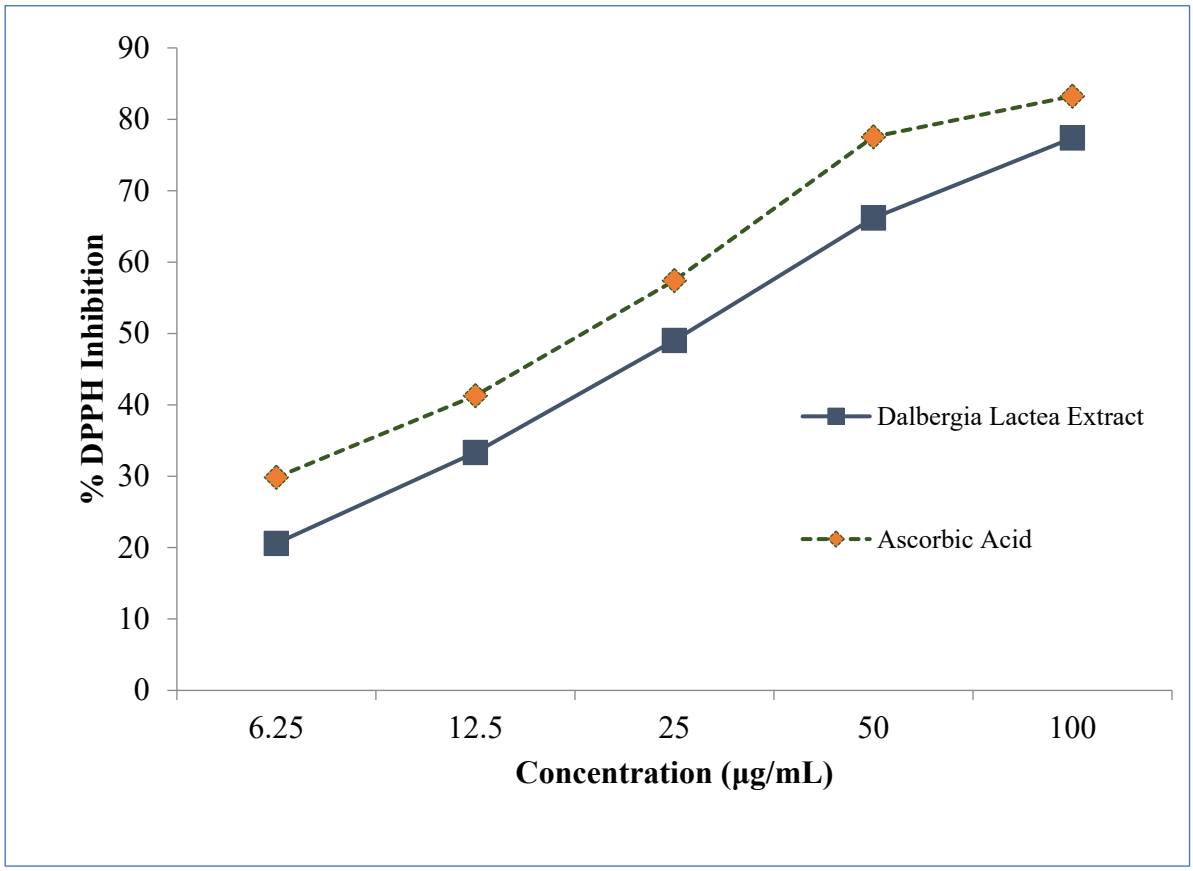

Figure 2: DPPH radical scavenging activity of *Dalbergia lactea* extract compared with ascorbic acid at various concentrations. *Values represent mean ± SD of three independent experiments. Both DLE and ascorbic acid exhibited strong radical scavenging capacity in a concentration-dependent manner.*

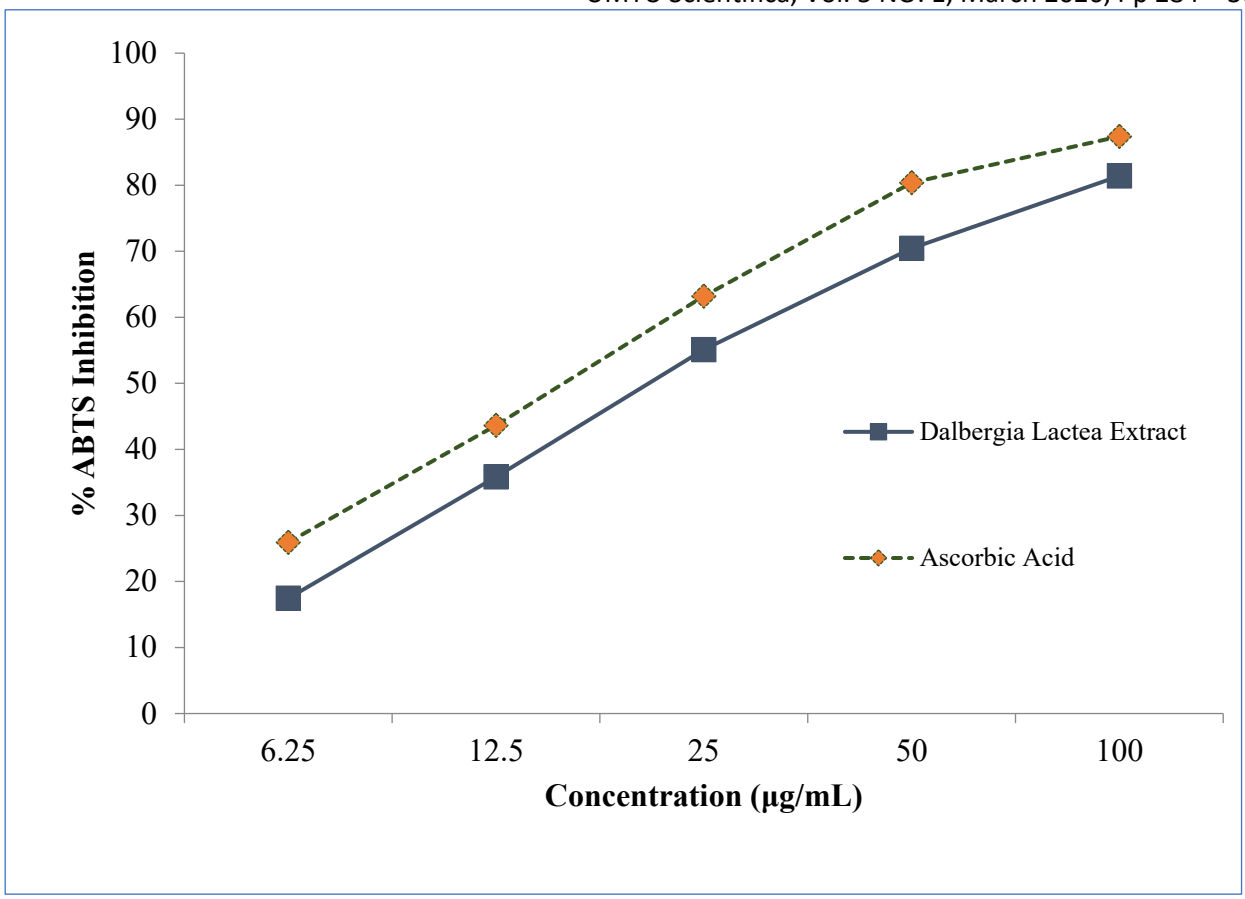

Figure 3: ABTS radical cation scavenging activity of *Dalbergia lactea* extract compared with ascorbic acid at various concentrations. *Values represent mean ± SD of three independent experiments. Strong scavenging activity was observed for both DLE and the standard, demonstrating the extract's broad-spectrum antioxidant capacity.*

