

Phytochemical Profile and Bioactivities of *Anisomeles malabarica* (L) R.Br Against SH-SY5Y Neuroblastoma Cells

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ABSTRACT

Phytochemicals are the compounds that can complement traditional therapies, with a promising approach in anticancer treatment. This study investigates antiproliferative and the antioxidant activity of *Anisomeles malabarica* methanolic leaves extract. Phytochemical evaluation was done by GC-MS analysis, antioxidant potential was observed by DPPH, CAT, ABTS, hydroxyl radical and nitric oxide scavenging assays, in vitro AChE inhibitory activity and antiproliferative activity against SH-SY5Y cells was evaluated by MTT assay. Bioactive components included in the extract, such as (Z)Isoeugenol, 4-Vinylphenol, Phytol, and Pyrogallol, improve its antioxidant capability. The extract showed dose-dependent antioxidant activity, with DPPH scavenging (IC₅₀= 65.5 µg/mL) then hydroxyl radical scavenging (IC₅₀= 61.4 µg/mL). The extract also showed AChE inhibitory activity (IC₅₀=164.57 µg/mL), suggesting that it have neuroprotective and antioxidant potential. MTT assay revealed concentration-dependent decrease of cell viability. Finally, this study indicates the phytochemical composition, antioxidant capacity, AChE inhibitory activity and cytotoxic effects, indicating that extract has potent free radical neutralizing activity.

Keywords: *Anisomeles malabarica*, antioxidant activity, GC-MS, AChE Inhibition, SH-SY5 cell line, MTT assay

How to cite this article: Varatharajan R, Kalamegam K., Phytochemical Profile and Bioactivities of *Anisomeles malabarica* (L) R.Br Against SH-SY5Y Neuroblastoma Cells. Int J Drug Deliv Technol. 2026;16(81s): 795-893; DOI: 10.25258/ijddt.16.81s.103

INTRODUCTION

Neuroblastoma (NB) is cancer develops in the child's nerve tissues most prevalent extra cranial solid tumour, making up about 8–10% of all paediatric malignancies, extremely aggressive metastatic disease which shows extraordinary biochemical and clinical heterogeneity (Arsenian 2024) (Johnsen and Kogner, 2024). Surgery, chemotherapy, radiation, stem cell transplantation, immunotherapy are the current risk-based therapeutic methods (Alkhalaz et al., 2025). Oxidative stress is the condition where there is an imbalance in ionic composition between the free radicals released by the cells and the insufficient antioxidants that can leads to cancer. The release of free oxygen molecules is the group of ROS (reactive oxygen species), which is a bundle of reactive molecules released from mitochondria, during its normal metabolic functions (Shukla et al., 2011). This will lead to normal cellular signaling and immune response whereas too much of ROS will make the cell unstable and make the cellular condition more stressed (Chong & Souayah, 2025). Excessive production of ROS leads to the production of high levels of oxidative stress will lead to cellular malfunctioning causing progressive cognitive impairment (Beal et al., 2005). Enzymatic and nonenzymatic antioxidants shields cells from oxidative stress, they convert the free oxygen molecules into stable molecules like water. Catalase, peroxidase, glutathione reductase, NADPH oxidase and superoxide dismutase are the enzymatic antioxidants studied widely in the ROS mechanism.

Over 86 billion neurons and 250–300 billion glial cells make up the human brain, which uses more than 20% of the oxygen. During the synthesis of adenosine triphosphate (ATP), the mitochondria in the brain convert O₂ to H₂O. Reactive oxygen species (ROS) escape from the mitochondria when there is too much of oxygen because the electron transport chain become inefficient and the brain's redox signaling system acts as an innate sensor to oxidative

stress leads to strong formation of reactive oxygen metabolites, low antioxidant levels, a low ability

for repair and leads to non-replicating neuronal brain tissue (Beal et al., 2005) (Cenini & Voos, 2020). Plants are the naturally occurring substances with secondary metabolites (Alam et al., 2022). having their antioxidant properties associated with their impact on the pathways of enzymes found in human mitochondria, which can slow down brain deterioration, and evidence suggests that oxidative stress substantially contribute to neurological deficits (Anwar et al., 2023). Secondary metabolites are beneficial for development of new drugs, due to distinct chemical constituents and biological activity.

Traditionally, *A. malabarica* is widely distributed plants species in India's Western ghats has been utilized in various folk remedies to treat various ailments (Santhanalakshmi et al., 2024). ROS remain neutralised and oxidative stress is decreased by natural products such as flavonoids and polyphenols (cheng et al., 2025). Polyphenols can reduce oxidative stress in neuronal cells (Mera et al., 2019) (De Almada-Vilhena et al., 2024). By increasing the antioxidant enzymes action these natural compounds shield neurones from oxidative damage and maintain cognitive function (Arias-Sanchez et al., 2023). Thus the current study summarizes phytochemical components of the methanolic leaf extract of *A. malabarica* using GC-MS analysis in order to evaluate the total phenolic and flavonoid contents, antioxidant activity, acetylcholinesterase inhibitory potential, and antiproliferative activity against SH-SY5Y human neuroblastoma cells.

MATERIALS AND METHODS

PLANT COLLECTION AND PREPARATION OF EXTRACTS

Fresh aerial parts of *A. malabarica* were collected from Salem district, Tamilnadu and authenticated at Botanical survey of India, Coimbatore with the number BSI/SRC/5/23/2024-25/Tech/533, collected leaves are shade-dried at room temperature for 3-5 days and powdered and stored in zip lock covers at room temperature until further usage.

METHANOL EXTRACTION

With the modified procedure of hanif *et al.*, 2023, powdered leaf sample of 10g dissolved in 100ml of methanol in airtight conical flask enclosed with cotton to prevent contamination and to obtain higher yield of concentration. The content was kept on mechanical orbital shaker for 24 hrs at room temperature and aqueous solution was filtered by muslin cloth and collected. Finally, the solvent, methanol was evaporated from the filtrates by keeping at 40 °C in oven, aqueous crude methanolic extract was concentrated for further analysis.

PHYTOCHEMICAL SCREENING

GC-MS ANALYSIS

To analyze the phytochemicals present in the methanolic leaves extract of *A. malabarica* Gas Chromatography-Mass Spectrometry (GC-MS) study was done using GCMSQP2010SE instrument, fortified with a capillary column with the instrumental conditions, column oven temperature initial: 50°C, final: 280°C, temperature program rate 60°C/min, injection temperature 250°C, split injection mode with pressure 68.1 kPa, total flow 16.2 ml/min with carrier gas helium (99.99% purity). The separated compounds with their respective peaks and retention time were obtained through a comparison of their mass spectra with the NIST/Wiley library database (Sparkman *et al.*, 2011).

QUANTITATIVE ESTIMATIONS OF TOTAL PHENOLS AND FLAVONOIDS

IDENTIFICATION OF TOTAL PHENOLS

With some modifications, the total phenolic compounds were examined using the Folin-Ciocalteu reagent method (Spanos and Wroslstad, 1990). 1ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water), and 100 µl of *A. malabarica* methanol extract (1 mg/ml) were combined then 1ml of a 20% Na₂CO₃ solution was added, after 30 minutes incubation in dark at 35°C, absorbance was measured using UV-vis spectrophotometer. The gallic acid is used as reference to express the total phenolic content.

DETERMINATION OF TOTAL FLAVONOIDS

Using a slightly altered methodology of Chang *et al.*, 2002 technique of aluminium chloride reagent method, 500µl of plant extract was added with 0.5 ml of methanol and 0.5 ml of (5% w/v) sodium nitrite solution followed by 1 ml of 1M NaOH and 0.5 ml of 10% aluminium chloride solution. The absorbance at 510 nm was measured after the mixture was incubated for 30 minutes at room temperature.

ANTIOXIDANT POTENTIAL ASSAYS (IN-VITRO)

FREE RADICAL SCAVENGING ACTIVITY

The DPPH radicals were measured in order to assess the ability of *A. malabarica* leaves extract to eliminate free radicals (Blois 1958). The samples at various concentrations (20, 40, 60, 80, and 100µg/ml) were added to 3 ml of DPPH solution (0.1 mM) thoroughly mixed and incubated at 20°C in the dark for 30 minutes. Following incubation, DPPH in methanol solution was employed as a negative control and ascorbic acid as a positive control for measuring absorbance at 517 nm using a UV-Vis spectrophotometer.

ESTIMATION OF CATALASE (CAT)

Catalase activity was measured by the method Sinha *et al.*, (1972) The plant sample (at concentrations

10-100 µg/ml) was added and make up to 1ml of 0.01M (pH.7.0) phosphate buffer and 0.4ml of 2M

H₂O₂. The reaction was stopped after 2-3 minutes by the addition of 2.0 ml of dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1.3 ratio). The concentration of catalase was assayed at 620 nm.

ABTS RADICAL SCAVENGING ABILITY

The ability of leaves extracts to scavenge ABTS radicals (2, 20 - Azinobis-3-ethyl benzothiazolone 6 sulfonic acid) was identified by the technique of Re *et al.*, (1999). ABTS solution was done by mixing 3.75 mM ABTS di-ammonium salt with 1.225 mM potassium persulphate solution, and it was incubated overnight at 30°C. ABTS cationic free radical solution was adjusted to the absorbance at 734 nm. Standard ABTS solution 200µl was assorted with diverse concentrations (10 – 100 µg/ml), and the absorbance was read at 734 nm for every 5 min up to 60 min.

HYDROXYL RADICAL SCAVENGING ABILITY

The scavenging ability of hydroxyl radicals (OH) was measured with the leaves extract (10 – 100µg/ml) was added to 1ml of ion-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5ml of EDTA solution (0.018%) and 1ml of DMSO (0.85% v/v in 0.1M phosphate buffer, pH 7.4). 0.5 ml of ascorbic acid (0.22%) was added, and the mixture was incubated in a water bath at 80–90°C for 15 minutes. Following incubation, 1 ml of ice-cold TCA (17.5%w/v) was added to stop the reaction. This was carried out by the addition of 3 ml of Nash reagent (7.5 grams of ammonium acetate, 0.3 ml of glacial acetic acid, and 0.2 ml of acetyl acetone, which were added together to make 100 ml with distilled water) and left 15 mins at room temperature. A UV Vis spectrophotometer was used to measure. The reaction mixture without sample was used as a negative control (Umamaheswari *et al.*, 2025) whereas; BHT (Butylated hydroxytoluene) was used as a positive control. Percentage of hydroxyl radical scavenging ability (HRSA) was calculated.

NITRIC OXIDE FREE RADICAL SCAVENGING ACTIVITY

Plant extracts with concentrations of 10 to 100 µl dissolved in DMSO were taken, and the volume was made up to 150 µl with methanol. The solutions were incubated at room temperature for 15 mins after 2.0 ml of sodium nitroprusside (10 mM) in phosphate buffer saline was added, then 5 ml of Greiss reagent added followed by the incubation, methanol was used as a blank. The IC₅₀ values were analyzed by evaluating absorbance at 546 nm (Conforti *et al.*, 2025).

INVITRO ACETYLCHOLINESTERASE INHIBITION ASSAY

The acetylcholinesterase (AChE) activity was estimated using a spectrophotometric method. The first cuvette that served as a blank to determine the non-enzymatic hydrolysis of acetylcholine contained 500 µL DTNB solution (3 mM in 0.1 M potassium phosphate buffer pH 8), 100 µL ACh (15 mM in water), 275 µL potassium phosphate buffer (0.1 M, pH 8), and 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml of plant leaves methanol extract solution. The reaction cuvette contained 0.16 µg/ml of AChE solution instead of 25 µl of buffer. A yellow colour compound develops when the thiocholine is formed during the hydrolysis of acetylcholine which rapidly reacts with DTNB. The absorbance was observed for five minutes at 405 nm (Ellman *et al.*, 1961).

CELL LINE AND MAINTENANCE

The human neuroblastoma cell line (SH-SY5Y) was attained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were preserved at 37°C, 5% CO₂, 95% air and 100% relative humidity.

CELL TREATMENT

Using 100 µL of culture media containing 5% FBS, SH-SY5Y cells (1 × 10⁴ cells/mL) were seeded onto 96-well plates and incubated under conventional culture conditions. The cells were

allowed to adhere to the wells at 37°C, 5% CO₂, 95% air, and 100% relative humidity. After 24-hours *A.malabarica* leaf extract was administered to the cells at concentrations ranging from 18.75 to 300 µg/mL. After 24 hours, the samples were dissolved in plain DMSO and then diluted with serum-free medium to a double the required final concentration. The plates were incubated for an additional 48 hours at 37 °C, 5% CO₂, 95% air, and 100% relative humidity after the sample was added (Kovalevich & Langford 2013).

MTT ASSAY

The yellow tetrazolium salt 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a soluble salt. This tetrazolium ring is broken by succinate-dehydrogenase, an enzyme

found in living cells' mitochondria, turning MTT into an insoluble purple formazan. Following 48 hours of incubation, each well received 15 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS), which was then incubated for 4 hours at 37°C. The MTT-containing solution was then turned off, and the formazan crystals that had formed were dissolved in 100 µl of DMSO before the absorbance at 570 nm was recorded (Riss *et al.*, 2016).

RESULTS AND DISCUSSION GC-MS ANALYSIS

The GC-MS chromatogram revealed several compounds, only the major bioactive components are shown in Table 1, due to their probable pharmacological significance. Particularly identified (Z)Isoeugenol being the predominant constituent (41.98%), together with 4-vinylphenol, phytol and pyrogallol (Fig 1).