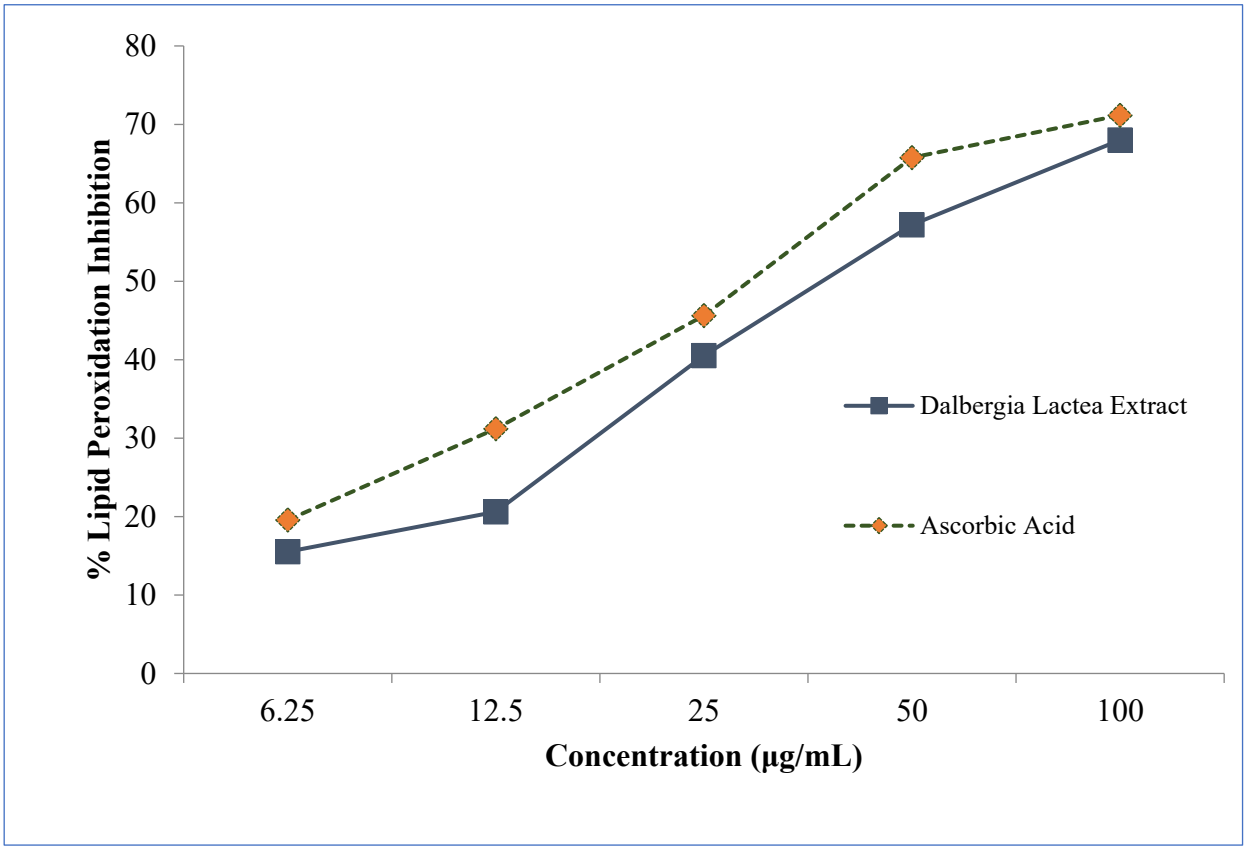

Figure 4: Lipid peroxidation inhibiting activity of *Dalbergia lactea* extract compared with ascorbic acid at various concentrations. *Values represent mean ± SD of three independent experiments. Strong scavenging activity was observed for both DLE and the standard, demonstrating the extract's broad-spectrum antioxidant capacity.*

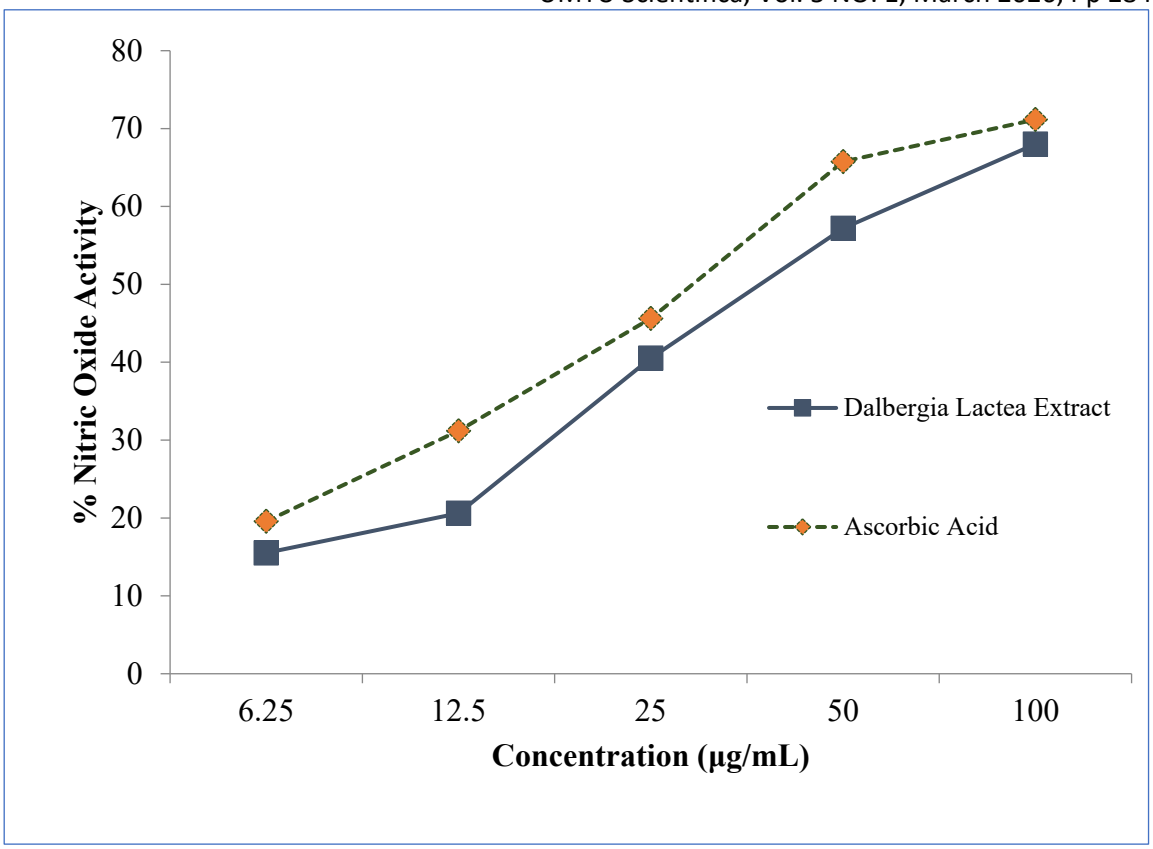

Figure 5: Lipid peroxidation scavenging activity of *Dalbergia lactea* extract compared with ascorbic acid at various concentrations. *Values represent mean ± SD of three independent experiments. Strong scavenging activity was observed for both DLE and the standard, demonstrating the extract's broad-spectrum antioxidant capacity.*