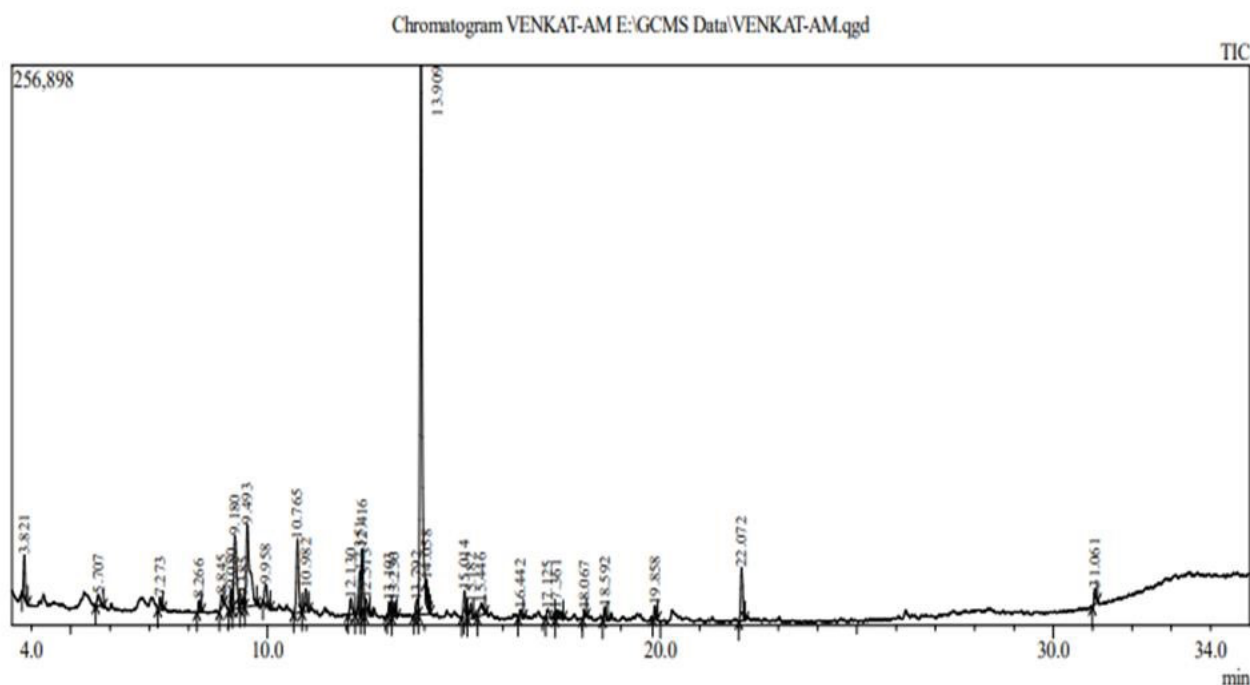


Figure 1: GC MS Chromatogram of aqueous methanolic extracts of *A.malabarica* leaves

Table 1: Bioactive Compounds Identified in *A. malabarica* leaves via GC-MS

		GC-MS ANALYSIS	
Peak	Retention Time (min)	Chemical Name	Common Name
9	9.493	4-Vinylphenol	p-Vinylphenol
19	13.792	Pyrogallol, 3TBDMS derivative	Pyrogallol derivative
20	13.909	Benzene, 1,2-dimethoxy-4-propenyl-, (Z)-	Isoeugenol (Z-isomer, high abundance)
31	22.072	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol

The GC-MS analysis of the *A.malabarica* methanolic extract revealed the occurrence of 32 phytochemical constituents with varying peak areas, the most evident compound known was Benzene, 1,2-dimethoxy-4-propenyl-, (Z)- (Peak 20, RT: 13.909 min) that is 41.98% of the total peak area. This compound is chemically known as (Z)-Isoeugenol, a phenylpropanoid. Isoeugenol is reported to possess antioxidant properties due to the presence of methoxy groups that donate electrons to stabilize free radicals and interact with cholinesterase binding sites (lobo *et al.*, 2005). Isoeugenol is a powerful phenolic antioxidant that scavenges superoxide anions produced by the xanthine–xanthine oxidase pathway, its conjugated double bond in its alkyl side chain, increases the stability of the phenoxyl radical through electron delocalization. Isoeugenol and eugenol have been shown to reduce inflammation, neuronal deficits, and oxidative stress mediators. (Alyami *et al.*, 2025; Damasceno *et al.*, 2024).

Another compound, 4-Vinylphenol (Peak 9, 9.83%) common phytochemical, is a phenolic compound that inhibits AChE via targeting the peripheral anionic site and catalytic active site of

AChE, contributing to its cytotoxic effect (Colovic *et al.*, 2013). Phytol (Peak 31, RT: 22.072 min, Area: 3.18%) is a diterpenoid that possesses anti-inflammatory and antioxidant activity. According to the study, phytol inhibits AChE through enhancing cholinergic transmission and interacting with AChE (Hung *et al.*, 2025).

A trihydroxybenzene was identified as pyrogallol (Peak 19, 1.16%). This acts by single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms. The ability of this compound to reduce oxidative stress in the neurons was demonstrated earlier by Liu *et al.*, 2012. A chromeno [2,3-b] pyridine derivative was identified as Peak 28 (0.35%). This is a minor component; however, it was previously reported as a pharmacologically active compound with dual-binding site AChE inhibitors properties, capable of forming hydrogen bonds and π - π interactions simultaneously at the catalytic and peripheral anionic sites (Cheung *et al.*, 2012).

QUANTITATIVE ESTIMATIONS OF TOTAL PHENOLS AND FLAVONOIDS

The total flavonoid content of *A. malabarica* was 59.78 ± 0.32 $\mu\text{g}/\text{mg}$, while the total phenolic content was 269.6 ± 0.39 $\mu\text{g}/\text{mg}$

(Fig 2). These findings denote that antioxidants were found to be higher because of the presence of increased phenolic and flavonoid contents. Plants are capable of producing a wide variety of secondary metabolites. Many of them are bioactive compounds used as defense chemicals by plants (Newman *et al.*, 2020).

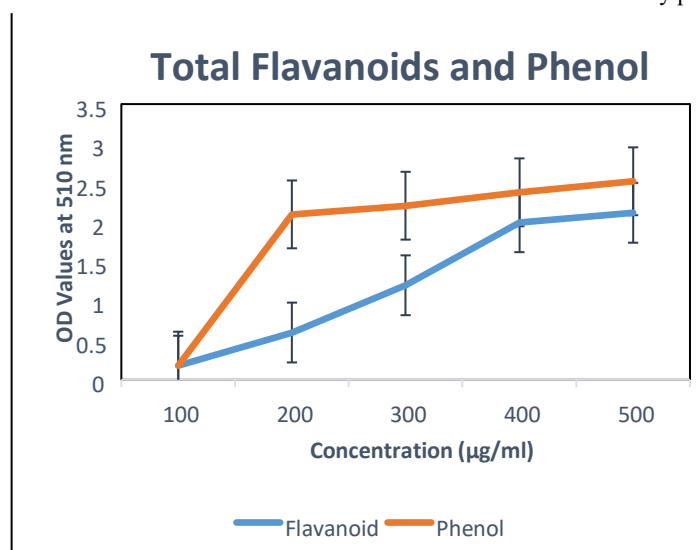


Figure 2: Total Flavonoid and Phenol content of leaves extracts of *A. malabarica*