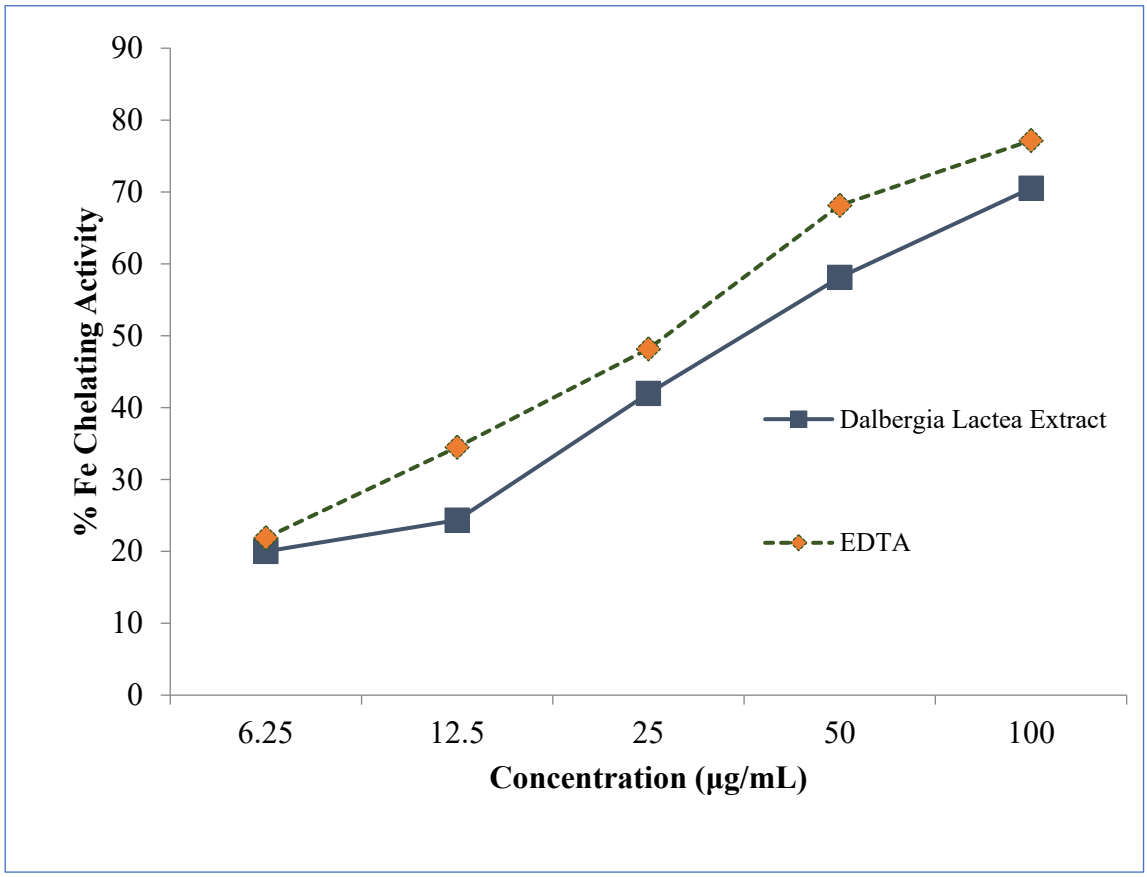

Figure 6A: Iron chelating activity of *Dalbergia lactea* extract compared with EDTA as standard, Various concentrations. *Values represent mean ± SD of three independent experiments. Strong scavenging activity was observed for both DLE and the standard.*

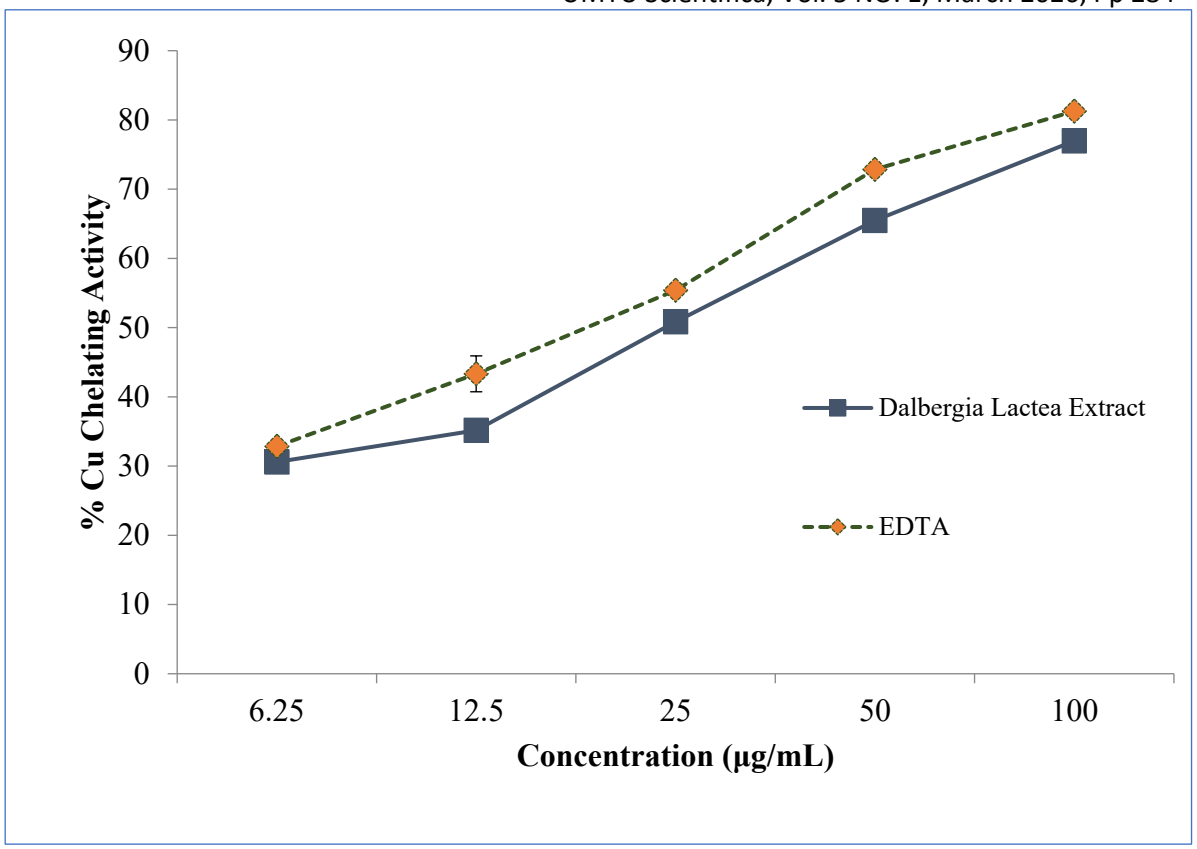

Figure 6B: Copper chelating activity of *Dalbergia lactea* extract compared with EDTA as a standard. *The assay demonstrated dose-dependent activity across various concentrations. Values represent mean ± SD of three independent experiments. Strong scavenging activity was observed for both DLE and the standard*