DPPH RADICAL SCAVENGING ACTIVITY

The ability of methanolic extracts of *A. malabarica* leaves to scavenge radicals against DPPH was determined spectrophotometrically. DPPH reacts with an antioxidant compound that can donate hydrogen and it gets reduced. The change in colour (from deep violet to light yellow) was measured. DPPH is a steady free radical and accepts an electron or hydrogen radical to become a stable molecule. The methanolic leaf extract of present study showed an increase in DPPH radical scavenging activity in the concentration dependent manner as evidenced by the gradual decrease in absorbance with increasing extract concentration. The percentage inhibition increased from 18.34 % at 20 $\mu\text{g}/\text{mL}$ to 58.98 % at 100 $\mu\text{g}/\text{mL}$ (Fig 3) which

showed increased free radical scavenging activity at higher concentrations. The extract has considerable antioxidant potential with an IC_{50} of 65.5 $\mu\text{g}/\text{mL}$. The lower the IC_{50} values, the better the antioxidant activity as less amount of extract is required to scavenge 50% of DPPH radicals (Gulcin, 2020). Reactive oxygen species are the reactive molecules that disrupt cellular processes by damaging lipids, proteins, and DNA, leading to cellular damage and death in particular dopaminergic neuronal cells including the SH-SY5Y cell line. (Riss *et al.*, 2016; Choudhari *et al.*, 2020). As an evidence of cytotoxicity, the current study demonstrated the loss of cell viability that were induced by *A. malabarica* leaf methanolic extract

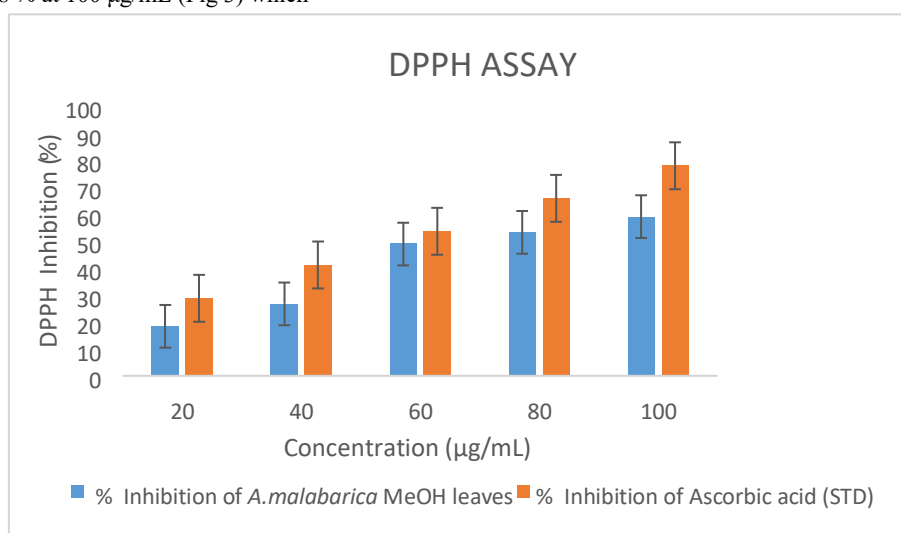


Figure 3: DPPH scavenging activity of leaves of *A. malabarica*

ESTIMATION OF CATALASE (CAT)

Catalase is an antioxidant enzyme that catalyzes the breakdown of hydrogen peroxide (H_2O_2) to water and oxygen, determined by measuring the reduction in absorbance of H_2O_2 at 240 nm using a UV-Visible spectrophotometer. The rate of decrease in absorbance is directly proportional to catalase activity, indicating its potential antioxidant enzyme activity. The extreme catalase action was observed at 72.85 $\mu\text{g}/\text{mL}$ (Fig 4), suggesting that

A. malabarica exhibits significant enzymatic antioxidant potential. The methanolic leaf extract showed a dose-dependent growth in catalase activity, confirming its potential to scavenge hydrogen peroxide to reduce oxidative stress. catalase enzyme converts hydrogen peroxide molecules into oxygen molecule hydrogen peroxide molecule is reduced in the first phase of the chemical pathway to create an intermediate product I, a covalent oxyferryll

species with a porphyrin π -cation radical in order to create the free enzyme, oxygen, and water in the second step reaction, chemical I is reduced by redox reactions by a two-electron transfer from an

electron donor (Von Ossowski *et al.*, 1993) (Deisseroth *et al.*, 1970)

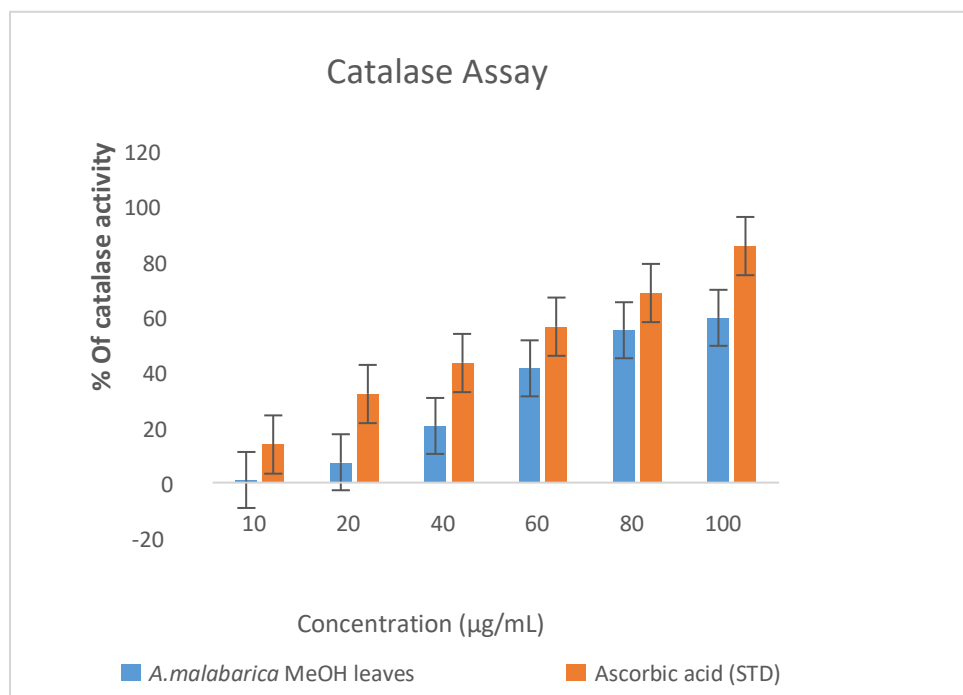


Figure 4: Effect of *A. malabarica* Leaf Extract on Catalase Activity

ABTS RADICAL SCAVENGING ABILITY

The scavenging activity of *A. malabarica* leaf extract on ABTS was found to increase with increase in concentration with an IC_{50} value 90.68 µg/mL. Low concentration of leaf extract (10 µg/ml) showed moderate scavenging effect while high concentration (100 µg/ml) showed high scavenging activity as compared with ascorbic acid. At 100 µg/mL, scavenged ABTS radicals by 58.98% (Fig 5), which was 24.20% lower than that of ascorbic

acid, a standard antioxidant (77.82%). The presence of antioxidant compounds like phenolics and flavonoids which donate electrons or hydrogen atoms to neutralize free radicals was determined by measuring scavenging ability. Hydrogen donation is known to be associated with DPPH and ABTS free radical scavenging, which is a measure of antioxidant activity (Lee *et al.*, 2015).

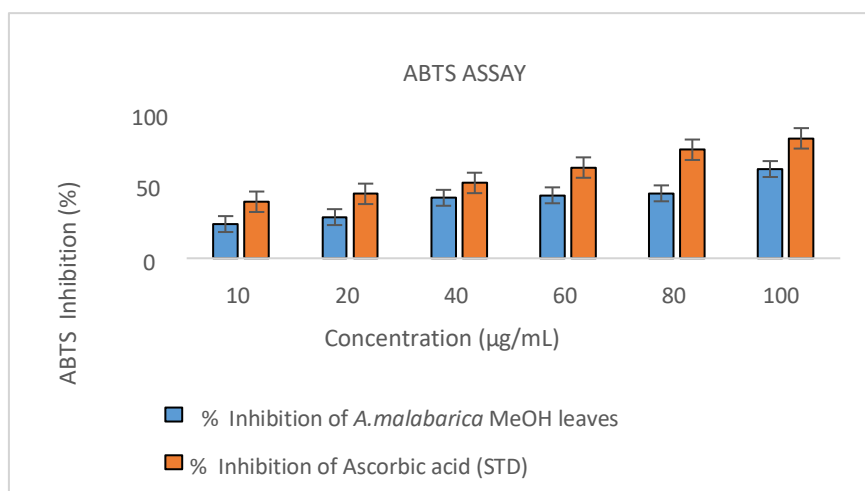


Figure 5: Effect of *A. malabarica* leaf extract on ABTS Activity

HYDROXYL RADICAL SCAVENGING ABILITY

The hydroxyl radical (OH) scavenging assay measures the ability of a compound to scavenge hydroxyl radicals (highly reactive ROS intermediates which can cause DNA, lipid, or protein damage) generated by the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} +$

$\bullet OH + OH^-$), and the reduction in the formation of degradation products (for example, deoxyribose or other molecules (treml *et al.*, 2016). *A. malabarica* leaf extract demonstrated a scavenging activity with an IC_{50} equal to 61.39 µg/ml (Fig 6), which is most prominent.

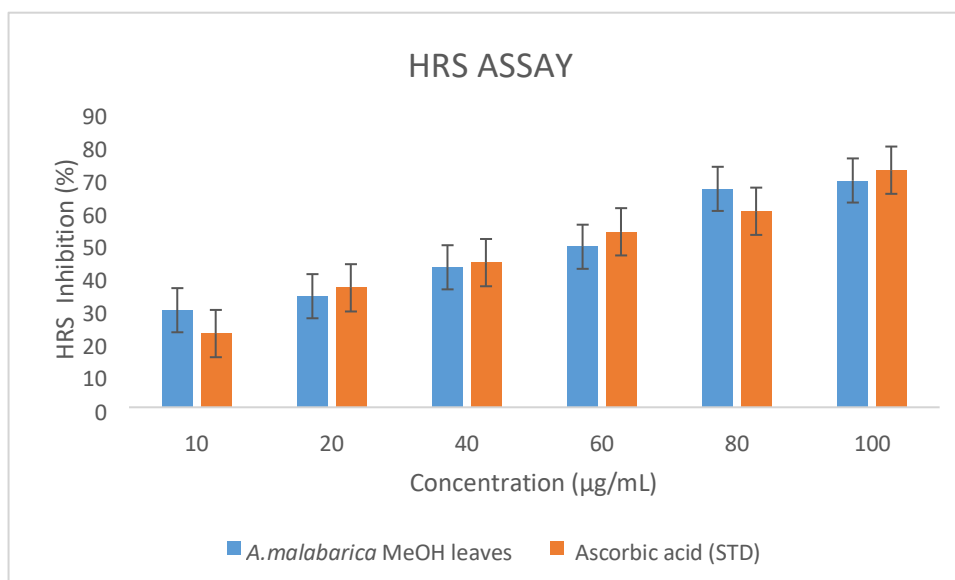


Figure 6: Effect of *A. malabarica* leaf extract on HRS Activity

NITRIC OXIDE FREE RADICAL SCAVENGING ACTIVITY

The scavenging activity of the leaf extract at the lowest concentration (10 µg/ml), moderate inhibition of NO radicals was observed, whereas the highest inhibition occurred at 100 µg/ml compared to standard, but the extract showed substantial radical

quenching ability, confirming its antioxidant potential demonstrating that the leaf extract with the IC_{50} value 141.30 µg/ml (Fig 7) possesses significant nitric oxide scavenging activity. Nitric oxide is a highly unstable, through the intermediates NO_2 , N_2O_4 , and N_3O_4 , it combines with O_2 to form the stable products nitrates and nitrite (Liang *et al.*, 2026)

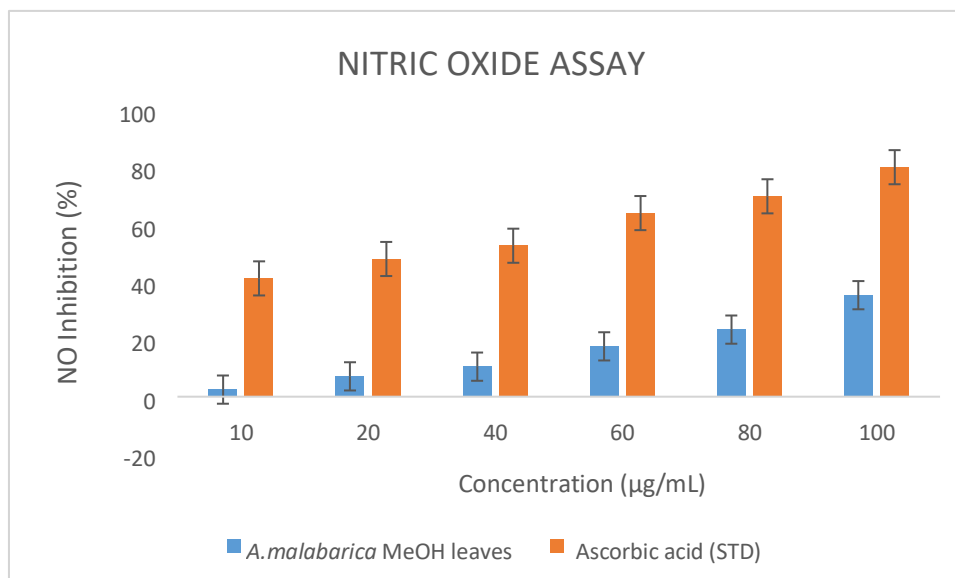


Figure 7: Effect of *A. malabarica* leaf extract on NO Activity

ACETYLCHOLINESTERASE (ACHE) INHIBITORY ACTIVITY AND CYTOTOXIC POTENTIAL

The bioactive potential of *A. malabarica* leaf extract was evaluated by the acetylcholinesterase (AChE) inhibitory activity (Ellman *et al.*, 1961). The extract showed the concentration dependent increase of AChE inhibition with the inhibition values from 15% at 50 µg/mL to 72% at 250 µg/mL. The consistent