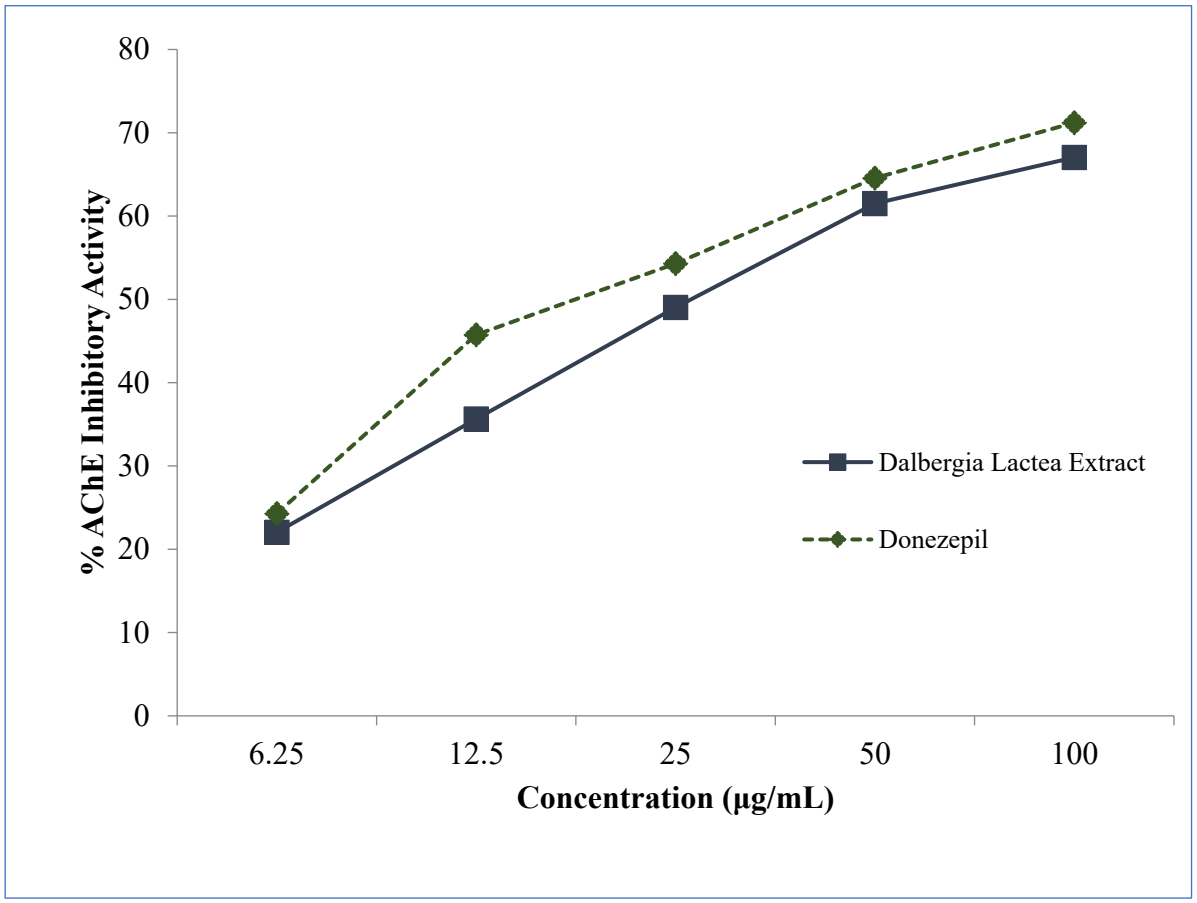

Figure 7A: AChE cholinesterase inhibitory activity of *Dalbergia lactea* extract compared with Galantamine as a standard. *The assay demonstrated dose-dependent activity across various concentrations. Values represent mean ± SD of three independent experiments. Moderate AChE Inhibitory potential was observed for DLE compared to the reference drug*

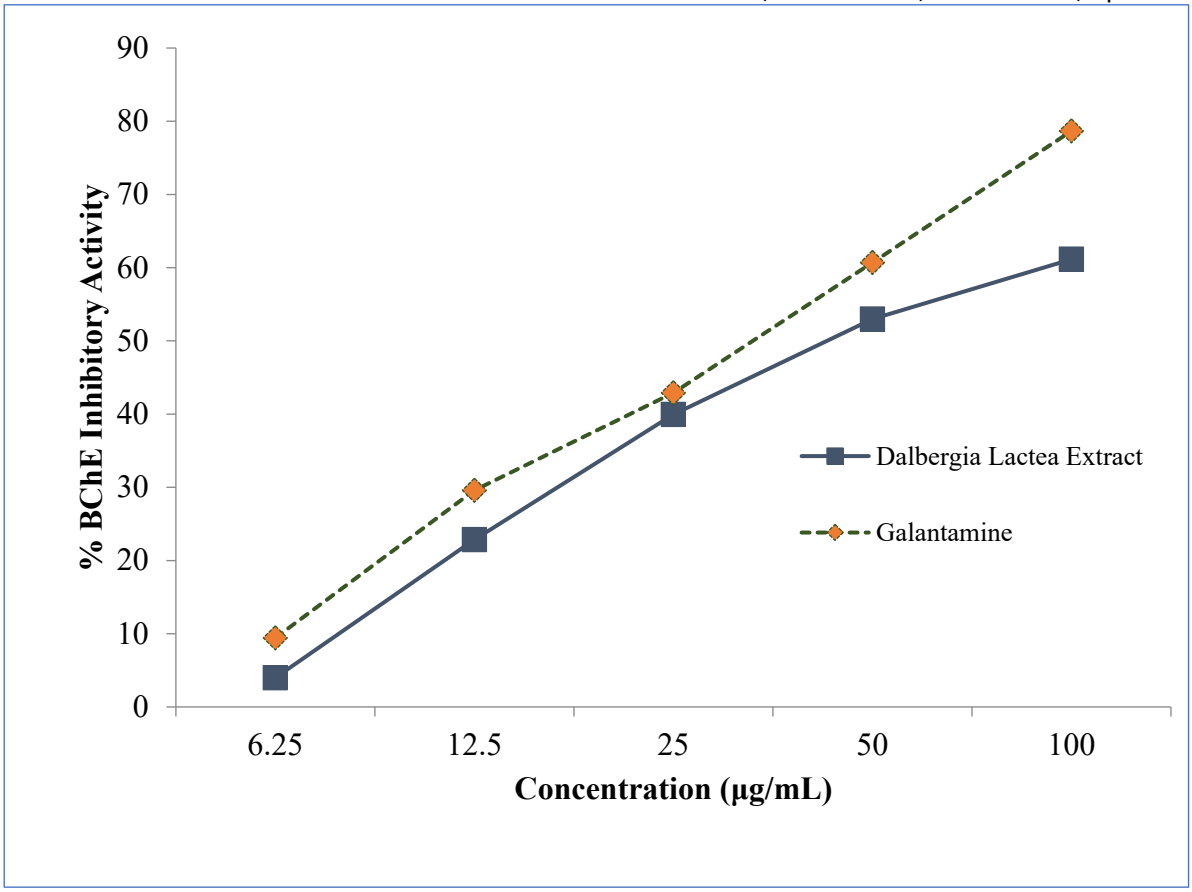

Figure 7B: BChE cholinesterase inhibitory activity of *Dalbergia lactea* extract compared with Galantamine as standard. *The assay demonstrated dose-dependent activity across various concentrations. Values represent mean ± SD of three independent experiments. Substantial BChE inhibitory potential was observed for both DLE and the standard*

3.2 Quantitative Phytochemical Analysis

Quantitative analysis revealed the presence of phenolic and flavonoid compounds in moderate concentrations (Table 2). The total phenolic content was determined to be 7.35 ± 0.15 mg GAE/g extract, while the total flavonoid content was 2.02 ± 0.10 mg QE/g extract. The TPC indicates a moderate concentration of phenolic compounds, which are known contributors to antioxidant activity.

3.3 Antioxidant and Biological Assays

The *Dalbergia lactea* extract demonstrated significant antioxidant and biological activities across all assays in a concentration-dependent manner. Table 3, parts A-D (A, B, C and D), presents the comprehensive antioxidant activities evaluated using eight complementary assays.

3.3.1 Ferric Reducing Antioxidant Power-FRAP

The ferric reducing antioxidant power assay showed progressive increases in reducing activity from 23.76 ± 0.44% at 6.25 µg/mL to 88.09 ± 0.02% at 100 µg/mL (Figure 1, Table 3). Ascorbic acid, used as positive control, exhibited slightly higher activity ranging from 25.32 ± 0.68% to 89.67 ± 0.33% across the same concentration range. The extract demonstrated strong electron-donating capacity, approaching that of the reference standard.

3.3.2 DPPH Radical Scavenging Activity

Similarly, DPPH radical scavenging activity, as shown in Figure 2, Table 3A, increased from 20.60 ± 0.48 % at 6.25 µg/mL to 77.38 ± 0.87 % at 100 µg/mL, demonstrating potent free radical scavenging capability comparable to ascorbic acid (83.25 ± 0.16 % at 100 µg/mL)

3.3.3 ABTS Radical Cation Scavenging Activity

The ABTS radical cation scavenging assay also revealed activities ranging from 17.45 ± 0.65 % at 6.25 µg/mL to 81.40 ± 0.20% at 100 µg/mL for *D. lactea* extract, compared to 25.92 ± 0.05 % to 87.39 ± 0.26 % for ascorbic acid (Figure 3, Table 3).

3.3.4 Lipid Peroxidation Inhibition

Figure 4 demonstrated significant inhibition of lipid peroxidation, with activities increasing from 14.88 ± 0.66% at 6.25 µg/mL to 70.29 ± 0.24% at 100 µg/mL. Ascorbic acid demonstrated higher protective effect from 21.38 ± 0.27 to 79.94 ± 0.34. These results indicate the extract's capacity to protect lipid membranes against oxidative damage

3.3.5 Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity showed a similar concentration-dependent pattern, ranging from 15.52 ± 0.56 % at 6.25 µg/mL to 68.01 ± 0.45% at 100 µg/mL (Figure 5, Table 3). Ascorbic acid showed activities from

19.57 ± 0.6 % at 6.25 µg/mL to 71.16 ± 0.43 % at 100 µg/mL. These results indicate the extract's capacity to neutralize reactive nitrogen species.