inhibition observed for galanthamine was in the range of 21% to 94% indicating the higher inhibitory efficiency of the standard compound. The IC_{50} value of *A. malabarica* leaf extract was calculated as 164.57 µg/mL while galanthamine had an IC_{50} value of 120.14 µg/mL (Fig 8). The increased IC_{50} of the extract indicates relatively lower enzyme inhibitory potency indicates the presence of active phytoconstituents contributing to its biological activity.

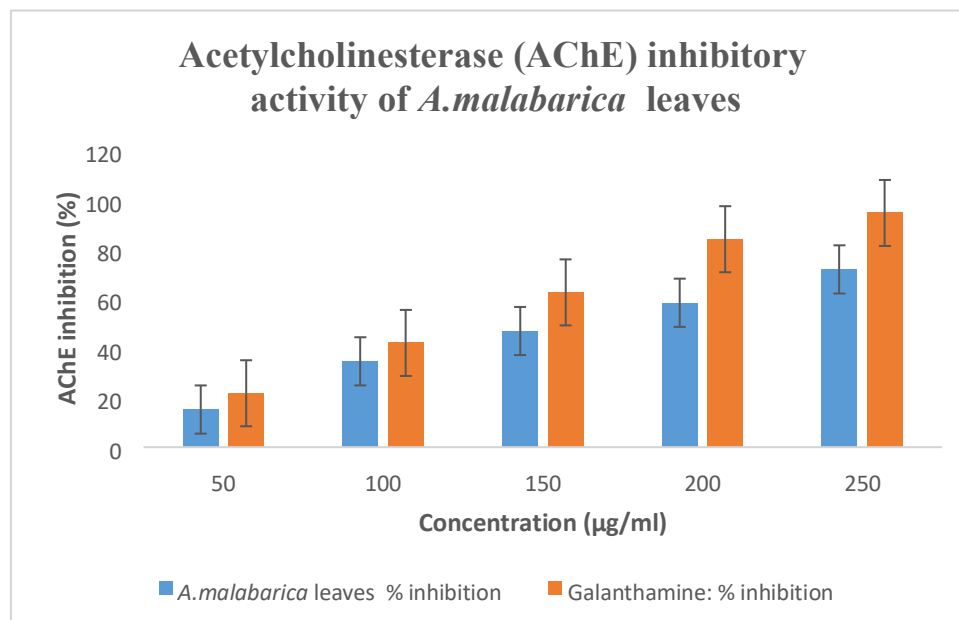


Figure 8: Acetylcholinesterase (AChE) inhibitory activity of *A. malabarica* leaves

The presence of secondary metabolites, phenolic and flavonoids are known to interact with enzymatic targets through hydrogen bonding, hydrophobic interactions, and modulation of oxidative pathways, inducing cellular response (Kumar *et al.*, 2021; Ghofrani *et al.*, 2022). As the hydroxyl group promote interaction with the AChE active site, phenolic-rich plant extracts have been shown to have acetylcholinesterase inhibitory activity (Ayaz *et al.*, 2020; Szwajgier *et al.*, 2021). It can cause oxidative stress modulation, mitochondrial dysfunction, and changes in cellular metabolic activity (Alam *et al.*, 2021; Zhang *et al.*, 2022).

MTT ASSAY

Cell viability was determined by MTT assay, which indicates the reducing ability of MTT to formazan, with reduced cell viability, suggesting its cytotoxic activity against SH-SY5Y neuronal cancer

cells. As shown in the figure below (Fig 8), cell viability decreased with increasing concentrations of methanolic plant crude extract, suggesting that it has more anti-proliferative potential with an IC_{50} value of 126.20 µg/ml (Gadade and Patil, 2024).

The values below 200 µg/ml were found to be biologically active (Naeem *et al.*, 2022). According to the phase-contrast microscopy, with the increasing of the extract concentration, the following signs of cell death were observed: cell shrinkage, reduction of cell density, membrane blebbing, and increased amount of cellular debris. Moreover, the strongest damage was induced by 300 µg/mL of the extract. The typical signs of apoptosis were closely related to the decrease in cell viability following the treatment with (IC_{50} = 126.20 µg/mL). Thus, the data proved the anti-proliferative effect of the herbal extract on SH-SY5Y cells (Granica *et al.*, 2022; Spagnuolo *et al.*, 2025).

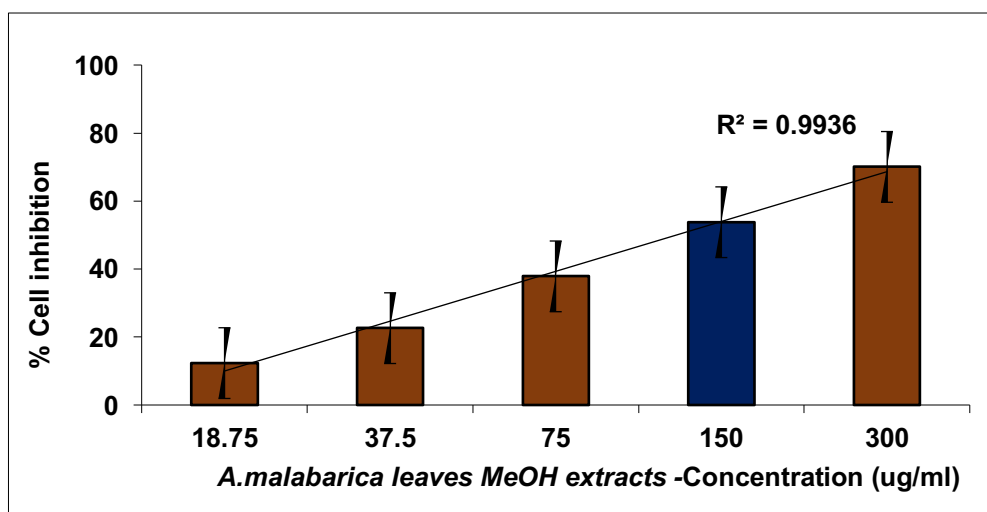


Figure 8: Dose-dependent percentage inhibition of SH-SY5Y cell viability by *A. malabarica* leaves MeOH extracts measured using MTT assay.

From the GC-MS analysis, it was recognized that Benzene, 1,2-dimethoxy-4-propenyl-, (Z)- ((Z)isoeugenol,) (Peak 20; RT: 13.909 min) was the major essential phytochemical isolated from the extract with a percent peak area of 41.98%. Reactive oxygen species (ROS) are highly reactive and destructive molecules that when present in high amounts, target various cellular macromolecules altering their physiological functions and causing

toxicity to dopaminergic neuronal cells such as the SH-SY5Y cell line (Riss *et al.*, 2016; Choudhari *et al.*, 2020).

The presence of (Z)-isoeugenol and the high concentration of phenolic and flavonoid contents in the extracts elucidate the anti-proliferative activity of on the SH-SY5Y cell line through the induction of apoptosis (Kamatou *et al.*, 2012; Khan *et al.*, 2019; Spagnuolo *et al.*, 2025). This is through the regulation of intracellular ROS, mitochondrial membrane potential

depolarization, caspase-dependent apoptotic pathways activation, or cell-cycle deregulation leading to chromatin condensation, cell shrinkage, membrane blebbing, and detachment from the culture flask (Fulda, 2010). Thus, based on the MTT assay and

morphological observation, it is concluded that *A. malabarica* possesses significant anti-proliferative potential against the SH-SY5Y neuroblastoma cell line.

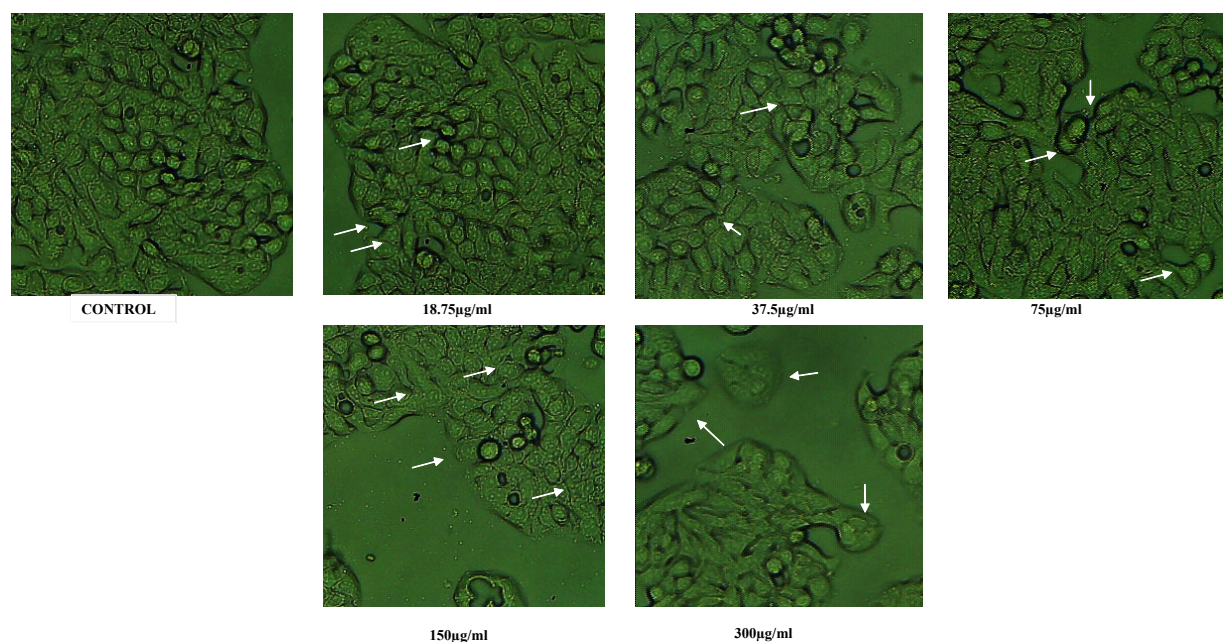


Figure 9: Representative phase-contrast microscopic images of SH-SY5Y neuroblastoma cells treated with *A. malabarica* methanolic leaf extract at concentrations of 18.75, 37.5, 75, 150, and 300 µg/mL for 24 h. The lowest concentration (18.75 µg/mL) showed cells with relatively normal morphology, whereas increasing extract concentrations induced progressive cytotoxic changes characterized by cell rounding, shrinkage, reduced cell density, membrane blebbing, loss of cell-to-cell adhesion, increased intercellular spaces, cellular detachment, and accumulation of cellular debris, scale bar 100µm. Arrows indicate the characteristic morphological alterations observed at each concentration.

CONCLUSION

In conclusion, methanolic leaf extracts of *A. malabarica* showed dose dependent cytotoxic effects on SH-SY5Y neuroblastoma cells. As (Z)-isoeugenol (Benzene, 1,2-dimethoxy-4-propenyl-) is the major phytoconstituent, this bioactive molecule is liable for the antiproliferative activity and this is the first report on the cytotoxic activity of this compound from the crude extract of *A. malabarica* and specifies its potential role for further anticancer drug discovery.

REFERENCES

- Alam S., Mohammad T., Padder R.A., Hassan M.I. and Husain M., Thymoquinone and quercetin induce enhanced apoptosis in non-small cell lung cancer in combination through the Bax/Bcl2 cascade, *J. Cell Biochem.*, 123, 259–274 (2022).
- Anwar S., Khan S., Shamsi A., Anjum F., Shafie A., Islam A., et al., Structure-based investigation of MARK4 inhibitory potential of Naringenin for therapeutic management of cancer and neurodegenerative diseases, *J. Cell Biochem.*, 122, 1445–1459 (2021).
- Arias-Sanchez R.A., Torner L. and Fenton Navarro B., Polyphenols and neurodegenerative diseases: potential effects and mechanisms of neuroprotection, *Molecules*, 28, 5415 (2023).
- Beal M.F., Oxidative damage as an early marker of Alzheimer's disease and mild cognitive impairment, *Neurobiol. Aging*, 26, 585–586 (2005).
- Blois M.S., Antioxidant determinations by the use of a stable free radical, *Nature*, 181, 1199–1200 (1958).
- Brooke D.N., Crookes M.J., Gray D. and Robertson S., Environmental risk assessment report: Decamethylcyclopentasiloxane, Environment Agency of England and Wales, Bristol, UK (2009).
- Cenini G. and Voos W., Mitochondria as potential targets in Alzheimer disease therapy: an update, *Front. Pharmacol.*, 10, 902 (2019).
- Chang C.C., Yang M.H., Wen H.M. and Chern J.C., Estimation of total flavonoid content in propolis by two complementary colorimetric methods, *J. Food Drug Anal.*, 10, 178–182 (2002).
- Cheng S., Gao H., Yang Y., Zhang M., Wang J., Li N., et al., Pharmacological diversity of flavonoids and their clinical application prospects in neurological disorders, *Phytother. Res.*, 39, 5222–5248 (2025).
- Cheung J., Rudolph M.J., Burshteyn F., Cassidy M.S., Gary E.N., Love J., et al., Structures of human acetylcholinesterase in complex with pharmacologically important ligands, *J. Med. Chem.*, 55, 10282–10286 (2012).
- Chong Z.Z. and Souayah N., Oxidative stress: Pathological driver in chronic neurodegenerative diseases, *Antioxidants*, 14, 696 (2025).
- Choudhari A.S., Mandave P.C., Deshpande M., Ranjekar P. and Prakash O., Phytochemicals in cancer treatment: From preclinical studies to clinical practice, *Front. Pharmacol.*, 10, 1614 (2020).
- Colovic M.B., Krstic D.Z., Lazarevic-Pasti T.D., Bondzic A.M. and Vasic V.M., Acetylcholinesterase inhibitors: pharmacology and toxicology, *Curr. Neuropharmacol.*, 11, 315–335 (2013).
- Conforti F., Statti G., Loizzo M.R., Sacchetti G., Poli F. and Menichini F., In vitro antioxidant effect and inhibition of α -amylase of two varieties of *Amaranthus caudatus* seeds, *Biol. Pharm. Bull.*, 28, 1098–1102 (2005).