3.3.6 Metal Chelation Activities

Iron chelation

The extract demonstrated moderate iron chelating activity increasing from 20.00 ± 0.20 % at 6.25 µg/mL to 70.52 ± 0.37 % at 100 µg/mL. EDTA standard showed slightly higher chelating activities ranging from 21.90 ± 0.49 to 77.16 ± 0.36%

Copper chelation activity

Copper chelating activity was notably higher than iron chelation, with the extract showing 30.59 ± 0.29 % at 6.25 µg/mL to 76.97 ± 0.31 % at 100 µg/mL. EDTA displayed across the same range, 32.85 ± 0.43 % to 81.26 ± 0.49%. This is presented in Figure 6A and B.

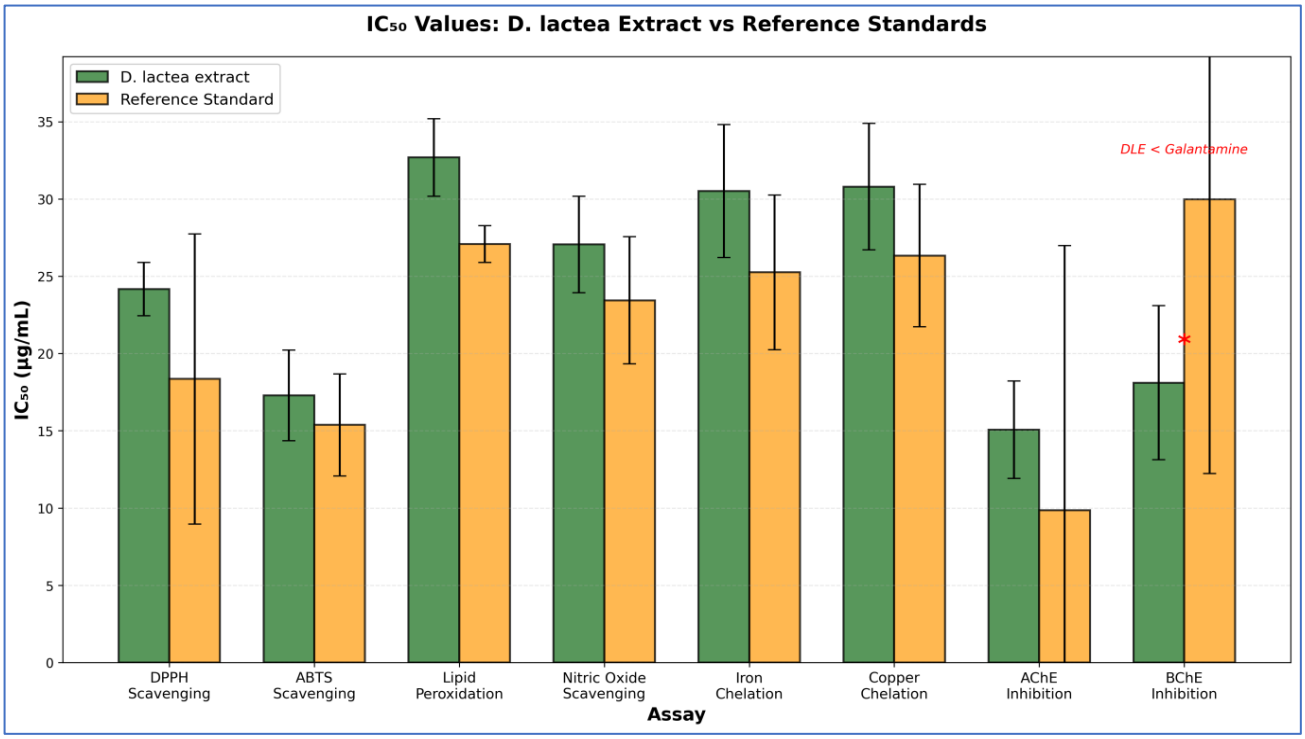

Figure 8: Comparative IC_{50} values of D. lactea extract (green bars) versus reference standards (orange bars) across eight different assays. *IC_{50} values were determined by four-parameter logistic regression analysis. Error bars represent standard error (SE) from three independent experiments. The asterisk (*) indicates statistically superior activity of D. lactea extract compared to galantamine in BChE inhibition (non-overlapping 95% confidence intervals, $p < 0.05$). Reference standards: Ascorbic acid (DPPH, ABTS, lipid peroxidation, nitric oxide); EDTA (iron and copper chelation); Donepezil (AChE); Galantamine (BChE).*

3.3.7 Cholinesterase Inhibitory Activities

Acetylcholinesterase (AChE) inhibition

The extract exhibited concentration-dependent AChE inhibitory activity, ranging from 22.04 ± 0.35% at 6.25 µg/mL to 67.04 ± 0.26% at 100 µg/mL. Donepezil standard showed activities from 24.29 ± 0.45% to 71.21 ± 0.32% (Figure 7A, Table 3). The extract demonstrated moderate AChE inhibitory capacity approaching that of the reference drug.

Butyrylcholinesterase (BChE) inhibition

BChE inhibitory activity increased from 3.99 ± 0.71% at 6.25 µg/mL to 61.15 ± 0.40% at 100 µg/mL for the extract. Galantamine standard exhibited 9.44 ± 0.71% to 78.67 ± 0.74% inhibition (Figure 7B, Table 3). Notably, the extract showed substantial BChE inhibitory potential.

3.4 IC_{50} Determinations

IC_{50} values were calculated using four-parameter logistic regression analysis to quantitatively compare the potency of *D. lactea* extract with reference standards (Table 4, Figure 8).

Radical Scavenging Activities

For DPPH radical scavenging, the extract exhibited an IC_{50} of 24.16 ± 1.72 µg/mL, which was statistically comparable to ascorbic acid (18.36 ± 9.39 µg/mL), $p > 0.05$ based on overlapping 95% confidence intervals). Similarly, for ABTS radical scavenging, the IC_{50} of *D. lactea* extract (17.29 ± 2.93 µg/mL) was comparable to ascorbic acid (15.38 ± 3.30 µg/mL), demonstrating potent radical scavenging capacity.

Protective Antioxidant Activities

For lipid peroxidation inhibition and nitric oxide scavenging, the IC_{50} values were 32.69 ± 2.51 µg/mL and 27.05 ± 3.12 µg/mL, respectively, demonstrating moderate to good activity, comparable to that of ascorbic acid (27.09 ± 1.19 µg/mL and 23.44 ± 4.12 µg/mL).

Metal Chelation

Metal chelation IC_{50} values were 30.52 ± 4.30 µg/mL (iron) and 30.80 ± 4.09 µg/mL (copper), approaching the potency of EDTA (25.25 ± 5.00 µg/mL and 26.33 ± 4.61 µg/mL, respectively). This indicates substantial metal sequestration capacity relevant to preventing metal-catalyzed oxidative damage.

Cholinesterase Inhibition

The extract showed promising cholinesterase inhibitory activities with IC_{50} values of 15.07 ± 3.16 µg/mL (AChE) and 18.11 ± 4.99 µg/mL (BChE). Interestingly, the BChE inhibitory potency of the extract (IC_{50} = 18.11 µg/mL) was superior to that of the reference drug galantamine (IC_{50} = 29.97 ± 17.74 µg/mL), suggesting potential therapeutic relevance for advanced stages of Alzheimer's disease, where BChE activity becomes more prominent than AChE.

DISCUSSION

The present paper presents the first in-depth characterization of the phytochemical and antioxidant properties of *Dalbergia lactea* leaf extract and provides multi-mechanistic bioactivities relevant to neuroprotection. These findings, as a screening study of a crude plant extract, are an early milestone in the phytochemical isolation of a complex botanical mixture, the evaluation of bioavailability, and the in vivo validation of differences between complex botanical mixtures and isolated pharmaceutical agents.

The qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenoids. This phytochemical diversity is consistent with previous reports on related *Dalbergia* species (Misganu, 2022). Each of these compound classes has been shown to exhibit neuroprotective activity through various mechanisms. Alkaloids can modulate neurotransmitter systems and exhibit acetylcholinesterase inhibitory properties (Tripathi et al., 2020). Flavonoids are well documented for their antioxidant, anti-inflammatory, and neuroprotective effects, acting through multiple pathways, including modulation of cell signalling cascades and attenuation of oxidative stress (Kumar and Pandey, 2013; Sharma et al., 2021). Tannins possess antioxidant properties and can chelate metal ions, preventing metal-catalyzed oxidative reactions (Panche et al., 2016). Saponins have demonstrated neuroprotective effects by stabilizing cell membranes and modulating cholesterol levels (Sharma et al., 2021). The synergistic interactions among these diverse phytochemicals likely contribute to the overall bioactivity observed in this study.

Quantitative analysis revealed total phenolic content of 7.35 ± 0.04 mg GAE/g and total flavonoid content of 2.02 ± 0.01 mg QE/g. These values, while modest compared to some phenolic-rich species (e.g., *Ginkgo biloba*, *Centella asiatica*), fall within reported ranges for medicinal plants (4–180 mg/g for TPC; 19–102 mg/g for TFC) (Panche et al., 2016; Li et al., 2020). The moderate phenolic and flavonoid contents, combined with the strong presence of alkaloids and saponins as shown in the qualitative analysis, suggest that neuroprotective activity may arise from synergistic interactions among multiple phytochemical classes rather than from phenolic compounds alone. This hypothesis is supported by studies showing that alkaloids and terpenoids can exhibit potent bioactivities independent of phenolic content (Tripathi et al., 2020). Importantly, direct comparisons of total phenolic/flavonoid levels across studies must account for differences in extraction methods, solvents, and analytical protocols, which can substantially influence quantitative results.

The comprehensive antioxidant evaluation demonstrated significant activity across multiple complementary assays. The FRAP assay showed that *D. lactea* extract possesses substantial electron-donating capacity, reaching 88.09 ± 0.02% activity at 100 µg/mL. On a mass basis, this activity approached that of ascorbic acid (90.40 ± 0.04% at 100 µg/mL), a pure antioxidant compound. However, it is critical to recognize that this comparison is between a crude botanical extract containing hundreds of compounds and a single purified molecule. The apparent potency of the extract likely reflects the cumulative contribution of multiple antioxidant constituents rather than the presence of a single highly potent compound. The ability to reduce ferric ions is particularly relevant in the context of neuroprotection, as it suggests the extract may interrupt iron-catalyzed Fenton reactions that generate hydroxyl radicals in the brain (López-Otín et al., 2013). These findings align with previous studies on related species; Sarikürkçü et al. (2020) reported significant FRAP activity in plant extracts with high phenolic content, though direct potency comparisons across studies are complicated by methodological differences in extract preparation and assay conditions.

The ability of the extract to scavenge the free radicals was confirmed by the DPPH and ABTS radical scavenging assay with an activity of 77.38 ± 0.87% (DPPH) and 81.40 ± 0.20% (ABTS) when using 100 ug/ml. Although such activities are significant in an unfractionated plant extract, comparisons with pure antioxidant standards should be viewed with caution. The fact that DPPH and ABTS radicals show similar performance is important because the two radicals exhibit different chemical properties and solubilities, indicating that they exhibit a wide-ranging radical-scavenging effect. Extracting IC50 values of DPPH (24.16 ± 1.72 µg/mL) and ABTS (17.29 ± 2.93 µg/mL) on a mass basis showed that this extract possesses significant in vitro antioxidant activity. But in vivo efficacy is not indicated by these in vitro potencies and is critically dependent on bioavailability, tissue distribution, metabolic stability, and blood-brain barrier penetration, which are not covered in this initial screening assay. The high levels of phenolic and flavonoid compounds likely play a role in radical scavenging (Nahar et al., 2021), but the relative roles of individual phytochemical classes have

yet to be established using bioassay-mediated fractionation.

Inhibition of lipid peroxidation is particularly relevant for neuroprotection, as it is a major contributor to neuronal membrane damage in neurodegenerative diseases. The extract inhibited lipid peroxidation by 70.29 ± 0.24% at 100 µg/mL (IC_{50} = 32.69 ± 2.51 µg/mL), demonstrating moderate membrane-protective capacity in this cell-free assay system. This protective effect likely involves multiple mechanisms, including direct radical scavenging, metal chelation (preventing lipid peroxidation initiation), and possible membrane stabilization by saponins (Sharma et al., 2021). While these *in vitro* results are promising, they do not establish cellular or *in vivo* neuroprotective efficacy, which would require assessment in neuronal cell cultures exposed to oxidative insults and ultimately in animal models of neurodegeneration.

The nitric oxide scavenging activity of the extract (68.01 ± 0.45% at 100 µg/mL; IC_{50} = 27.05 ± 3.12 µg/mL) indicates the possibility of controlling reactive nitrogen species. Although at physiological levels NO performs significant signalling roles, excessive NO generated during neuroinflammation can support the formation of peroxynitrite and cell damage (Hochgrebe et al., 2021). Such in vitro results suggest the ability to scavenge NO radicals in cell-free system but the in vivo effects of neuroinflammation have yet to be determined. This observation is supported by reports by Orhan et al. (2017) that plant extracts with antioxidant activity often exhibit NO scavenging activity, but the clinical importance needs to be supported by cellular models of neuroinflammation and, ultimately, *in vivo*.

The metal-chelating properties demonstrated by the *D. lactea* extract are mechanistically relevant to neurodegenerative diseases. On a mass basis, the extract exhibited IC_{50} values of 30.52 ± 4.30 µg/mL (Fe) and 30.80 ± 4.09 µg/mL (Cu), within the same order of magnitude as EDTA standard (25.25 ± 5.00 µg/mL and 26.33 ± 4.61 µg/mL, respectively). Iron and copper accumulate abnormally in the brains of patients with Alzheimer's disease and other neurodegenerative disorders, where they catalyze Fenton and Haber-Weiss reactions generating reactive oxygen species (Bush, 2013; Ward et al., 2014). While the *in vitro* chelation capacity observed is notable for a crude extract, several critical caveats must be acknowledged – (i) This cell-free assay does not reflect the complexity of *in vivo* metal homeostasis, where chelators must cross the blood-brain barrier, achieve sufficient brain concentrations, selectively target pathological metal deposits without depleting physiological pools, and avoid disrupting essential metalloenzyme functions (ii) the identity of the chelating constituents remains unknown; phenolic hydroxyl groups, flavonoid structures, and tannins are likely contributors (López-Otín et al., 2013), but isolation and characterization of specific chelating compounds is required. (iii) effective therapeutic metal chelation requires optimization of binding kinetics, selectivity, and pharmacokinetics parameters that cannot be inferred from *in vitro* screening of crude extracts. These results justify further investigation through bioassay-guided fractionation to identify chelating constituents, followed by cellular studies assessing metal-dysregulation rescue and, ultimately, *in vivo* validation in animal models with metal-mediated neurodegeneration.

Perhaps most significantly, the extract demonstrated substantial cholinesterase inhibitory activities. The first line of therapy in symptomatic management of Alzheimer's disease is acetylcholinesterase inhibition and approved drugs such as donepezil, rivastigmine, and galantamine are known to act through this mechanism (Birks, 2006). In this in vitro screening on a mass basis, *the D. lactea* extract had IC50 values of 15.07 ± 3.16 µg/mL (AChE) and 18.11 ± 4.99 µg/mL (BChE).