- de Almada-Vilhena A.O., Dos Santos O.V., Machado M.D.A., Nagamachi C.Y. and Pieczarka J.C., Prospecting pharmacologically active biocompounds from the Amazon rainforest: in vitro approaches, mechanisms of action based on chemical structure, and perspectives on human therapeutic use, *Pharmaceuticals*, 17, 1449 (2024).
- Deisseroth and Dounce A.L., Catalase: physical and chemical properties, mechanism of catalysis, and physiological role, *Physiol. Rev.*, 50, 319–375 (1970).
- Ellman G.L., Courtney K.D., Andres V. Jr. and Featherstone R.M., A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.*, 7, 88–95 (1961).
- Gadade J.P. and Patil S.A., Evaluation of cytotoxic activity of plant extracts against SH-SY5Y neuroblastoma cell line, *J. Cancer Res. Ther.*, 20, 1–6 (2024).
- Hanif A., Ibrahim A.H., Ismail S., Al-Rawi S.S., Ahmad J.N., Hameed M., et al., Cytotoxicity against A549 human lung cancer cell line via the mitochondrial membrane potential and nuclear condensation effects of *Nepeta paulesenii* Briq., a perennial herb, *Molecules*, 28, 2812 (2023).
- Hanif A., Nisar M., Khan M.A., Mushtaq Z. and Zia M., Phytochemical profiling and biological evaluation of medicinal plant extracts using methanolic extraction techniques, *J. Appl. Res. Med. Aromat. Plants*, 32, 100451 (2023).
- Houldsworth A., Role of oxidative stress in neurodegenerative disorders: a review of reactive oxygen species and prevention by antioxidants, *Brain Commun.*, 6, fcd356 (2024).
- Hung L.W., Sanbonmatsu K.Y., Williams R.F. and Chen J.C.H., Acetylcholinesterase: Structure, dynamics, and interactions with organophosphorus compounds, *Protein Sci.*, 34, e70297 (2025).
- Ivancich H., Jouve H., Sartor B. and Gaillard J., EPR investigation of compound I in *Proteus mirabilis* and bovine liver catalases: formation of porphyrin and tyrosyl radical intermediates, *Biochemistry*, 36, 9356–9364 (1997).
- Jabar L.A., et al., MTT-based evaluation of plant-derived phenolics: Antiproliferative effects in cancer models, *Int. J. Adv. Biochem. Res.*, 9, 112–120 (2025).
- Kovalevich J. and Langford D., Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology, *Methods Mol. Biol.*, 1078, 9–21 (2013).
- Lee K.J., Oh Y.C., Cho W.K. and Ma J.Y., Antioxidant and anti-inflammatory activity determination of one hundred kinds of pure chemical compounds using offline and online screening HPLC assay, *Evid. Based Complement. Alternat. Med.*, 2015, 165457 (2015).
- Liang J., Huang T., Li J., Yang Z., Ni J. and Wang Y., The chemistry of the nitrate–nitrite–nitric oxide pathway: regulating muscle oxygenation and exercise performance, *RSC Adv.*, 16, 15723–15735 (2026).
- Lobo V., Patil A., Phatak A. and Chandra N., Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacogn. Rev.*, 4, 118–126 (2010).
- Beal M.F., Mitochondria take center stage in aging and neurodegeneration, *Ann. Neurol.*, 58, 495–505 (2005).
- Mera I.F.G., González D.E.G. and Morera Córdova V., Secondary metabolites in plants: Main classes, phytochemical analysis and pharmacological activities, *Rev. Bionatura*, 4, 1000–1009 (2019).
- Naeem A., et al., Natural products as anticancer agents: Current status and future perspectives, *Molecules*, 27, 836 (2022).
- Newman D.J. and Cragg G.M., Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019, *J. Nat. Prod.*, 83, 770–803 (2020).
- Phaniendra A., Jestadi D.B. and Periyasamy L., Free radicals: properties, sources, targets, and their implication in various diseases, *Indian J. Clin. Biochem.*, 30, 11–26 (2015).
- Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C., Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.*, 26, 1231–1237 (1999).
- Riss T.L., Moravec R.A., Niles A.L., Duellman S., Benink H.A., Worzella T.J. and Minor L., Cell viability assays, *Assay Guidance Manual*, Bethesda (MD), Eli Lilly & Company and the National Center for Advancing Translational Sciences (2016).
- Santhanalakshmi B., Sivanandhan G., Manojkumar S., Anitha S., Sharmistha G., Selvaraj N. and Kapildev G., Ethnopharmacological potential of *Anisomeles malabarica*: a systematic review on traditional uses and bioactive compounds, *Plant Biosyst.*, 158, 925–941 (2024).
- Shukla V., Mishra S.K. and Pant H.C., Oxidative stress in neurodegeneration, *Adv. Pharmacol. Pharm. Sci.*, 2011, 572634 (2011).
- Sinha A.K., Colorimetric assay of catalase, *Anal. Biochem.*, 47, 389–394 (1972).
- Spanos G.A. and Wrolstad R.E., Influence of processing and storage on the phenolic composition of Thompson seedless grape juice, *J. Agric. Food Chem.*, 38, 1565–1571 (1990).
- Sparkman O.D., Penton Z. and Kitson F.G., *Gas Chromatography and Mass Spectrometry: A Practical Guide*, Academic Press (2011).
- Trembl J. and Šmejkal K., Flavonoids as potent scavengers of hydroxyl radicals, *Compr. Rev. Food Sci. Food Saf.*, 15, 720–738 (2016).
- Umamaheswari J., Ilamaram M.C., Prabhu M.C., Abirami M. and Nadaraj V., Eco-Friendly Microwave Synthesis and Antioxidant Study of Bioactive Quinoline Scaffolds, *IJSAT-Int. J. Sci. Technol.*, 16, 1–8 (2025).
- Von Ossowski G., Hausner G. and Loewen P.C., Molecular evolutionary analysis based on the amino acid sequence of catalase, *J. Mol. Evol.*, 37, 71–76 (1993).