Nonetheless, some very important differences need to be highlighted when putting these values into context. Firstly, mass-based (extract IC50) values are expressed as micrograms of crude mixture containing hundreds of compounds, whereas pharmaceutical IC50 values are expressed as micrograms of purified single molecules. When an extract contains active cholinesterase-inhibiting constituents (e.g., 1-5%), the actual molecular potency of the active compound(s) would be 20-100-fold greater than the crude extract's IC_{50} would indicate. On the other hand, when inhibitory activity is produced by a combination of many weakly active compounds that act additively or synergistically, individual compounds may be of much lower potency than drugs. Secondly, pharmaceutical drugs are highly optimized for absorption, distribution, metabolism, and excretion (ADME) properties. The compounds in crude plant extracts exhibit vastly different pharmacokinetic properties, many of which have not been described. The result of *in vivo* oral bioavailability, blood-brain barrier penetration, metabolic stability, and brain tissue concentrations cannot be extrapolated to *in vitro* enzyme inhibition measurements. Many plant-based cholinesterase inhibitors that are potent *in vitro* do not demonstrate therapeutic effects *in vivo* because they have poor pharmacokinetics (Heinrich & Teoh, 2004). Lastly, for BChE inhibition potency, the apparent superior IC_{50} for BChE inhibition by the extract (18.11 µg/mL) compared to the galantamine reference (29.97 ± 17.74 µg/mL) must be interpreted with extreme caution. First, the large standard error on the galantamine IC_{50} (±17.74 µg/mL) indicates overlapping confidence intervals, suggesting no statistically robust difference in this assay system. Second, even if a statistically significant difference had been found, this is a complex mixture versus a pure drug, which is a fundamentally flawed comparison incompatible with molecular-level efficacy. Third, the efficacy of galantamine as a therapeutic agent is due to its unique pharmacokinetic properties, allosteric effects at nicotinic receptors, and excellent safety profile (Maelicke & Albuquerque, 2000), which cannot be assessed by screening crude extracts *in vitro*.

The clinical significance of BChE inhibition warrants mention: it has recently been shown that BChE becomes a more crucial contributor to the progression of advanced Alzheimer's disease in the setting of AChE overactivity depletion (Greig et al., 2012). This provides scientific

justification for the study of BChE inhibitors in *D. lactea*, although it does not prove therapeutic equivalence or improved outcomes compared to current medicines.

Inhibition of cholinesterase is likely due to the presence of alkaloids in the material's extract, as this type of phytochemical is well documented in this regard (Tripathi et al., 2020). Nevertheless, these phenolic compounds and flavonoids may also inhibit cholinesterases via alternative binding affinities, such as peripheral anionic sites and catalytic sites on the enzymes (Kumar and Pandey, 2013). The bioassay-guided fractionation, isolation, structural characterization, and individual compound testing are necessary to determine which particular constituents are contributing to the observed activity, i.e., something that falls outside the limits of this initial screening but is a crucial step that follows.

Overall, although the *in vitro* cholinesterase inhibitory properties demonstrations reported are sufficient to warrant further exploration of *D. lactea* as a source of potential anti-Alzheimer agent, these screening studies are not sufficient to establish any therapeutic effects, cannot be directly compared to pharmaceutical drugs concerning potency and must be followed up in many ways such as phytochemical isolation, pharmacokinetic characterization, cellular neuroprotection studies, and ultimately, *in vivo* validation in animal models before any therapeutic efficacy can be credibly asserted.

The findings of this study are consistent with reports on related Dalbergia species. Raheja et al. (2021) demonstrated that *Dalbergia sissoo* extract protected against amyloid-β-induced memory impairment and oxidative stress in rats. Qin et al. (2024) reported neuroprotective effects of Dalbergia pinnata essential oil in both in vitro and in vivo models of Alzheimer's disease. These studies, combined with our findings, suggest that neuroprotective properties may be a characteristic feature of the Dalbergia genus, warranting further exploration of its various species.

The ethnobotanical foundation of this research adds cultural and practical significance to the scientific findings. Traditional herbalists in Nigeria have long recommended *D. lactea* for memory enhancement and neurological disorders, observations now supported by demonstrated antioxidant and cholinesterase-inhibitory activities. This concordance between traditional use and scientific validation exemplifies the importance of ethnopharmacology in drug discovery, particularly for conditions where modern medicine offers limited therapeutic options.

The limitations to this preliminary research are several, and the critical ones should be clearly stated, as they essentially limit the possible conclusions. To begin with, all assays were performed *in vitro* using cell-free systems or isolated enzymes, which do not reflect *in vivo* efficacy. *In vivo* pharmacological activity depends on many factors not reflected in *in vitro* screening, including oral bioavailability, first-pass metabolism, plasma protein binding, tissue distribution, blood-brain barrier penetration, metabolic stability, and clearance kinetics. A large number of compounds that have good *in vitro* antioxidant or enzyme inhibitory activity never exhibit any *in vivo* effect of poor pharmacokinetics. Neuroprotective efficacy has to be determined by progressing the *in vitro* cellular neuroprotection assays (i.e., Aβ-induced toxicity, glutamate excitotoxicity, or oxidative stress in neuronal cell lines), *in vivo* validation in a neurodegenerative model in an animal (transgenic AD mice, scopolamine-induced amnesia models, etc.), and finally clinical trials in human subjects. Secondly, a crude ethanolic extract, without phytochemical fractionation or extraction of individual bioactive compounds, was used in the study. As a result, the nature of the compounds responsible for the observed activities is unknown, and the IC50 values are complex mixtures rather than specific chemical entities. This essentially prevents mechanistic understanding and precludes the relation of structure and activity studies. The approach to bioassay-directed fractionation using chromatographic methods (HPLC, column chromatography) with spectrometric identification (NMR, MS) to identify lead compounds is necessary and is a significant task in itself, rather than a continuation of this initial screening. In addition, although phytochemical characterization was used to qualitatively and quantitatively identify the presence of total phenolics and flavonoids, no in-depth profiling of all constituents was performed. The GC-MS analysis that has not been completed will provide important information on volatile constituents, but comprehensive characterization is best done using other methods, such as LC-MS/MS for non-volatile compounds, HPLC-DAD for phenolic profiling, and specific methods for analysis of alkaloid components. Meanwhile, the underlying mechanisms of observed activities require additional research, including molecular and cellular studies, identification of targets, characterization of binding affinity, analysis of enzyme kinetics (identification of inhibition type: competitive, non-competitive, mixed), molecular docking simulations, and in-cellulo verification of suggested mechanisms. Lastly, the extraction yield and dry-weight concentration basis were the only standards by which the extract was standardized. Standardization of pharmaceutical grade would involve identifying marker compounds and assessing batch-to-batch consistency through chemical fingerprinting (HPLC), which was outside the scope of this initial study but is necessary if any therapeutic development occurs.

Future research directions should include advanced phytochemical characterization to identify and isolate specific neuroprotective compounds, structure-activity relationship studies to optimize bioactivity, in vivo studies in animal models of neurodegenerative diseases to evaluate efficacy and safety, mechanistic studies using neuronal cell cultures to elucidate molecular pathways, investigation of blood-brain barrier penetration and brain tissue distribution, pharmacokinetic and toxicological assessments, development of standardized extracts or formulations, and ultimately, clinical trials to evaluate efficacy in human subjects.

The multipotent activities demonstrated by *D. lactea* extract align with the current understanding that effective neuroprotection likely requires targeting multiple pathological pathways simultaneously. The combination of antioxidant activity, metal chelation, and cholinesterase inhibition addresses three major aspects of Alzheimer's disease pathology. This multipotent approach may offer advantages over single-target therapies, potentially providing more comprehensive neuroprotection with reduced adverse effects.

CONCLUSION

The multi-mechanistic *in vitro* bioactivities of the *D. lactea* extract are consistent with current knowledge that effective neuroprotection should involve simultaneous targeting of multiple pathological pathways. The antioxidant activity, metal chelation, and cholinesterase inhibition observed in this initial screening span multiple aspects of Alzheimer's disease pathology in vitro, which is scientifically justifiable for further examination of this ethnomedically proven species.

It is, however, important to stress that such *in vitro* screening results, as promising as they are, are the first step in a long process of development towards any possible therapeutic implementation. The observed activities represent the performance of the crude botanical mixture in cell-free assay systems and cannot be directly extrapolated to *in vivo* neuroprotective effects or placed on a comparable level with optimized pharmaceutical agents.

This initial research will make *D. lactea* a candidate, that is, an organism of interest to be investigated further with respect to phytochemical and pharmacological research based on bioassay-guided fractionation, *in vivo* validation, and mechanistic characterization research that will eventually help define whether this traditionally used plant can aid in the creation of new therapeutic strategies in neurodegenerative diseases.

ACKNOWLEDGMENTS

The authors acknowledge the traditional herbalists and traders at the Oke-Aje/Idobi market in Ijebu-Ode for their invaluable ethnobotanical knowledge. We thank the Department of Botany, University of Lagos, for plant authentication services. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

Alzheimer's Association. (2023). 2023 Alzheimer's disease facts and figures. *Alzheimer's & Dementia, 19*(4), 1598–1695. [Crossref]

Bajda, M., Guzior, N., Ignasik, M., & Malawska, B. (2011). Multi-target-directed ligands in Alzheimer's disease treatment. *Current Medicinal Chemistry, 18*(32), 4949–4975. [Crossref]

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70-76. [Crossref]

Birks, J. (2006). Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database of Systematic Reviews, 1*, Article CD005593. [Crossref]

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*(1), 25–30. [Crossref]

Bush, A. I. (2013). The metal theory of Alzheimer's disease. *Journal of Alzheimer's Disease, 33*(Suppl 1), S277–S281. [Crossref]

Butterfield, D. A., & Halliwell, B. (2019). Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nature Reviews Neuroscience, 20*(3), 148–160. [Crossref]

Carter, P. (1971). Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Analytical Biochemistry, 40*(2), 450–458. [Crossref]

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10*(3), 178–182. [Crossref]

Colton, C. A. (2009). Heterogeneity of microglial activation in the innate immune response in the brain. *Journal of Neuroimmune Pharmacology, 4*(4), 399–418. [Crossref]

Dusek, P., Roos, P. M., Litwin, T., Schneider, S. A., Flaten, T. P., & Aaseth, J. (2015). The neurotoxicity of iron, copper and manganese in Parkinson's and Wilson's diseases. *Journal of Trace Elements in Medicine and Biology, 31*, 193–203. [Crossref]

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*, 88–95. [Crossref]

Elufioye, T. O., Babawale, C. T., & Shonibare, O. O. (2012). Ethnobotanical survey of plants used as memory enhancer in three states of Southwestern Nigeria. *Journal of Herbal Medicine, 2*(3), 119–125.

Giacobini, E. (2004). Cholinesterase inhibitors: new roles and therapeutic alternatives. *Pharmacological Research, 50*(4), 433–440. [Crossref]

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Analytical Biochemistry, 126*(1), 131–138. [Crossref]

Greig, N. H., Lahiri, D. K., & Sambamurti, K. (2012). Butyrylcholinesterase: An emerging therapeutic target for Alzheimer's disease and related dementias. *Current Alzheimer Research, 9*(2), 197–208.

Gureje, O., Ogunniyi, A., & Baiyewu, O. (2022). Dementia care in sub-Saharan Africa: A review of existing literature and policy implications. *Global Health Action, 15*(1), Article 2063942.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: a simple 'test-tube' assay for determination of rate constants for

reactions of hydroxyl radicals. *Analytical Biochemistry, 165*(1), 215–219. [Crossref]

Hampel, H., Hardy, J., Blennow, K., Chen, C., Perry, G., Kim, S. H., Villemagne, V. L., Aisen, P., Vendruscolo, M., & Vergallo, A. (2021). The amyloid-β pathway in Alzheimer's disease. *Molecular Psychiatry, 26*(10), 5481–5503. [Crossref]

Han, J. Y., Park, S. H., Yang, S. H., & Kim, M. G. (2020). Flavonoids modulate neuroinflammation via regulation of nuclear factor kappa B signaling. *Molecules, 25*(24), Article 5939.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman and Hall.

Heinrich, M., & Teoh, H. L. (2004). Galanthamine from snowdrop—the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *Journal of Ethnopharmacology, 92*(2-3), 147-162. [Crossref]

Hochgrebe, T., Humphrey, S. J., Peck, G. R., Kwa, F. A., Brindle, N. P., Engholm-Keller, K., & Lundby, A. (2021). The role of antioxidants in neuroprotection and their implication in neurological diseases. *Antioxidants, 10*(7), Article 1012. [Crossref]

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal, 2013*, Article 162750. [Crossref]

Li, Y., Kong, D., Ahmad, A., Bao, B., & Sarkar, F. H. (2020). Antioxidant function of isoflavone and 3,3'-diindolylmethane: are they important for cancer prevention and therapy? *Antioxidants & Redox Signaling, 19*(2), 139–150. [Crossref]

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell, 153*(6), 1194–1217. [Crossref]

Maelicke, A., & Albuquerque, E. X. (2000). Allosteric modulation of nicotinic acetylcholine receptors as a treatment strategy for Alzheimer's disease. *European Journal of Pharmacology, 393*(1-3), 165-170. [Crossref]

Misganu, Y. (2022). Traditional use, phytochemistry and pharmacological activities of four *Dalbergia* species: A review. *International Research Journal of Pure and Applied Chemistry, 23*(6), 42–53.

Nahar, L., Sarker, S. D., & Delazar, A. (2021). Phytochemicals and their potential roles in neuroprotection. *Phytotherapy Research, 35*(8), 4195–4214.

Onyeka, T. C., Ugochukwu, C., & Nwankwo, B. (2023). Current trends in dementia management in Nigeria: Bridging treatment gaps. *Nigerian Journal of Clinical Practice, 26*(3), 287–293.

Orhan, I. E., Senol, F. S., Demirci, B., & Baser, K. H. (2017). Profiling of in vitro neurobiological effects and phenolic acids of selected endemic *Salvia* species. *Pure and Applied Chemistry, 89*(4), 541–555.

Ouahhoud, S., Mamri, S., Morjane, I., Laaroussi, H., Elbouzidi, A., Aanniz, T., El Moussaoui, A., & Bouyahya, A. (2022). Evaluation of the antioxidant and antibacterial properties of extracts from Moroccan medicinal plants. *Scientific African, 15*, Article e01099.

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315. [Crossref]

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science, 5*, Article e47. [Crossref]

Qin, S., Fang, J., He, X., Yu, G., Yi, F., & Zhu, G. (2024). The therapeutic potential of *Dalbergia pinnata* essential oil in Alzheimer's disease: EEG signal analysis in vivo, SH-SY5Y model in vitro, and network pharmacology. *Biology, 13*(7), Article 544. [Crossref]

Raheja, S., Girdhar, A., Kamboj, A., Lather, V., & Pandita, D. (2021). Protective effect of *Dalbergia sissoo* extract against amyloid-β(1-42)-induced memory impairment, oxidative stress, and neuroinflammation in rats. *Turkish Journal of Pharmaceutical Sciences, 18*(1), 104–110. [Crossref]

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231–1237. [Crossref]

Sarikürkçü, C., Zengin, G., & Uysal, S. (2020). Screening of phenolic compounds and antioxidant properties of selected *Medicago* L. species collected from Turkey. *Molecules, 25*(2), Article 305.

Sharma, B., Singh, S., & Siddiqi, N. J. (2021). Bioactive compounds of *Dalbergia sissoo*: Comprehensive review on ethnopharmacology, phytochemistry and pharmacological activities. *Journal of Ethnopharmacology, 267*, Article 113515.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178. [Crossref]

Sultana, R., Perluigi, M., & Butterfield, D. A. (2021). Oxidative stress and inflammation in Alzheimer's disease: A review. *Journal of Alzheimer's Disease, 82*(3), 1137–1150.

Tenchov, R., Sasso, J. M., & Zhou, Q. A. (2024). Alzheimer's disease: exploring the landscape of cognitive decline. *ACS Chemical Neuroscience, 15*(21), 3800–3827. [Crossref]

Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Saunders Publishers.

Tripathi, A., Misra, R., & Singh, R. (2020). Pharmacological potential of alkaloids from medicinal plants: A review. *Journal of Pharmacy Research, 14*(3), 227–233.

Ward, R. J., Zucca, F. A., Duyn, J. H., Crichton, R. R., & Zecca, L. (2014). The role of iron in brain ageing and neurodegenerative disorders. *Lancet Neurology, 13*(10), 1045–1060. [Crossref]